



UNIVERSIDADE
ESTADUAL DE LONDRINA

INGRID CAROLINE PINTO

**AVALIAÇÃO DO EFEITO TERAPÊUTICO DA RESOLVINA
D2 NA INFLAMAÇÃO E ESTRESSE OXIDATIVO INDUZIDOS
PELA RADIAÇÃO UVB EM CAMUNDONGOS**

Londrina
2019

INGRID CAROLINE PINTO

**AVALIAÇÃO DO EFEITO TERAPÊUTICO DA RESOLVINA
D2 NA INFLAMAÇÃO E ESTRESSE OXIDATIVO INDUZIDOS
PELA RADIAÇÃO UVB EM CAMUNDONGOS**

Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Mestre em Ciências da Saúde.

Orientadora: Prof^a. Dr^a. Rúbia Casagrande.

Londrina
2019

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Pinto, Ingrid Caroline.

Avaliação do efeito terapêutico da resolvina D2 na inflamação e estresse oxidativo induzidos pela radiação UVB em camundongos / Ingrid Caroline Pinto. - Londrina, 2019. 102 f. : il.

Orientador: Rúbia Casagrande.

Dissertação (Mestrado em Ciências da Saúde) - Universidade Estadual de Londrina, Centro de Ciências da Saúde, Programa de Pós-Graduação em Ciências da Saúde, 2019. Inclui bibliografia.

1. Fotocarcinogênese - Tese. 2. Inflamação - Tese. 3. Estresse oxidativo - Tese. 4. Resolvina - Tese. I. Casagrande, Rúbia. II. Universidade Estadual de Londrina. Centro de Ciências da Saúde. Programa de Pós-Graduação em Ciências da Saúde. III. Título.

INGRID CAROLINE PINTO

**AVALIAÇÃO DO EFEITO TERAPÊUTICO DA RESOLVINA D2 NA
INFLAMAÇÃO E ESTRESSE OXIDATIVO INDUZIDOS PELA
RADIAÇÃO UVB EM CAMUNDONGOS**

Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Mestre em Ciências da Saúde.

BANCA EXAMINADORA

Orientadora: Prof^a. Dr^a. Rúbia Casagrande
Universidade Estadual de Londrina – UEL

Prof^a. Dr^a. Graziela Sciantti Ceravolo
Universidade Estadual de Londrina – UEL

Prof^a. Dr^a. Sandra Regina Georgetti
Universidade Estadual de Londrina – UEL

Londrina, 28 de março de 2019.

Dedico à minha família, que me deu todo o suporte e incentivo para a realização desta conquista.

AGRADECIMENTOS

À Profa. Dra. Rúbia Casagrande, agradeço pela oportunidade, orientação, paciência e todo o aprendizado adquirido nesses dois anos que contribuíram muito para a minha formação científica e acadêmica. Muito obrigada!

Ao Prof. Dr. Waldiceu Verri Jr, não só por disponibilizar seu laboratório para realização de parte dos experimentos, mas também por todos os ensinamentos e o exemplo profissional.

Ao prof. Décio Sabbatini Barbosa pelo auxílio e suporte prestados para o uso dos equipamentos.

Aos membros da banca Graziela Scialanti Ceravolo, Sandra Regina Georgetti, Renata Micheli Martinez e Décio Sabbatini Barbosa, por terem aceitado o convite de participarem da banca examinadora da minha dissertação.

Aos meus companheiros de laboratório Renata, Clóvis, Camilla, Cristina e David pela ajuda, boas conversas e conhecimento adquirido de bancada. Agradeço em especial à Priscila pela amizade e também pela disponibilidade durante todo esse tempo.

Aos colegas do Laboratório de Dor, Inflamação, Neuropatia e Câncer pelo auxílio e aprendizado prestados na realização dos experimentos.

Aos meus pais Ivete e Sinésio, meu irmão Wilian e toda minha família. Agradeço por todo amor, exemplo, apoio e companheirismo, que se fazem presentes diariamente mesmo com toda a distância. Isso que me motiva a cada dia. Ao Bruno, pela parceria que adquirimos durante todos esses anos. E também agradeço às minhas amigas, que compartilharam comigo bons momentos e tornaram esta minha caminhada mais leve, principalmente nos dias difíceis. Sou muito grata por tudo!

Ao corpo docente do Programa de Pós-Graduação em Ciências da Saúde pelas aulas que contribuíram para a nossa formação.

Às funcionárias da Secretaria de Pós-Graduação em Ciências da Saúde por todo auxílio prestado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Fundação Araucária pelo apoio financeiro.

Enfim, meus sinceros agradecimentos a todos aqueles que contribuíram direta e indiretamente para realização deste trabalho e a conclusão desta etapa acadêmica.

PINTO, Ingrid Caroline. **Avaliação do efeito terapêutico da resolvina D2 na inflamação e estresse oxidativo induzidos pela radiação UVB em camundongos.** 2019. 102 f. Dissertação (Mestrado em Ciências da Saúde) – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

A pele é um órgão altamente metabólico e complexo e que representa uma barreira física protetora contra agentes externos. A radiação ultravioleta B (UVB) é o fator principal para o desenvolvimento de fotocarcinogênese, resultando em dano direto ao ácido desoxirribonucleico (DNA) com ocorrência de mutações. Além de causar danos extensos à pele diretamente, a radiação UVB também pode induzir lesão por mecanismos indiretos que envolvem a produção de radicais livres e intensa resposta inflamatória. Os mecanismos envolvidos na inflamação e estresse oxidativo induzidos pela radiação UVB são mecanismos passíveis de serem alvos de mediadores lipídicos como a resolvina D2 (RvD2). Estudos demonstram atividade terapêutica em alguns modelos de doença, porém não há conhecimento da ação da RvD2 no modelo de exposição à UVB. As resolvinas, de uma maneira geral, inibem a resposta inflamatória por meio da redução da produção de citocinas e recrutamento de células inflamatórias e, também, podem reprimir a ação do fator nuclear kappa B (NFκB), que está intimamente ligado à inflamação e ao estresse oxidativo, além de também induzirem a expressão de moléculas antioxidantes. Neste contexto, o objetivo desse trabalho foi avaliar o efeito da RvD2 no modelo de lesão cutânea induzido pela radiação UVB. O tratamento intraperitoneal com a RvD2 protegeu a pele contra os danos inflamatórios induzidos pela radiação UVB, pois reduziu significativamente os parâmetros de edema, atividade da mieloperoxidase e metaloproteinase-9, degradação das fibras colágenas, espessamento da epiderme, apoptose dos queratinócitos, número de mastócitos e produção de citocinas pró-inflamatórias e anti-inflamatórias. A RvD2 também foi capaz de diminuir significativamente os parâmetros oxidativos induzidos pela radiação UVB como a produção de ânion superóxido e hidroperóxidos lipídicos. Além disso, a capacidade antioxidante da pele foi recuperada de acordo com os resultados dos ensaios de níveis de glutathiona reduzida (GSH), poder antioxidante redutor de ferro (FRAP), capacidade redutora do radical 2,2' azinobis (3-etilbenzotiazolina-6-ácido sulfônico) (ABTS) e atividade da catalase. Esses resultados sugerem que o mediador lipídico RvD2 possui grande potencial para o controle da resposta inflamatória e do estresse oxidativo induzidos pela radiação UVB.

Palavras-chave: Fotocarcinogênese. Anti-inflamatório. Mediador lipídico. Resolvina. Antioxidante.

PINTO, Ingrid Caroline. **Evaluation of the therapeutic effect of resolvin D2 on UVB irradiation-induced inflammation and oxidative stress in mice.** 2019. 102 p. Dissertation (Master's degree in Health Science) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

The skin is a highly metabolic and complex organ and represents a protective physical barrier against external agents. Ultraviolet B (UVB) radiation is the main factor for the development of photocarcinogenesis, resulting in direct damage to deoxyribonucleic acid (DNA) with occurrence of mutations. In addition to causing extensive damage to the skin directly, UVB radiation can also induce injury by indirect mechanisms involving the production of free radicals and intense inflammatory response. The mechanisms involved in inflammation and oxidative stress induced by UVB irradiation can be targeted by lipid mediators such as resolvin D2 (RvD2). Studies have demonstrated therapeutic activity of these mediators in some disease models, but there is no knowledge of the role of RvD2 in the UVB exposure model. In general, resolvins inhibit the inflammatory response by reducing cytokine production and recruitment of inflammatory cells and may also suppress the action of nuclear factor kappa B (NFκB), which is closely linked to inflammation and oxidative stress, besides inducing the expression of antioxidant molecules. Thus, the objective of this study was to evaluate the effect of RvD2 on the model of skin lesion induced by UVB radiation. Systemic intraperitoneally treatment with RvD2 protected the skin against inflammatory damage induced by UVB radiation, as it significantly reduced the parameters of edema, myeloperoxidase and metalloproteinase-9 activity, collagen fibers degradation, epidermal thickness, keratinocyte apoptosis, number of mast cells and pro-inflammatory and anti-inflammatory cytokines production. RvD2 was also able to significantly decrease oxidative parameters induced by UVB radiation such as the production of superoxide anion and lipid hydroperoxides. In addition, the antioxidant capacity of the skin was recovered according to the results of the reduced glutathione levels (GSH), ferric reducing antioxidant power (FRAP), reductive capacity of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) and catalase activity. These results suggest that the lipid mediator RvD2 has great potential of use for the control of inflammatory response and oxidative stress UVB radiation-induced.

Keywords: Photocarcinogenesis. Anti-inflammatory. Lipid Mediator. Resolvin. Antioxidant.

LISTA DE ILUSTRAÇÕES

| | |
|---|----|
| Figura 1 – Espectro da radiação ultravioleta | 15 |
| Figura 2 – Representação esquemática da redução do oxigênio molecular até a formação de água e geração de EROs | 17 |
| Figura 3 – Estrutura química da RvD2 | 23 |
| Figura 4 – Fluxograma dos tempos de tratamento e coleta das peles dos animais de acordo com os protocolos experimentais pré-estabelecidos para avaliação da eficácia da RvD2 administrada via intraperitoneal através de parâmetros inflamatórios e oxidativos | 29 |

LISTA DE TABELAS

| | |
|--|----|
| Tabela 1 – Componentes do gel de separação e do gel de concentração | 31 |
|--|----|

LISTA DE ABREVIATURAS E SIGLAS

| | |
|--------------------------------|--|
| ABTS | 2,2' azinobis (3-etilbenzotiazolina-6-ácido sulfônico) |
| ANOVA | Análise de variância |
| AP-1 | Ativador de proteína-1 |
| ARE | Elemento de resposta antioxidante |
| CaCl ₂ | Cloreto de cálcio |
| CAT | Catalase |
| CEUA | Comissão de Ética no Uso de Animais |
| COX-2 | Ciclooxigenase-2 |
| CPD | Dímeros de ciclobutano |
| DHA | Ácido docosahexaenoico |
| DNA | Ácido desoxirribonucleico |
| DTNB | Ácido 5,5'-ditio-bis-(2-nitrobenzóico) |
| EDTA | Ácido etilenodiamino tetra-acético |
| ELISA | Ensaio imunoenzimático |
| EPM | Erro padrão da média |
| ERK | Quinase regulada por sinal extracelular |
| EROs | Espécies reativas de oxigênio |
| FRAP | Poder antioxidante de redução férrica |
| Gp91phox | Glutaciona peroxidase 91fox |
| GPCR | Receptor acoplado à proteína G |
| GPx | Glutaciona peroxidase |
| GR | Glutaciona redutase |
| GSH | Glutaciona reduzida |
| GSSG | Glutaciona oxidada |
| H ₂ O ₂ | Peróxido de hidrogênio |
| H ₃ PO ₄ | Ácido ortofosfórico |
| HCl | Ácido clorídrico |
| HTAB | Brometo de hexadecil trietil amônio |
| i.p. | Intraperitoneal |
| IL | Interleucina |
| INCa | Instituto Nacional de Câncer |
| IκB | Fator inibidor de κB |

| | |
|------------------------------|---|
| JNK | Quinase c-Jun N-terminal |
| KCl | Cloreto de potássio |
| LOOH | Hidroperóxidos lipídicos |
| MAPK | Proteína ativada por mitógeno |
| MMP | Metaloproteinase |
| MPO | Mieloperoxidase |
| NaCl | Cloreto de sódio |
| NADPH | Nicotinamida adenina dinucleotídeo fosfato |
| NBT | <i>Nitroblue tetrazolium</i> |
| NF-κB | Fator nuclear kappa B |
| NOX2 | NADPH oxidase 2 |
| NQO1 | NADPH quinona oxidoreductase 1 |
| Nrf2 | Fator nuclear [derivado eritroide-2] tipo 2 |
| O ₂ | Oxigênio molecular |
| O ₂ ^{•-} | Ânion superóxido |
| OH [•] | Radical hidroxila |
| OPD | Ortofenilenodiamina |
| p53 | Proteína citoplasmática de massa molecular 53 kDa |
| RNA | Ácido ribonucleico |
| RUV | Radiação ultravioleta |
| RvD1 | Resolvina D1 |
| RvD2 | Resolvina D2 |
| SDS | Dodecil sulfato de sódio |
| TCA | Ácido tricloroacético |
| TGF-β | Fator de transformação do crescimento beta |
| TNF-α | Fator de necrose tumoral alfa |
| TPTZ | 2,4,6 tripiridil-S-triazina |
| TRIS | Hidroximetil aminometano |
| UVB | Radiação ultravioleta B |

SUMÁRIO

| | | |
|----------|---|----|
| 1 | INTRODUÇÃO | 13 |
| 1.1 | A PELE | 13 |
| 1.2 | RADIAÇÃO ULTRAVIOLETA E SUAS CONSEQUÊNCIAS NA PELE | 14 |
| 1.3 | MECANISMOS FISIOPATOLÓGICOS DA RADIAÇÃO UVB: ESTRESSE OXIDATIVO E INFLAMAÇÃO..... | 16 |
| 1.4 | MEDIADOR LIPÍDICO PRÓ-RESOLUÇÃO: RESOLVINA D2..... | 21 |
| 2 | OBJETIVOS | 25 |
| 2.1 | OBJETIVO GERAL..... | 25 |
| 2.2 | OBJETIVOS ESPECÍFICOS..... | 25 |
| 3 | MATERIAIS E MÉTODOS | 26 |
| 3.1 | MATERIAIS | 26 |
| 3.1.1 | Reagentes | 26 |
| 3.1.2 | Equipamentos..... | 26 |
| 3.2 | MÉTODOS..... | 27 |
| 3.2.1 | Modelo de indução da lesão cutânea pela radiação UVB..... | 27 |
| 3.2.2 | Protocolo experimental de avaliação do potencial terapêutico da resolvina D2 em camundongos | 27 |
| 3.2.3 | Avaliação do edema cutâneo..... | 29 |
| 3.2.4 | Avaliação da atividade da enzima MPO | 29 |
| 3.2.5 | Determinação da atividade/secreção de metaloproteinase-9 (MMP-9) | 30 |
| 3.2.6 | Avaliação do poder antioxidante redutor de ferro (FRAP) | 32 |
| 3.2.7 | Avaliação do poder antioxidante pelo ensaio de sequestro do radical ABTS (2,2' azinobis (3-etilbenzotiazolina-6-ácido sulfônico) | 32 |
| 3.2.8 | Avaliação dos níveis do antioxidante endógeno GSH | 33 |
| 3.2.9 | Avaliação da atividade da CAT..... | 33 |
| 3.2.10 | Avaliação da produção de $O_2^{\cdot-}$ | 34 |
| 3.2.11 | Avaliação da produção de LOOH | 34 |
| 3.2.12 | Dosagem das citocinas pró-inflamatórias TNF- α , IL-33, IL-1 β e citocinas anti-inflamatórias TGF- β e IL-10 | 35 |
| 3.2.13 | Avaliação histopatológica por microscopia óptica..... | 35 |

| | | |
|----------|--|-----------|
| 3.3 | Análise estatística dos resultados..... | 36 |
| 4 | RESULTADOS E DISCUSSÃO – ARTIGO CIENTÍFICO | 37 |
| 4.1 | Resolvin D2 inhibits UV irradiation-induced inflammation and oxidative stress in the skin of hairless mice | 37 |
| 5 | CONSIDERAÇÕES FINAIS | 69 |
| 6 | REFERÊNCIAS | 70 |
| | ANEXOS | 80 |

1. INTRODUÇÃO

1.1. A PELE

De acordo com os dados do Instituto Nacional de Câncer (INCa), o câncer de pele do tipo não melanoma é o mais comum no Brasil e representa cerca de 30% dos tumores malignos. A estimativa para o ano de 2018 foi de aproximadamente 165 mil novos casos, sendo distribuídos em 85.170 casos para o sexo masculino e 80.410 casos para o sexo feminino. Sua ocorrência se deve principalmente à exposição solar excessiva e apesar de causar baixa mortalidade, a sua alta incidência pode justificar o número de óbitos. Além disso, o câncer de pele não melanoma pode apresentar chances elevadas de cura com o diagnóstico precoce. Entretanto, se o tratamento não ocorrer adequadamente, as lesões podem aumentar de tamanho e resultar em mutilações bastante expressivas, trazendo um grande impacto psicológico para os pacientes (INCa, 2018).

O câncer de pele não melanoma é comumente dividido em dois subtipos, basocelular e espinocelular, conforme a localização epidérmica do tumor e sua progressão. O carcinoma basocelular se origina a partir de células basais da camada da epiderme e possui crescimento lento, sendo o subtipo mais comum e com raros casos de metástase. Já o carcinoma espinocelular ocorre em células da parte externa epidérmica, com rápido desenvolvimento e uma capacidade maior de proliferação em outros tecidos (AMERICAN CANCER SOCIETY, 2016; INCa, 2018).

A pele, juntamente com seus anexos (glândulas, pelos e unhas), pertence ao sistema tegumentar e é o órgão responsável por revestir a superfície do nosso corpo, exercendo múltiplas funções como proteção contra agentes externos, a desidratação e o atrito, além de colaborar com a termorregulação e a excreção de várias substâncias. Sua camada mais externa é chamada de epiderme, constituída por epitélio estratificado pavimentoso queratinizado, que fornece uma barreira física direta para a proteção e que sofre constante renovação celular através de intensa atividade mitótica. Ademais, é o local onde se encontram vários tipos celulares como os melanócitos, queratinócitos, células de Langerhans e de Merkel e que desempenham funções importantes. Os melanócitos são células que sintetizam a melanina, a qual confere a pigmentação cutânea. Os queratinócitos são as células mais abundantes da epiderme e armazenam os grânulos de melanina em seu citoplasma, oferecendo proteção ao ácido desoxirribonucleico (DNA) através do bloqueio da radiação solar. Por fim, as células de Langerhans possuem como função a captação e processamento de antígenos e as células de Merkel são sensoriais, atuando como mecanorreceptores (DAMIANI; ULLRICH, 2016; JUNQUEIRA; CARNEIRO, 2013).

A camada intermediária que se liga à epiderme é a derme, que é composta por tecido conjuntivo contendo colágeno, vasos sanguíneos e linfáticos e terminações nervosas, além de anexos que são oriundos da epiderme como os folículos pilosos e as glândulas sudoríparas e sebáceas. É responsável por oferecer suporte mecânico, nutrição e oxigenação para a epiderme. Células como fibroblastos estão presentes na derme e são responsáveis pela síntese de componentes da matriz extracelular como o colágeno, e que juntamente com os mastócitos e queratinócitos epidérmicos, exercem função imunológica. A hipoderme, camada mais profunda da pele, é o tecido adiposo que funciona como reserva de energia e sustentação para órgãos subjacentes (FRIEDMANN et al., 1993; GELSE; PÖSCHL; AIGNER, 2003; HEATH; CARBONE, 2013; JUNQUEIRA; CARNEIRO, 2013).

Diante disso, a pele é um órgão altamente metabólico e complexo e funciona como uma interface entre o ambiente e o nosso organismo, e por isso é frequentemente exposta a fatores que ameaçam a sua integridade e função. A radiação ultravioleta (RUV) é o principal fator de risco, sendo bastante agressiva e podendo gerar danos irreversíveis à pele que, a longo prazo, podem resultar em fotocarcinogênese (ANANTHASWAMY; PIERCEALL, 1990). Além disso, em uma exposição aguda, já é possível gerar uma diversidade de efeitos prejudiciais e que são amplamente descritos como queimaduras solares, hiperpigmentação, edema, eritema e imunossupressão (AFAQ; KATIYAR, 2011; D'ORAZIO et al., 2013; HIRAMOTO et al., 2012). Cabe ainda destacar que, o uso de protetores solares não é totalmente efetivo contra a RUV, visto que pode promover a geração de espécies reativas de oxigênio (EROs) e causar reações alérgicas (HANSON; GRATTON; BARDEEN, 2006; HEURUNG; RAJU; WARSHAW, 2014).

Nos últimos anos, a destruição da camada de ozônio e o aumento da expectativa de vida ocasionaram uma elevada exposição a RUV e conseqüentemente uma ampliação no número de casos novos de doenças relacionadas à pele e por isso, existe a necessidade de compreender os mecanismos envolvidos na RUV e buscar novas alternativas terapêuticas que revertam/previnam os danos cutâneos causados nesse processo (AFAQ; ADHAMI; MUKHTAR, 2005; DIEPGEN; MAHLER, 2002; URBACH, 1991).

1.2. RADIAÇÃO ULTRAVIOLETA E SUAS CONSEQUÊNCIAS NA PELE

A luz solar é um espectro eletromagnético que compõe a RUV, e que em baixas quantidades, pode agir positivamente através da síntese de vitamina D (importante na homeostasia do cálcio) e liberação de endorfina (JUZENIENE; MOAN, 2012). Todavia, a exposição excessiva a RUV pode desencadear efeitos deletérios provenientes da intensa reação inflamatória e do aumento de radicais livres (CASAGRANDE et al., 2006; MARTINEZ

et al., 2018; SAITO et al., 2018).

A RUV abrange três faixas, de acordo com seu comprimento de onda. A primeira região e a mais prejudicial é a UVC (280-100 nm), sendo a mais energética, porém, o seu efeito é bloqueado pela camada de ozônio da Terra. As outras faixas correspondem à UVB (315-280 nm) e UVA (400-315 nm), que conseguem atravessar a atmosfera e são responsáveis por causar distúrbios na pele, incluindo o câncer de pele (AFAQ; ADHAMI; MUKHTAR, 2005; D'ORAZIO et al., 2013; SCHUCH et al., 2017), conforme ilustrado na figura 1.

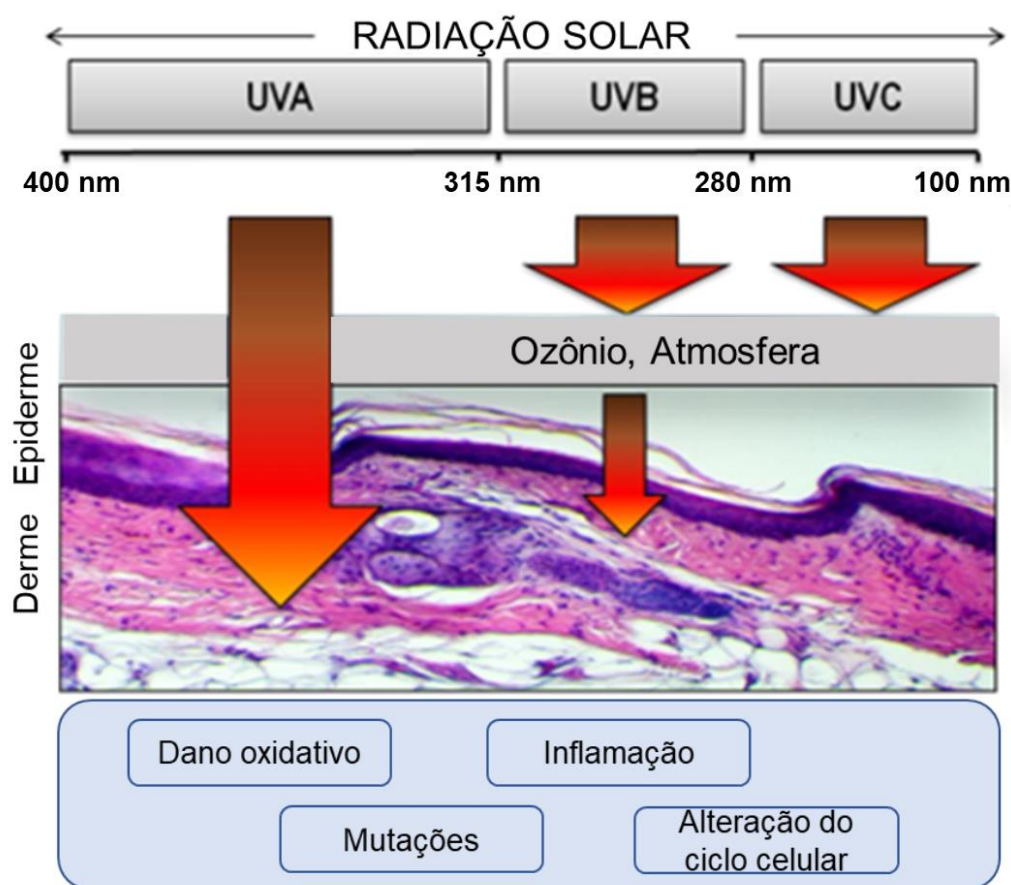


Figura 1: Espectro da radiação ultravioleta (Adaptado de D'ORAZIO et al. 2013)

Ao entrar em contato com a pele, a RUV é absorvida por estruturas chamadas de cromóforos, que convertem essa energia eletromagnética em energia química. Os cromóforos são estruturas com dupla ou tripla ligações entre carbonos e são considerados a primeira linha de defesa contra a RUV, e são eles: DNA, melanina, ácido urânico, ácido ribonucleico (RNA), proteínas, entre outros. Esses cromóforos atingem um estado de maior energia (excitado) e quando voltam para seu estado de origem, liberam energia e é nessa fase que pode ocorrer mudanças químicas em suas estruturas, reagindo com outras moléculas e gerando EROs (BRAND et al., 2018; GIBBS; NORVAL, 2013; MADDODI;

JAYANTHY; SETALURI, 2012).

Apesar de ser quase totalidade na composição da RUV que é absorvida (aproximadamente 90%), o espectro de radiação UVA é responsável somente por cerca de 10% da dose carcinogênica da luz solar e provoca dano oxidativo em camadas mais profundas, principalmente na derme (AFAQ; ADHAMI; MUKHTAR, 2005; BREM; GUVEN; KARRAN, 2017; KAPPES et al., 2006; MATSUMURA; ANANTHASWAMY, 2004).

A radiação UVB é a principal causadora dos efeitos nocivos da luz solar, pois seu alvo primordial é a camada epidérmica, acometendo diretamente o DNA dos queratinócitos por meio de reações químicas que resultam em fotoprodutos mutagênicos entre as pirimidinas, existentes na forma de dímeros de ciclobutano (CPDs) e fotoprodutos de pirimidina (6-4) (ANANTHASWAMY; KANJILAL, 1996; BEISSERT; LOSER, 2008).

Em casos de dano grave ao DNA, ocorre uma proliferação de células danificadas e conseqüentemente o surgimento de neoplasias. Para que uma lesão ao DNA seja reparada, existe um sistema que inclui a proteína p53, a qual possui importante papel na sinalização celular e que é ativada após a exposição à radiação UVB, impedindo o crescimento celular e a progressão do tumor através de mecanismos que envolvem a parada do ciclo para o reparo e em última instância, a indução da apoptose. Na pele, os queratinócitos apoptóticos são chamados de “sunburn cells” (BRENNER; HEARING, 2008; NAKANISHI et al., 2009; SREEVIDYA et al., 2010).

1.3. MECANISMOS FISIOPATOLÓGICOS DA RADIAÇÃO UVB: ESTRESSE OXIDATIVO E INFLAMAÇÃO

Além de causar danos extensos à pele diretamente, a radiação UVB também pode induzir lesão por mecanismos indiretos que envolvem a produção de EROs e a resposta inflamatória, tornando-se importante para a fotocarcinogênese (IVAN et al., 2014; MARTINEZ et al., 2018; SAITO et al., 2018).

As EROs são radicais livres oriundos do metabolismo do oxigênio (O_2), o qual possui grande capacidade para receber elétrons. Radicais livres são espécies que possuem existência independente, com um ou mais elétrons desemparelhados em sua última camada de valência, sendo bastante instáveis e podendo reagir com ácidos nucleicos, proteínas e lipídios. Em condições normais e em baixas quantidades, os radicais livres são sintetizados na respiração aeróbica, na ativação de fagócitos como mecanismo de defesa, ou de forma exógena, como na exposição à radiação UVB. Durante a redução do oxigênio à água no processo respiratório, radicais intermediários podem ser formados como o ânion superóxido ($O_2^{\cdot-}$), hidroxila (OH^{\cdot}) e espécies não radicalares como o peróxido de hidrogênio (H_2O_2)

(HALLIWELL; GUTTERIDGE, 2007; RAY; HUANG; TSUJI, 2013), conforme ilustrado na figura 2.

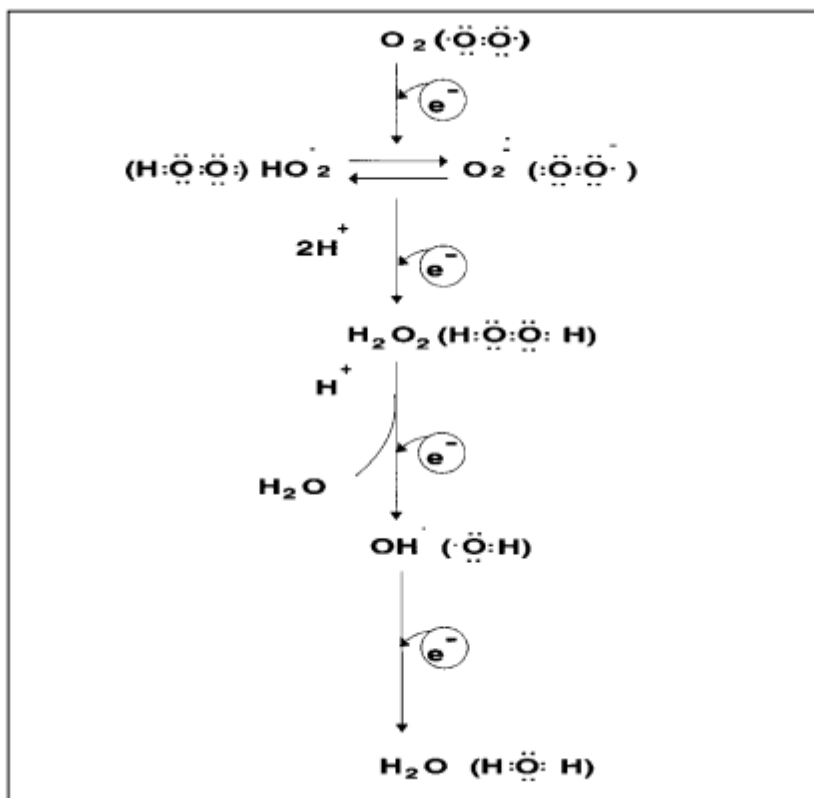


Figura 2: Representação esquemática da redução do oxigênio molecular até a formação de água e geração de EROs (FERREIRA; MATSUBARA, 1997).

O H_2O_2 , apesar de não ser um radical livre, pode ser bastante prejudicial porque participa da reação que sintetiza o radical hidroxila, considerado o mais tóxico das EROs. Este, por sua vez, na presença de metais pode levar à mutação no DNA e inativação de proteínas e lipídios (FERREIRA; MATSUBARA, 1997). Já o $\text{O}_2^{\cdot-}$ é um radical que é formado na primeira redução do O_2 , e sofre ação da enzima superóxido dismutase (SOD), produzindo O_2 e H_2O_2 (FERREIRA; MATSUBARA, 1997).

Entretanto, estas espécies radiculares podem ser geradas adicionalmente por meio do complexo enzimático da nicotinamida adenina dinucleotídeo fosfato (NADPH) oxidase (NOX). A família de proteínas NOX catalisa a transferência de elétrons através das membranas para o O_2 e é composta por isoformas expressas em vários tecidos, regulando a proliferação e diferenciação celular (SHARMA et al., 2018; VEITH et al., 2019). NOX1-3 e NOX5 estão envolvidas principalmente com a produção de $\text{O}_2^{\cdot-}$ e são expressas em diversas células como musculares e endoteliais, fagocitárias, neurais, adipócitos e espermatozoides,

entre outras (ANDUEZA et al., 2018; MAZZONETTO et al., 2019; TOUYZ et al., 2019). Ao passo que a NOX4 produz especialmente H_2O_2 e tem sido implicada no contexto de estudos com fibroblastos e células epiteliais (JIANG et al., 2014; VEITH et al., 2019). A NOX2, especificamente, está presente na membrana plasmática de leucócitos e possui subunidades como a gp91phox, que está associada diretamente à síntese de ânion superóxido induzida pela radiação UVB (HIRAMOTO et al., 2012; RYU et al., 2010).

O estresse oxidativo também pode afetar lipídios através do dano a membranas biológicas, evento chamado de peroxidação lipídica. As membranas biológicas possuem em sua composição grandes quantidades de ácidos graxos poli-insaturados que conferem múltiplas funções. Na presença de oxigênio, os radicais de ácidos graxos são formados, estes por sua vez podem reagir com mais radicais, ocorrendo a propagação da oxidação para diversas moléculas de ácidos graxos. Isso pode acarretar em sua degradação por meio de modificações em sua estrutura, perda da função das proteínas que estão incrustadas nessas membranas e aumento da permeabilidade, gerando uma desorganização e consequente liberação de produtos como o ácido araquidônico, que resulta na inflamação cutânea por meio do aumento de moléculas inflamatórias como a cicloxigenase-2 (COX-2) (GODAR, 1999; HALLIDAY, 2005).

Com o objetivo de combater os possíveis danos causados pelos radicais livres, os queratinócitos epidérmicos possuem um sistema antioxidante que tem como função impedir a oxidação de substratos. Esse sistema pode ser dividido em enzimático e não enzimático. O sistema enzimático é composto por enzimas como a catalase (CAT), glutathione peroxidase (GPx) e SOD. Já no sistema não enzimático contém a glutathione reduzida (GSH) e outras substâncias provenientes da dieta como o ácido ascórbico (vitamina C) e o alfa-tocoferol (vitamina E) (AFAQ; MUKHTAR, 2001; SIES; BERNDT; JONES, 2017; VALKO et al., 2006). Quando há uma deficiência da capacidade antioxidante e um excesso da produção de radicais livres, gera um desequilíbrio pró-oxidante/oxidante, o que caracteriza o estresse oxidativo, que pode estar associado a diversas manifestações celulares resultando em morte celular (AFAQ; ADHAMI; MUKHTAR, 2005; HALLIWELL; GUTTERIDGE, 2007).

A GSH é considerada o principal marcador para o estresse oxidativo induzido pela radiação UVB, pois é produzida em maior quantidade pelas células epidérmicas (CASAGRANDE et al., 2006; MARTINEZ et al., 2018; SAITO et al., 2018). Ela é um oligopeptídeo composto por cisteína, glicina e glutamina e possui um grupamento tiol, que é responsável pela neutralização das substâncias oxidantes. Ao doar elétrons para moléculas reativas, a GSH na forma reduzida (GSH) é convertida para sua forma oxidada (GSSG). Porém, GSSG pode ser reduzida novamente para seu estado basal sob ação da glutathione reductase (GR), tornando um ciclo. Outro meio de neutralizar oxidantes é através da ação da

CAT e GPx, que convertem o H₂O₂ e impedem a formação de radicais bastante tóxicos como a hidroxila. Alterações nos níveis desses antioxidantes são alvos de investigações para determinação de respostas cutâneas à UVB (BICKERS; ATHAR, 2006; D'ORAZIO et al., 2013; SCHALLREUTER et al., 1999).

O aumento de EROs pode ativar diversas vias de sinalização e alterar a transcrição de determinados genes, provocando modificações em sua expressão. Uma das principais respostas celulares contra essa alteração é o estímulo para produção de maior quantidade de antioxidantes através da indução do fator de transcrição Nrf2 (fator nuclear [derivado eritróide-2] tipo 2), que está presente no citoplasma. Em condições normais, a proteína Keap1 forma um complexo com Nrf2, regulando sua ação e impedindo sua ida para o núcleo mediada por ubiquitinação e posterior degradação através do sistema proteassomal. Após a exposição à UVB, esse complexo é dissociado e o Nrf2 é deslocado para o núcleo, ligando-se ao elemento de resposta antioxidante (ARE). A interação entre Nrf2 e ARE leva a ativação da transcrição de genes que codificam proteínas com ações antioxidantes/detoxificantes como a hemeoxigenase-1 (HO-1), NADPH quinona oxidoreductase 1 (NQO1), GR e GPx. A HO-1 é uma enzima que exerce importante papel anti-inflamatório e antioxidante ao realizar a quebra do grupamento heme, resultando em biliverdina, ferro e monóxido de carbono. Neste contexto, o Nrf2 tem apresentado efeito protetor por meio da regulação de moléculas com ações benéficas, garantindo a sobrevivência da célula através da eliminação de substâncias oxidantes (KOBAYASHI et al., 2004; LEE; CHAU, 2002; MOTOHASHI; YAMAMOTO, 2007).

Em contrapartida, quando a capacidade antioxidante é superada pelo aumento de radicais livres, o fator nuclear kappa B (NF-κB) pode reprimir a atividade do Nrf2 (LI et al., 2009; LIU; QU; SHEN, 2008). Em resposta ao excesso de EROs, a via de sinalização do NF-κB é ativada, promovendo a transcrição de genes associados com a resposta inflamatória como a COX-2 e a produção de citocinas inflamatórias. O NF-κB geralmente é inibido pela proteína IκB. Quando esse inibidor é degradado através do sistema ubiquitina-proteassoma, ele se dissocia do NF-κB e este exerce sua ação na resposta inflamatória (KUMAR et al., 2004; SONG et al., 2018).

O NF-κB é uma das principais vias que ativam fatores inflamatórios e ela está associada com a secreção de citocinas pelos queratinócitos epidérmicos como a TNF-α e IL-1β (ABEYAMA et al., 2000; MUTHUSAMY; PIVA, 2010). Os queratinócitos exercem função importante na resposta imune através da liberação de mediadores chamados de citocinas e quimiocinas, amplificando sua resposta para outras células imunes cutâneas como as células de Langerhans e mastócitos. Esses mediadores, por sua vez, atraem o recrutamento de leucócitos para o local da lesão (CHEN; DIPIETRO, 2017; RYU et al., 2019). Além disso,

a ativação de citocinas inflamatórias expressas nessas células desencadeia uma série de manifestações clínicas incluindo o aumento da permeabilidade vascular, resultando em edema (KAJIYA; HIRAKAWA; DETMAR, 2006; NICHOLS; KATIYAR, 2010; MONTES DE OCA et al., 2017).

Os queratinócitos possuem mecanismos adaptativos contra a radiação UVB. Um deles é chamado de hiperqueratose, o qual os queratinócitos se proliferam no local da lesão mediados por fatores de crescimento, resultando no espessamento da camada epidérmica com o objetivo de melhorar a proteção contra os raios UV (D'ORAZIO et al., 2013; MUTHUSAMY; PIVA, 2010; SCOTT et al., 2012). Quando essas células não conseguem evitar os danos causados, os queratinócitos ativam vias apoptóticas com participação da proteína p53, conforme citado anteriormente, e sofrem o fenômeno apoptótico que microscopicamente é caracterizado por núcleos picnóticos no interior dessas células (BAYERL et al., 1995).

Embora o efeito da radiação UVB tenha predominância na camada epidérmica, ela pode atingir a derme através das papilas dérmicas superiores, que são projeções que separam a derme da epiderme, acarretando em danos indiretos aos componentes dérmicos (MICHALAK et al., 2018). Após a exposição a UVB, mastócitos se proliferam e liberam produtos de degranulação como a histamina, exercendo importante papel na imunossupressão (CLYDESDALE; DANDIE; MULLER, 2001; MUTHUSAMY; PIVA, 2010).

As fibras de colágeno, componentes da matriz extracelular dérmica, também podem sofrer danos induzidos pela radiação UVB (DAI et al., 2007; MICHALAK et al., 2018; SANCHES SILVEIRA; PEDROSO, 2014). Isso ocorre devido à ação de metaloproteinases (MMP), que são proteases dependentes de zinco e sintetizadas principalmente por neutrófilos. Essas células são capazes de responder a mediadores como a citocinas, incluindo IL-1 β e TNF- α , e posteriormente liberam as MMPs de grânulos específicos de armazenamento para o meio extracelular (NAVARRO et al., 2006). A MMP-2 e MMP-9 são enzimas proteolíticas que degradam o colágeno do tipo IV, componente dérmico em abundância. A MMP-9 efetua um papel importante na deterioração e remodelamento da matriz extracelular, estando envolvida em manifestações patológicas como artrite, doença cardiovascular e câncer. Além disso, ela pode estar associada a metástase no processo carcinogênico (CHOI et al., 2010; JOHN; TUSZYNSKI, 2001).

Os neutrófilos são as primeiras células que são atraídas para o foco inflamatório. A enzima mieloperoxidase (MPO) é um dos principais marcadores para detectar o recrutamento de neutrófilos. Esta enzima é armazenada em seus grânulos azurófilos e produz ácido hipocloroso para combater agentes infecciosos. O mecanismo de defesa de neutrófilos responsável pela produção de oxidantes também depende da enzima NADPH

oxidase. Dessa forma, como não existe um microrganismo nesse processo, os neutrófilos atuam como uma fonte adicional de EROs e os danos teciduais são acentuados (ABBAS et al., 2016; BRADLEY et al., 1982; CLYDESDALE; DANDIE; MULLER, 2001; HIRAMOTO et al., 2012).

A via de sinalização MAPK também é ativada devido ao excesso de EROs. Essa via pertence a família de proteínas quinases ativadas por mitógeno, que é subdividida em proteínas ERK (quinase regulada por sinal extracelular), JNK (quinase c-Jun N-terminal) e quinase p38, as quais estão associadas com eventos celulares como a diferenciação, proliferação, apoptose e promoção de tumores (AFAQ; ADHAMI; MUKHTAR, 2005; PEUS et al., 1998, 1999). A ativação dessa via proporciona a transmissão do sinal para uma variedade de fatores de transcrição como o NF- κ B e ativador de proteína-1 (AP-1), que regulam a expressão de diversos genes como as citocinas. A AP-1 é um complexo formado por dímeros c-Fos e c-Jun que medeia a inflamação, proliferação celular, tumorigênese e também interfere na expressão de MMP responsáveis pela deterioração da matriz extracelular (MONTES DE OCA et al., 2017).

Para limitar a reação inflamatória, o NF- κ B também ativa a produção de citocinas anti-inflamatórias como a IL-10 e o fator de transformação do crescimento beta (TGF- β) que regulam negativamente a resposta imune. A IL-10 é sintetizada principalmente por macrófagos infiltrados na camada epidérmica induzidos pela RUV (CLYDESDALE; DANDIE; MULLER, 2001; COOPER et al., 1992). TGF- β desempenha um papel relevante no reparo tecidual, pois estimula a produção de colágeno pelos fibroblastos (PENN; GROBBELAAR; ROLFE, 2012; SAITO et al., 2018). A inibição concomitante da produção de citocinas pró e anti-inflamatórias sugere reverter a amplificação do processo inflamatório para restabelecer a homeostase.

1.4. MEDIADOR LIPÍDICO PRÓ-RESOLUÇÃO: RESOLVINA D2

Considerando as consequências da exposição cutânea à radiação UVB mencionadas anteriormente, observa-se que a inflamação e estresse oxidativo estão intimamente ligados, pois a produção excessiva de EROs ativa diversas vias de sinalização, inclusive para produção de citocinas e quimiocinas. Estas, por sua vez, recrutam células inflamatórias para o local, o que acaba resultando em uma produção adicional de radicais livres como mecanismo de defesa, tornando esse processo um ciclo vicioso e que gera danos teciduais expressivos. Neste contexto, alternativas terapêuticas para o controle do processo inflamatório e estresse oxidativo induzidos pela radiação UVB devem ter como alvo seus mecanismos fisiopatológicos, uma vez que o sistema antioxidante que protege a pele contra os danos oxidativos após a exposição crônica ou excessiva à radiação é

deficiente (CASAGRANDE et al., 2006; MARTINEZ et al., 2018; SAITO et al., 2018; VICENTINI et al., 2011).

A inflamação é um processo gerado como resposta contra agentes infecciosos ou o estímulo que a desencadeou no intuito de eliminar e reparar o dano tecidual causado. Geralmente, é uma resposta adequada e controlada, com liberação de mediadores para a restauração do local, restabelecendo a homeostase. Quando essa resposta é exacerbada, o dano pode ser irreversível, favorecendo o desenvolvimento de diversas doenças crônicas (CALDER et al., 2009; SERHAN, 2014).

Citocinas pró-inflamatórias, prostaglandinas e leucotrienos interagem entre si amplificando o processo inflamatório e essa condição pode ser atenuada através da participação ativa de mediadores lipídicos pró-resolução, que são produzidos principalmente por neutrófilos e macrófagos. Esses mediadores são chamados de mediadores pró-resolução especializados (SPMs) e podemos citar as lipoxinas, resolvinas, protectinas e maresinas, que auxiliam na resolução da inflamação por meio do estímulo à fagocitose de microrganismos e células apoptóticas (fenômeno chamado de eferocitose) e remoção de detritos celulares. Suas ações reduzem a quantidade de células inflamatórias, a produção de citocinas e conseqüentemente de EROs, promovendo a redução da magnitude e duração do processo inflamatório, e por fim, estimulando a cicatrização e regeneração tecidual (CALDER et al., 2009; SERHAN, 2014; SERHAN; CHIANG; VAN DYKE, 2008).

Dentre os mediadores lipídicos citados, destacam-se as resolvinas, que de maneira geral, inibem a produção de citocinas, recrutamento de células pró-inflamatórias, aumentam o recrutamento de células anti-inflamatórias, inibem a ativação do NFκB, inibem a expressão/atividade da NADPH oxidase e aumentam a expressão de moléculas antioxidantes como o Nrf2 e HO-1 (CROASDELL et al., 2015; LEE; SURH, 2013; POSSO et al., 2018; YE et al., 2018). As resolvinas da série D, bem como epímeros de resolvina induzidos pela aspirina como a 17(R) resolvina D1, são gerados como resposta a uma inflamação a partir da conversão enzimática do ácido docosahexaenoico (DHA), pertencente à família do ômega-3 (CALDER, 2013; SEKI et al., 2010).

O ômega-3 é composto por ácidos graxos poli-insaturados (PUFAs) e contém em sua estrutura química duas ou mais ligações duplas entre carbonos e possui a primeira insaturação entre o terceiro e quarto carbono. Ele é encontrado especialmente na dieta (GAMMONE et al., 2019; PINEDA-PEÑA et al., 2018).

O termo resolvina é denominado como produto de interação da fase de resolução, pois foi originalmente encontrada na fase resolutiva da inflamação aguda. As resolvinas se diferenciam em suas estruturas químicas por meio de grupos di ou trihidroxila com ligações duplas conjugadas, apresentando a capacidade de doar elétrons e estabilizar compostos

como radicais livres (SEKI et al., 2010). As resolvinas efetuam suas ações terapêuticas potenciais por meio da ligação a superfamília de receptores acoplados a proteína G (GPCR). A Resolvina D2 (RvD2), especificamente, interage com os receptores GPR18, intermediando a fagocitose de células apoptóticas e remoção de detritos, ou seja, estimulando a resolução do processo inflamatório (CHIANG et al., 2015).

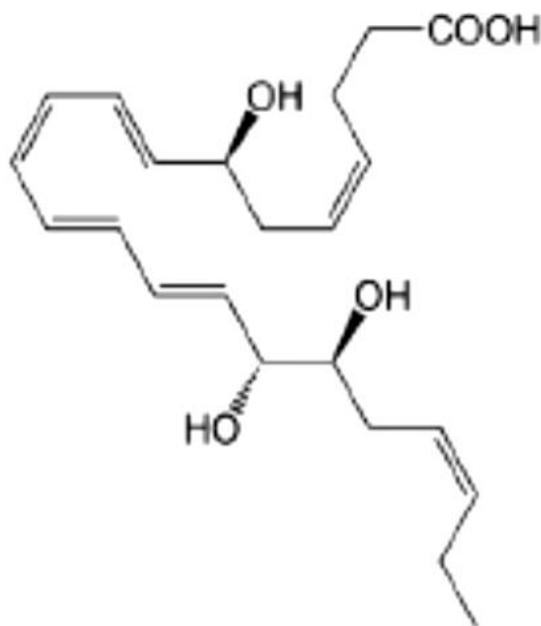


Figura 3: Estrutura química da RvD2 (Adaptado de CHIURCHIU et al. 2016).

De acordo com alguns estudos, a RvD2 tem demonstrado ação anti-inflamatória em modelos experimentais. Em um modelo de sepse microbiana induzida por ligadura e punção cecal, o tratamento com a RvD2 foi capaz de inibir o recrutamento de leucócitos, e também de diminuir a produção de citocinas pró-inflamatórias como TNF- α e IL-1 e a anti-inflamatória IL-10 (SPITE et al., 2009). Já em um modelo de colite experimental feita em camundongos, foi avaliada a atividade da mieloperoxidase (MPO) e COX-2, enzimas participativas da resposta inflamatória, e que tiveram sua atividade reduzida associada ao tratamento com a RvD2 (BENTO et al., 2011).

As resolvinas, no geral, também demonstraram ação antioxidante em outros modelos experimentais. Em um modelo de lesão hepática aguda induzida por tetracloreto de carbono, observou-se uma melhora da capacidade antioxidante com o grupo tratado com a resolvina D1 (RvD1) através do aumento dos níveis de GSH, principal marcador epidermal (CHEN et al., 2016). Já em um modelo de cultura celular de macrófagos, o tratamento com a

RvD1 inibiu a NOX2, complexo enzimático envolvido na produção adicional de EROs (LEE; SURH, 2013). Recentemente, foi demonstrado que a RvD1 inibiu o processo oxidativo e inflamatório em modelo de inflamação e estresse oxidativo cutâneo induzidos pela radiação UVB (SAITO et al., 2018). Ainda, o tratamento com outro mediador lipídico pró-resolução lipoxina A4 apresentou eficácia nesse mesmo modelo de exposição à UVB (MARTINEZ et al., 2018). Por fim, em um outro estudo, a RvD2 foi capaz de reduzir parâmetro de estresse oxidativo diminuindo os níveis de proteínas carboniladas no modelo de exposição à fumaça de cigarro (CROASDELL et al., 2015).

Em suma, é importante dizer que apesar do estabelecimento do papel endógeno e efeito anti-inflamatório/pró-resolução em alguns modelos de doença com a atual existência de poucos estudos associados com a RvD2 especificamente, não há conhecimento do efeito terapêutico desse mediador lipídico na inflamação e estresse oxidativo induzidos pela radiação UVB. Logo, esses dados indicam a RvD2 como alternativa promissora para o controle do processo inflamatório e melhora da capacidade antioxidante da pele nesse modelo.

2. OBJETIVOS

2.1. OBJETIVO GERAL

O objetivo geral deste trabalho foi avaliar a eficácia terapêutica da RvD2 na inflamação e estresse oxidativo induzidos pela radiação UVB.

2.2. OBJETIVOS ESPECÍFICOS

Avaliar o efeito da RvD2 quando administrada por via intraperitoneal em camundongos sem pelo, no modelo de lesão cutânea induzida pela radiação UVB com enfoque na (o):

- Edema de pele;
- Migração do infiltrado leucocitário (ensaio da mieloperoxidase);
- Atividade/secreção de metaloproteinase-9;
- Avaliação da capacidade antioxidante global cutânea por meio dos ensaios de FRAP (poder antioxidante de redução férrica) e ABTS (2,2' azinobis (3-etilbenzotiazolina-6-ácido sulfônico);
- Níveis do antioxidante endógeno GSH e atividade da catalase;
- Produção de ânion superóxido e hidroperóxidos lipídicos;
- Produção de citocinas pró-inflamatórias (IL-1 β , IL-33 e TNF- α) e anti-inflamatórias (TGF- β e IL-10);
- Avaliação histopatológica pelas colorações de hematoxilina-eosina (H&E), azul de toluidina e tricrômico de masson.

3. MATERIAIS E MÉTODOS

3.1. MATERIAIS

3.1.1. Reagentes

RvD2 com pureza $\geq 95\%$ foi obtida da Cayman Chemical (Ann Arbor, Michigan, USA); terc-butil hidropéroxido da Acros (Pittsburgh, PA, USA) ; azul brilhante R, glutathiona reduzida (GSH), 2,2' azinobis (3-etilbenzotiazolina-6-ácido sulfônico) (ABTS), 2,4,6 tripiridil-S-triazina (TPTZ), Trolox, ácido 5,5'-ditio-bis-(2-nitrobenzóico) (DTNB), brometo de hexadeciltrimetil amônio (HTAB), o-dianisidina, *nitroblue tetrazolium* (NBT) e bisacrilamida foram obtidos da Sigma-Aldrich (St Louis, MO, USA). Hidroximetil aminometano (TRIS) da Amresco (Solon, OH, USA). Acrilamida e dodecil sulfato de sódio (SDS) da Invitrogen. Kits de ensaio imunoenzimático (ELISA) para dosagem de citocinas da eBioscience (San Diego, CA, USA). Todos os outros reagentes utilizados foram de grau analítico.

3.1.2. Equipamentos

Agitador mecânico, Fisatom®; Balança analítica, HR-120, A&d®, precisão de 4 casas; Banho-maria, 314/2 DN, Nova Ética®; Banho-maria 100, Fanem; Centrífuga refrigerada, Rotina 46R, Hettich Zentrifugen®; Câmara de madeira projetada para irradiação; Deionizador de água, Purebal Option-Q, Elga®; Estufa 0-120°C, De Leo & Cia®; Fonte elétrica para eletroforese MS 300V, Major Science®; Homogeneizador de tecidos Tissue-Tearor (Biospec 985370); Lâmpada ultravioleta fluorescente PHILIPS TL/12 40W RS-UVB, MedicalHolland®; Leitor de microplaca, Asys Expert Plus, Biochrom®; Leitor de microplaca, Enspire, Perkin Elmer®; Leitor de microplaca, Multiskan GO, Thermo Scientific; pHmetro, Tec-3MP, TECNAL®; Radiômetro IL 1700 Research Radiometer. Detectores: SED240 – filtro UVB (290nm), SED005 – filtro UV (350nm); Sistema de eletroforese Mini Vertical, Mini-Protean® Tetra System, Bio-RAD®; Spray drier, model SD-05, Lab Plant®; Ultra-som, TSO, Thornton®.

3.2. MÉTODOS

3.2.1. Modelo de indução da lesão cutânea pela radiação UVB

Foram utilizados camundongos sem pelo da linhagem HRS/J, de ambos os sexos e adultos, pesando em média 30g. Os camundongos foram mantidos em uma sala com temperatura controlada (22 ± 2 °C) localizada no Biotério do Centro de Ciências da Saúde/UEL, utilizando ciclo de claro/escuro (12/12 h) com água e ração *ad libitum*. O projeto foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Estadual de Londrina, com seguimento de suas normas (registro no CEUA sob o nº 017/2015, processo nº 1447.2015.10).

A fonte de luz utilizada nos experimentos de indução de estresse oxidativo e processo inflamatório agudo foi uma lâmpada UVB fluorescente modelo PHILIPS TL/12 40W RS (MEDICAL-HOLANDA). A lâmpada emite radiação na faixa de 270 a 400 nm com pico máximo de emissão em torno de 313 nm e foi acoplada no interior de uma caixa retangular de madeira desenvolvida especificamente para a indução da lesão cutânea, com capacidade máxima para 6 caixas de polipropileno com dimensão de 30x19x13 cm para acondicionamento de camundongos. Os camundongos foram distribuídos nas caixas e cobertos com tela plástica para que eles permanecessem dentro delas, além de estarem completamente expostos à luz ultravioleta. Ainda, os animais foram colocados a 20 cm de distância da lâmpada UVB, movimentando-se livremente em suas caixas e para que a mesma dose de radiação fosse distribuída para todos os animais, foi realizada uma rotatividade entre as caixas, já que podem ocorrer variações de emissão de luz ao longo da lâmpada. A medida da irradiância desta lâmpada foi realizada utilizando-se um radiômetro (IL 1700) com detectores para radiação UV (SED 005) e, especialmente, para UVB (SED 240) (CARINI et al., 2000; CASAGRANDE et al., 2006), tornando-se possível a posterior padronização da dose de radiação para $4,14 \text{ J/cm}^2$ durante o período de exposição de cinco horas e trinta minutos (CAMPANINI et al., 2013; IVAN et al., 2014).

3.2.2. Protocolo experimental de avaliação do potencial terapêutico da resolvina D2 em camundongos

Os animais foram distribuídos aleatoriamente em cinco grupos experimentais, contendo 6 animais cada:

(1) grupo não radiado e tratado com salina;

- (2) grupo radiado e tratado com salina;
- (3) grupo radiado e tratado com a dose 0,3 ng/animal de RvD2;
- (4) grupo radiado e tratado com a dose 1,0 ng/animal de RvD2;
- (5) grupo radiado e tratado com a dose 3,0 ng/animal de RvD2.

Camundongos foram tratados com 200 μ L de diferentes doses de RvD2 (0,3; 1,0 e 3,0 ng/animal) ou apenas o veículo (salina estéril) utilizado para diluir o mediador lipídico, via intraperitoneal (i.p), 1h antes do início da radiação. As doses de RvD2 foram selecionadas baseando nos efeitos terapêuticos descritos previamente (BERRUETA et al., 2016; MARTINEZ et al., 2018; SAITO et al., 2018; SPITE et al., 2009) e nos experimentos de dose-resposta realizados neste trabalho. Os animais foram submetidos a eutanásia com 5% de isoflurano e amostras de pele da região dorsal foram coletadas 12 horas após a radiação para os testes de edema, atividade da MPO, MMP-9, histologia, avaliação do FRAP, ABTS e níveis de GSH. Em outra série de experimentos, as amostras foram coletadas após 2 horas do fim da exposição à UVB para os testes de CAT e produção de ânion superóxido (NBT). Por último, citocinas e hidroperóxidos lipídicos (LOOH) foram analisados por meio das amostras de pele coletadas 4 horas após a radiação. Nestes últimos tempos citados (2 e 4 horas), os animais foram anestesiados com 5% de isoflurano e decaptados. Cada parâmetro foi analisado em um tempo específico de coleta após determinação prévia em outros trabalhos do grupo (CAMPANINI et al., 2013; MARTINEZ et al., 2015).

A melhor dose da RvD2 foi determinada através dos resultados obtidos dos testes de edema, atividade da MPO, MMP-9, FRAP, ABTS e GSH e selecionada para os demais parâmetros avaliados.

As amostras de pele do dorso dos animais coletadas foram divididas para os diferentes testes e armazenadas a -80 °C para as análises, exceto para o teste de edema cutâneo, que foi realizado logo após a coleta, e para a avaliação histopatológica, que foram coletadas e adicionadas em formol a 10% até serem processadas.

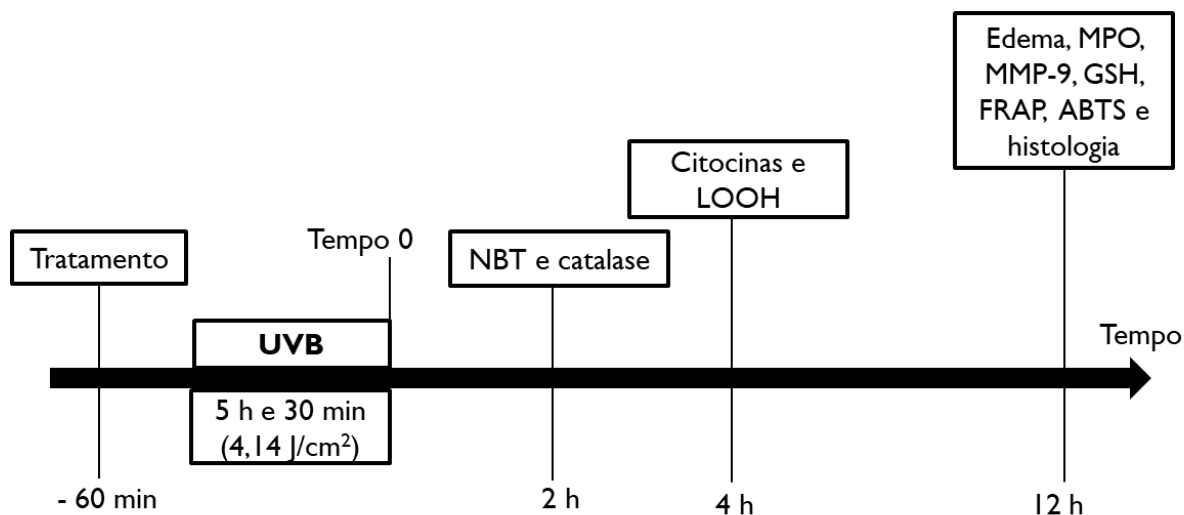


Figura 4: Fluxograma dos tempos de tratamento e coletas das peles dos animais de acordo com os protocolos experimentais pré-estabelecidos para avaliação da eficácia da RvD2 administrada via intraperitoneal através de parâmetros inflamatórios e oxidativos. Foi realizado um pré-tratamento com a RvD2 1 h antes da radiação com as doses de 0,3; 1,0 e 3,0 ng/animal. Os animais foram submetidos à radiação UVB por um tempo previamente padronizado de 5 horas e 30 minutos ($4,14 \text{ J/cm}^2$). Os animais passaram por eutanásia e as amostras de pele dorsal foram coletadas em tempos distintos previamente determinados de acordo com cada teste: 2, 4 e 12 horas após o fim da radiação UVB.

3.2.3. Avaliação do edema cutâneo

A exposição excessiva da radiação UVB sobre a pele induz a formação de edema através do aumento da permeabilidade vascular e consequente extravasamento do líquido intersticial na epiderme (KAJIYA; HIRAKAWA; DETMAR, 2006). Para esse teste, as amostras de pele dorsal dos animais foram coletadas com auxílio de um molde com área fixa (5 mm de diâmetro). O edema foi mensurado pela variação do peso de pele, comparando os diferentes grupos e os resultados foram expressos em mg de pele (IVAN et al., 2014).

3.2.4. Avaliação da atividade da enzima MPO

A atividade da enzima MPO representa um ensaio indireto para o recrutamento de leucócitos após a radiação UVB (CAMPANINI et al., 2013; IVAN et al., 2014).

As amostras de pele foram coletadas e homogeneizadas com o tampão fosfato 50 mM (pH 6,0) contendo 0,5% de brometo de hexadeciltrimetil amônio (HTAB). Elas foram homogeneizadas com o auxílio do homogeneizador de tecidos Tissue-Tearor (Biospec

985370) e posteriormente centrifugadas (16.100 g por 2 min a 4 °C). Para a reação colorimétrica, foram adicionados 30 µL do sobrenadante de cada amostra juntamente com 200 µL de uma solução contendo 10% de tampão fosfato 0,05 M (pH 6.0), 0,0167 % de *orto*-dianisidina e 0,05% de H₂O₂ em água deionizada. A atividade da MPO das amostras foi determinada espectrofotometricamente em 450 nm (EnSpire, Perkin Elmer) e comparada a uma curva padrão de neutrófilos com concentração conhecida. Os resultados obtidos foram expressos em números de neutrófilos/mg de pele (CASAGRANDE et al., 2006).

3.2.5. Determinação da atividade/secreção de metaloproteinase-9 (MMP-9)

A MMP-9 é uma enzima proteolítica que degrada componentes importantes da matriz extracelular como o colágeno e sua expressão é aumentada através da exposição a RUV (CUELHO et al., 2018; SAITO et al., 2016). Para determinação da atividade da MMP-9, foi utilizado o ensaio de zimografia em gel de poliacrilamida com dodecil sulfato de sódio (SDS-PAGE), o qual permite a detecção de enzimas que degradam a gelatina presente no gel (FONSECA et al., 2011).

Foi feito um pool das amostras de pele de cada grupo e foram homogeneizadas com auxílio do Tissue-Tearor (Biospec 985370), na proporção 1:4 em tampão fosfato Tris/HCl 50 mM (pH 7,4) com cloreto de cálcio (CaCl₂) e 1% de inibidores de proteinases (fenantrolina, fluoreto de fenilmetilsulfonila e N-etilmaleimida). Os homogenatos foram duplamente centrifugados a 12.000 g por 10 min a 4 °C e os sobrenadantes foram utilizados para o ensaio. 30 µL do sobrenadante das amostras foi diluído em 6 µL de tampão Tris/HCl (pH 6,8) contendo 20% de glicerol, 4% de dodecil sulfato de sódio (SDS) e 0,001% de azul de bromofenol. Posteriormente, estas amostras foram colocadas em banho-maria a 37 °C durante 8 minutos imediatamente antes de ser aplicada no gel de eletroforese.

O gel contém 1 mm de espessura e é composto por duas partes, um gel de separação e de concentração, preparados conforme a tabela 1.

Tabela 1: Componentes do gel de separação e do gel de concentração.

| Substância | Quantidade (μL) | |
|---|------------------------------|---------------------|
| | Gel de separação | Gel de concentração |
| Água miliQ | 5870 | 4060 |
| Tampão Tris/HCl 1 M (pH 8,8) com 0,4% SDS | 3750 | - |
| Tampão Tris/HCl 0,5 M (pH 6,8) com 0,4% SDS | - | 1670 |
| Acrilamida: bis-acrilamida (30:0,8) | 5000 | 860 |
| Gelatina 10% | 375 | - |
| Persulfato de amônio 10% | 50 | 33 |
| Temed 20% | 10 | 6,6 |

Após o preparo da solução contendo os dois géis, os mesmos foram aplicados um por vez no aparato do sistema de eletroforese Mini Vertical (Bio-RAD®). Posteriormente, o gel foi acoplado no suporte que fica no interior da cuba de eletroforese, preenchida com tampão Tris/glicina 190 mM (pH 8.3) contendo 0,1% de SDS. Antes da aplicação das amostras, o gel foi submetido a uma pré-corrída de 10 mA por 15 minutos. Em seguida, foram aplicados 15 μL de cada amostra. Durante a eletroforese a corrente aplicada foi de 10 mA para o gel de concentração e 13 mA para o gel de separação, sendo que a última corrente foi mantida constante por 15 minutos após a saída do corante do gel de separação.

Após o término da corrida com as amostras, o gel de poliacrilamida foi retirado da cuba e submetido à lavagem, sob agitação constante, com a solução de Triton X-100 2% durante 1 hora. Posteriormente, o gel foi incubado por 16 horas a 37 °C em tampão Tris/HCl 50 mM (pH 7.4) contendo CaCl_2 10 mM e 0,02 de azida sódica. Na etapa seguinte, o gel foi corado com uma solução contendo 0,25% de azul brilhante, 10% de ácido acético e 50% de metanol em água deionizada. Para visualização das bandas o gel foi descorado com ácido acético 20% (FONSECA et al., 2011). Zonas de atividade enzimática foram detectadas como regiões de coloração negativa contra um fundo escuro. A atividade proteolítica foi analisada semi-quantitativamente por meio da comparação das diferenças de densidades de cor entre as bandas de cada grupo pelo programa Image J® (NIH, Bethesda, MD, USA).

3.2.6. Avaliação do poder antioxidante redutor de ferro (FRAP)

O ensaio de FRAP mede a redução férrica do 2,4,6 tripiridil-S-triazina (TPTZ) para um produto colorido por meio da ação de antioxidantes que doam elétrons para a reação (KATALINIC et al., 2005). O ensaio foi adaptado e utilizado para avaliar o poder antioxidante de amostras da pele (MARTINEZ et al., 2015).

As amostras de pele dos animais dos diferentes grupos foram coletadas e homogeneizadas em 400 μ L de KCl (1,15%) com auxílio do Tissue-Tearor (Biospec 985370) e centrifugadas a 1.000 g por 10 min a 4°C. Para a reação foram utilizados 30 μ L do sobrenadante do homogenato da amostra e 150 μ L de reagente FRAP. O reagente de FRAP foi preparado adicionando 2,5 mL de uma solução 10 mM de TPTZ em HCl 40 mM com 2,5 mL de cloreto de ferro hexahidratado 20 mM e 25 mL de tampão acetato 0,3 mM (pH 3.6), e esta solução foi incubada a 37 °C por 30 min antes do uso. Em seguida, foi realizada a leitura em 595 nm (EnSpire, Perkin Elmer). Soluções padrão com diferentes concentrações de trolox (0,5 a 20 nmol) (antioxidante análogo da vitamina E) foram utilizadas para calibração. Os resultados foram expressos como nmol equivalente de Trolox/mg de pele (KATALINIC et al., 2005).

3.2.7. Avaliação do poder antioxidante pelo ensaio de sequestro do radical ABTS (2,2' azinobis (3-etilbenzotiazolina-6-ácido sulfônico)

A capacidade antioxidante de cada amostra é medida através do decaimento da coloração do radical cátion ABTS⁺ quando ocorre doação de elétrons a ele feita pelo antioxidante. Para a reação de ABTS, as amostras foram homogeneizadas em 400 μ L de KCl a 1,15% com auxílio do homogeneizador de tecidos Tissue-Tearor (Biospec 985370) e centrifugadas a 1.000 g por 10 min a 4°C, posteriormente o sobrenadante foi utilizado para análise. A solução de ABTS foi preparada após reagir 7 mM da solução de ABTS com 2,45 mM de persulfato de potássio resultando no cátion ABTS⁺. A mistura foi armazenada em frasco âmbar por no mínimo 16 horas antes do uso. Após as 16 horas, a solução de ABTS foi misturada com tampão fosfato até atingir a absorvância de 0,8 em 730 nm. Foram adicionados em uma placa de 96 poços 7 μ L do sobrenadante e 200 μ L da solução de ABTS diluída. O branco era composto apenas do tampão. Após 6 minutos de reação, foi realizada a leitura em 730 nm (EnSpire, Perkin Elmer). Uma curva padrão foi preparada com diferentes concentrações de trolox (0,01 a 20 nmol) e os resultados foram expressos em nmol equivalente de trolox/mg de pele (KATALINIC et al., 2005; MARTINEZ et al., 2015).

3.2.8. Avaliação dos níveis do antioxidante endógeno GSH

A GSH é considerada o principal marcador para o estresse oxidativo induzido pela radiação UVB, pois é produzida em maior quantidade pelas células epidérmicas (CASAGRANDE et al., 2006). O seu grupamento sulfidril é responsável pela neutralização de substâncias oxidantes, doando elétrons para essas moléculas reativas (D'ORAZIO et al., 2013). Neste ensaio, GSH doa elétrons para o ácido 5',5'-ditio-bis-(2-nitrobenzóico) (DTNB), convertendo em um produto colorido (SRINIVASAN; SABITHA; SHYAMALADEVI, 2007).

As amostras de pele foram diluídas (1:4) em EDTA 0,02 M e trituradas utilizando o Tissue-Tearor (Biospec 985370). Ao homogenato foi adicionado ácido tricloroacético (TCA) 50% na proporção de 1:0,2 de EDTA e TCA, respectivamente. Em seguida, a mistura foi centrifugada a 2.700 g por 10 minutos a 4 °C. O sobrenadante foi retirado e centrifugado novamente a 2.700 g por mais 15 minutos a 4 °C. O sobrenadante final foi removido para análise. Para a quantificação dos níveis de GSH, foram adicionados 50 µL do sobrenadante da segunda centrifugação em microplaca juntamente com 100 µL de tampão Tris 0,4 M (pH 8,9) e 5 µL de uma solução de 1,9 mg/mL de DTNB em metanol. Após 5 minutos de incubação, a leitura da microplaca foi realizada no espectrofotômetro (EnSpire, Perkin Elmer) em 405 nm. A curva padrão foi preparada com 5 a 150 µM de GSH. Os resultados foram expressos em µM de GSH/mg de pele (SRINIVASAN; SABITHA; SHYAMALADEVI, 2007).

3.2.9. Avaliação da atividade da CAT

A enzima CAT tem como função converter o H_2O_2 em água e oxigênio. O princípio da técnica se baseia na decomposição do H_2O_2 seguida da diminuição de absorvância. A diferença de absorvância por unidade de tempo mede a atividade da enzima (AEBI, 1984).

As amostras de pele dos animais foram homogeneizadas em 500 µL de EDTA 0,02 M com auxílio do Tissue-Tearor (Biospec 985370). Em seguida, o homogenato foi centrifugado a 2.700 g por 10 minutos a 4 °C. O sobrenadante foi centrifugado novamente a 2.700 g por 10 minutos a 4 °C, e o sobrenadante final foi retirado para análise. Para a reação, foram adicionados 10 µL amostra, 160 µL tampão Tris-HCl 1M com EDTA 5 mM pH 8,0, 20 µL água deionizada e 20 µL H_2O_2 200 mM. Foi incluído um branco para cada amostra preparado com 10 µL do sobrenadante da amostra, 180 µL de tampão Tris-HCl 1M com EDTA 5 mM pH 8,0 e 20 µL de água deionizada. A velocidade com que o H_2O_2 é reduzido pela ação da CAT foi avaliada através da diminuição no valor da absorvância pela diferença entre a leitura inicial e a leitura 30 segundos após a adição do H_2O_2 200 mM. A

leitura foi realizada em espectrofotômetro de microplacas (EnSpire, Perkin Elmer) a 240 nm com temperatura mantida em 25 °C. Os valores de catalase foram expressos como unidade de catalase/mg de pele/minuto (AEBI, 1984).

3.2.10. Avaliação da produção de $O_2^{\cdot-}$

O ânion superóxido é um radical que é formado na primeira redução do oxigênio na respiração aeróbica e sua produção pode ser acentuada induzida pela radiação UVB (FERREIRA; MATSUBARA, 1997; HIRAMOTO et al., 2012). Este método foi baseado na redução do *nitroblue tetrazolium* (NBT). As amostras de pele foram homogeneizadas com o Tissue-Tearor (Biospec 985370) em EDTA 0,02 M e centrifugadas (2.000 g por 20 segundos a 4°C). Primeiramente, 50 µL do sobrenadante de cada amostra foi adicionado em microplaca e incubado por 1 hora. Posteriormente, o sobrenadante foi removido e foi adicionado o NBT (1 mg/mL) ao meio. Depois de incubar por 15 minutos, o sobrenadante foi retirado e ao precipitado foram adicionados 20 µL de metanol 100% para fixar, 120 µL de KOH 2 M e 140 µL de dimetilsulfóxido (DMSO) para solubilizar o composto formazan (NBT reduzido). A formação desse composto foi medida em espectrofotômetro (EnSpire, Perkin Elmer) a 620 nm e os resultados foram apresentados como densidade óptica (OD)/10 mg de pele (CAMPANINI et al., 2013).

3.2.11. Avaliação da produção de LOOH

A produção de hidroperóxidos lipídicos é baseada no método de quimiluminescência iniciada pelo tert-butil hidroperóxido descrito anteriormente (GONZALEZ FLECHA; LLESUY; BOVERIS, 1991). Esse teste foi adaptado por Martinez et al. (2015). As amostras de pele foram coletadas e homogeneizadas em 800 uL de tampão fosfato (pH 7.4) com o homogeneizador de tecidos Tissue-Tearor (Biospec 985370) e posteriormente centrifugadas a 700 g por 2 minutos a 4 °C. Para o ensaio, 250 µL do sobrenadante foram adicionados a 1730 µL de meio de reação (KCl 120 mM, tampão fosfato pH 7,4 30 mM) e 20 µL de tert-butil hidroperóxido 3 mM. Este ensaio foi realizado em contador β marca Beckman® LS 6000 (FULLERTON, CA, EUA) em uma faixa de contagem não coincidente com a resposta entre 300 e 620 nm. Todo o experimento foi realizado ao abrigo da luz para evitar a fosforescência dos frascos, a 30 °C, durante 120 minutos. Os resultados foram medidos em contagem por minuto (cpm) por mg de pele.

3.2.12. Dosagem das citocinas pró-inflamatórias TNF- α , IL-33, IL-1 β e citocinas anti-inflamatórias TGF- β e IL-10

A quantificação das citocinas foi realizada por meio da técnica de enzimaímmunoensaio (ELISA) do tipo sanduíche com diferentes kits comerciais disponíveis de acordo com as instruções de uso do fabricante e conforme descrito previamente (VERRI et al., 2008).

As amostras de pele foram coletadas em salina (500 μ L) e homogeneizadas com o Tissue-Tearor (Biospec 985370). Logo após, os homogenatos foram centrifugados a 2.000 g por 15 min a 4 °C e os sobrenadantes foram coletados para a análise. Para a realização do teste, primeiramente as placas de 96 poços foram incubadas *overnight* à 4 °C com os anticorpos de captura contra as proteínas de interesse. Depois dessa etapa, as placas foram submetidas a lavagens com PBS com 0,05% de Tween 20 e incubadas a temperatura ambiente durante 1 hora com a solução de bloqueio contendo 1% de albumina bovina. Posteriormente, a curva padrão e as amostras específicas para cada citocina foram adicionadas e incubadas a 4 °C por 16 h. As placas foram lavadas com o tampão de lavagem e os anticorpos policlonais biotinilados (anticorpo de detecção) foram adicionados e incubados por mais 1 hora. Novamente, as placas foram lavadas com o tampão e logo após a enzima avidina-peroxidase foi adicionada. As placas foram incubadas por 30 minutos à temperatura ambiente, lavadas, e adicionou-se 100 μ L do substrato contendo 0,04% de ortofenilenodiamina (OPD) e 0,04% de H₂O₂ em tampão fosfato, a placa foi mantida no escuro em temperatura ambiente por 15 minutos. A reação enzimática foi interrompida com uma solução de H₃PO₄ 1 M e as absorvâncias foram determinadas em 450 nm (Multiskan GO, Thermo Scientific). Os resultados foram obtidos comparando as densidades ópticas das amostras com as densidades das respectivas curvas padrões das citocinas e expressos em picogramas (pg) de citocina/mg de pele.

3.2.13. Avaliação histopatológica por microscopia óptica

Para a análise histopatológica da pele, as amostras foram coletadas 12 horas após UVB, fixadas em formol a 10% até serem processadas. Posteriormente, foram desidratadas em banhos de soluções de etanol com concentrações crescentes (70%, 95% e 100%) e diafanizadas com xilol para inclusão de parafina. Foram preparadas secções de 5 μ m de amostras de pele e coradas com hematoxilina-eosina (H&E), sendo examinadas utilizando microscopia óptica a ampliações de 40 e 100x para determinação da espessura epidérmica (DENG et al., 2015) e número de queratinócitos apoptóticos (SCHWARZ et al., 1995),

respectivamente.

Além disso, secções dos tecidos também foram coradas com o azul de toluidina para determinação de mastócitos e foram observados por microscopia óptica com o aumento de 40x. As análises foram feitas com o Infinity Analyze (Lumenera[®] Software). Por fim, secções dos tecidos foram coradas com tricrômico de masson para análise de possíveis alterações nas fibras de colágeno (ampliação de 10x) e a intensidade da coloração azul nas áreas dérmicas da pele foi analisada pelo software Image J[®] (NIH) (SONG et al., 2016).

3.3. Análise estatística dos resultados

As análises estatísticas foram realizadas utilizando o software GraphPad Prism 7 (GraphPad Software Inc., San Diego, EUA). Os resultados foram analisados estatisticamente por análise de variância (ANOVA) com um fator seguido do teste de comparações múltiplas de Tukey e apresentados pela média \pm erro padrão da média (EPM) de mensurações feitas com 6 animais em cada grupo por experimento. Os resultados foram representativos de 2 experimentos separados e foram considerados significativamente diferentes para $p < 0,05$.

4. RESULTADOS E DISCUSSÃO – ARTIGO CIENTÍFICO

4.1. Resolvin D2 inhibits UV irradiation-induced inflammation and oxidative stress in the skin of hairless mice

Journal of Photochemistry and Photobiology B: Biology

Ingrid Caroline Pinto^a, Priscila Saito^a, Camilla C. A. Rodrigues^a, Renata M. Martinez^a, Cristina P. B. Melo^a, Clovis M. Kumagai^a, David L. Vale^a, Allan J. C. Bussmann^b, Marcela M. Baracat^a, Sandra R. Georgetti^a, Waldiceu A. Verri Jr.^b, Rúbia Casagrande^{a*}

^aDepartamento de Ciências Farmacêuticas, Universidade Estadual de Londrina-UEL, Avenida Robert Koch, 60, Hospital Universitário, 86039-440 Londrina, Paraná, Brazil

^bDepartamento de Ciências Patológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina-UEL, Rodovia Celso Garcia Cid, Km 80, PR445, Cx. Postal 10.011, 86057-970, Londrina, Brazil

*Corresponding author. Address: Avenida Robert Koch, 60, Vila Operária, CEP 86039-440 Londrina, Paraná, Brazil. Tel.: +55 43 33712475. E-mail address: rubiacasa@yahoo.com.br (R. Casagrande).

Highlights

- RvD2 protected the skin from the deleterious effects of UVB irradiation.
- RvD2 inhibited UVB-induced inflammatory edema, MPO and MMP-9 activity.
- RvD2 reduced the secretion of cytokines.
- RvD2 inhibited UVB-induced production of hydroperoxides and superoxide anion.

Abbreviations

| | |
|-------------------------------|---|
| ABTS | 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) |
| CAT | catalase |
| DHA | docosahexaenoic acid |
| DNA | deoxyribonucleic acid |
| DTNB | 5,5'-dithiobis(2-nitrobenzoic acid) |
| FRAP | ferric reducing antioxidant power |
| GPR18 | G protein-coupled receptor 18 |
| GSH | reduced glutathione |
| H ₂ O ₂ | hydrogen peroxide |
| HTAB | hexadecyltrimethylammonium bromide |
| IL | interleukin |
| KCl | potassium chloride |
| LOOH | lipid hydroperoxides |
| MMP | matrix metalloproteinase |
| MPO | myeloperoxidase |
| NaCl | sodium chloride |
| NF-κB | nuclear factor-κB |
| NBT | nitroblue tetrazolium |
| O ₂ | oxygen |
| ROS | reactive oxygen species |
| RvD1 | resolvin D1 |
| RvD2 | resolvin D2 |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| SEM | standard error mean |

| | |
|---------------|----------------------------------|
| TGF- β | transforming growth factor beta |
| TNF- α | tumor necrosis factor |
| TPTZ | 2,4,6-Tris(2-pyridyl)-s-triazine |
| UV | ultraviolet |

Abstract

Ultraviolet (UV) irradiation is the main risk factor for the development of photocarcinogenesis. The mechanisms involved in UVB irradiation-induced inflammation and oxidative stress can be targeted by lipid mediators such as resolvin D2 (RvD2). Studies have demonstrated potential activity of these mediators in some disease models, but there is no knowledge of the role of RvD2 in the UVB exposure model. In general, resolvins inhibit the inflammatory response by reducing cytokine production and recruitment of inflammatory cells and may also suppress the action of nuclear factor kappa B (NFκB), which is closely linked to inflammation and oxidative stress. Thus, the objective of this study was to evaluate the effect of RvD2 on the model of skin lesion induced by UVB irradiation. Systemic treatment with RvD2 protected the skin against inflammatory damage induced by UVB irradiation, as it significantly reduced the parameters of edema, myeloperoxidase, metalloproteinase-9 activity, collagen fibers degradation, epidermal thickness, keratinocyte apoptosis, number of mast cells and production of different cytokines. RvD2 was also able to significantly decrease oxidative damage induced by UVB irradiation by reducing the oxidative parameters of superoxide anion and hydroperoxides production. In addition, the antioxidant capacity of the skin was recovered according to the results of the reduced glutathione (GSH), ferric reducing antioxidant power (FRAP), reductive capacity of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) and catalase activity. These results suggest that the lipid mediator RvD2 has great potential of use for the control of UVB irradiation-induced inflammatory response and oxidative stress.

Keywords: Resolvin D2, oxidative stress, inflammation, antioxidant, UVB irradiation.

1. Introduction

Ultraviolet (UV) irradiation is the main risk factor for the development of photocarcinogenesis and its occurrence is mainly due to exacerbated solar exposure [1,2]. Ultraviolet irradiation comprises three bands with different wavelengths: UVC (280-100 nm), UVB (315-280 nm) and UVA (400-315 nm) [2–4]. UVB irradiation is also the main cause of the harmful effects of sunlight, not only because it causes direct damage through DNA mutations, but also because it induces cutaneous damage by indirect mechanisms that involve the production of reactive oxygen species (ROS) and inflammatory response intense [5–8].

Excessive ROS production that overlaps the endogenous antioxidant capacity, leads to an imbalance known as oxidative stress, which threatens the integrity and function of cellular structures. In addition, oxidative stress leads to the activation of various signaling pathways such as nuclear factor- κ B (NF- κ B), thus promoting the transcription of genes associated with the inflammatory response such as the production of cytokines and the recruitment of inflammatory cells to the lesion site. The installation of the inflammatory process leads to an additional production of free radicals, making this process a vicious cycle that generates expressive tissue damages [9,10].

In this context, since the antioxidant system that protects the skin against oxidative damages after chronic or excessive exposure to irradiation is deficient, therapeutic alternatives for the control of the UVB induced inflammatory and oxidative stress should target their pathophysiological mechanisms. Among them, we highlight resolvin D2 (RvD2), a lipid mediator identified in the resolution phase of inflammation and synthesized from the enzymatic conversion of docosahexaenoic acid (DHA), which belongs to the family of the omega-3 polyunsaturated fatty acids that find mainly from the diet [11,12].

Resolvins exert beneficial biological effects and are widely described in experimental models, including inflammatory diseases. For example, RvD2 showed a neuroprotective effect in a model *in vivo* of ischemia/reperfusion [13]. The same effect occurred in a study with experimental model of Parkinson's disease induced by lipopolysaccharide, since treatment with RvD2 inhibited the expression of inflammatory cytokines such as TNF- α and IL-1 β and the production of ROS [14]. In addition, resolvin D1 promoted the regeneration of corneal epithelium in diabetic mice streptozocin-induced and was related to the reduction of inflammatory cytokines and the expression of the enzymatic complex NADPH oxidase responsible for the additional production of free radicals, besides improving the antioxidant capacity by increasing the expression of glutathione [15]. Recently, we demonstrated that

resolvin D1 (RvD1) reduces UVB irradiation-induced skin inflammation and oxidative stress [8].

Thus, the objective of this study was to evaluate RvD2 therapeutic potential *in vivo* for the control of inflammatory response and oxidative stress in the UVB irradiation-induced cutaneous lesion model, since there is no knowledge of the effect of this lipid mediator in this model.

2. Materials and methods

2.1. Chemicals

Resolvin D2 purity $\geq 95\%$ was obtained from Cayman Chemical (Ann Arbor, Michigan, USA); tert-butyl hydroperoxide from Acros (Pittsburgh, PA, USA); Brilliant blue R, reduced glutathione (GSH), 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-S-triazine (TPTZ), Trolox, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), o-dianisidine, nitroblue tetrazolium (NBT) and bisacrylamide were obtained from Sigma-Aldrich (St Louis, MO, USA). Hydroxymethyl aminomethane (Tris) from Amresco (Solon, OH, USA). Acrylamide, sodium dodecyl sulfate (SDS) were obtained from Invitrogen. Immunoenzymatic assay kits (ELISA) for cytokine dosing from eBioscience (San Diego, CA, USA).

2.2. Experimental groups

The experiments were performed in sex matched hairless mice (HRS/J), adults and weighing on average 30g. Mice were kept in a room with controlled temperature ($22 \pm 2^\circ\text{C}$) located in the University Hospital of Londrina State University. The animals were housed under 12/12h light/dark cycles with water and food at will. The project was approved by the Animal Ethics Committee (CEUA) of the State University of Londrina, following its standards (registration in the CEUA under no. 017/2015, case n^o 1447.2015.10).

The animals were randomly assigned to five experimental groups containing 6 animals each:

- (1) non-irradiated and saline treated group;
- (2) irradiated and saline treated group;
- (3) irradiated and treated group at the 0.3 ng/mouse dose of RvD2;
- (4) irradiated and treated group at the 1.0 ng/mouse dose of RvD2;
- (5) irradiated and treated group at 3.0 ng/mouse dose of RvD2.

Mice were treated with 200 μl of different concentrations of RvD2 (0.3, 1.0 and 3.0 ng/mouse) or only the vehicle (sterile saline) used to dilute the lipid mediator intraperitoneally (i.p.) 1 h before beginning of irradiation. The dose of RvD2 was selected based on the therapeutic effects previously described [7,8,16,17] and in the dose-response experiments performed in this study.

2.3. Model of induction of skin lesion by UVB irradiation

A fluorescent UVB lamp model PHILIPS TL/12 40W RS (MEDICAL-NETHERLANDS) was used to induce acute inflammatory process and oxidative stress. The lamp emits radiation in the range of 270 to 400 nm with maximum emission peak around 313 nm and was coupled inside a rectangular wooden box developed specifically for the induction of cutaneous lesion, and was placed at 20 cm above the animals. The irradiance of this lamp was measured using a radiometer (IL 1700) with detectors for UV radiation (SED 005) and especially for UVB (SED 240) [18,19], making it possible to standardize the dose of radiation to 4,14 J/cm² during the exposure period of five hours and thirty minutes [6,20].

The animals were terminally anesthetized with 5% isoflurane and dorsal skin samples were collected 12 hours after the exposure to UVB irradiation for edema, MPO, MMP-9 activity, histology, FRAP, ABTS and GSH levels. Samples were collected after 2 hours of exposure to UVB for the tests of catalase and superoxide anion production (NBT). Finally, samples collected 4 hours after the end of the irradiation were tested for cytokines and hydroperoxide production. In these last-mentioned times (2 and 4 hours), the animals were anesthetized with 5% isoflurane followed by decapitation [20,21]. Skin samples to be analyzed were stored separated for each different test at -80 °C, except for the skin edema test, which was performed shortly after collection, and for the histopathological evaluation, for which the samples were stored in 10% formaldehyde solution after collected, until they were processed.

2.4. Skin edema

The UVB excessive exposure of the skin induces edema formation by increasing vascular permeability and consequent leakage of interstitial fluid into the epidermis [22]. For this test, mice dorsal skin samples were collected using a fixed area mold (5 mm diameter). For each of the groups, edema was measured by the variation of the skin weight before and after UVB exposure. The groups tested were statistically compared and the results were expressed in mg of skin [6].

2.5. Myeloperoxidase (MPO) activity

The activity of the enzyme MPO represents an indirect test of leukocytes recruitment to the lesion site after UVB irradiation [6,20]. Skin samples were homogenized in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (HTAB)

with the aid of the Tissue-Tearor homogenizer (Biospec 985370) and subsequently centrifuged (16.100 g for 2 min at 4 °C). For the colorimetric reaction, 30 µL of the supernatant from each sample was added together with 200 µL of a solution containing 10% 0.05 M phosphate buffer (pH 6.0), 0.0167% ortho-dianisidine and 0.05% H₂O₂ in deionized water. MPO activity of the samples was determined spectrophotometrically at 450 nm (EnSpire, Perkin Elmer) and compared to a standard curve of neutrophils with known concentration. The results obtained were expressed in numbers of neutrophils/mg of skin [19].

2.6. Determination of the activity / secretion of metalloproteinase-9 (MMP-9)

MMP-9 is a proteolytic enzyme that degrades important components of the extracellular matrix such as collagen, and its expression is increased through exposure to RUV [23,24]. The activity of this protease is determined by the previously described sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) zymography assay [25]. Samples were homogenized (Tissue-Tearor-Biospec 985370) in a ratio of 1:4 in 50 mM Tris/HCl phosphate buffer (pH 7.4) with calcium chloride (CaCl₂) and 1% proteinase inhibitors (phenanthroline, phenylmethylsulfonyl fluoride and N-ethylmaleimide). The homogenates were doubly centrifuged at 12.000 g for 10 min at 4 °C. Aliquots of 30 µL of the supernatant from the samples were diluted in 6 µL of Tris/HCl buffer (pH 6.8) containing 20% glycerol, 4% sodium dodecyl sulfate (SDS) and 0.001% bromophenol blue and applied gel (13.5% acrylamide and 0.025% gelatin). After completion of the run, the polyacrylamide gel washed with 2% Triton X-100 solution for 1 hour and then incubated for 16 hours at 37 °C in 50 mM Tris/HCl buffer (pH 7.4) containing 10 mM CaCl₂ and 0.02% sodium azide. In the next step, the gel was stained with 0,5% brilliant blue solution and washed with a 20% acetic acid solution for visualization of the bands. The proteolytic activity was analyzed semi-quantitatively by comparing the intensities of the bands between the groups by ImageJ® program (NIH, Bethesda, MD, USA).

2.7. Evaluation of ferric reducing antioxidant power (FRAP)

FRAP assay measures the ability of the skin sample of reducing ferric 2,4,6-tripyridyl-S-triazine (TPTZ) to a colored product through the action of antioxidants that donate electrons to the reaction [26]. Skin samples were homogenized in 400 µL of KCl (1.15%) with the aid of Tissue-Tearor (Biospec 985370) and centrifuged at 1000 g for 10 min at 4 °C. For the reaction, 30 µL of the homogenate supernatant and 150 µL FRAP reagent (10 mM TPTZ

in 40 mM HCl with 20 mM hexahydrate ferric chloride and 0.3 mM acetate buffer pH 3.6) were used. This solution was incubated at 37 °C for 30 min and then read at 595 nm (EnSpire, Perkin Elmer). Standard solutions with different concentrations of trolox (0.5 to 20 nmol) were used for calibration and the results were expressed as nmol equivalent of Trolox/mg of skin.

2.8. Evaluation of the antioxidant power by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical sequestration test

The antioxidant capacity of each sample is measured by the decaying of the color of the ABTS⁺ cation radical when it receives an electron from an antioxidant. For the ABTS reaction, the samples were homogenized in 400 µL of 1.15% KCl with the aid of Tissue-Tearor (Biospec 985370) and centrifuged at 1000 g for 10 min at 4 °C, after which the supernatant was used for analysis. 7 µL of the supernatant and 200 µL of the diluted ABTS solution (7 mM of the ABTS solution with 2.45 mM potassium persulfate) were added to a plate. After 6 minutes, the absorbance was read at 730 nm (EnSpire, Perkin Elmer) and compared with a standard curve of trolox (0.01 to 20 nmol) and the results were expressed nmol of Trolox / mg of skin [21,26].

2.9. Evaluation of endogenous antioxidant levels (GSH)

GSH is considered the main marker for UVB irradiation-induced oxidative stress because it is produced in greater quantity by the epidermal cells [19]. Skin samples were diluted (1:4) in 0.02 M EDTA and triturated using Tissue-Tearor (Biospec 985370). To the homogenate it was added 50% trichloroacetic acid (TCA) in the ratio of 1: 0.2 EDTA and TCA, respectively. Then, the mixture was centrifuged twice at 2.700 g at 4 °C for 10 minutes for the first time and 15 minutes and the final supernatant was removed for analysis. For quantitation of GSH levels, 50 µL of the supernatant was added to 100 µL of 0.4 M Tris buffer (pH 8.9) and 5 µL of a 1.9 mg/mL solution of DTNB in methanol was combined to the reaction. After 5 minutes of incubation, the samples were read at 405 nm (EnSpire, Perkin Elmer), and the results were compared with a GSH standard curve (5 to 150 µM) and expressed as µM GSH/mg skin [27].

2.10. Assay for catalase activity (CAT)

The principle of the technique is based on the decomposition of H_2O_2 followed by the decrease of absorbance. The difference in absorbance per unit of time measures the activity of the enzyme [28]. Skin samples were homogenized in 500 μ L of 0.02 M EDTA with the aid of Tissue-Tearor (Biospec 985370). Then the homogenate was centrifuged twice at 2.700 g for 10 minutes at 4 °C and the final supernatant was removed for analysis. For the reaction, 10 μ L sample, 160 μ L 1M Tris-HCl buffer with 5 mM EDTA pH 8.0, 20 μ L deionized water and 20 μ L of 200 mM H_2O_2 were added. The reaction was read 1 and 30 seconds after the addition of H_2O_2 at 240 nm (EnSpire, Perkin Elmer). Catalase values were expressed as catalase unit/mg skin/minute [28].

2.11. Evaluation of the superoxide anion production

Superoxide anion is a free radical formed in the first reduction of oxygen to water during aerobic respiration and its production can be accentuated by UVB irradiation [29,30]. This method is based on the reduction of nitroblue tetrazolium (NBT) to formazan. Skin samples were homogenized with Tissue-Tearor (Biospec 985370) in 0.02 M EDTA and centrifuged (2000 g for 20 seconds at 4 °C). First, 50 μ L of the supernatant from each sample was added and incubated for 1 hour. Subsequently, the supernatant was removed and NBT (1mg/mL) was added to the medium. After 15 minutes of incubation, the supernatant was removed and 20 μ L of 100% methanol was added to fix. Then 120 μ L of 2 M KOH and 140 μ L of dimethylsulfoxide (DMSO) were added to solubilize the formazan compound (reduced NBT). The concentration of formazan formed was measured spectrophotometrically (EnSpire, Perkin Elmer) at 620 nm and the results were presented as optical density (OD)/10 mg of skin [20].

2.12. Lipid Hydroperoxide (LOOH) assay

The production of hydroperoxides is measured by a chemiluminescence method and lipoperoxydation is initiated by addition of tert-butyl hydroperoxide as previously described [31] and adapted [21]. Skin samples were collected and homogenized (Tissue-Tearor Biospec 985370) in 800 μ L of phosphate buffer (pH 7.4) and then centrifuged at 700 g for 2 minutes at 4 °C. For the assay, 250 μ L of the supernatant was added to 1730 μ L of reaction medium (120 mM KCl, 30 mM phosphate buffer pH 7.4) and 20 μ L of 3 mM tert-butyl hydroperoxide. This assay was performed on a Beckman® LS 6000 β counter

(FULLERTON, CA, USA) in a count range not coincident with the response between 300 and 620 nm. The reaction must be carried on in the dark at 30 °C for 120 minutes and the results were measured in counts per minute (cpm) per mg of skin.

2.13. Dosage of pro-inflammatory cytokines TNF- α , IL-33, IL-1 β and anti-inflammatory cytokines TGF- β and IL-10

Cytokines quantification was performed using sandwich type enzyme-linked immunosorbent assay (ELISA) technique and performed according to the manufacturer's instructions [32]. The reaction was determined spectrophotometrically at 450 nm (Multiskan GO, Thermo Scientific) and the results were compared to a standard calibration curve and expressed as pg of cytokines/mg skin.

2.14. Histopathological evaluation by optical microscopy

For the histopathological analysis of the skin, the samples were collected and fixed in 10% formaldehyde until they were processed. Subsequently, they were dehydrated in baths of ethanol solutions with increasing concentrations (70%, 95% and 100%), diaphanized with xylol, and embedded in paraffin wax. Hematoxylin-eosin (H & E) stained sample sections of 5 μ m were analysed using light microscopy at 40 and 100x magnification for determination of epidermal thickness [33] and number of apoptotic keratinocytes [34], respectively. In addition, tissue sections were also stained with toluidine blue for determination of mast cells with a 40x magnification. Analyzes were done with Infinity Analyze (Lumenera® Software). Finally, sections of the tissues were stained with masson's trichrome for analysis of possible changes in collagen fibers (10x magnification) and were analyzed by Image J software (NIH) [35].

2.15. Statistical analysis

Statistical analyzes were performed using GraphPad Prism 7 software (GraphPad Software Inc., San Diego, USA). Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test. Results were presented as mean \pm standard error (SEM) of measurements made with 6 animals in each group per experiment. The results were representative of 2 separate experiments and were considered significantly different at $p < 0.05$.

3. Results

3.1. Resolvin D2 reduces UVB irradiation-induced skin edema, MPO and MMP-9 activity

Exposure to UVB irradiation resulted in skin edema and increased MPO activity in the irradiated group compared to non-irradiated group. Treatment with RvD2 reduced edema and MPO activity when compared with irradiated group. There was a reduction of cutaneous edema with doses of 1.0 and 3.0 ng/mouse and MPO activity with the three doses tested (0.3, 1.0 e 3.0 ng/mouse) when compared to irradiated group (Figure 1A-B). For the secretion/activity of MMP-9, it was observed an increase in the irradiated group compared to the non-irradiated group after the exposure to UVB radiation. The treatment with RvD2 significantly reduced enzyme activity with the dose of 3.0 ng/mouse (Figure 1C).

3.2. Resolvin D2 maintains antioxidant capacity and reduces production of LOOH and superoxide anion

The antioxidant capacity was evaluated initially through FRAP and ABTS assays and the dosage of the endogenous GSH antioxidant. It was observed that UVB irradiation-induced exposure was able to significantly reduce all the parameters tested in the irradiated group, comparing with the non-irradiated group. In addition, treatment with RvD2 was able to inhibit the depletion of skin antioxidant capacity with significant effects with the dose of 3.0 ng/mouse. On the other hand, the doses of 0.3 and 1.0 ng/mouse did not maintain the antioxidant capacity of the skin as shown through the results of all the parameters analyzed (Figure 2A-C) except by the dose of 1.0 ng/mouse in the FRAP assay that also induced a significant antioxidant activity. Therefore, 3.0 ng/mouse was selected as the best dose for the next set of tests.

UVB irradiation reduced CAT activity and increased both superoxide anion (NBT assay) and LOOH production in the irradiated group compared to the non-irradiated group. Treatment with RvD2 (3.0 ng/mouse) significantly inhibited the depletion of CAT assay and the production of superoxide anion and hydroperoxides when compared to irradiated group (Figure 2D-F).

3.3. Resolvin D2 inhibits UVB irradiation-induced skin inflammation by reducing cytokine production

The results demonstrated that there was a significant increase in the production of all cytokines evaluated after the exposure to UVB irradiation, including the pro-inflammatory TNF- α , IL-33 and IL-1 β and the anti-inflammatory agents TGF- β and IL-10. Treatment with

RvD2 (3.0 ng/mouse) was able to inhibit increased production of all cytokines cited above (Figure 3A-E).

3.4. Resolvin D2 reduces UVB irradiation-induced inflammatory parameters by decreasing the epidermal thickness and apoptosis of keratinocytes

The epidermal thickness of the dorsal skin had a significant increase in the group exposed to UVB irradiation compared to the non-irradiated control group. In contrast, treatment with RvD2 (3.0 ng/mouse) was able to reduce this epidermal hypertrophy (Figure 4A). Furthermore, another consequence of exposure to UVB irradiation is the presence of apoptotic keratinocytes - epidermal cells with eosinophilic cytoplasm and condensed nucleus - which were observed in greater number in the irradiated group and reduced in the group treated with RvD2 (Figure 4B).

3.5. Resolvin D2 reduces UVB irradiation-induced damage to collagen fibers

Epidermal tissue sections were stained with Masson's trichrome for visualization of collagen fibers and increase in their color intensity were observed in the irradiated control group compared to the non-irradiated control group. In addition, treatment with RvD2 (3.0 ng/mouse) prevented UVB damage to collagen fibers (Figure 5).

3.6. Resolvin D2 reduces UVB irradiation-induced mast cell proliferation

After exposure to UVB irradiation, there was a significant increase in the number of dermal mast cells, which are cells participating in the immunomodulation. Furthermore, samples from animals treated with RvD2 (3.0 ng/mouse) showed a reduction in the proliferation of these cells when compared to samples from the irradiated control group (Figure 6).

4. Discussion

UV irradiation is an external factor that generates expressive skin damage, including DNA damage that can lead in to skin cancer [30,36]. It is agreed that the pathophysiological mechanisms of UVB irradiation involve the association between oxidative stress and the production of inflammatory mediators [37]. It should also be noted that the use of sunscreens is not totally effective against UVB radiation, since it can further promote the generation of ROS and cause allergic reactions [38,39]. In addition, the antioxidant system that protects the skin against oxidative damage is deficient under stress conditions and therefore, the use of therapies with exogenous antioxidants would be a valuable alternative [7,8,19]. Pro-resolution lipid mediators are generated to attenuate the amplification of the inflammatory response and consequently of ROS, stimulating healing and tissue regeneration [40,41,42]. RvD2 is a lipid mediator derived from the enzymatic conversion of DHA that has shown therapeutic potential in several other disease models [11,12].

In the present study, systemic treatment with RvD2 protected UVB irradiation-induced skin against inflammatory damage, significantly reducing the following parameters analyzed: edema, MPO, MMP-9 activity, collagen fiber degradation, epidermal thickness, number of mast cells and apoptosis of keratinocytes. In addition, RvD2 anti-inflammatory action is also be associated with reduced production of pro-inflammatory cytokines IL-1 β , TNF- α and IL-33, responsible for positively regulating the immune response, and anti-inflammatory IL-10 and TGF- β , which decrease the inflammatory process. IL-10 is an anti-inflammatory cytokine that is co-released with pro-inflammatory cytokines to limit the inflammatory reaction [43]. Concomitant inhibition of pro and anti-inflammatory cytokine suggests that RvD2 blocks the release of these cytokines.

Other studies with different experimental models show that RvD2 reduces the inflammation, corroborating with our findings. In model of microbial sepsis induced by cecal ligation and puncture, treatment with RvD2 was able to inhibit leukocyte recruitment, and also to decrease the production of proinflammatory cytokines such as TNF- α and IL-1 and the anti-inflammatory IL-10, since the tissue damage was repaired [16]. MPO activity was also evaluated in a model of experimental colitis in mice as an indirect test for the analysis of neutrophil infiltration, and its activity was reduced by the treatment with RvD2 [44]. Excessive exposure to UVB irradiation promotes synthesis of cytokines that recruit neutrophils and mediate the action of their enzymes such as metalloproteinases and MPO, enzymes associated with the degradation of collagen fibers and additional production of ROS respectively [45,46,47].

Additionally, activation of cytokines triggers other cellular events. Keratinocytes are epidermal cells that have adaptive mechanisms to enhance protection against UVB irradiation. One is the proliferation of the cells at the site of the lesion, resulting in increased epidermal thickness and also in skin edema [2,22,48]. When these cells fail to prevent damage caused by UV irradiation, keratinocytes undergo apoptosis, which are known as sunburn cells and characterized microscopically by pyknotic nucleus [49,50]. After exposure to UVB, proliferation of mast cells plays an important role in immunosuppression [51]. These data are consistent with our findings, once these cells were reduced with treatment with RvD2 at the dose of 3.0 ng/mouse. Corroborating, RvD1, another DHA-derived metabolite, was responsible for decreasing these inflammatory parameters at the dose of 30 ng/mouse in the model UVB-induced of skin lesion [8].

UVB irradiation generates ROS, such as superoxide anion, which can react with lipids promoting the event called lipid peroxidation [37,52]. Furthermore, increased production of ROS leads to an imbalance in the antioxidant system, resulting in the depletion of molecules belonging to this system, such as GSH and CAT [6,19,53]. GSH neutralizes oxidants by the action of the sulfhydryl group of the molecule and CAT converts hydrogen peroxide to water (H₂O) and oxygen (O₂), preventing the formation of toxic radicals such as hydroxyl [54]. In this work, the systemic treatment with RvD2 was able to significantly reduce oxidative stress induced by UVB irradiation by reducing superoxide anion and hydroperoxide production, as well as maintaining the levels of the GSH and CAT endogenous antioxidants. In addition, RvD2 protected the skin against oxidative damage through its ferric reduction ability and the ABTS radical, parameters evaluated for the overall antioxidant capacity of the tissue [26].

Resolvins in general have demonstrated antioxidant activity in other experimental models as, for instance a model of osteoarthritis, in which an improvement of the antioxidant capacity was observed by maintaining basal levels of GSH in the group treated with RvD1 [55]. In another work, RvD2 was able to reduce oxidative stress by decreasing levels of carbonylated proteins in the model of exposure to cigarette smoke [56]. Furthermore, treatment with RvD2 decreased TNF α -induced superoxide anion production in mouse aortic smooth muscle cell. Inflammatory cytokines such as TNF- α are mediators that intensify the local response, resulting in the additional production of ROS and amplification of tissue damage [57].

Recently, we demonstrated that RvD1, reduces UVB irradiation-induced skin oxidative stress, because it improved the antioxidant system (GSH and CAT assays), and inhibited hydroperoxide formation and superoxide anion production [8], in accordance with the results of this work. However, it should be noted that the antioxidant and anti-inflammatory activities of RvD1 were demonstrated at a dose of 30 ng/mouse, dose ten times

higher than the dose of RvD2 used in the present study (3.0 ng/mouse), indicating that treatment with RvD2 can be even more potent in the model UVB irradiation exposure.

In this context, the possible explanation for such biological actions of RvD2 is the interaction with the receptor coupled to G protein, denominated G protein-coupled receptor 18 (GPR18), which promotes the phagocytosis of apoptotic cells and the removal of cell debris for the resolution of inflammation [58]. Resolution of inflammation is an endogenous mechanism that reduces the magnitude of the inflammatory response with the reestablishment of homeostasis, resulting in decreased cytokine production, leukocyte infiltration and extracellular ROS [41]. In conclusion, these findings demonstrated for the first time the great potential of RvD2 as a treatment molecule for the control and prevention of inflammatory damage and oxidative stress induced by UVB irradiation.

Acknowledgements

This study was supported by Brazilian grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Araucária and Parana State Government. Authors declare no conflict of interest.

References

- [1] M. Brenner, V.J. Hearing, The Protective Role of Melanin Against UV Damage in Human Skin, *Photochem Photobiol.* 84 (2008) 539–549. doi:10.1111/j.1751-1097.2007.00226.x.The.
- [2] J. D’Orazio, S. Jarrett, A. Amaro-ortiz, T. Scott, UV Radiation and the Skin, *Int. J. Mol. Sci.* 14 (2013) 12222–12248. doi:10.3390/ijms140612222.
- [3] F. Afaq, V.M. Adhami, H. Mukhtar, Photochemoprevention of ultraviolet B signaling and photocarcinogenesis, *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* 571 (2005) 153–173. doi:10.1016/j.mrfmmm.2004.07.019.
- [4] A.P. Schuch, N.C. Moreno, N.J. Schuch, C.F.M. Menck, C.C.M. Garcia, A, Sunlight damage to cellular DNA: Focus on oxidatively generated lesions, *Free Radic. Biol. Med.* 107 (2017) 110–124. doi:10.1016/j.freeradbiomed.2017.01.029.
- [5] S. Beissert, K. Loser, Molecular and cellular mechanisms of photocarcinogenesis, *Photochem. Photobiol.* 84 (2008) 29–34. doi:10.1111/j.1751-1097.2007.00231.x.
- [6] A.L.M. Ivan, M.Z. Campanini, R.M. Martinez, V.S. Ferreira, V.S. Steffen, F.T.M.C. Vicentini, F.M.P. Vilela, F.S. Martins, A.C. Zarpelon, T.M. Cunha, M.J.V. Fonseca, M.M. Baracat, S.R. Georgetti, W.A. Verri, R. Casagrande, Pyrrolidine dithiocarbamate inhibits UVB-induced skin inflammation and oxidative stress in hairless mice and exhibits antioxidant activity in vitro, *J. Photochem. Photobiol. B Biol.* 138 (2014) 124–133. doi:10.1016/j.jphotobiol.2014.05.010.
- [7] R.M. Martinez, V. Fattori, P. Saito, C.P.B. Melo, S.M. Borghi, I.C. Pinto, A.J.C. Bussmann, M.M. Baracat, S.R. Georgetti, W.A. Verri Jr, R. Casagrande, Lipoxin A4 inhibits UV radiation-induced skin inflammation and oxidative stress in mice, *J. Dermatol. Sci.* 91 (2018) 164–174. doi:10.1016/j.jdermsci.2018.04.014.
- [8] P. Saito, C.P.B. Melo, R.M. Martinez, V. Fattori, T.L.C. Cezar, I.C. Pinto, A.J.C. Bussmann, J.A. Vignoli, S.R. Georgetti, M.M. Baracat, W.A. Verri, R. Casagrande, The Lipid Mediator Resolvin D1 Reduces the Skin Inflammation and Oxidative Stress Induced by UV Irradiation in Hairless Mice, *Front. Pharmacol.* 9 (2018) 1–15. doi:10.3389/fphar.2018.01242.
- [9] A. Kumar, Y. Takada, A. Boriak, B.B. Aggarwal, Nuclear factor- κ B: its role in health and disease, *J. Mol. Med.* 82 (2004) 434–448. doi:10.1007/s00109-004-0555-y.

- [10] J. Song, W. Zhang, J. Wang, H. Yang, X. Zhao, Q. Zhou, H. Wang, L. Li, G. Du, Activation of Nrf2 signaling by salvianolic acid C attenuates NF- κ B mediated inflammatory response both in vivo and in vitro, *Int. Immunopharmacol.* 63 (2018) 299–310. doi:10.1016/j.intimp.2018.08.004.
- [11] H. Seki, T. Sasaki, T. Ueda, M. Arita, Resolvins as Regulators of the Immune System, *Sci. World J.* 10 (2010) 818–831. doi:10.1100/tsw.2010.72.
- [12] P.C. Calder, Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?, *Br. J. Clin. Pharmacol.* 75 (2013) 645–662. doi:10.1111/j.1365-2125.2012.04374.x.
- [13] G. Zuo, D. Zhang, R. Mu, H. Shen, X. Li, Z. Wang, H. Li, G. Chen, Resolvin D2 protects against cerebral ischemia/reperfusion injury in rats, *Mol. Brain.* 11 (2018) 1–13. doi:10.1186/s13041-018-0351-1.
- [14] Y. Tian, Y. Zhang, R. Zhang, S. Qiao, J. Fan, Resolvin D2 recovers neural injury by suppressing inflammatory mediators expression in lipopolysaccharide-induced Parkinson's disease rat model, *Biochem. Biophys. Res. Commun.* 460 (2015) 799–805. doi:10.1016/j.bbrc.2015.03.109.
- [15] Z. Zhang, X. Hu, X. Qi, G. Di, Y. Zhang, Q. Wang, Q. Zhou, Resolvin D1 promotes corneal epithelial wound healing and restoration of mechanical sensation in diabetic mice, *Mol. Vis.* 24 (2018) 274–285. <http://www.molvis.org/molvis/v24/274>.
- [16] M. Spite, L. V. Norling, L. Summers, R. Yang, D. Cooper, N.A. Petasis, R.J. Flower, M. Perretti, C.N. Serhan, Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis, *Nature.* 461 (2009) 1287–1291. doi:10.1038/nature08541.
- [17] L. Berrueta, I. Muskaj, S. Olenich, T. Butler, G.J. Badger, R.A. Colas, M. Spite, C.N. Serhan, H.M. Langevin, Stretching Impacts Inflammation Resolution in Connective Tissue, *J. Cell. Physiol.* 231 (2016) 1621–1627. doi:10.1002/jcp.25263.
- [18] M. Carini, G. Aldini, M. Piccone, R.M. Facino, Fluorescent probes as markers of oxidative stress in keratinocyte cell lines following UVB exposure, *Il Farm.* 55 (2000) 526–534. doi:10.1016/S0014-827X(00)00037-9.
- [19] R. Casagrande, S.R. Georgetti, W.A. Verri, D.J. Dorta, A.C. dos Santos, M.J.V. Fonseca, Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice, *J. Photochem. Photobiol. B Biol.* 84 (2006) 21–27. doi:10.1016/j.jphotobiol.2006.01.006.

- [20] M.Z. Campanini, F.A. Pinho-Ribeiro, A.L.M. Ivan, V.S. Ferreira, F.M.P. Vilela, F.T.M.C. Vicentini, R.M. Martineza, A.C. Zarpelon, M.J.V. Fonseca, T.J. Faria, M.M. Baracat, W.A. Verri, S.R. Georgetti, R. Casagrande, Efficacy of topical formulations containing *Pimenta pseudocaryophyllus* extract against UVB-induced oxidative stress and inflammation in hairless mice, *J. Photochem. Photobiol. B Biol.* 127 (2013) 153–160. doi:10.1016/j.jphotobiol.2013.08.007.
- [21] R.M. Martinez, F.A. Pinho-Ribeiro, V.S. Steffen, C. V. Caviglione, J.A. Vignoli, D.S. Barbosa, M.M. Baracat, S.R. Georgetti, W.A. Verri, R. Casagrande, Naringenin Inhibits UVB Irradiation-Induced Inflammation and Oxidative Stress in the Skin of Hairless Mice, *J. Nat. Prod.* 78 (2015) 1647–1655. doi:10.1021/acs.jnatprod.5b00198.
- [22] K. Kajiya, S. Hirakawa, M. Detmar, Vascular Endothelial Growth Factor-A Mediates Ultraviolet B-Induced Impairment of Lymphatic Vessel Function, 169 (2006) 1496–1503. doi:10.2353/ajpath.2006.060197.
- [23] M. Saito, M. Tanaka, E. Misawa, R. Yao, K. Nabeshima, K. Yamauchi, F. Abe, Y. Yamamoto, F. Furukawa, Oral administration of aloe vera gel powder prevents uvb-induced decrease in skin elasticity via suppression of overexpression of mmps in hairless mice, *Biosci. Biotechnol. Biochem.* 80 (2016) 1416–1424. doi:10.1080/09168451.2016.1156480.
- [24] C.H.F. Cuelho, G. de A.D. Alves, M.O. Lovatto, I.F. Bonilha, F. Barbisan, I.B.M. da Cruz, S.M. Oliveira, R. Fachinetto, G.S. do Canto, M.P. Manfron, Topical formulation containing *Ilex Paraguariensis* extract increases metalloproteinases and myeloperoxidase activities in mice exposed to UVB radiation, *J. Photochem. Photobiol. B Biol.* 189 (2018) 95–103. doi:10.1016/j.jphotobiol.2018.10.004.
- [25] M.J.V. Fonseca, Y.M. Fonseca, F. Marquele-Oliveira, F.T.M.C. Vicentini, N.A.J.C. Furtado, J.P.B. Sousa, Y.M. Lucisano-Valim, Evaluation of the potential of Brazilian propolis against UV-induced oxidative stress, *Evidence-Based Complement. Altern. Med.* 2011 (2011) 1–8. doi:10.1155/2011/863917.
- [26] V. Katalinic, D. Modun, I. Music, M. Boban, Gender differences in antioxidant capacity of rat tissues determined by reducing antioxidant power (FRAP) assays, *Comp. Biochem. Physiol.* 140 (2005) 47–52. doi:10.1016/j.cca.2005.01.005.
- [27] P. Srinivasan, K.E. Sabitha, C.S. Shyamaladevi, Attenuation of 4-Nitroquinoline 1-oxide induced in vitro lipid peroxidation by green tea polyphenols, *Life Sci.* 80 (2007) 1080–1086. doi:10.1016/j.lfs.2006.11.051.

- [28] H. Aebi, Catalase in Vitro, *Methods Enzym.* 105 (1984) 121–126. doi:10.1016/S0076-6879(84)05016-3.
- [29] A.L.A. Ferreira, L.S. Matsubara, Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo, *Rev Ass Med Bras.* 43 (1997) 61–68. doi:10.1590/S0104-42301997000100014.
- [30] K. Hiramoto, H. Kobayashi, Y. Yamate, M. Ishii, E.F. Sato, Intercellular pathway through hyaluronic acid in UVB-induced inflammation, *Exp. Dermatol.* 21 (2012) 911–914. doi:10.1111/exd.12032.
- [31] B.Gonzalez Flecha, S.Llesuy, A. Boveris, Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle, *Free Radic. Biol. Med.* 10 (1991) 93–100. doi:10.1016/0891-5849(91)90002-K.
- [32] W.A. Verri, A.T.G. Guerrero, S.Y. Fukada, D.A. Valerio, T.M. Cunha, D. Xu, S.H. Ferreira, F.Y. Liew, F.Q. Cunha, IL-33 mediates antigen-induced cutaneous and articular hypernociception in mice, *Proc. Natl. Acad. Sci.* 105 (2008) 2723–2728. doi:10.1073/pnas.0712116105.
- [33] Y. Deng, A. Ediriwickrema, F. Yang, J. Lewis, M. Girardi, W.M. Saltzman, A Sunblock Based On Bioadhesive Nanoparticles, *Nat Mater.* 14 (2015) 1278–1285. doi:10.1038/nmat4422.A.
- [34] A. Schwarz, R. Bhardwaj, Y. Aragane, K. Mahnke, H. Riemann, D. Metze, T.A. Luger, T. Schwarz, Ultraviolet-B-Induced Apoptosis of Keratinocytes: Evidence for Partial Involvement of Tumor Necrosis Factor- α in the Formation of Sunburn Cells, *J. Invest. Dermatol.* 104 (1995) 922–927. doi:10.1111/1523-1747.ep12606202.
- [35] J.A.E.H. Song, M.E.I.J. Piao, X.I.A. Han, K.A.H. Kang, H.E.E.K. Kang, W.J. Yoon, M.I.H.E.E. Ko, N.A.M.H.O. Lee, M.I.Y. Lee, S. Chae, J.I.N.W.O.N. Hyun, Anti-wrinkle effects of *Sargassum muticum* ethyl acetate fraction on ultraviolet B-irradiated hairless mouse skin and mechanistic evaluation in the human HaCaT keratinocyte cell line, *Mol. Med. Rep.* 14 (2016) 2937–2944. doi:10.3892/mmr.2016.5655.
- [36] F. Afaq, S.K. Katiyar, Polyphenols: Skin Photoprotection and Inhibition of Photocarcinogenesis, *Mini Rev Med Chem* 11 (2011) 1200–1215.
- [37] G.M. Halliday, Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis, *Mutat. Res.* 571 (2005) 107–120. doi:10.1016/j.mrfmmm.2004.09.013.

- [38] K.M. Hanson, E. Gratton, C.J. Bardeen, Sunscreen enhancement of UV-induced reactive oxygen species in the skin, *Free Radic. Biol. Med.* 41 (2006) 1205–1212. doi:10.1016/j.freeradbiomed.2006.06.011.
- [39] A.R. Heurung, S.I. Raju, E.M. Warshaw, Adverse reactions to sunscreen agents: Epidemiology, responsible irritants and allergens, clinical characteristics, and management, *Dermatitis*, 2014. doi:10.1097/DER.0000000000000079.
- [40] P.C. Calder, R. Albers, J. Antoine, S. Blum, G.A. Ferns, G. Folkerts, Inflammatory Disease Processes and Interactions with Nutrition, *Br. J. Nutr.* 101 (2009). doi:10.1017/S0007114509377867.
- [41] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology, *Nature*. 510 (2014) 92–101. doi:10.1038/nature13479.
- [42] C.N. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation : dual lipid mediators, *Nat. Rev. Immunol.* 8 (2008) 349–361. doi:10.1038/nri2294.
- [43] V. Fattori, F.A. Pinho-Ribeiro, S.M. Borghi, J.C. Alves-Filho, T.M. Cunha, F.Q. Cunha, R. Casagrande, W.A. Verri, Curcumin inhibits superoxide anion-induced pain-like behavior and leukocyte recruitment by increasing Nrf2 expression and reducing NF- κ B activation, *Inflamm. Res.* 64 (2015) 993–1003. doi:10.1007/s00011-015-0885-y.
- [44] A.F. Bento, R.F. Claudino, R.C. Dutra, R. Marcon, J.B. Calixto, Omega-3 Fatty Acid-Derived Mediators 17(R)-Hydroxy Docosahexaenoic Acid, Aspirin-Triggered Resolvin D1 and Resolvin D2 Prevent Experimental Colitis in Mice, *J. Immunol.* 187 (2011) 1957–1969. doi:10.4049/jimmunol.1101305.
- [45] V.P. Navarro, P. Nelson-Filho, L.A.B. Silva, A.C. Freitas, A participação das metaloproteinases da matriz nos processos fisiológicos da cavidade bucal, *Rev. Odontol. Da UNESP* 35 (2006) 233–248. ISSN 1807-2577.
- [46] K.M. Michalak, T. Biedermann, E. Reichmann, M. Meuli, A.S. Klar, Induction of angiogenic and inflammation-associated dermal biomarkers following acute UVB exposure on bio-engineered pigmented dermo-epidermal skin substitutes in vivo, *Pediatr. Surg. Int.* 35 (2018) 129–136. doi:10.1007/s00383-018-4384-4.
- [47] S. Abbas, S. Alam, A. Pal, M. Kumar, D. Singh, K.M. Ansari, UVB exposure enhanced benzanthrone-induced inflammatory responses in SKH-1 mouse skin by activating the expression of COX-2 and iNOS through MAP kinases / NF- κ B / AP-1 signalling pathways, *Food Chem. Toxicol.* 96 (2016) 183–190. doi:10.1016/j.fct.2016.07.034.

- [48] T.L. Scott, P.A. Christian, M. V. Kesler, K.M. Donohue, B. Shelton, K. Wakamatsu, I. Shosuke, J. D'Orazio, Pigment-independent cAMP-mediated epidermal thickening protects against cutaneous UV injury by keratinocyte proliferation, *Exp. Dermatol.* 21 (2012) 771–777. doi:10.1111/exd.12012.
- [49] C. Bayerl, S. Taake, I. Moll, E.G. Jung, Characterization of sunburn cells after exposure to ultraviolet light, *Photodermatol. Photoimmunol. Photomed.* 11 (1995) 149–154. doi:10.1111/j.1600-0781.1995.tb00157.x.
- [50] V. Muthusamy, T.J. Piva, The UV response of the skin: A review of the MAPK, NF κ B and TNF α signal transduction pathways, *Arch. Dermatol. Res.* 302 (2010) 5–17. doi:10.1007/s00403-009-0994-y.
- [51] N.K. Gibbs, M. Norval, Photoimmunosuppression: a brief overview, *Photodermatol Photoimmunol Photomed.* 29 (2013) 57–64. doi: 10.1111/phpp.12021.
- [52] D.E. Godar, UVA1 Radiation Triggers Two Different Final Apoptotic Pathways, *J. Invest. Dermatol.* 112 (1999) 3–12. doi:10.1046/j.1523-1747.1999.00474.x.
- [53] R.M. Martinez, F.A. Pinho-Ribeiro, D.L. Vale, V.S. Steffen, F.T.M.C. Vicentini, J.A. Vignoli, M.M. Baracat, S.R. Georgetti, W.A. Verri, R. Casagrande, Trans-chalcone added in topical formulation inhibits skin inflammation and oxidative stress in a model of ultraviolet B radiation skin damage in hairless mice, *J. Photochem. Photobiol. B Biol.* 171 (2017) 139–146. doi:10.1016/j.jphotobiol.2017.05.002.
- [54] D.R. Bickers, M. Athar, Oxidative Stress in the Pathogenesis of Skin Disease, *J. Invest. Dermatol.* 126 (2006) 2565–2575. doi:10.1038/sj.jid.5700340.
- [55] H. Benabdoune, E.P. Rondon, Q. Shi, J. Fernandes, P. Ranger, H. Fahmi, M. Benderdour, The role of resolvin D1 in the regulation of inflammatory and catabolic mediators in osteoarthritis, *Inflamm. Res.* 65 (2016) 635–645. doi:10.1007/s00011-016-0946-x.
- [56] A. Croasdell, T.H. Thatcher, R.M. Kottmann, R.A. Colas, J. Dalli, C.N. Serhan, P.J. Sime, R.P. Phipps, Resolvins attenuate inflammation and promote resolution in cigarette smoke- exposed human macrophages, *Am J Physiol Lung Cell Mol Physiol.* 309 (2015) L888–L901. doi:10.1152/ajplung.00125.2015.
- [57] D. Akagi, M. Chen, R. Toy, A. Chatterjee, M.S. Conte, Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice, *FASEB J.* 29 (2015) 2504–2513. doi:10.1096/fj.14-265363.

- [58] N. Chiang, J. Dalli, R.A. Colas, C.N. Serhan, Identification of resolvin D2 receptor mediating resolution of infections and organ protection, *J. Exp. Med.* 212 (2015) 1203–1217. doi:10.1084/jem.20150225.

Figures

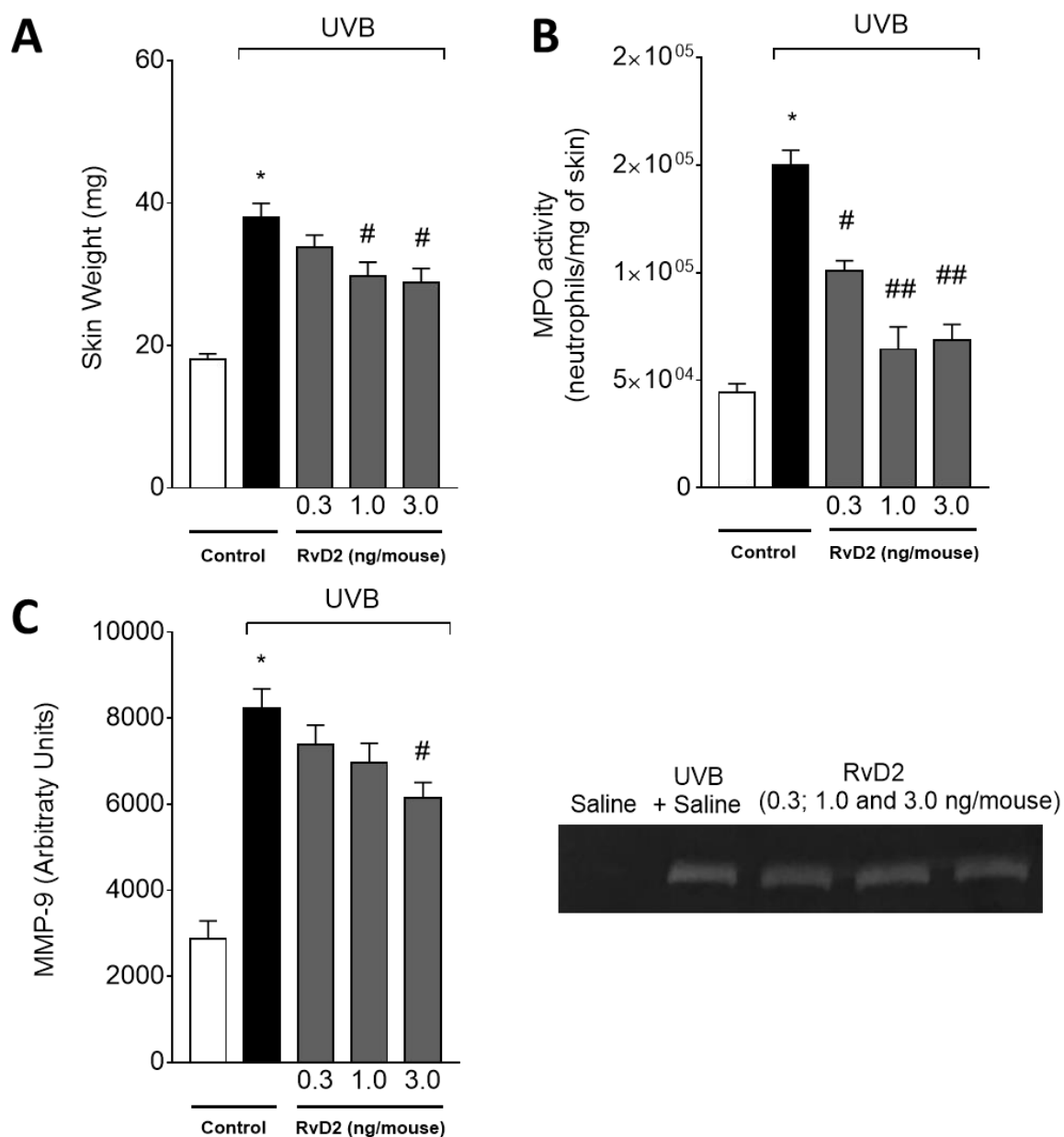


Figure 1 – RvD2 reduces UVB irradiation-induced skin edema, MPO and MMP-9 activity. The skin inflammation was determined in samples collected 12 h after the end of irradiation. Skin edema (A), MPO activity (B) and MMP-9 activity and representative image of gelatin zymography (C). Bars represent means \pm SEM of 6 mice per group and are representative of two separate experiments. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. [* $p < 0.05$ compared to the non-irradiated group; # $p < 0.05$ compared to the irradiated group (vehicle); ## $p < 0.05$ compared to the irradiated group and treated group at 0.3 ng/mouse].

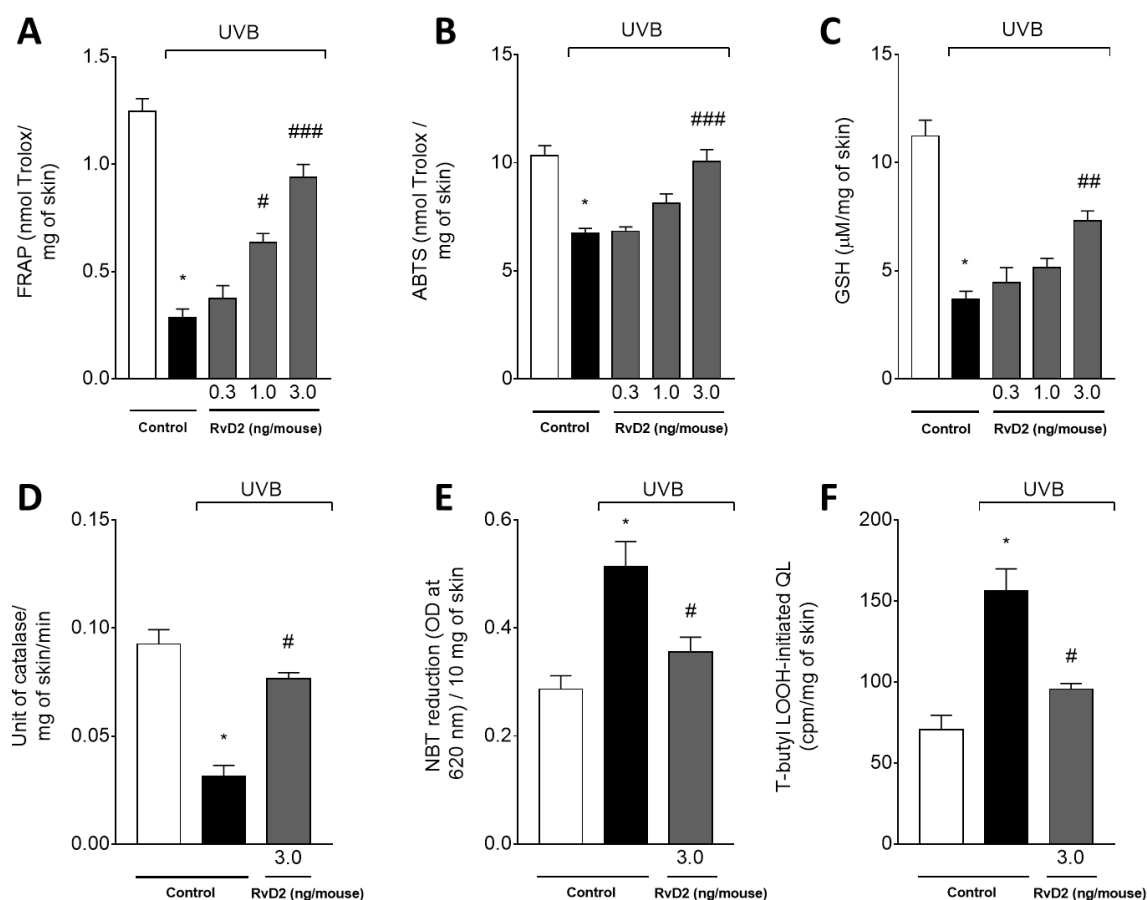


Figure 2 – RvD2 maintains antioxidant capacity and reduces production of hydroperoxides (LOOH) and superoxide anion. The antioxidant capacity was determined by FRAP (A), ABTS (B), GSH (C) assays in samples collected 12 h after the end of irradiation. The catalase activity (D) and superoxide anion production (E) were determined in samples collected 2 h after the end of irradiation. Lipid hydroperoxides production (F) was determined in samples collected 4 h after the end of irradiation. Bars represent means \pm SEM of 6 mice per group per experiment and are representative of two separated experiments. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. [* p <0.05 compared to the non-irradiated group; # p <0.05 compared to the irradiated group (vehicle); ### p <0.05 compared to the irradiated group and treated group at 0.3 ng/mouse; #### p <0.05 compared to the irradiated group and treated group at 1.0 ng/mouse].

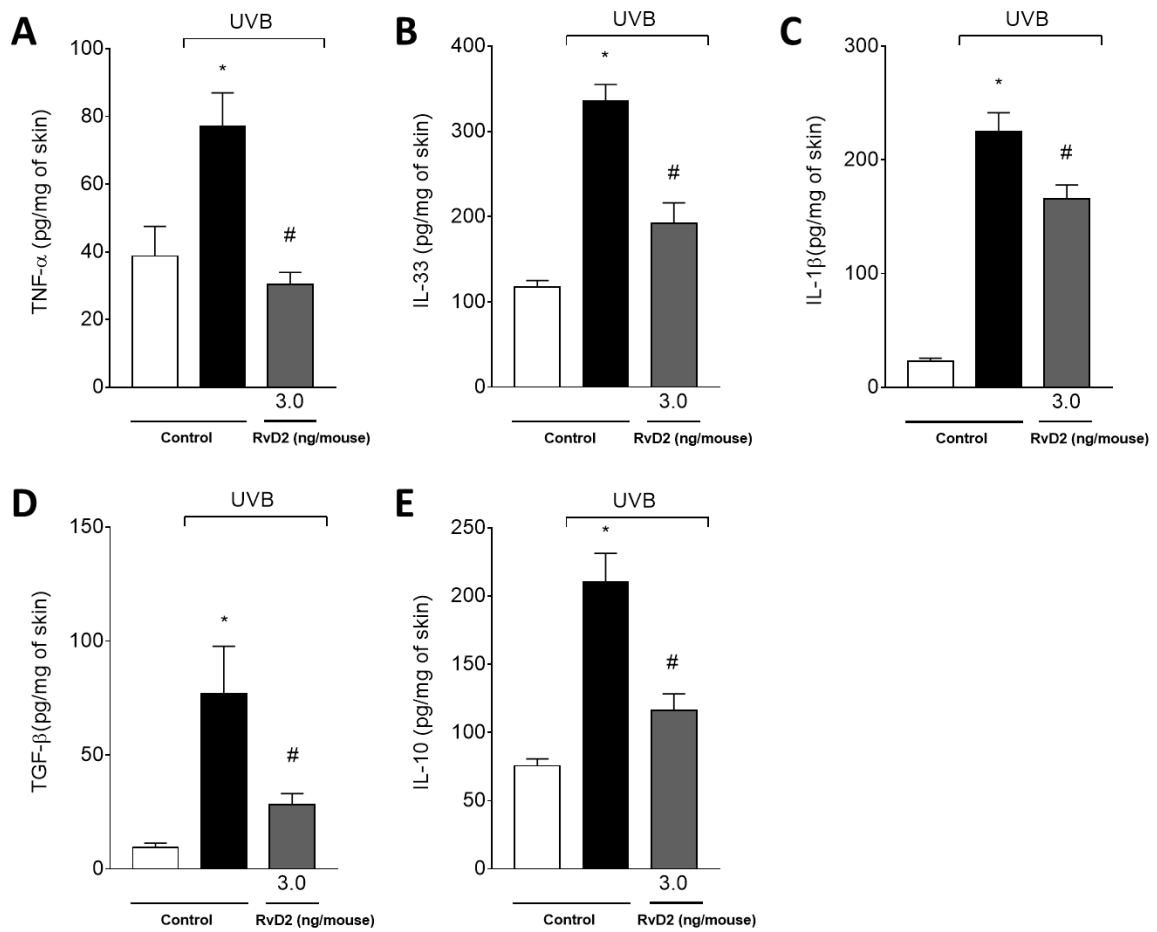


Figure 3 – RvD2 inhibits UVB irradiation-induced pro-inflammatory TNF- α (A), IL-33 (B) and IL-1 β (C) and anti-inflammatory TGF- β (D) and IL-10 (E) cytokines production. Cytokines were determined in skin samples collected 4 h after the end of irradiation. Bars represent means \pm SEM of 6 mice per group per experiment and are representative of two separated experiments. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. [* $p < 0.05$ compared to the non-irradiated group and # $p < 0.05$ compared to the irradiated group (vehicle)].

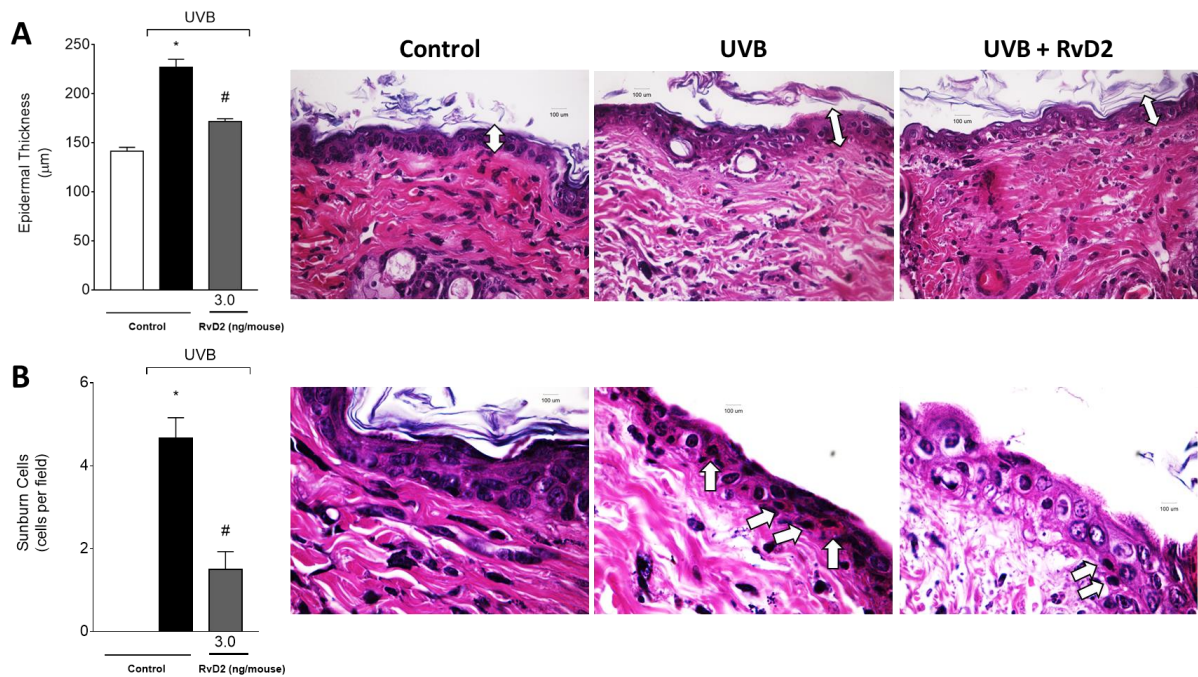


Figure 4 – RvD2 reduces UVB irradiation-induced inflammatory parameters by decreasing the epidermal thickness and apoptosis of keratinocytes (sunburn cells). Epidermal thickness and sunburn cells were evaluated using hematoxylin and eosin (H&E) staining in skin samples collected 12 h after the end of irradiation. Epidermal thickness (μm) (A) and the number of sunburn cells (B). The sections stained with H&E were examined using light microscopy at 40x (A) magnification and 100x (B) respectively. Bars represent means \pm SEM of 6 mice per group per experiment and are representative of two separated experiments. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. [$*p < 0.05$ compared to the non-irradiated group and $\#p < 0.05$ compared to the irradiated group (vehicle)].

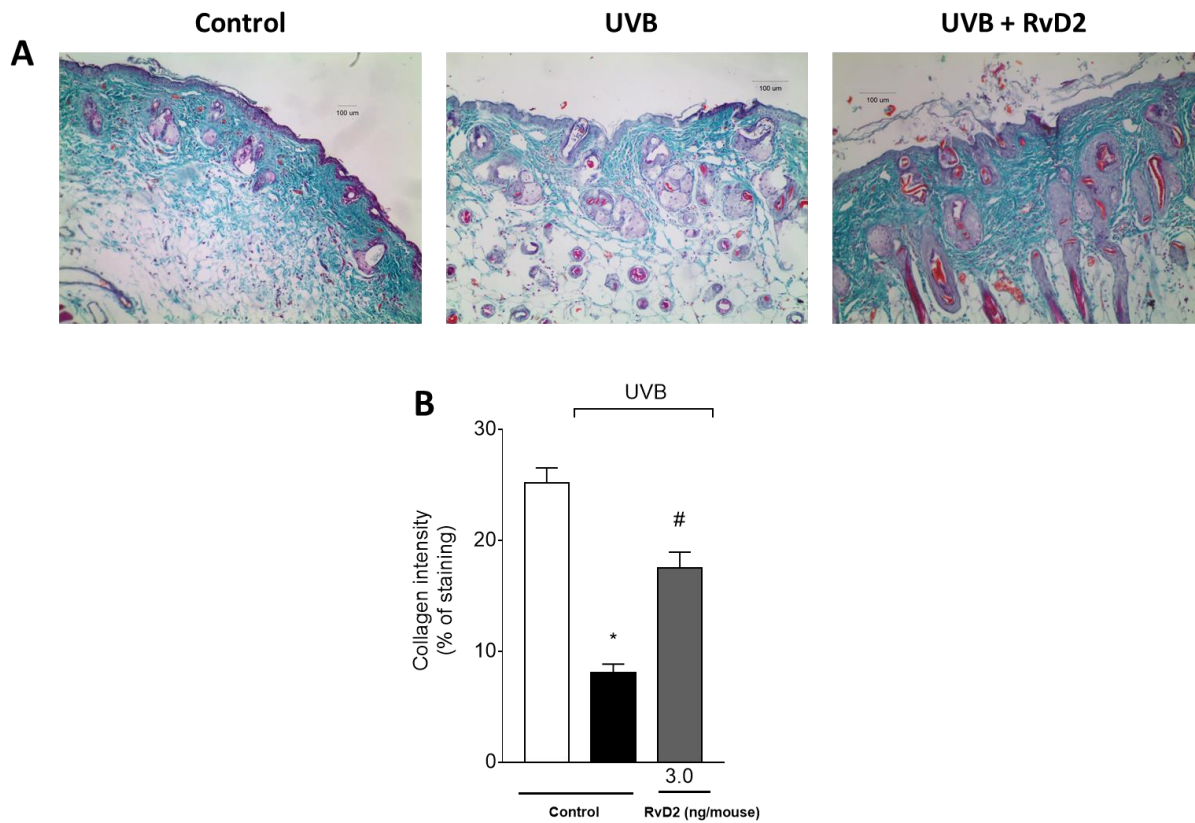


Figure 5 – RvD2 reduces UVB irradiation-induced collagen fibers damage. Collagen fiber formation was evaluated using Masson's trichrome staining in skin samples collected 12 h after the end of irradiation. Collagen fiber intensity and bundles shown in blue was analyzed by Image J Program (10x magnification). Bars represent means \pm SEM of 6 mice per group per experiment and are representative of two separated experiments. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. [$*p < 0.05$ compared to the non-irradiated group and $\#p < 0.05$ compared to the irradiated group (vehicle)].

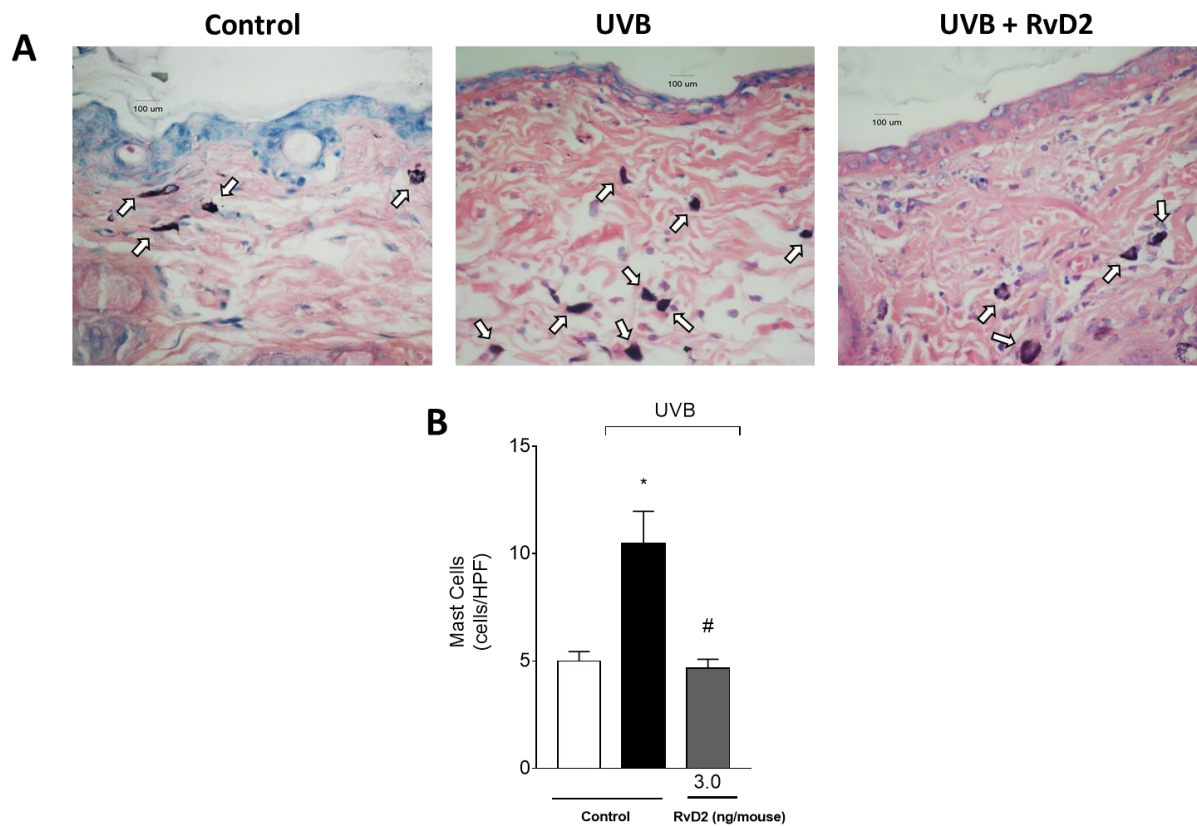


Figure 6 – RvD2 reduces UVB irradiation-induced mast cell proliferation. Mast cells were evaluated using blue toluidine in skin samples collected 12 h after the end of irradiation. Sections stained with blue toluidine were examined using light microscopy at 40x magnification (A) and number of mast cells (B). Bars represent means \pm SEM of 6 mice per group per experiment and are representative of two separated experiments. Statistical analysis was performed by one-way ANOVA followed by Tukey’s test. [* $p < 0.05$ compared to the non-irradiated group and # $p < 0.05$ compared to the irradiated group (vehicle)].

5. CONSIDERAÇÕES FINAIS

A exposição à radiação ultravioleta é uma das principais causas de danos na pele, que pode resultar em lesões cancerosas e aceleração do envelhecimento cutâneo. Isto ocorre devido ao excesso de radicais livres, levando a depleção do sistema antioxidante endógeno. Aliado a esse fator, existe a grande produção de moléculas pró-inflamatórias como as citocinas, via fatores de transcrição, e recrutamento de leucócitos, que são responsáveis por gerar adicionalmente mais radicais livres, resultando em danos teciduais ainda mais expressivos. Neste contexto, alternativas terapêuticas podem ser estabelecidas baseando-se na melhora da capacidade antioxidante e no controle da lesão induzida pela radiação UVB com a administração de mediadores lipídicos anti-inflamatórios/pró-resolução. Estudos anteriores constataram efeito protetor do uso da RvD2 contra danos oxidativos e inflamatórios em outros modelos experimentais. Todavia, além da atual existência de poucos trabalhos com a RvD2 especificamente, não há conhecimento da sua ação no modelo de exposição à UVB em camundongos.

Os resultados *in vivo* obtidos nesse estudo demonstraram que o efeito antioxidante da RvD2 está relacionado a uma redução da produção de hidroperóxidos lipídicos e de ânion superóxido, somados a uma melhora na capacidade antioxidante da pele através dos ensaios de FRAP e ABTS, bem como da manutenção dos níveis de GSH e atividade da CAT. Além disso, o dano inflamatório também foi atenuado, pois a RvD2 foi capaz de inibir parâmetros inflamatórios de edema cutâneo, recrutamento de neutrófilos, atividade da MMP-9 e produção de citocinas inflamatórias (IL-1 β , IL-33 e TNF- α) e anti-inflamatórias (TGF- β e IL-10). Em conjunto, a RvD2 também reduziu a espessura epidérmica, o número de queratinócitos apoptóticos, de mastócitos e a degradação de fibras de colágeno induzidos pela radiação UVB.

Cabe ainda destacar que, RvD2 apresenta atividade farmacológica na escala de nanogramas, tornando-se um grande atrativo para a indústria farmacêutica. Desta forma, nossos achados sugerem que a RvD2 pode ser uma abordagem promissora para o controle e/ou prevenção dos danos cutâneos causados pela radiação UVB.

6. REFERÊNCIAS

- ABBAS, S. et al. UVB exposure enhanced benzanthrone-induced inflammatory responses in SKH-1 mouse skin by activating the expression of COX-2 and iNOS through MAP kinases / NF- κ B / AP-1 signalling pathways. **Food and Chemical Toxicology**, v. 96, p. 183–190, 2016.
- ABEYAMA, K. et al. A role for NF- κ B-dependent gene transactivation in sunburn. **Journal of Clinical Investigation**, v. 105, n. 12, p. 1751–1759, 2000.
- AEBI, H. Catalase *in vitro*. **Methods Enzymol**, v. 105, p. 121–126, 1984.
- AFAQ, F.; ADHAMI, V. M.; MUKHTAR, H. Photochemoprevention of ultraviolet B signaling and photocarcinogenesis. **Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis**, v. 571, p. 153–173, 2005.
- AFAQ, F.; KATIYAR, S. K. Polyphenols: Skin Photoprotection and Inhibition of Photocarcinogenesis. v. 11, n. 14, p. 1200–1215, 2011.
- AFAQ, F.; MUKHTAR, H. Effects of solar radiation on cutaneous detoxification pathways. **Journal of Photochemistry and Photobiology B: Biology**, v. 63, p. 61–69, 2001.
- AMERICAN CANCER SOCIETY. **About Basal and Squamous Cell Skin Cancer**. Disponível em: <<https://www.cancer.org/cancer/basal-and-squamous-cell-skin-cancer/about.html>>. Acesso em: 20 dez. 2018.
- ANANTHASWAMY, H. N.; KANJILAL, S. Oncogenes and Tumor Suppressor Genes in Photocarcinogenesis. **Photochemistry and Photobiology**, v. 63, n. 4, p. 428–432, 1996.
- ANANTHASWAMY, H. N.; PIERCEALL, W. E. Yearly Review Molecular Mechanisms of Ultraviolet. **Photochemistry and Photobiology**, v. 52, n. 6, p. 1119–1136, 1990.
- ANDUEZA, A. et al. NADPH oxidase 5 promotes proliferation and fibrosis in human hepatic stellate cells. **Free Radical Biology and Medicine**, v. 126, p. 15–26, 2018.
- BAYERL, C. et al. Characterization of sunburn cells after exposure to ultraviolet light. **Photodermatology, Photoimmunology & Photomedicine**, v. 11, n. 4, p. 149–154, 1995.
- BEISSERT, S.; LOSER, K. Molecular and cellular mechanisms of photocarcinogenesis. **Photochemistry and Photobiology**, v. 84, n. 1, p. 29–34, 2008.

BENTO, A. F. et al. Omega-3 Fatty Acid-Derived Mediators 17(R)-Hydroxy Docosaehaenoic Acid, Aspirin-Triggered Resolvin D1 and Resolvin D2 Prevent Experimental Colitis in Mice. **The Journal of Immunology**, v. 187, p. 1957–1969, 2011.

BERRUETA, L. et al. Stretching Impacts In fl ammation Resolution in Connective Tissue. **Journal of Cellular Physiology**, v. 231, p. 1621–1627, 2016.

BICKERS, D. R.; ATHAR, M. Oxidative Stress in the Pathogenesis of Skin Disease. **Journal of Investigative Dermatology**, v. 126, p. 2565–2575, 2006.

BRADLEY, P. P. et al. Measurement of Cutaneous Inflammation: Estimation of Neutrophil Content with an Enzyme Marker. **Journal of Investigative Dermatology**, v. 78, n. 3, p. 206–209, 1982.

BRAND, R. M. et al. Targeting mitochondrial oxidative stress to mitigate UV-induced skin damage. **Frontiers in Pharmacology**, v. 9, n. AUG, p. 1–10, 2018.

BREM, R.; GUVEN, M.; KARRAN, P. Oxidatively-generated damage to DNA and proteins mediated by photosensitized UVA. **Free Radical Biology and Medicine**, v. 107, n. August 2016, p. 101–109, 2017.

BRENNER, M.; HEARING, V. J. The Protective Role of Melanin Against UV Damage in Human Skin. **Photochem Photobiol**, v. 84, n. 3, p. 539–549, 2008.

CALDER, P. C. et al. Inflammatory Disease Processes and Interactions with Nutrition. **British Journal of Nutrition**, v. 101, n. S1, 2009.

CALDER, P. C. Omega-3 polyunsaturated fatty acids and inflammatory processes : nutrition or pharmacology? **British Journal of Clinical Pharmacology**, v. 75, n. 3, p. 645–662, 2013.

CAMPANINI, M. Z. et al. Efficacy of topical formulations containing Pimenta pseudocaryophyllus extract against UVB-induced oxidative stress and inflammation in hairless mice. **Journal of Photochemistry and Photobiology B: Biology**, v. 127, p. 153–160, 2013.

CARINI, M. et al. Fluorescent probes as markers of oxidative stress in keratinocyte cell lines following UVB exposure. **Il Farmaco**, v. 55, p. 526–534, 2000.

CASAGRANDE, R. et al. Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. **Journal of Photochemistry and Photobiology B: Biology**, v. 84, n. 1, p. 21–27, 2006.

CHEN, L.; DIPIETRO, L. A. Toll-Like Receptor Function in Acute Wounds. **Advances in Wound Care**, v. 6, n. 10, p. 344–355, 2017.

CHEN, X. et al. Resolvin D1 attenuates CCl₄-induced acute liver injury involving up-regulation of HO-1 in mice. **Immunopharmacol Immunotoxicol**, v. 38, p. 1–7, 2016.

CHIANG, N. et al. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. **The Journal of Experimental Medicine**, v. 212, n. 8, p. 1203–1217, 2015.

CHIURCHIÙ, V. et al. Pro-resolving lipid mediators Resolvin D1, Resolvin D2 and Maresin 1 are critical in modulating T cell responses. **Sci Transl Med**, v. 8, n. 353, p. 1–24, 2016.

CHOI, J. H. et al. Suppression of PMA-induced tumor cell invasion and metastasis by aqueous extract isolated from *Prunella vulgaris* via the inhibition of NF- κ B-dependent MMP-9 expression. **Food and Chemical Toxicology**, v. 48, n. 2, p. 564–571, 2010.

CLYDESDALE, G. J.; DANDIE, G. W.; MULLER, H. K. Ultraviolet light induced injury: Immunological and inflammatory effects. **Immunology and Cell Biology**, v. 79, p. 547–568, 2001.

COOPER, K. D. et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, CD1a-DR⁺ epidermal macrophage induction, and Langerhans cell depletion. **Proceedings of the National Academy of Sciences**, v. 89, n. 18, p. 8497–8501, 1992.

CROASDELL, A. et al. Resolvins attenuate inflammation and promote resolution in cigarette smoke-exposed human macrophages. **Am J Physiol Lung Cell Mol Physiol**, v. 309, p. L888–L901, 2015.

CUELHO, C. H. F. et al. Topical formulation containing *Ilex Paraguariensis* extract increases metalloproteinases and myeloperoxidase activities in mice exposed to UVB radiation. **Journal of Photochemistry and Photobiology B: Biology**, v. 189, n. October, p. 95–103, 2018.

D'ORAZIO, J. et al. UV Radiation and the Skin. **International Journal of Molecular Sciences**, v. 14, p. 12222–12248, 2013.

DAI, G. et al. Chronic Ultraviolet B Irradiation Causes Loss of Hyaluronic Acid from Mouse Dermis Because of Down-Regulation of Hyaluronic Acid Synthases. **The American Journal of Pathology**, v. 171, n. 5, p. 1451–1461, 2007.

DAMIANI, E.; ULLRICH, S. E. Understanding the connection between platelet-activating factor, a UV-induced lipid mediator of inflammation, immune suppression and skin cancer. **Progress in Lipid Research**, v. 63, p. 14–27, 2016.

DENG, Y. et al. A Sunblock Based On Bioadhesive Nanoparticles. **Nat Mater**, v. 14, n. 12, p. 1278–1285, 2015.

DIEPGEN, T. L.; MAHLER, V. The epidemiology of skin cancer. **Br J Dermatol**, v. 61, p. 1–6, 2002.

FERREIRA, A. L. A.; MATSUBARA, L. S. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. **Rev Ass Med Brasil**, v. 43, n. 1, p. 61–68, 1997.

FONSECA, M. J. V. et al. Evaluation of the potential of Brazilian propolis against UV-induced oxidative stress. **Evidence-based Complementary and Alternative Medicine**, v. 2011, p. 1–8, 2011.

FRIEDMANN, P. S. et al. Early time course of recruitment of immune surveillance in human skin after chemical provocation. **Clin Exp Immunol**, v. 91, n. 3, p. 351–36., 1993.

GAMMONE, M. A. et al. Omega-3 Polyunsaturated Fatty Acids : Benefits and Endpoints in Sport. **Nutrients**, v. 11, n. 46, p. 1–16, 2019.

GELSE, K.; PÖSCHL, E.; AIGNER, T. Collagens - Structure, function, and biosynthesis. **Advanced Drug Delivery Reviews**, v. 55, n. 12, p. 1531–1546, 2003.

GIBBS, N. K.; NORVAL, M. Photoimmunosuppression : a brief overview. **Photodermatol Photoimmunol Photomed**, v. 29, p. 57–64, 2013.

GODAR, D. E. UVA1 Radiation Triggers Two Different Final Apoptotic Pathways. **Journal of Investigative Dermatology**, v. 112, n. 1, p. 3–12, 1999.

GONZALEZ FLECHA, B.; LLESUY, S.; BOVERIS, A. Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. **Free Radical Biology & Medicine**, v. 10, p. 93–100, 1991.

HALLIDAY, G. M. Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis. **Mutation Research**, v. 571, p. 107–120, 2005.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free Radicals in Biology and Medicine**. 4^a ed. Oxford: Oxford University Press, 2007.

HANSON, K. M.; GRATTON, E.; BARDEEN, C. J. Sunscreen enhancement of UV-induced reactive oxygen species in the skin. **Free Radical Biology and Medicine**, v. 41, n. 8, p. 1205–1212, 2006.

HEATH, W. R.; CARBONE, F. R. The skin-resident and migratory immune system in steady state and memory: Innate lymphocytes, dendritic cells and T cells. **Nature Immunology**, v. 14, n. 10, p. 978–985, 2013.

HEURUNG, A. R.; RAJU, S. I.; WARSHAW, E. M. Adverse reactions to sunscreen agents: Epidemiology, responsible irritants and allergens, clinical characteristics, and management. **Dermatitis**, v. 25, n. 6, p. 289-326, 2014.

HIRAMOTO, K. et al. Intercellular pathway through hyaluronic acid in UVB-induced inflammation. **Experimental Dermatology**, v. 21, n. 12, p. 911–914, 2012.

INCa, I. N. DE C. **Câncer de pele não melanoma**. Disponível em: <<https://www.inca.gov.br/tipos-de-cancer/cancer-de-pele-nao-melanoma>>. Acesso em: 20 dez. 2018.

IVAN, A. L. M. et al. Pyrrolidine dithiocarbamate inhibits UVB-induced skin inflammation and oxidative stress in hairless mice and exhibits antioxidant activity in vitro. **Journal of Photochemistry and Photobiology B: Biology**, v. 138, p. 124–133, 2014.

JIANG, F. et al. NADPH oxidase-dependent redox signaling in TGF- β -mediated fibrotic responses. **Redox Biology**, v. 2, p. 267–272, 2014.

JOHN, A.; TUSZYNSKI, G. The Role of Matrix Metalloproteinases in Tumor Angiogenesis and Tumor Metastasis. **Pathology Oncology Research**, v. 7, n. 1, p. 14–23, 2001.

JUNQUEIRA; CARNEIRO. **Histologia Básica**. 12. ed. Rio de Janeiro: Guanabara Koogan, 2013.

JUZENIENE, A.; MOAN, J. Beneficial effects of UV radiation other than via vitamin D production. **Dermato-Endocrinology**, v. 4, n. 2, p. 109–117, 2012.

KAJIYA, K.; HIRAKAWA, S.; DETMAR, M. Vascular Endothelial Growth Factor-A Mediates Ultraviolet B-Induced Impairment of Lymphatic Vessel Function. **The American Journal of Pathology**, v. 169, n. 4, p. 1496–1503, 2006.

KAPPES, U. P. et al. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. **Journal of Investigative Dermatology**, v. 126, n. 3, p. 667–675, 2006.

KATALINIC, V. et al. Gender differences in antioxidant capacity of rat tissues determined by reducing antioxidant power (FRAP) assays. **Comparative Biochemistry and Physiology**, v. 140, p. 47–52, 2005.

KOBAYASHI, A. et al. Oxidative Stress Sensor Keap1 Functions as an Adaptor for Cul3-Based E3 Ligase To Regulate Proteasomal Degradation of Nrf2. **Molecular and Cellular Biology**, v. 24, n. 16, p. 7130–7139, 2004.

KUMAR, A. et al. Nuclear factor- κ B: its role in health and disease. **Journal of Molecular Medicine**, v. 82, n. 7, p. 434–448, 2004.

LEE, H.-N.; SURH, Y.-J. Resolvin D1-mediated NOX2 inactivation rescues macrophages undertaking efferocytosis from oxidative stress-induced apoptosis. **Biochemical Pharmacology**, v. 86, n. 6, p. 759–769, 2013.

LEE, T.; CHAU, L. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. **Nature Medicine**, v. 8, n. 3, p. 240–246, 2002.

LI, W. et al. Activation of Nrf2-antioxidant signaling attenuates NF- κ B-inflammatory response and elicits apoptosis. **Biochem Pharmacol**, v. 76, n. 11, p. 1485–1489, 2009.

LIU, G. H.; QU, J.; SHEN, X. NF- κ B/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. **Biochimica et Biophysica Acta - Molecular Cell Research**, v. 1783, n. 5, p. 713–727, 2008.

MADDODI, N.; JAYANTHY, A.; SETALURI, V. Shining Light on Skin Pigmentation: The Darker and the Brighter Side of Effects of UV Radiation. **Photochem Photobiol**, v. 88, n. 5, p. 1075–1082, 2012.

MARTINEZ, R. M. et al. Naringenin Inhibits UVB Irradiation-Induced Inflammation and Oxidative Stress in the Skin of Hairless Mice. **Journal of Natural Products**, v. 78, n. 7, p. 1647–1655, 2015.

MARTINEZ, R. M. et al. Lipoxin A4 inhibits UV radiation-induced skin inflammation and oxidative stress in mice. **Journal of Dermatological Science**, v. 91, p. 164–174, 2018.

MATSUMURA, Y.; ANANTHASWAMY, H. N. Toxic effects of ultraviolet radiation on the skin. **Toxicology and Applied Pharmacology**, v. 195, n. 3, p. 298–308, 2004.

MAZZONETTO, P. C. et al. Mutation in NADPH oxidase 3 (NOX3) impairs SHH signaling and increases cerebellar neural stem/progenitor cell proliferation. **Biochim Biophys Acta Mol Basis Dis**, v. 3, 2019.

MICHALAK, K. M. et al. Induction of angiogenic and inflammation-associated dermal biomarkers following acute UVB exposure on bio-engineered pigmented dermo-epidermal skin substitutes in vivo. **Pediatric Surgery International**, v. 35, n. 1, p. 129–136, 2018.

MONTES DE OCA, M. K. et al. Phytochemicals for the Prevention of Photocarcinogenesis. **Photochemistry and Photobiology**, v. 93, n. 4, p. 956–974, 2017.

MOTOHASHI, H.; YAMAMOTO, M. Carcinogenesis and transcriptional regulation through Maf recognition elements. **Cancer Sci**, v. 98, n. 2, p. 135–139, 2007.

MUTHUSAMY, V.; PIVA, T. J. The UV response of the skin: A review of the MAPK, NF κ B and TNF α signal transduction pathways. **Archives of Dermatological Research**, v. 302, n. 1, p. 5–17, 2010.

NAKANISHI, M. et al. DNA damage responses in skin biology — Implications in tumor prevention and aging acceleration. **Journal of Dermatological Science journal**, v. 56, p. 76–81, 2009.

NAVARRO, V. P. et al. A participação das metaloproteinases da matriz nos processos fisiológicos da cavidade bucal. **Revista de Odontologia da UNESP**, v. 35, p. 233–248, 2006.

NICHOLS, J. A.; KATIYAR, S. K. Skin photoprotection by natural polyphenols: Anti-inflammatory, anti-oxidant and DNA repair mechanisms. **Arch Dermatol Res**, v. 302, n. 2, p. 71–83, 2010.

PENN, J. W.; GROBBELAAR, A. O.; ROLFE, K. J. The role of the TGF- β family in wound healing, burns and scarring: a review. **Int J Burn Trauma**, v. 2, n. 1, p. 18–28, 2012.

PEUS, D. et al. H₂O₂ is an important mediator of UVB-induced EGF-receptor phosphorylation in cultured keratinocytes. **Journal of Investigative Dermatology**, v. 110, n. 6, p. 966–971, 1998.

PEUS, D. et al. UVB activates ERK1/2 and p38 signaling pathways via reactive oxygen species in cultured keratinocytes. **Journal of Investigative Dermatology**, v. 112, n. 5, p. 751–756, 1999.

PINEDA-PENÑA, E. A. et al. Participation of the anti-inflammatory and antioxidative activity of docosahexaenoic acid on indomethacin-induced gastric injury model. **European Journal of Pharmacology**, v. 818, n. 239, p. 585–592, 2018.

POSSO, S. V. et al. AT-RVD1 repairs mouse lung after cigarette smoke-induced emphysema via downregulation of oxidative stress by Nrf2/Keap1 pathway. **International Immunopharmacology**, v. 56, p. 330–338, 2018.

RAY, P. D.; HUANG, B.-W.; TSUJI, Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. **Cell Signal**, v. 24, n. 5, p. 981–990, 2013.

RYU, H. et al. UVB Radiation Induces Apoptosis in Keratinocytes by Activating a Pathway Linked to “ BLT2-Reactive Oxygen Species ”. **Journal of Investigative Dermatology**, v. 130, p. 1095–1106, 2010.

RYU, Y. S. et al. Particulate matter induces inflammatory cytokine production via activation of NF κ B by TLR5-NOX4-ROS signaling in human skin keratinocyte and mouse skin. **Redox Biology**, v. 21, n. October 2018, p. 101080, 2019.

SAITO, M. et al. Oral administration of aloe vera gel powder prevents uvb-induced decrease in skin elasticity via suppression of overexpression of mmps in hairless mice. **Bioscience, Biotechnology and Biochemistry**, v. 80, n. 7, p. 1416–1424, 2016.

SAITO, P. et al. The Lipid Mediator Resolvin D1 Reduces the Skin Inflammation and Oxidative Stress Induced by UV Irradiation in Hairless Mice. **Frontiers in Pharmacology**, v. 9, n. October, p. 1–15, 2018.

SANCHES SILVEIRA, J. E. P.; PEDROSO, D. M. M. UV light and skin aging. **Rev Environ Health**, v. 29, n. 3, p. 243–254, 2014.

SCHALLREUTER, K. U. et al. In Vivo and In Vitro Evidence for Hydrogen Peroxide (H₂O₂) Successful Removal by a UVB-Activated Pseudocatalase. **Journal of Investigative Dermatology Symposium Proceedings**, v. 4, n. 1, p. 91–96, 1999.

SCHUCH, A. P. et al. Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. **Free Radical Biology and Medicine**, v. 107, p. 110–124, 2017.

SCHWARZ, A. et al. Ultraviolet-B-Induced Apoptosis of Keratinocytes: Evidence for Partial; Involvement of Tumor Necrosis Factor- α in the Formation of Sunburn Cells. **The Journal of Investigative Dermatology**, v. 104, n. 6, p. 922–927, 1995.

SCOTT, T. L. et al. Pigment-independent cAMP-mediated epidermal thickening protects against cutaneous UV injury by keratinocyte proliferation. **Experimental Dermatology**, v. 21, n. 10, p. 771–777, 2012.

SEKI, H. et al. Resolvins as Regulators of the Immune System. **The Scientific World Journal**, v. 10, p. 818–831, 2010.

SERHAN, C. N. Pro-resolving lipid mediators are leads for resolution physiology. **Nature**, v. 510, p. 92–101, 2014.

SERHAN, C. N.; CHIANG, N.; VAN DYKE, T. E. Resolving inflammation: dual lipid mediators. **Nature Reviews Immunology**, v. 8, p. 349–361, 2008.

SHARMA, H. et al. Role of NADPH oxidase in cooperative reactive oxygen species generation in dopaminergic neurons induced by combined treatment with dieldrin and lindane. **Toxicology Letters**, v. 299, p. 47–55, 2018.

SIES, H.; BERNDT, C.; JONES, D. P. Oxidative Stress. **Annu. Rev. Biochem.**, v. 86, p. 715–748, 2017.

SONG, J. et al. Activation of Nrf2 signaling by salvianolic acid C attenuates NF- κ B mediated inflammatory response both in vivo and in vitro. **International Immunopharmacology**, v. 63, p. 299–310, 2018.

SONG, J. A. E. H. et al. Anti-wrinkle effects of Sargassum muticum ethyl acetate fraction on ultraviolet B-irradiated hairless mouse skin and mechanistic evaluation in the human HaCaT keratinocyte cell line. **Molecular Medicine Reports**, v. 14, p. 2937–2944, 2016.

SPITE, M. et al. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. **Nature**, v. 461, n. 7268, p. 1287–1291, 2009.

SREEVIDYA, C. S. et al. Agents that Reverse UV-Induced Immune Suppression and Photocarcinogenesis Affect DNA Repair. **Journal of Investigative Dermatology**, v. 130, n. 5, p. 1428–1437, 2010.

SRINIVASAN, P.; SABITHA, K. E.; SHYAMALADEVI, C. S. Attenuation of 4-Nitroquinoline 1-oxide induced in vitro lipid peroxidation by green tea polyphenols. **Life Sciences**, v. 80, p. 1080–1086, 2007.

TOUYZ, R. M. et al. Nox5: Molecular biology and pathophysiology. **Exp Physiol**, 2019.

URBACH, F. Potential health effects of climatic change: Effects of increased ultraviolet radiation on man. **Environmental Health Perspectives**, v. 96, p. 175–176, 1991.

VALKO, M. et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. **Chem Biol Interact**, v. 160, n. 1, p. 1–40, 2006.

VEITH, C. et al. Redox Imbalance in Idiopathic Pulmonary Fibrosis: A Role for Oxidant Cross-Talk Between NOX Enzymes and Mitochondria. **Antioxid Redox Signal**, v. 4, p. 1–69, 2019.

VERRI, W. A. et al. IL-33 mediates antigen-induced cutaneous and articular hypernociception in mice. **Proceedings of the National Academy of Sciences**, v. 105, n. 7, p. 2723–2728, 2008.

VICENTINI, F. T. M. C. et al. Quercetin inhibits UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing NF- κ B pathway. **Journal of Dermatological Science**, v. 61, n. 3, p. 162–168, 2011.

YE, Y. et al. Anti-cancer and analgesic effects of resolvin D2 in oral squamous cell carcinoma. **Neuropharmacology**, v. 139, p. 182–193, 2018.

ANEXOS

ANEXO I – Carta de aprovação do Comitê de Ética



Universidade
Estadual de Londrina

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OF. CIRC. CEUA Nº 017/2015

Londrina, 19 de Março de 2015.

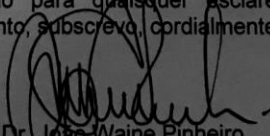
Prezada Pesquisadora,

A CEUA/UEL reunida em 10 de Março de 2015 avaliou o projeto de pesquisa intitulado “Potencial terapêutico de lipídeos anti-inflamatório/pró-resolução para o controle da inflamação e estresse oxidativo induzidos por radiação UVB em camundongos”, registrado sob o processo CEUA nº1447.2015.10, pesquisa do Centro de Ciências da Saúde, desenvolvido sob sua responsabilidade, julgando-o **aprovado** para execução entendendo-se que os princípios éticos postulados pelo Conselho Nacional de Controle de Experimentação Animal estão respeitados.

Serão utilizados 1.720 camundongos HRS/L (Hairless), com 3 meses de idade, pesando aproximadamente 20g e provenientes do Biotério do Centro de Ciências da Saúde da UEL. O projeto tem como objetivo avaliar o efeito anti-inflamatório/pró-resolução dos lipídeos lipoxina A4, 15-epi-lipoxina A4, resolvina D1, resolvina D2, 17(R) resolvina D1 e 7(S) maresina 1 na radiação UVB, bem como a participação endógena da lipoxina A4 na radiação UVB e da 15-epi-lipoxina A4 no efeito da aspirina no modelo de radiação UVB em camundongos sem pelo. Para tanto, será realizada a avaliação do efeito da lipoxina A4 e papel endógeno na inflamação e estresse oxidativo induzidos pela radiação UVB, a avaliação do efeito da 15-epi-lipoxina A4 e papel no mecanismo de ação da aspirina na inflamação e estresse oxidativo induzidos pela radiação UVB, a avaliação do efeito das resolvinas D1, D2 e 17 (R) resolvina D1 na inflamação e estresse oxidativo induzidos pela radiação UVB, a avaliação do efeito da 7(S) maresina 1 na inflamação e estresse oxidativo induzidos pela radiação UVB e a avaliação do efeito tópico dos lipídeos anti-inflamatórios/pró-resolução na inflamação e estresse oxidativo induzidos pela radiação UVB. Os protocolos experimentais estão aprovados para execução em 36 meses.

Cumpra orientar que caso pretendam-se quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação da CEUA/UEL anteriormente à execução das modificações.

Coloco-me à disposição para quaisquer esclarecimentos que se fizerem necessária. Sem mais para o momento, subscrevo, cordialmente,



Prof. Dr. João Waine Pinheiro
Vice-Coordenador da CEUA/UEL

Ilma. Sra.

Prof. Dra. Rubia Casagrande

Coordenadora do Projeto

Departamento de Ciências Farmacêuticas

Centro de Ciências da Saúde

Com cópia para Sra. Edilamar dos Anjos (Chefe da DCA/PROPPG), Chefe do Departamento de Ciências Farmacêuticas e Diretor(a) do Centro de Ciências da Saúde.

ANEXO II - Informações sobre submissão do artigo para a revista Journal of Photochemistry and Photobiology B: Biology

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.

To find out more, please visit the Preparation section below.

INTRODUCTION

The Journal of Photochemistry and Photobiology B: Biology (JPPB) welcomes Invited review articles and original papers not previously published

The journal provides a forum for the publication of papers relating to the various aspects of photobiology, as well as a means for communication in this multidisciplinary field. The scope includes:

- bioluminescence
- chronobiology
- DNA repair
- environmental photobiology
- nanotechnology in photobiology
- photocarcinogenesis
- photochemistry of biomolecules
- photomedicine
- photomorphogenesis
- photomovement
- photoreception
- photosensitization
- photosynthesis
- phototechnology
- spectroscopy of biological systems
- UV and visible radiation effects
- vision

This journal is cited by the following Abstracting Services: BIOSIS, Cambridge Scientific Abstracts, Chemical Abstracts, Current Contents, Elsevier BIOBASE/Current Awareness in Biological Sciences, EMBASE/Excerpta Medica, Embiology, Engineering Index, Medlar's System, Metals Abstracts, PASCAL/ CNRS, Physics Abstracts, Physikalische Berichte, Polymer Contents, Research Alert™, Science Citation Index, Scopus.

Contact Details for Submission

Authors are requested to submit their article to the Editor responsible for the topic involved:

Dr. Laszlo Matyus: Biophysics, Biomolecular spectroscopy, Photodynamic Therapy, Dermatology, Photomedicine.

Dr. Robert Carpentier: Photosynthesis, Environmental photobiology, Photosensitisers, Oxygen radicals, DNA repair, UV/VIS effects.

Dr. Ramaiah: Photochemistry of biomolecules, Photodynamic therapy, Photosensitization, Molecular probes, Phototechnology, Nanotechnology in photobiology.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address

All necessary files have been uploaded:

Manuscript:

- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

Further considerations

- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interests statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration

for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Changes to authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the gold open access publication fee. Details of existing agreements are available online.

Open access

This journal offers authors a choice in publishing their research:

Subscription

- Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.
- No open access publication fee payable by authors.
- The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peerreviewed research in journal publications. The embargo period for this journal can be found below.

Gold open access

- Articles are freely available to both subscribers and the wider public with permitted reuse.
- A gold open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)

Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they

do not alter or modify the article.

The gold open access publication fee for this journal is USD 3300, excluding taxes. Learn more about Elsevier's pricing policy: <https://www.elsevier.com/openaccesspricing>.

Green open access

Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our green open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.

This journal has an embargo period of 24 months.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the

Editor's decision and requests for revision, is sent by e-mail.

For submitting your manuscript to JPPB please go to our Elsevier Editorial System (EES) Website at: <http://ees.elsevier.com/jphotochem/> For technical support during the submission process, please visit www.elsevier.com/submissionsupport. For general questions, contact Dr. Robert Carpentier (Robert.Carpentier@uqtr.ca), Dr. Laszlo Matyus (lmatyus@med.unideb.hu), or Dr. Dominic Robinson (d.robinson@erasmusmc.nl).

Referees

Please submit, with the manuscript, the names and addresses of 5 potential referees. The referees should not be editorial board members or from the same institute or geographical region as the authors.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or layout that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction,

Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

LaTeX

You are recommended to use the Elsevier article class `elsarticle.cls` to prepare your manuscript and BibTeX to generate your bibliography.

Our LaTeX site has detailed submission instructions, templates and other information.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section

numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Essential title page information

- Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the

country name and, if available, the e-mail address of each author.

- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.
- Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images and in accordance with all technical requirements.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view

example Highlights on our information site.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and Units

Nomenclature and units. Follow internationally accepted rules and conventions: use the

international system of units (SI). The only exception is the use of min, h, d and a as units for elapsed time, though never when combined algebraically with other units. If other quantities are mentioned, give their equivalent in SI. Consult the IUPAC "Glossary of Terms Used in Photochemistry" for the correct terminology (source: The Spectrum, Bowling Green, Vol.16, 1, 2003, p 16), (online: www.bgsu.edu/departments/photochem), (cited link: http://www.aspjournals.com/auth_instruct.html)

UV ranges. The official UV ranges as defined by the CIE must be used:

UV-A: 400-315 nm

UV-B: 315-280 nm

UV-C: 280-100 nm

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files. • Indicate per figure if it is a single, 1.5 or 2-column fitting image.
- For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
- Please note that individual figure files larger than 10 MB must be provided in separate source files. A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
- Supply files that are too low in resolution.
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online

only. Further information on the preparation of electronic artwork.

Figure captions

Ensure that each illustration has a caption. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Text graphics

Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX and have such features embedded in the text, these can be left. See further under Electronic artwork.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. *Journal of Geophysical Research*, <https://doi.org/10.1029/2001JB000884>. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley and Zotero, as well as EndNote. Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:

<http://open.mendeley.com/use-citation-style/journal-of-photochemistry-and-photobiology-b-biology>

When preparing your manuscript, you will then be able to select this style using the Mendeley plugins for Microsoft Word or LibreOffice.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference Style

Text: Indicate references by number(s) in square brackets in line with the text. The actual Authors can be referred to, but the reference number(s) must always be given. *Example:* "..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result...." *List:* Number the references (numbers in square brackets) in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:

[1] B.K. Armstrong, A. Krickler, The epidemiology of UV induced skin cancer, J. Photochem. Photobiol. B 63 (2001) 8-18.

Reference to a book:

[2] W. Strunk Jr., E.B. White, The Elements of Style, third ed., Macmillan, New York, 1979.

Reference to a chapter in an edited book:

[3] G.R. Mettam, L.B. Adams, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing, Inc., New York, 1994, pp. 281-304.

[4] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. <http://dx.doi.org/10.17632/xwj98nb39r.1>

Video

Elsevier accepts video material and animation sequences to support and enhance your

scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful

materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to Mendeley Data. The datasets will be listed and directly accessible to readers next to your published article online. For more information, visit the Mendeley Data for journals page.

Data in Brief

You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your

data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for Data in Brief as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to Data in Brief where it will be editorially reviewed and published in the open access data journal, Data in Brief. Please note an open access fee of 500 USD is payable for publication in Data in Brief. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

MethodsX

You have the option of converting relevant protocols and methods into one or multiple MethodsX articles, a new kind of article that describes the details of customized research methods. Many researchers spend a significant amount of time on developing methods to fit their specific needs or setting, but often without getting credit for this part of their work. MethodsX, an open access journal, now publishes this information in order to make it searchable, peer reviewed, citable and reproducible. Authors are encouraged to submit their MethodsX article as an additional item directly alongside the revised version of their manuscript. If your research article is accepted, your methods article will automatically be transferred over to MethodsX where it will be editorially reviewed. Please note an open access fee is payable for publication in MethodsX. Full details can be found on the MethodsX website. Please use this template to prepare your MethodsX article.

Data statement

To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by

allowing you to directly type your corrections, eliminating the potential introduction of errors. If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch. You can also check the status of your submitted article or find out when your accepted article will be published.