TELMA SARAIVA DOS SANTOS

AVALIAÇÃO E MECANISMOS DE AÇÃO DO MEDIADOR LIPÍDICO PRÓ-RESOLUÇÃO LIPOXINA A4 EM MODELO MURINO DE ARTRITE INDUZIDA POR DIÓXIDO DE TITÂNIO

Londrina
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Dissertação de mestrado apresentada ao Programa de Pós Graduação em Patologia Experimental da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Mestre em Patologia Experimental.

Orientação: Prof. Dr. Waldiceu Aparecido Verri Júnior.

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Dificuldades preparam pessoas
comuns para destinos extraordinários.

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RESUMO

A artroplastia total articular do quadril e joelho é um procedimento ortopédico comum eficaz para o tratamento de pacientes com artrite, aliviando o desconforto e melhorando o estado funcional. Apesar disso, cerca de 15% desses procedimentos tendem a falhar devido à liberação de nanopartículas metálicas, como o dióxido de titânio (TiO_2) gerando um microambiente pró-inflamatório articular, que resulta em rejeição da prótese e necessidade de revisão cirúrgica. Atualmente, as terapias disponíveis para pacientes com artrite incluem medicamentos como anti-inflamatórios não esteroidais, corticosteroides e opióides, que apresentam eficácia limitada e oferecem efeitos adversos intensos, tendo isso em vista, uma alternativa terapêutica é a lipoxina A4 (LXA_4), um mediador lipídico pró-resolução gerado a partir do ácido araquidônico (AA) que atua na faixa de nanogramas, com propriedades analgésica, anti-inflamatória e resolutiva. Portanto, o presente estudo tem por objetivo avaliar os efeitos protetores da LXA_4 em modelo experimental de artrite induzida por TiO_2. Para isso, camundongos foram tratados com LXA_4 (0,1, 1 ou 10 ng/animal) ou veículo (etanol) 24 h após a injeção de TiO_2 intra-articular, durante 30 dias. Os resultados obtidos demonstram que a LXA_4 reduziu a hiperalgesia mecânica induzida por TiO_2 de maneira dose-dependente, além de atuar na redução de edema e recrutamento de leucócitos induzidos por TiO_2, sem induzir toxicidade. A LXA_4 atuou na modulação de citocinas como TNF-α, IL-1β, IL-6 e IL-10, bem como reestabeleceu a capacidade antioxidante (níveis de GSH e ABTS), induziu expressão de Nrf2 e reduziu a expressão de RANK. Desta forma, o presente resultado sugere que a LXA_4 possui propriedade antioxidante, anti-inflamatória e analgésica no modelo de artrite induzida por desgaste de prótese.

Palavras-chave: Mediador lipídico; Artrite; Artroplastia; Resolução da inflamação; dor.

Santos, Telma Saraiva. **Evaluation and mechanisms of action of the lipid mediator pro-resolution lipoxin A4 in murine model of arthritis induced by titanium dioxide**. 2019. 68 pages. Master's degree dissertation (Experimental Pathology) –Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

Total joint arthroplasty and knee is a common orthopedic procedure effective for the treatment of patients with arthritis, relieving discommodity and improving functional state. Despite this, about of 15% of these proceedings tend to fail due to the release of metallic nanoparticles, such as titanium dioxide (TiO_2) that creates a pro inflammatory microenvironment, which results in rejection of the prothesis and the need for surgical revision. Currently therapies available to patients with arthritis include drugs such as non-steroidal anti-inflammatory drugs, corticosteroids and opioids, that have limited efficacy and offer intense adverse effects. In view of this, a therapeutic alternative is lipoxin A4 (LXA_4), a pro-resolution lipid mediator generated from arachidonic acid (AA) that acts in the range of nanograms, with analgesic, anti-inflammatory and resolutive properties. Therefore, the present study aims to evaluate the protective effects of LXA_4 in experimental model of TiO_2-induced arthritis. For this, mice were treated with LXA_4 (0.1, 1 or 10 ng/animal) or vehicle (ethanol) 24h after intra-articular injection of TiO_2 for 30 days. The results demonstrate that LXA_4 reduced TiO_2-induced mechanical hiperalgesia in a dose-dependent manner, in addition acting to reduce TiO_2-induced edema and recruitment of leukocytes, without inducing toxicity. LXA_4 acted on the modulation of cytokines such as TNF-α, IL-1β, IL-6 and IL-10, as well as the expression of IL-1β and IL-10. Furthermore, the LXA_4 reestablished the antioxidant capacity (levels of GSH and ABTS), induced Nrf2 expression and reduced RANK expression. Therefore, the present result suggests that LXA_4 possess antioxidant, anti-inflammatory and analgesic activity in a model of prosthesis wear process-induced arthritis.

Key words: Lipid mediator; Arthritis; Implant; Arthroplasty; Resolution of inflammation; Pain.

LISTA DE ILUSTRAÇÕES

LISTA DE ABEVRIATURAS E SIGLAS

AA	Ácido araquidônico
AhR	Receptor nuclear hidrocarboneto de arila
AINEs	Anti-inflamatórios não esteroidais
ALX/FPR2	Receptor de peptídeo formilado tipo 2 ou receptor de LXA_4
ATP	Trifosfato de adenosina
COX-2	Ciclo-oxigenase-2
DAMPs	Padrões moleculares associados a danos
DHA	Àcido docosaexaenoico
DNA	Ácido desoxirribonucleico
EPA	Ácido eicosapentaenoico
EROs	Espécies reativas de oxigênio
fMLP	Peptídeos formilados
GPCRs	Receptores acoplado a proteína G
GRD	Gânglios da raiz dorsal
IFN-γ	Interferon-gamma
IL-1β	Interleucina-1β
IL-33	Interleucina-33
IL-6	Interleucina-6
IL-8	Interleucina-8
LOX	Lipoxigenase
LPS	Lipopolissacarídeos
LTB_4	Leucotrieno B_4
LXA_4	Lipoxina A_4
LXB_4	Lipoxina B_4
LXs	Lipoxinas
MAPK	MAP quinase
MHC	Complexo principal de histocompatibilidade
MLPI	Mediadores lipídicos pró-inflamatórios
MLPR	Mediadores lipídicos pró-resolução
NF-κB	Fator nuclear κappa B

NO	Oxido nítrico
Nrf2	Fator nuclear eritróide relacionado ao fator 2
PAMPs	Padrões moleculares associados a patógenos
PGE_2	Prostaglandinas E2
PGI_2	Prostaglandinas I2
PMN	Polimorfonucleares
PRRs	Receptores de reconhecimento padrão
RANKL	Receptor ativador do fator nuclear κB ligante
Th1	T helper 1
Th2	T helper 2
TiO_2	Dióxido de titânio
TNF-α	Fator de necrose tumoral-alpha

SUMÁRIO

1 INTRODUÇÃO

1.1. INFLAMAÇÃO

A inflamação é uma resposta imunológica do hospedeiro frente a estímulos e/ou circunstâncias nocivas, como infecção causada por patógenos, corpos estranhos ou injúria tecidual (Medzhitov, 2008). Essa resposta protetora do organismo visa à neutralização, remoção de estímulos lesivos, remodelamento e reparação tecidual (Flower, 2006; Medzhitov, 2008; Samuelsson, 2012).

O processo inflamatório é altamente regulado, autolimitado e indispensável para a manutenção da saúde, promovendo a homeostase (Serhan, 2007). Classicamente a resposta inflamatória aguda é caracterizada por cinco sinais cardinais: eritema (rubor), edema, calor e dor (algia) que refletem no aumento da permeabilidade do endotélio vascular com extravasamento de células imunes e componentes séricos. Quando exacerbados, esses sinais podem culminar na destruição de tecidos, fibrose e perda da função tecidual (Takeuchi e Akira, 2010).

A inflamação inicia-se por meio do reconhecimento de padrões moleculares associados a patógenos (PAMPs) e moléculas endógenas liberadas por células danificadas, denominadas padrões moleculares associados a danos (DAMPs), por receptores de reconhecimento padrão (PRRs) presentes em leucócitos, dando início aos eventos vasculares e celulares da inflamação. Após este reconhecimento ocorre uma sequência de sinalizações intracelulares que culminam no aumento da transcrição de genes de citocinas pró-inflamatórias como fator de necrose tumoral-α (TNF-α), Interleucina-1β (IL-1β) e interleucina-6 (IL-6), quimiocinas e proteínas envolvidas na modulação da sinalização de PRRs (Medzhitov, 2008; Takeuchi e Akira, 2010).

A princípio ocorrem eventos vasculares inflamatórios, mediados por aminas vasoativas como histamina e serotonina, que consistem na alteração do fluxo sanguíneo com aumento do extravasamento de líquidos e permeabilidade endotelial, guiando neutrófilos para o foco inflamatório (Mcdonald *et al.*, 2010). A partir desses eventos ocorre a fase celular da inflamação, caracterizada pela marginalização de leucócitos que entram em contato com células endoteliais ativadas por meio de moléculas de adesão, promovendo rolamento, adesão e transmigração de leucócitos do vaso sanguíneo em direção ao foco inflamatório (Spector e Willoughby, 1964). A atração e direcionamento dos neutrófilos ao foco inflamatório infeccioso ou não infeccioso é dependente de moléculas quimioatraentes locais como C5a e peptídeos formilados (fMLP), bem como moléculas adjacentes (provenientes do endotélio vascular, por exemplo), como interleucina-8 (IL-8) e leucotrieno B4 (LTB4) (Foxman *et al.*, 1997).

A resposta inflamatória pode ser dividida em duas fases, a iniciação e a resolução. Os estudos iniciais se concentravam no esclarecimento dos mecanismos e moléculas que são produzidas durante a fase da iniciação, demonstrando a geração de várias classes de moléculas, como citocinas, quimiocinas e eicosanoides (Cotran *et al.,* 1999). Os mediadores lipídicos pró-inflamatórios (MLPI) e eicosanoides produzidos a partir do ácido araquidônico (AA), como prostaglandinas E2 e I2 (PGE_2 e PGI_2) (Flower, 2006) e LTB4 (Malawista *et al.*, 2008), em conjunto com mediadores locais como histamina, produtos do complemento (C5a, C3b) e as quimiocinas atuam como quimiotáticos para neutrófilos que respondem através da diapedese das vênulas para combater lesões e agentes invasores (Flower, 2006; Malawista *et al.*, 2008).

Apesar da identificação de MLPI terem aberto um novo caminho para o desenvolvimento de ferramentas terapêuticas usadas no tratamento de doenças inflamatórias, essas moléculas também demonstraram diversos efeitos colaterais, como por exemplo: os anti-inflamatórios não esteroidais (AINEs) que aumentam a incidência de sangramentos gastrointestinais (Goldstein e Cryer, 2015) e anti-TNFs que aumentam a incidência de infecções (Minozzi *et al.*, 2016).

Posteriormente foi demonstrado que o AA era substrato na biossíntese de moléculas protetoras e anti-inflamatórias como as lipoxinas (LXs), e não apenas na síntese de prostaglandinas e leucotrienos, trazendo à tona um novo conceito de que a resolução é um processo ativo (Serhan *et al.*, 1984b; Levy *et al.*, 2001).

O processo denominado resolução, dentre os possíveis resultados da inflamação, ocorre quando a lesão é limitada e de curta duração, ou quando há pouca destruição tecidual, restaurando o local da inflamação aguda e retornando à normalidade (Cotran *et al.,* 1999). A presença de células apoptóticas, como neutrófilos, associados à produção de PGE_2 induz a troca de classe de MLPI para mediadores lipídicos pró-resolução (MLPR) (Levy *et al.*, 2001). A resolução envolve o decaimento espontâneo dos mediadores químicos locais com retorno da permeabilidade vascular normal, encerramento da infiltração leucocitária, morte celular (apoptose de neutrófilos, por exemplo), remoção de edema e de agentes estranhos e debris celulares no sítio inflamatório (Cotran *et al.,* 1999).

Quando a resposta protetora não é controlada e não elucida o processo inflamatório este pode culminar em diversas doenças de ampla ocorrência, como doenças cardiovasculares, metabólicas e doenças inflamatórias clássicas, como artrite e doença periodontal, bem como câncer (Nathan e Ding, 2010; Serhan, 2010).

1.2. DOR INFLAMATÓRIA

A nocicepção confere ao indivíduo a capacidade de autopreservação, onde a percepção dolorosa permite a identificação de situações que possam causar danos ou controlar aquelas já existentes em uma lesão. As sensações nociceptivas agudas, e principalmente as crônicas causam diminuição na qualidade de vida e sua persistência é fator de risco consistente na saúde pública (Calati *et al.*, 2015). Portanto, é de suma importância o estudo e entendimento da compreensão da dor, bem como os mecanismos fisiológicos e seus agentes envolvidos, principalmente na dor inflamatória.

De acordo com a Associação Internacional para o Estudo da Dor, ela é definida como “uma experiência sensorial e emocional desagradável associada a dano tecidual real ou potencial” (D'mello e Dickenson, 2008). A dor pode ser classificada como: fisiológica, inflamatória e dor neuropática. A dor fisiológica é

gerada por um estímulo nocivo, a dor inflamatória é resultante de injúria tecidual e/ou ativação das células imunes e a dor neuropática está associada a uma lesão e má adaptação do sistema nervoso. As dores com origem inflamatória compõem transtornos clínicos que afetam pacientes, podendo cronificar-se e permanecem sem terapia realmente eficazes na grande maioria dos casos. Esses processos são caracterizados por estados de hipersensibilidade no foco ou gatilho da lesão e na área adjacente (Woolf e Salter, 2000).

A percepção consciente da dor envolve interação do sistema nervoso central e periférico, por meio da ativação de receptores específicos e vias neuroanatômicas que promovem essa comunicação (Peirs e Seal, 2016). O início da percepção sensorial acontece na periferia, onde verifica-se a resposta a estímulos por meio de neurônios aferentes primários e posteriormente transdução destas informações para o corno dorsal da medula espinal, onde terminam as extremidades dessas fibras. Os corpos celulares desses neurônios encontram-se nos gânglios trigeminais e nos gânglios da raiz dorsal (GRD), dependendo da localização da interação estímulo/sistema. Os três principais neurônios/ fibras axonais sensoriais do sistema nervoso periférico são as fibras Aβ, Aδ e C que respondem e transmitem informações sensoriais (D'mello e Dickenson, 2008; Milligan e Watkins, 2009).

As fibras Aδ (delta) e C estão associadas à inflamação e após sensibilização podem transduzir em impulsos elétricos, estímulos mecânicos, térmicos ou químicos que são transmitidos ao sistema nervoso central (Rang *et al.*, 1991). Deste modo, estímulos dolorosos agudos são detectados inicialmente pelos nociceptores e retransmitidos para os níveis espinais, supraespinais e para múltiplas áreas do córtex que estão associadas com a percepção consciente da dor (Milligan e Watkins, 2009). Apesar dos nociceptores desempenharem um papel na percepção e transmissão da informação dolorosa suas funções extrapolam seu papel primário. Moléculas como trifosfato de adenosina (ATP), fMLP e lipopolissacarídeos (LPS) ativam diretamente a atividade nociceptiva (Chiu *et al.*, 2013).

A dor é um sintoma presente na maioria das doenças inflamatórias, podendo até mesmo levar à perda da função de tecidos e/ou órgãos afetados. Durante a inflamação ocorre a ativação de mecanismos responsáveis pela indução de edema, migração de leucócitos e hiperalgesia (resposta aumentada a estímulos dolorosos). A produção de espécies reativas de oxigênio (EROs) e citocinas pró-inflamatórias

possuem participação na resposta inflamatória e no desenvolvimento da dor (Serhan, 2014).

A dor inflamatória tem como origem a interação do tecido comprometido com os neurônios sensoriais nociceptivos periféricos (Hardy *et al.*, 1950) que são sensibilizados por mediadores pró-inflamatórios, como IL-1β, TNF- α, LTB4, PGE_2, ATP e C5a, que por sua vez são liberados pelas células lesionadas após reconhecimento de agentes estranhos por células de defesa residentes como macrófagos (Ferreira, 1993; Ribeiro *et al.*, 2000). A associação desses eventos, coopera com o aumento da sensibilidade neuronal a estímulos que em condições naturais produzem dor moderada (hiperalgesia) ou dor alguma (alodinia). Essa sensibilização do neurônio é uma característica importante da dor inflamatória, e o bloqueio deste fenômeno representa o principal mecanismo de ação dos analgésicos e anti-inflamatórios comercializados (Cunha *et al.*, 1992; Ferreira, 1993; Watkins *et al.*, 1995; Lorenzetti *et al.*, 2002; Sachs *et al.*, 2002).

Os neutrófilos desempenham um papel essencial na manutenção da dor inflamatória (Cunha *et al.*, 2008), produzindo citocinas pró-inflamatórias como IL-1β, TNF-α e interleucina-33 (IL-33) (Verri *et al.*, 2006). Após o estímulo nocivo, uma cascata de citocinas pró-inflamatórias antecedem a liberação de aminas simpáticas e PGE_2 que levam a sensibilização dos nociceptores (Scholz e Woolf, 2002; Braz *et al.*, 2014).

A dor decorrente de processos inflamatórios pode ser associada a diversas doenças, onde a exacerbação ou persistência da dor é a principal causa de procura por atendimento médico (Mogil *et al.*, 2000; Woolf e Salter, 2000; Scholz e Woolf, 2002; Verri *et al.*, 2006; Guerrero *et al.*, 2008; Braz *et al.*, 2014).

1.3. ARTRITE INDUZIDA POR DIÓXIDO DE TITÂNIO

O processo inflamatório articular crônico pode acarretar em alterações morfológicas caracterizadas pela destruição da superfície de suporte articular, evoluindo de maneira a necessitar da substituição parcial ou total da articulação, cirurgia denominada artroplastia, com o objetivo de melhorar a qualidade de vida do paciente (Verri *et al.*, 2010; Maioli *et al.*, 2015; Ferreira *et al.*, 2017).

A artroplastia total articular do quadril e joelho é um procedimento ortopédico comum (Kane *et al.*, 2005) e eficaz para o tratamento de pacientes com osteoartrite,

artrite reumatoide, fraturas e necrose avascular que apresentam níveis altos de dor (Nunez *et al.*, 2007), aliviando o desconforto e melhorando o estado funcional (Callahan *et al.*, 1994). O crescimento da demanda por cirurgias de substituição articular nos últimos anos deve-se à alta prevalência de artrite e maior necessidade de mobilidade e qualidade de vida (Maradit Kremers *et al.*, 2015). Em 2010, cerca de 7 milhões de estadunidenses já haviam realizado a substituição total do joelho ou quadril e acredita-se que até 2030 cerca de 11 milhões irão realizar tais procedimentos (Agency for Healthcare Research and Quality- AHRQ, 2016).

Estima-se que aproximadamente 189.457 cirurgias de quadril e joelho são realizadas a cada três anos pelo Sistema Único de Saúde (SUS-Brasil), gerando grandes gastos econômicos anuais (Verri *et al.*, 2010; Maioli *et al.*, 2015); (Ferreira *et al.*, 2017). Apesar da artroplastia ser um procedimento de sucesso na medicina moderna, cerca de 10-15% das artroplastias tendem a falhar (Harris, 2001).

As próteses podem apresentar efeito nocivo, devido à liberação de nanopartículas metálicas, que ativam macrófagos residentes no espaço periprotético, estimulam a produção de mediadores por essas células, como o ligante do receptor ativador do fator nuclear κB ligante (RANKL), citocinas pró-inflamatórias (TNF-α, IL-1β e IL-6), bem como produção de EROs. Estes mediadores promovem a ativação do fator nuclear κB (NF-κB), o qual é responsável pela manutenção do processo inflamatório asséptico no tecido periprotético (Wooley *et al.*, 2002; Wang *et al.*, 2010; Cobelli *et al.*, 2011). O microambiente pró-inflamatório induz osteoclastogênese e ativação de osteoclastos, promovendo reabsorção óssea e osteólise em 5-20% dos pacientes com artroplastia (Harris, 2001). A ativação do sistema imune pelas nanopartículas metálicas resulta na rejeição da prótese e necessidade de um novo procedimento cirúrgico. Clinicamente, as terapias mais utilizadas são os AINEs, corticosteroides e opióides; medicamentos que apresentam eficácia limitada e oferecem efeitos adversos intensos (Wooley *et al.*, 2002; Cobelli *et al.*, 2011).

As nanopartículas são partículas com diâmetros inferiores a 100 nm que foram rapidamente incorporadas pelo mercado de consumo, dentre elas a nanopartícula de dióxido de titânio (TiO_2). Esta é extensamente fabricada, sendo caracterizada como um pó branco e inodoro amplamente usadas em alimentos, bebidas, cosméticos e medicamentos devido às suas propriedades químicas e físicas únicas (Weir *et al.*, 2012; Martirosyan e Schneider, 2014).

A forma como o TiO_2 afeta as funções imunes até então é pouco esclarecida. Sabe-se que macrófagos são as primeiras células da linha de defesa na tentativa de limpar o TiO_2 por meio da fagocitose, protegendo contra potenciais danos (Frohlich, 2015). Estudos demonstram que a exposição a TiO_2 pode causar ativação da resposta imune, com aumento da produção de EROs e fatores inflamatórios como NF-κB, IL-1β, TNF-α e interferon-γ (IFN-γ) (Schanen *et al.*, 2009; Sang *et al.*, 2012).

Estudos *in vitro* comprovam a citotoxicidade e genotoxicidade do TiO_2 pela indução da apoptose, inflamação e indução de EROs. *In vivo* também foi evidenciado que nanopartículas de TiO_2 podem se acumular em órgãos importantes como baço, fígado, rim, pulmão, cérebro e coração causando lesões (Chang *et al.*, 2013; Shi *et al.*, 2013). Liu *et al.* (2010) demonstraram que a estimulação intratecal com TiO_2 promove aumento da capacidade quimiotáxica, expressão de complexo principal de histocompatibilidade (MHC) classe 2 em células superficiais e aumento da secreção de oxido nítrico (NO) e TNF-α em macrófagos pulmonares. Em estudo toxicológico de nanopartículas de TiO_2 duas vias de sinal de imunidade são mais amplamente estudadas, o NF-κB e MAP quinase (MAPK) (Wu e Tang, 2018). As nanopartículas de TiO_2 também causam lesão após atravessar a membrana celular, produzindo estresse oxidativo e inflamação, danificando o ácido desoxirribonucleico (DNA) e levando a apoptose (Shi *et al.*, 2013; Makumire *et al.*, 2014).

O modelo de artrite crônica relacionada à prótese induzida por TiO_2 foi recentemente padronizado por Borghi *et al.* (2018), onde a administração intra-articular de 3 mg de TiO_2 induz dor crônica durante 30 dias e intenso recrutamento de neutrófilos com degradação de proteoglicanos, estresse oxidativo e produção de citocinas inflamatórias. Com isso em vista, o modelo de artrite induzida por TiO_2 é promissor, podendo assim proporcionar melhor compreensão dos mecanismos celulares e moleculares relacionado ao processo inflamatório asséptico mediado por partículas liberadas no espaço periprotético.

1.4. MEDIADORES LIPÍDICOS PRÓ-RESOLUÇÃO

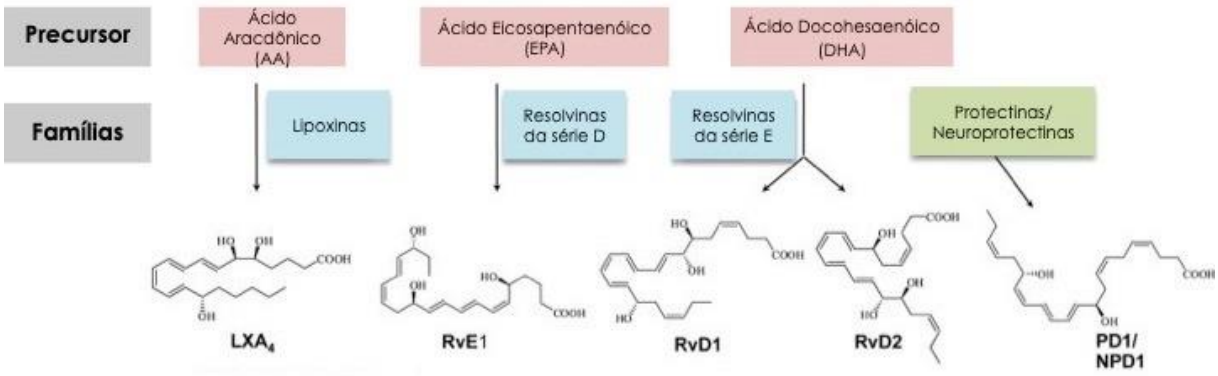
A resposta inflamatória em excesso é vista atualmente como componente frequente de doenças crônicas a qual preocupa a saúde pública, como síndromes metabólicas, doenças vasculares e outras (Serhan, 2014). Os pontos endógenos de

controle da inflamação trazem uma nova perspectiva a respeito de novas abordagens terapêuticas (Serhan, 2014).

A identificação de mediadores lipídicos biossintetizados a partir do ácido graxo essencial ômega-3 com propriedade pró-resolução evidenciou que a resolução da resposta inflamatória aguda pode ser considerada uma resposta ativa programada e não simplesmente um processo passivo de diluição de quimioatraentes (Serhan *et al.*, 2000; Serhan *et al.*, 2002).

Os MLPR abrangem diversas famílias de mediadores incluindo mediadores biossintetizados a partir do AA como as LXs, a partir do ácido eicosapentaenoico (EPA), como as resolvinas da série E, e a partir do ácido docosaexaenoico (DHA), como resolvinas, protectinas e maresinas, que apresentam estruturas químicas, receptores e mecanismos de ação distintos (Serhan, 2010; Chiang e Serhan, 2017) (Figura 1).

Figura 1- Perfil dos mediadores especializados pró-resolução.



Fonte: adaptado de Serhan (2010).

Charles Serhan foi o primeiro a descrever os MLPR e seus impactos na resolução da inflamação (Serhan, 2014; 2017). A descoberta dos MLPR possibilitou o interesse nas vias de resolução e nos mecanismos imunes envolvidos na homeostase, uma vez que essas moléculas atuam como agonistas que estimulam os eventos celulares da resolução, como a interrupção no influxo de polimorfonucleares (PMN) e remoção de restos apoptóticos por macrófagos (Maderna e Godson, 2009), além de reduzir os eicosanoides pró-inflamatórios (Serhan e Chiang, 2013).

Além das características analgésicas, anti-inflamatórias, resolutivas e imunomoduladora, esses MLPR não possuem atividade imunossupressora e apesar da meia-vida curta em meio aquoso (Aursnes *et al.*, 2015), apresentam efeito biológico duradouro, na faixa de dias (Serhan *et al.*, 2012), fazendo destas moléculas fortes candidatas a testes clínicos. De fato a resolvina E1 derivada do EPA alcançou a clínica como RX-10045® para testes no tratamento da síndrome do olho seco, contribuindo para melhora significativa dos pacientes de maneira dose-dependente (Clinicaltrials.gov identificação NCT00799552) (Lee, 2012; Norling e Perretti, 2013).

A dose necessária de MLPR para cessar a inflamação está na faixa de pico a nanomolar (Serhan *et al.*, 2015; Serhan, 2017). Esses mediadores atuam pela ativação de receptores acoplado a proteína G (GPCRs) e fosforilação de moléculas para transdução de sinal (Serhan, 2014).

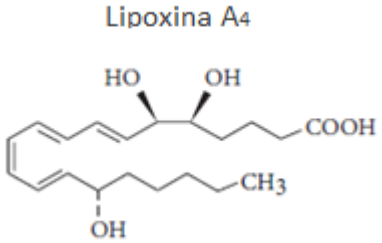
Dentre os mecanismos de ação dos MLPR estão à inibição de NF-κB (Buckley *et al.*, 2014), com consequente diminuição de marcadores inflamatórios, como proteína C reativa, fibrinogênio, IL-1β, IL-6 e TNF-α (Buffon *et al.*, 1999; Liuzzo *et al.*, 1999) e quimiocinas como CXCL1 e CXCL3 (Buckley *et al.*, 2014), indução mudança de classe de macrófagos do fenótipo M1 para M2, resposta que promove mudança do padrão T helper 1 (Th1) para T helper 2 (Th2), gerando um ambiente pró-resolução (Buckley *et al.*, 2014; Dalli e Serhan, 2016). Ademais, modulam a dor, promovem remodelação tecidual e reduzem lesão tecidual (Chiang e Serhan, 2017; Serhan *et al.*, 2018). Tendo em vista as propriedades, mecanismos de ação, efeitos e estudos em animais de experimentação, os MLPR são fortes candidatos terapêuticos para diversas doenças inflamatórias e uso em testes clínicos (Fattori *et al.*, 2016).

1.5. LIPOXINA A4

A Lipoxina A4 (LXA_4) (Figura 2) e a Lipoxina B4 (LXB_4) foram os primeiros mediadores lipídicos reconhecidos (Serhan, 2005), sendo descritos pela primeira vez por Serhan em 1984 (Serhan, 2014). As LXs são eicosanoides derivados de lipoxigenases a partir do AA; um ácido graxo ômega-6 liberado e mobilizado no processo inflamatório (Samuelsson *et al.*, 1987; Shimizu, 2009). Sua biossíntese se

dá via eventos metabólicos transcelulares durante a interação de leucócitos com células da mucosa e dentro dos vasos durante interações plaqueta-leucócitos (Serhan, 2005; 2007).

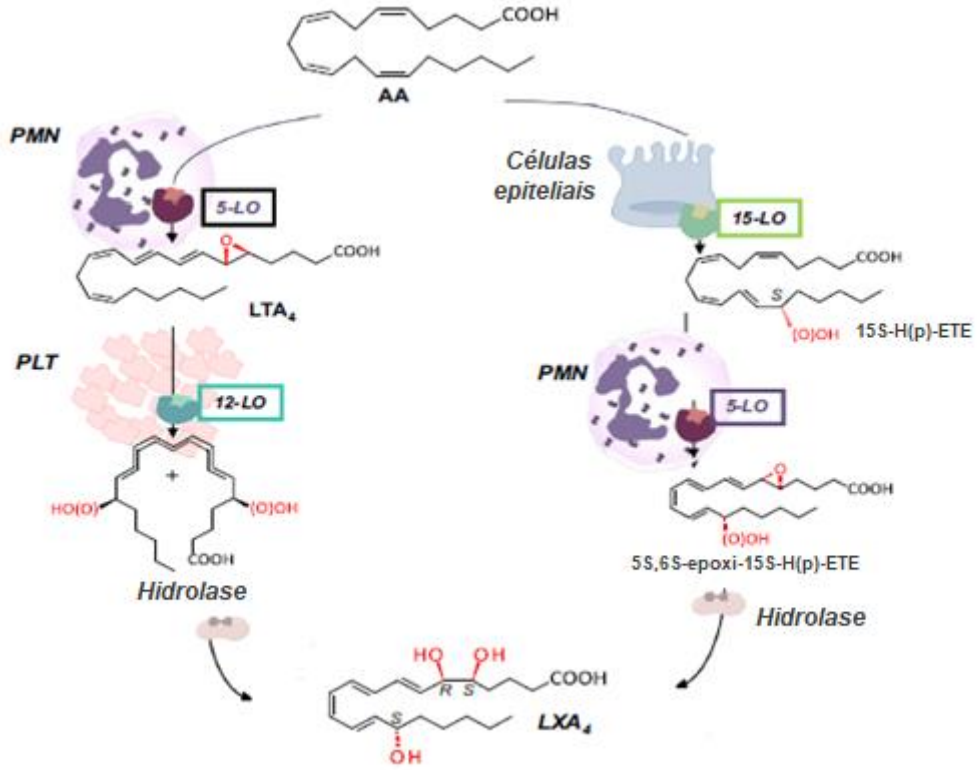
Figura 2- Estrutura química da Lipoxina A_4 ($C_{20}H_{32}O_5$).



Fonte: Adaptado de Han *et al.* (2016).

A formação da LXA_4 pode acontecer através de duas vias de biossíntese diferentes, de forma independente ou simultânea na circulação sanguínea (Fiore e Serhan, 1990). A primeira via acontece em eosinófilos, monócitos ou células epiteliais, e nessa via ocorre a inserção de O_2 ao grupo hidroxila do carbono 15 do AA, reação catalisada pela 15-Lipoxigenase. O produto intermediário 15S-H-(p)-ETE é liberado destas células, entrando em PMN ou monócitos, onde 5-Lipoxigenase gera 5S,6S, 15S-epoxitetraeno que então é hidrolisado dentro destas células por LXA_4 hidrolase, dando origem a LXA_4 bioativa (Serhan *et al.*, 1984a). Outra via de biossíntese da LXA_4 resulta da geração de LTA4 a partir do AA por meio de 5-Lipoxigenase nos leucócitos ou células epiteliais que posteriormente é liberada, captada pelas plaquetas e sofre metabolismo pela 12-Lipoxigenase para produção de LXA_4 (Edenius *et al.*, 1988; Fiore e Serhan, 1990) (Figura 3).

Figura 3- Vias de biossíntese da LXA_4. PMN (neutrófilos polimorfonucleares); PLT (plaquetas); LO (lipoxigenase).



Fonte: Adaptado de Romano *et al.* (2015).

A LX atua na faixa de pico e nanogramas, possui ações como limitação do recrutamento, da quimiotaxia e da adesão de PMN, cessando a lesão tecidual causada por essas células (Serhan, 2005; Morris *et al.*, 2009). Os membros da família das LXs atuam mediante interação ligante-receptor, sinalizando através de dois receptores, um receptor nuclear hidrocarboneto de arila (AhR) ou o GPCR conhecido como receptor de peptídeo formilado tipo 2 ou receptor de LXA_4 (ALX/FPR2), que foi o primeiro receptor eicosanoide derivado da lipoxigenase (LOX), isolado e clonado em tecidos humanos e animais (Serhan, 1997). Em humanos o receptor ALX está presente em PMN, monócitos, células T e células residentes como macrófagos, sinoviais, fibroblastos e células epiteliais intestinais (Chiang *et al.*, 2006). De fato, em modelo de artrite induzida por soro K/BxN, camundongos que não expressam o receptor ALX/ FPR2 exibem maior gravidade da doença (Dufton *et al.*, 2010)

O papel da LXA_4 na resolução dos sinais de dor induzida pela inflamação já foi demonstrado por Svensson *et al.* (2007) em modelos murinos, onde houve

diminuição da hiperalgesia térmica com baixas doses de 10 µg/kg administrados por via intravenosa (i.v.) ou 0,3 nmol intratecal (i.t.). Posteriormente Abdelmoaty *et al.* (2013) demonstraram que a LXA_4 atenua liberação de citocinas pelos astrócitos espinhais e suprime a hiperalgesia mecânica no mesmo modelo, com administração de 0,1-1 µl i.t. de LXA_4.

A LXA_4 possui ação anti-inflamatória, inibindo a quimiotaxia e diapedese de neutrófilos em vênulas pós capilares e consequente entrada em tecidos inflamados (Serhan, 1999), já tendo sido demonstrada a inibição da aglomeração de integrinas e motilidade de neutrófilos, reduzindo a inflamação aguda (Patcha *et al.*, 2004). Em contrapartida, atua na estimulação da quimiotaxia e aderência de monócitos para o local da inflamação, sem a indução de degranulação de neutrófilos ou liberação de EROs, eventos esses essenciais para o processo de cicatrização e depuração do local inflamado, característico de um estado pró-resolutivo (Maddox *et al.*, 1997; Godson *et al.*, 2000). Em modelos de doenças inflamatórias crônicas a LXA_4 reduz o influxo de PMN e expressão de ciclo-oxigenase-2 (COX-2) em modelo de periodontite (Pouliot *et al.*, 2000) e possui efeito protetor no controle da destruição óssea mediada por inflamação também em modelo de periodontite em coelhos com maior expressão de 15-lipoxigenase do tipo 1 (Serhan *et al.*, 2003). Conjuntamente, já foi demonstrado seu papel na redução de fibrose renal (Borgeson *et al.*, 2011).

Trabalhos confirmam que a LXA_4 pode suprimir funções como a apresentação de antígeno e promover mudança do padrão de citocinas Th1 para Th2 (Parkinson, 2006; Liao et al., 2013; Shi et al., 2017), bem como inibir a translocação nuclear de NF-κB (Shi et al., 2017). Além do mais, possui forte papel antioxidante com inibição do estresse oxidativo (Cui et al., 2018), pela indução da expressão de Fator nuclear eritróide relacionado ao fator 2 (Nrf2) e heme-oxigenase, corroborando com trabalhos que apontam uma neuroproteção com redução de escore neurológico (Wu et al., 2013). Outro achado indica uma proteção da LXA_4 na perda de memória induzida pelo peptídeo β-amiloide (Pamplona et al., 2012; Pruss et al., 2013).

Existem evidencias de que a LX pode ter impacto nas doenças articulares inflamatórias, pois foi observado que a LXA_4 inibe a liberação de metaloproteinases da matriz e citocinas por fibroblastos sinoviais humanos (Sodin-Semrl *et al.*, 2000). Também já foi observado aumento da expressão de ALX/FPR2 em pacientes acometidos por artrite reumatoide (Hashimoto *et al.*, 2007), além do tratamento com LXA_4 atenuar a artrite experimental induzida por zimosan (Conte *et al.*, 2010).

Portanto, os diversos estudos a respeito da LXA_4 e seu efeito em modelos inflamatórios levaram a avaliação da eficácia da LXA_4 no modelo murino de artrite induzida de TiO_2.

2. OBJETIVOS

2.1. OBJETIVOS GERAIS

Avaliar os efeitos e mecanismos analgésicos e anti-inflamatórios da LXA_4 em modelo de artrite induzida por dióxido de titânio.

2.2. OBJETIVOS ESPECÍFICOS

Avaliar o efeito da LXA_4 nos seguintes parâmetros:

- Hiperalgesia mecânica e edema articular;
- Toxicidade hepática e renal;
- Lesão gástrica;
- Recrutamento leucocitário para a cavidade articular;
- Expressão de citocinas (pró-IL-1β, IL-10);
- Produção de citocinas (TNF-α, IL-1β, IL-6 e IL-10);
- Parâmetros de estresse oxidativo (ensaios de GSH e ABTS; expressão de Nrf2);
- Degradação óssea (expressão de RANK);

3. REFERÊNCIAS

ABBAS, A. K., LICHTMAN, A. H., PILLAI, S. **Imunologia celular e molecular**. 7 ed. Rio de Janeiro: Elsevier, 2011.

ABDELMOATY, S. et al. Spinal actions of lipoxin A4 and 17(R)-resolvin D1 attenuate inflammation-induced mechanical hypersensitivity and spinal TNF release. **PLoS One,** v. 8, n. 9, p. e75543, 2013. ISSN 1932-6203 (Electronic) 1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24086560 >.

AGENCY FOR HEALTHCARE RESEARCH AND QUALITY (AHRQ). **AHRQ Study: Joint Replacement To Become the Most Common Elective Surgical Procedure in the Next Decades | Agency for Healthcare Research & Quality.** 2016.

AURSNES, M. et al. Synthesis of the 16S,17S-Epoxyprotectin Intermediate in the Biosynthesis of Protectins by Human Macrophages. **J Nat Prod,** v. 78, n. 12, p. 2924-31, Dec 24 2015. ISSN 1520-6025 (Electronic) 0163-3864 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26580578 >.

BORGESON, E. et al. Lipoxin A(4) and benzo-lipoxin A(4) attenuate experimental renal fibrosis. **FASEB J,** v. 25, n. 9, p. 2967-79, Sep 2011. ISSN 1530-6860 (Electronic) 0892-6638 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21628447 >.

BORGHI, S. M. et al. The flavonoid quercetin inhibits titanium dioxide (TiO2)-induced chronic arthritis in mice. **J Nutr Biochem,** v. 53, p. 81-95, Mar 2018. ISSN 1873-4847 (Electronic) 0955-2863 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29197723 >.

BRAZ, J. et al. Transmitting pain and itch messages: a contemporary view of the spinal cord circuits that generate gate control. **Neuron,** v. 82, n. 3, p. 522-36, May 7 2014. ISSN 1097-4199 (Electronic) 0896-6273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24811377 >.

BUCKLEY, C. D.; GILROY, D. W.; SERHAN, C. N. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. **Immunity,** v. 40, n. 3, p. 315-27, Mar 20 2014. ISSN 1097-4180 (Electronic) 1074-7613 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24656045 >.

BUFFON, A. et al. Preprocedural serum levels of C-reactive protein predict early complications and late restenosis after coronary angioplasty. **J Am Coll Cardiol,** v. 34, n. 5, p. 1512-21, Nov 1 1999. ISSN 0735-1097 (Print) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10551701 >.

CALATI, R. et al. The impact of physical pain on suicidal thoughts and behaviors: Meta-analyses. **J Psychiatr Res,** v. 71, p. 16-32, Dec 2015. ISSN 1879-1379 (Electronic) 0022-3956 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26522868 >.

CALLAHAN, C. M. et al. Patient outcomes following tricompartmental total knee replacement. A meta-analysis. **JAMA,** v. 271, n. 17, p. 1349-57, May 4 1994. ISSN 0098-7484 (Print) 0098-7484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8158821 >.

CHANG, X. et al. Health effects of exposure to nano-TiO2: a meta-analysis of experimental studies. **Nanoscale Res Lett,** v. 8, n. 1, p. 51, Jan 25 2013. ISSN 1931-7573 (Print) 1556-276X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23351429 >.

CHIANG, N.; SERHAN, C. N. Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. **Mol Aspects Med,** v. 58, p. 114-129, Dec 2017. ISSN 1872-9452 (Electronic) 0098-2997 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28336292 >.

CHIANG, N. et al. The lipoxin receptor ALX: potent ligand-specific and stereoselective actions in vivo. **Pharmacol Rev,** v. 58, n. 3, p. 463-87, Sep 2006. ISSN 0031-6997 (Print) 0031-6997 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16968948 >.

CHIU, I. M. et al. Bacteria activate sensory neurons that modulate pain and inflammation. **Nature,** v. 501, n. 7465, p. 52-7, Sep 5 2013. ISSN 1476-4687 (Electronic) 0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23965627 >.

COBELLI, N. et al. Mediators of the inflammatory response to joint replacement devices. **Nat Rev Rheumatol,** v. 7, n. 10, p. 600-8, Sep 6 2011. ISSN 1759-4804 (Electronic) 1759-4790 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21894210 >.

CONTE, F. P. et al. Lipoxin A(4) attenuates zymosan-induced arthritis by modulating endothelin-1 and its effects. **Br J Pharmacol,** v. 161, n. 4, p. 911-24, Oct 2010. ISSN 1476-5381 (Electronic) 0007-1188 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20860668 >.

COTRAN, R. S., KUMAR, V., COLLINS, T. **Bases patológicas da doença de Robbins.** WB Saunders: Filadélfia, 1999.

CUI, K. et al. Lipoxin A4 improves erectile dysfunction in rats with type I diabetes by inhibiting oxidative stress and corporal fibrosis. **Asian J Androl,** v. 20, n. 2, p. 166-172, Mar-Apr 2018. ISSN 1745-7262 (Electronic) 1008-682X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29111541 >.

CUNHA, F. Q. et al. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. **Br J Pharmacol,** v. 107, n. 3, p. 660-4, Nov 1992. ISSN 0007-1188 (Print) 0007-1188 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1472964 >.

CUNHA, T. M. et al. Crucial role of neutrophils in the development of mechanical inflammatory hypernociception. **J Leukoc Biol,** v. 83, n. 4, p. 824-32, Apr 2008. ISSN 0741-5400 (Print) 0741-5400 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18203872 >.

D'MELLO, R.; DICKENSON, A. H. Spinal cord mechanisms of pain. **Br J Anaesth,** v. 101, n. 1, p. 8-16, Jul 2008. ISSN 1471-6771 (Electronic) 0007-0912 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18417503 >.

DALLI, J.; SERHAN, C. Macrophage Proresolving Mediators-the When and Where. **Microbiol Spectr,** v. 4, n. 3, Jun 2016. ISSN 2165-0497 (Electronic) 2165-0497 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27337457 >.

DUFTON, N. et al. Anti-inflammatory role of the murine formyl-peptide receptor 2: ligand-specific effects on leukocyte responses and experimental inflammation. **J Immunol,** v. 184, n. 5, p. 2611-2619, Mar 1 2010. ISSN 1550-6606 (Electronic) 0022-1767 (Linking). Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20107188 >.

EDENIUS, C.; HAEGGSTROM, J.; LINDGREN, J. A. Transcellular conversion of endogenous arachidonic acid to lipoxins in mixed human platelet-granulocyte suspensions. **Biochem Biophys Res Commun,** v. 157, n. 2, p. 801-7, Dec 15 1988. ISSN 0006-291X (Print) 0006-291X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/2849436 >.

FATTORI, V.; AMARAL, F. A.; VERRI, W. A., JR. Neutrophils and arthritis: Role in disease and pharmacological perspectives. **Pharmacol Res,** v. 112, p. 84-98, Oct 2016. ISSN 1096-1186 (Electronic)1043-6618 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26826283 >.

FERREIRA, C. C. et al. Improvement of Titanium Corrosion Resistance by Coating with Poly-Caprolactone and Poly-Caprolactone/Titanium Dioxide: Potential Application in Heart Valves. **Materials Research,** v. 20, p. 126-133, 2017. ISSN 1516-1439. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-14392017000700126&nrm=iso >.

FERREIRA, S. H. The role of interleukins and nitric oxide in the mediation of inflammatory pain and its control by peripheral analgesics. **Drugs,** v. 46 Suppl 1, p. 1-9, 1993. ISSN 0012-6667 (Print) 0012-6667 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7506142 >.

FIORE, S.; SERHAN, C. N. Formation of lipoxins and leukotrienes during receptor-mediated interactions of human platelets and recombinant human granulocyte/macrophage colony-stimulating factor-primed neutrophils. **J Exp Med,** v. 172, n. 5, p. 1451-7, Nov 1 1990. ISSN 0022-1007 (Print) 0022-1007 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/2172436 >.

FLOWER, R. J. Prostaglandins, bioassay and inflammation. **Br J Pharmacol,** v. 147 Suppl 1, p. S182-92, Jan 2006. ISSN 0007-1188 (Print) 0007-1188 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16402103 >.

FOXMAN, E. F.; CAMPBELL, J. J.; BUTCHER, E. C. Multistep navigation and the combinatorial control of leukocyte chemotaxis. **J Cell Biol,** v. 139, n. 5, p. 1349-60, Dec 1 1997. ISSN 0021-9525 (Print) 0021-9525 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9382879 >.

FROHLICH, E. Value of phagocyte function screening for immunotoxicity of nanoparticles in vivo. **Int J Nanomedicine,** v. 10, p. 3761-78, 2015. ISSN 1178-2013 (Electronic) 1176-9114 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26060398 >.

GODSON, C. et al. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. **J Immunol,** v. 164, n. 4, p. 1663-7, Feb 15 2000. ISSN 0022-1767 (Print) 0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10657608 >.

GOLDSTEIN, J. L.; CRYER, B. Gastrointestinal injury associated with NSAID use: a case study and review of risk factors and preventative strategies. **Drug Healthc Patient Saf,** v. 7, p. 31-41, 2015. ISSN 1179-1365 (Print) 1179-1365 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25653559 >.

GUERRERO, A. T. et al. Involvement of LTB4 in zymosan-induced joint nociception in mice: participation of neutrophils and PGE2. **J Leukoc Biol,** v. 83, n. 1, p. 122-30, Jan 2008. ISSN 0741-5400 (Print) 0741-5400 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17913976 >.

HAN, X. et al. Lipoxin A4 Preconditioning Attenuates Intestinal Ischemia Reperfusion Injury through Keap1/Nrf2 Pathway in a Lipoxin A4 Receptor Independent Manner. **Oxid Med Cell Longev,** v. 2016, p. 9303606, 2016. ISSN 1942-0994 (Electronic) 1942-0994 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27375835 >.

HARDY, J. D.; WOLFF, H. G.; GOODELL, H. Experimental evidence on the nature of cutaneous hyperalgesia. **J Clin Invest,** v. 29, n. 1, p. 115-40, Jan 1950. ISSN 0021-9738 (Print) 0021-9738 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15399521 >.

HARRIS, W. H. Wear and periprosthetic osteolysis: the problem. **Clin Orthop Relat Res**, n. 393, p. 66-70, Dec 2001. ISSN 0009-921X (Print) 0009-921X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11764372 >.

HASHIMOTO, A. et al. Antiinflammatory mediator lipoxin A4 and its receptor in synovitis of patients with rheumatoid arthritis. **J Rheumatol,** v. 34, n. 11, p. 2144-53, Nov 2007. ISSN 0315-162X (Print) 0315-162X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17918787 >.

KANE, R. L. et al. The functional outcomes of total knee arthroplasty. **J Bone Joint Surg Am,** v. 87, n. 8, p. 1719-24, Aug 2005. ISSN 0021-9355 (Print) 0021-9355 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16085610 >.

LEE, C. H. Resolvins as new fascinating drug candidates for inflammatory diseases. **Arch Pharm Res,** v. 35, n. 1, p. 3-7, Jan 2012. ISSN 0253-6269 (Print) 0253-6269 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22297737 >.

LEVY, B. D. et al. Lipid mediator class switching during acute inflammation: signals in resolution. **Nat Immunol,** v. 2, n. 7, p. 612-9, Jul 2001. ISSN 1529-2908 (Print) 1529-2908 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11429545 >.

LIAO, W. et al. Lipoxin A4 attenuates acute rejection via shifting TH1/TH2 cytokine balance in rat liver transplantation. **Transplant Proc,** v. 45, n. 6, p. 2451-4, Jul-Aug 2013. ISSN 1873-2623 (Electronic) 0041-1345 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23742835 >.

LIU, R. et al. Small-sized titanium dioxide nanoparticles mediate immune toxicity in rat pulmonary alveolar macrophages in vivo. **J Nanosci Nanotechnol,** v. 10, n. 8, p. 5161-9, Aug 2010. ISSN 1533-4880 (Print) 1533-4880 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21125865 >.

LIUZZO, G. et al. Enhanced inflammatory response in patients with preinfarction unstable angina. **J Am Coll Cardiol,** v. 34, n. 6, p. 1696-703, Nov 15 1999. ISSN 0735-1097 (Print) 0735-1097 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10577559 >.

LORENZETTI, B. B. et al. Cytokine-induced neutrophil chemoattractant 1 (CINC-1) mediates the sympathetic component of inflammatory mechanical hypersensitivitiy in rats. **Eur Cytokine Netw,** v. 13, n. 4, p. 456-61, Oct-Dec 2002. ISSN 1148-5493 (Print) 1148-5493 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12517731 >.

MADDOX, J. F. et al. Lipoxin A4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A4 receptor. **J Biol Chem,** v. 272, n. 11, p. 6972-8, Mar 14 1997. ISSN 0021-9258 (Print) 0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9054386 >.

MADERNA, P.; GODSON, C. Lipoxins: resolutionary road. **Br J Pharmacol,** v. 158, n. 4, p. 947-59, Oct 2009. ISSN 1476-5381 (Electronic) 0007-1188 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19785661 >.

MAIOLI, N. A. et al. The superoxide anion donor, potassium superoxide, induces pain and inflammation in mice through production of reactive oxygen species and cyclooxygenase-2. **Braz J Med Biol Res,** v. 48, n. 4, p. 321-31, Apr 2015. ISSN 1414-431X (Electronic) 0100-879X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25714890 >.

MAKUMIRE, S. et al. Immunomodulatory activity of zinc peroxide (ZnO(2)) and titanium dioxide (TiO(2)) nanoparticles and their effects on DNA and protein integrity. **Toxicol Lett,** v. 227, n. 1, p. 56-64, May 16 2014. ISSN 1879-3169 (Electronic) 0378-4274 (Linking). Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/24631018 >.

MALAWISTA, S. E. et al. Tonic inhibition of chemotaxis in human plasma. **Proc Natl Acad Sci U S A,** v. 105, n. 46, p. 17949-54, Nov 18 2008. ISSN 1091-6490 (Electronic) 0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18997012 >.

MARADIT KREMERS, H. et al. Prevalence of Total Hip and Knee Replacement in the United States. **J Bone Joint Surg Am,** v. 97, n. 17, p. 1386-97, Sep 2 2015. ISSN 1535-1386 (Electronic) 0021-9355 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26333733 >.

MARTIROSYAN, A.; SCHNEIDER, Y. J. Engineered nanomaterials in food: implications for food safety and consumer health. **Int J Environ Res Public Health,** v. 11, n. 6, p. 5720-50, May 28 2014. ISSN 1660-4601 (Electronic) 1660-4601 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24879486 >.

MCDONALD, B. et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. **Science,** v. 330, n. 6002, p. 362-6, Oct 15 2010. ISSN 1095-

9203 (Electronic) 0036-8075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20947763 >.

MEDZHITOV, R. Origin and physiological roles of inflammation. **Nature,** v. 454, n. 7203, p. 428-35, Jul 24 2008. ISSN 1476-4687 (Electronic) 0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18650913 >.

MILLIGAN, E. D.; WATKINS, L. R. Pathological and protective roles of glia in chronic pain. **Nat Rev Neurosci,** v. 10, n. 1, p. 23-36, Jan 2009. ISSN 1471-0048 (Electronic) 1471-003X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19096368 >.

MINOZZI, S. et al. Risk of infections using anti-TNF agents in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis: a systematic review and meta-analysis. **Expert Opin Drug Saf,** v. 15, n. sup1, p. 11-34, Dec 2016. ISSN 1744-764X (Electronic) 1474-0338 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27924643 >.

MOGIL, J. S.; YU, L.; BASBAUM, A. I. Pain genes?: natural variation and transgenic mutants. **Annu Rev Neurosci,** v. 23, p. 777-811, 2000. ISSN 0147-006X (Print) 0147-006X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10845081 >.

MORRIS, T. et al. Effects of low-dose aspirin on acute inflammatory responses in humans. **J Immunol,** v. 183, n. 3, p. 2089-96, Aug 1 2009. ISSN 1550-6606 (Electronic) 0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19597002 >.

NATHAN, C.; DING, A. Nonresolving inflammation. **Cell,** v. 140, n. 6, p. 871-82, Mar 19 2010. ISSN 1097-4172 (Electronic) 0092-8674 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20303877 >.

NORLING, L. V.; PERRETTI, M. The role of omega-3 derived resolvins in arthritis. **Curr Opin Pharmacol,** v. 13, n. 3, p. 476-81, Jun 2013. ISSN 1471-4973 (Electronic) 1471-4892 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23434193 >.

NUNEZ, M. et al. Health-related quality of life in patients with osteoarthritis after total knee replacement: factors influencing outcomes at 36 months of follow-up. **Osteoarthritis Cartilage,** v. 15, n. 9, p. 1001-7, Sep 2007. ISSN 1063-4584 (Print) 1063-4584 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17428689 >.

PAMPLONA, F. A. et al. Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. **Proc Natl Acad Sci U S A,** v. 109, n. 51, p. 21134-9, Dec 18 2012. ISSN 1091-6490 (Electronic) 0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23150578 >.

PARKINSON, J. F. Lipoxin and synthetic lipoxin analogs: an overview of anti-inflammatory functions and new concepts in immunomodulation. **Inflamm Allergy Drug Targets,** v. 5, n. 2, p. 91-106, Apr 2006. ISSN 1871-5281 (Print) 1871-5281 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16613568 >.

PATCHA, V. et al. Differential inside-out activation of beta2-integrins by leukotriene B4 and fMLP in human neutrophils. **Exp Cell Res,** v. 300, n. 2, p. 308-19, Nov 1

2004. ISSN 0014-4827 (Print) 0014-4827 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15474996 >.

PEIRS, C.; SEAL, R. P. Neural circuits for pain: Recent advances and current views. **Science,** v. 354, n. 6312, p. 578-584, Nov 4 2016. ISSN 1095-9203 (Electronic) 0036-8075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27811268 >.

POULIOT, M. et al. Lipoxin A(4) analogues inhibit leukocyte recruitment to Porphyromonas gingivalis: a role for cyclooxygenase-2 and lipoxins in periodontal disease. **Biochemistry,** v. 39, n. 16, p. 4761-8, Apr 25 2000. ISSN 0006-2960 (Print) 0006-2960 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10769133 >.

PRUSS, H. et al. Proresolution lipid mediators in multiple sclerosis - differential, disease severity-dependent synthesis - a clinical pilot trial. **PLoS One,** v. 8, n. 2, p. e55859, 2013. ISSN 1932-6203 (Electronic) 1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23409068 >.

RANG, H. P.; BEVAN, S.; DRAY, A. Chemical activation of nociceptive peripheral neurones. **Br Med Bull,** v. 47, n. 3, p. 534-48, Jul 1991. ISSN 0007-1420 (Print) 0007-1420 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1724403 >.

RIBEIRO, R. A. et al. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. **Eur J Pharmacol,** v. 387, n. 1, p. 111-8, Jan 3 2000. ISSN 0014-2999 (Print) 0014-2999 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10633169 >.

ROMANO, M. et al. Lipoxins and aspirin-triggered lipoxins in resolution of inflammation. **Eur J Pharmacol,** v. 760, p. 49-63, Aug 5 2015. ISSN 1879-0712 (Electronic) 0014-2999 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25895638 >.

SACHS, D. et al. Tumour necrosis factor-alpha, interleukin-1beta and interleukin-8 induce persistent mechanical nociceptor hypersensitivity. **Pain,** v. 96, n. 1-2, p. 89-97, Mar 2002. ISSN 0304-3959 (Print) 0304-3959 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11932065 >.

SAMUELSSON, B. Role of basic science in the development of new medicines: examples from the eicosanoid field. **J Biol Chem,** v. 287, n. 13, p. 10070-80, Mar 23 2012. ISSN 1083-351X (Electronic) 0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22318727 >.

SAMUELSSON, B. et al. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. **Science,** v. 237, n. 4819, p. 1171-6, Sep 4 1987. ISSN 0036-8075 (Print) 0036-8075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/2820055 >.

SANG, X. et al. The chronic spleen injury of mice following long-term exposure to titanium dioxide nanoparticles. **J Biomed Mater Res A,** v. 100, n. 4, p. 894-902, Apr 2012. ISSN 1552-4965 (Electronic) 1549-3296 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22275130 >.

SCHANEN, B. C. et al. Exposure to titanium dioxide nanomaterials provokes inflammation of an in vitro human immune construct. **ACS Nano,** v. 3, n. 9, p. 2523-32, Sep 22 2009. ISSN 1936-086X (Electronic) 1936-0851 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19769402 >.

SCHOLZ, J.; WOOLF, C. J. Can we conquer pain? **Nat Neurosci,** v. 5 Suppl, p. 1062-7, Nov 2002. ISSN 1097-6256 (Print) 1097-6256 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12403987 >.

SERHAN, C. N. Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? **Prostaglandins,** v. 53, n. 2, p. 107-37, Feb 1997. ISSN 0090-6980 (Print) 0090-6980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9112289 >.

SERHAN, C. N. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. **Prostaglandins Leukot Essent Fatty Acids,** v. 73, n. 3-4, p. 141-62, Sep-Oct 2005. ISSN 0952-3278 (Print) 0952-3278 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16005201 >.

SERHAN, C.N. **Aspirin and aspirin-triggered 15-epi-lipoxins. Inflamation: Basic principles and correlates**. 3rd ed. Chapter 24, 373. eds. John J. Gallin and Ralph Snyderman, Lipincott Williams and Wilkins, Philadelphia, 1999.

SERHAN, C. N. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. **Annu Rev Immunol,** v. 25, p. 101-37, 2007. ISSN 0732-0582 (Print) 0732-0582 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17090225 >.

SERHAN, C. N. Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? **Am J Pathol,** v. 177, n. 4, p. 1576-91, Oct 2010. ISSN 1525-2191 (Electronic) 0002-9440 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20813960 >.

SERHAN, C. N. Pro-resolving lipid mediators are leads for resolution physiology. **Nature,** v. 510, n. 7503, p. 92-101, Jun 5 2014. ISSN 1476-4687 (Electronic) 0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24899309 >.

SERHAN, C. N. Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. **Mol Aspects Med,** v. 58, p. 1-11, Dec 2017. ISSN 1872-9452 (Electronic) 0098-2997 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28263773 >.

SERHAN, C. N.; CHIANG, N. Resolution phase lipid mediators of inflammation: agonists of resolution. **Curr Opin Pharmacol,** v. 13, n. 4, p. 632-40, Aug 2013. ISSN 1471-4973 (Electronic) 1471-4892 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23747022 >.

SERHAN, C. N.; CHIANG, N.; DALLI, J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. **Semin Immunol,** v. 27, n. 3, p. 200-15, May 2015. ISSN 1096-3618 (Electronic) 1044-5323 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25857211 >.

SERHAN, C. N. New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. **Mol Aspects Med,** v. 64, p. 1-17, Dec 2018.

ISSN 1872-9452 (Electronic) 0098-2997 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28802833 >.

SERHAN, C. N. et al. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. **J Exp Med,** v. 192, n. 8, p. 1197-204, Oct 16 2000. ISSN 0022-1007 (Print) 0022-1007 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11034610 >.

SERHAN, C. N. et al. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. **FASEB J,** v. 26, n. 4, p. 1755-65, Apr 2012. ISSN 1530-6860 (Electronic) 0892-6638 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22253477 >.

SERHAN, C. N.; HAMBERG, M.; SAMUELSSON, B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. **Proc Natl Acad Sci U S A,** v. 81, n. 17, p. 5335-9, Sep 1984a. ISSN 0027-8424 (Print) 0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/6089195 >.

SERHAN, C. N. Trihydroxytetraenes: a novel series of compounds formed from arachidonic acid in human leukocytes. **Biochem Biophys Res Commun,** v. 118, n. 3, p. 943-9, Feb 14 1984b. ISSN 0006-291X (Print) 0006-291X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/6422933 >.

SERHAN, C. N. et al. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. **J Exp Med,** v. 196, n. 8, p. 1025-37, Oct 21 2002. ISSN 0022-1007 (Print) 0022-1007 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12391014 >.

SERHAN, C. N. et al. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. **J Immunol,** v. 171, n. 12, p. 6856-65, Dec 15 2003. ISSN 0022-1767 (Print) 0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14662892 >.

SHI, H. et al. Titanium dioxide nanoparticles: a review of current toxicological data. **Part Fibre Toxicol,** v. 10, p. 15, Apr 15 2013. ISSN 1743-8977 (Electronic) 1743-8977 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23587290 >.

SHI, Y. et al. Lipoxin A4 mitigates experimental autoimmune myocarditis by regulating inflammatory response, NF-kappaB and PI3K/Akt signaling pathway in mice. **Eur Rev Med Pharmacol Sci,** v. 21, n. 8, p. 1850-1859, Apr 2017. ISSN 2284-0729 (Electronic) 1128-3602 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28485793 >.

SHIMIZU, T. Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. **Annu Rev Pharmacol Toxicol,** v. 49, p. 123-50, 2009. ISSN 0362-1642 (Print) 0362-1642 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18834304 >.

SODIN-SEMRL, S. et al. Lipoxin A4 inhibits IL-1 beta-induced IL-6, IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances

synthesis of tissue inhibitors of metalloproteinases. **J Immunol,** v. 164, n. 5, p. 2660-6, Mar 1 2000. ISSN 0022-1767 (Print) 0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10679106 >.

SPECTOR, W. G.; WILLOUGHBY, D. A. Vasoactive Amines in Acute Inflammation. **Ann N Y Acad Sci,** v. 116, p. 839-46, Aug 27 1964. ISSN 0077-8923 (Print) 0077-8923 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14212856 >.

SVENSSON, C. I.; ZATTONI, M.; SERHAN, C. N. Lipoxins and aspirin-triggered lipoxin inhibit inflammatory pain processing. **J Exp Med,** v. 204, n. 2, p. 245-52, Feb 19 2007. ISSN 0022-1007 (Print) 0022-1007 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17242163 >.

TAKEUCHI, O.; AKIRA, S. Pattern recognition receptors and inflammation. **Cell,** v. 140, n. 6, p. 805-20, Mar 19 2010. ISSN 1097-4172 (Electronic) 0092-8674 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20303872 >.

VERRI, W. A., JR. et al. IL-15 mediates immune inflammatory hypernociception by triggering a sequential release of IFN-gamma, endothelin, and prostaglandin. **Proc Natl Acad Sci U S A,** v. 103, n. 25, p. 9721-5, Jun 20 2006. ISSN 0027-8424 (Print) 0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16766656 >.

VERRI, W. A., JR. et al. IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. **Ann Rheum Dis,** v. 69, n. 9, p. 1697-703, Sep 2010. ISSN 1468-2060 (Electronic) 0003-4967 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20472598 >.

WANG, C. T. et al. Over-expression of receptor activator of nuclear factor-kappaB ligand (RANKL), inflammatory cytokines, and chemokines in periprosthetic osteolysis of loosened total hip arthroplasty. **Biomaterials,** v. 31, n. 1, p. 77-82, Jan 2010. ISSN 1878-5905 (Electronic) 0142-9612 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19781765 >.

WATKINS, L. R. et al. Mechanisms of tumor necrosis factor-alpha (TNF-alpha) hyperalgesia. **Brain Res,** v. 692, n. 1-2, p. 244-50, Sep 18 1995. ISSN 0006-8993 (Print) 0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8548310 >.

WEIR, A. et al. Titanium dioxide nanoparticles in food and personal care products. **Environ Sci Technol,** v. 46, n. 4, p. 2242-50, Feb 21 2012. ISSN 1520-5851 (Electronic) 0013-936X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22260395 >.

WOOLEY, P. H. et al. Inflammatory responses to orthopaedic biomaterials in the murine air pouch. **Biomaterials,** v. 23, n. 2, p. 517-26, Jan 2002. ISSN 0142-9612 (Print) 0142-9612 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11761173 >.

WOOLF, C. J.; SALTER, M. W. Neuronal plasticity: increasing the gain in pain. **Science,** v. 288, n. 5472, p. 1765-9, Jun 9 2000. ISSN 0036-8075 (Print) 0036-8075 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10846153 >.

WU, L. et al. Lipoxin A4 ameliorates cerebral ischaemia/reperfusion injury through upregulation of nuclear factor erythroid 2-related factor 2. **Neurol Res,** v. 35, n. 9, p.

968-75, Nov 2013. ISSN 1743-1328 (Electronic) 0161-6412 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23880501 >.

WU, T.; TANG, M. The inflammatory response to silver and titanium dioxide nanoparticles in the central nervous system. **Nanomedicine (Lond),** v. 13, n. 2, p. 233-249, Jan 2018. ISSN 1748-6963 (Electronic) 1743-5889 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29199887 >.

4. ARTIGO PARA PUBLICAÇÃO (LIFE SCIENCES)

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The Lipid Mediator Lipoxin A_4 ameliorates titanium dioxide (TiO_2)-induced arthritis by reducing pain and inflammation in mice

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Abstract

Aims: To evaluate the effect and mechanisms of Lipoxin A4 in TiO_2-induced chronic arthritis in mice, a model resembling prosthesis and implant inflammation.

Methods: Mice were stimulated with 3 mg of TiO_2 on the knee joint. After 24h, mice were treated with LXA_4 (0.1, 1 or 10 ng/animal) or vehicle (ethanol) over 30 days. The disease phenotype, treatment toxicity, leukocytes recruitment, oxidative stress, cytokines production and mRNA of receptor activator of nuclear fator kappaB (RANK) were evaluated (2^{nd} and 30^{th} days of treatment).

Key Findings: LXA_4 reduced TiO_2-induced mechanical hiperalgesia in a dose-dependent manner. LXA_4 reduced the TiO_2-induced edema and recruitment of leukocytes, without inducing toxicity. LXA_4 modulates cytokine such as TNF-α, IL-1β, IL-6 and IL-10. Furthermore, the LXA_4 reestablished the antioxidant capacity (GSH and ABTS assays) by increasing expression of Nrf2 and reducing bone resorption through mRNA RANK expression.

Significance: Lipoxin A4 ameliorates titanium dioxide (TiO_2)-induced arthritis by reducing pain and inflammation in mice with reduction of leukocytes migration cytokines production and increment of antioxidant capacity.

Key words: Lipid mediator; Arthritis; Implant; Arthroplasty; Resolution of inflammation; Pain.

INTRODUCTION

Several inflammatory, autoimmune, infectious or traumatic conditions may cause destruction of bone surfaces. The occurrence of these processes on major joints, responsible for large-bodied movements, may lead to arthritis. In this sense, it is expected that many patients worldwide require medical procedures consisting on total or partial joint replacement by metal prostheses (arthroplasty) [1]. Arthroplasty is a successful orthopedic procedure that enables improvement in the life quality of patients suffering from osteoarthritis, inflammatory arthritis or tumors, which relieves pain and improve the functional state [2; 3]. Studies have shown that, approximately, 11 million patients will perform an arthroplasty until 2030 [4]. Although arthroplasty is considered a successful surgical technique, 10-15% of the procedures tend to fail due to the release of metallic nanoparticles in the periprosthetic space, which results in rejection of the prosthesis and need of a novel surgery and prosthesis replacement [5].

The titanium dioxide (TiO_2) nanoparticle is a white, odorless powder widely used in the production of orthopedic prostheses and various other products [6]. This molecule is the major component involved on prosthesis wear process-induced arthritis. This condition occurs mainly by recognition of TiO_2 debris by macrophages its activation and consequent release of tumor necrosis factor alpha (TNF-α), as demonstrated in a case report [7]. In fact, Borghi *et al.* [8] demonstrated in a mice experimental model that intra-articular administration of TiO_2 induces chronic arthritis. In fact, this model allows the understanding of cellular and molecular mechanisms involved in TiO_2-induced mechanical hyperalgesia, edema, histopathological alterations, degradation of proteoglycans, oxidative stress and production of inflammatory cytokines.

Currently, the available therapies for patients with prosthesis-induced arthritis are non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and opioids; drugs that have limited efficacy and offer intense adverse effects [9; 10], affecting, significantly the life quality and generating great economic expenses [11; 12]. Therefore, the investigation of novel drugs is necessary for the treatment of prosthesis-induced arthritis.

Lipoxin A4 (LXA_4) is a specialized pro resolving lipid mediator (SPRLM) derived from arachidonic acid by sequential actions of lipoxygenases [13]. This

molecule plays an important and relevant anti-inflammatory and resolutive role on inflammatory processes [14]. LXA_4 is described as active on the range of nanograms by limitation of cellular recruitment, chemotaxis and polymorphonuclear adhesion, therefore, ceasing the tissue damage induced by these cells [15]. In addition, LXA_4 reduced serum levels of cytokines such as TNF-α and interleukin-6 (IL-6) [16]. Furthermore, LXA_4 reduced inflammatory-induced pain by suppressing mechanical [17] and thermal hyperalgesia [18] with small doses of LXA_4. This lipid mediator is also described as having strong anti-oxidative activity by blocking the generation of reactive oxygen species (ROS) [19] and increasing nuclear factor erythroid 2-related factor 2 (Nrf2) and heme-oxygenase (HO) expression levels [20]. Another important role of LXA_4 is the stimulation of chemotaxis and adhesion of monocytes for clearance and healing of the inflamed site [21], in addition to the regulation of Th2 response [22] and inhibition of nuclear factor kappa B (NF-κB) activation in different cell types [23; 24; 25]. In this sense, LXA_4 is a promising candidate with analgesic and anti-inflammatory properties. Therefore, the aim of our study was to evaluate the analgesic, anti-inflammatory and antioxidants proprieties of LXA_4 on TiO_2-induced arthritis in mice.

MATERIAL AND METHODS

Experimental Procedures

In the first part of experiments, mice (n = 6 per group per experiment) were stimulated in the right joint with an intra-articular (i.a.) injection of TiO_2 (3 mg/10µl, i.a.) per knee joint as described by Borghi *et al.* [8]. Twenty-four hours after TiO_2 stimulus, mice were treated with LXA_4 at 0.1ng, 1ng, 10 ng or vehicle (3.2% ethanol plus saline) (100µl per animal, intraperitoneal, i.p.). Mechanical hyperalgesia and edema were evaluation started 24h after TiO_2 stimulus, before and after LXA_4 treatment (1h, 3h, 5h, 7h and 24h after LXA_4 treatment in the first day and every two days from 2^{nd} to the 30^{th} days) [8]. The dose and time of treatment were chosen base on the results of mechanical hyperalgesia. LXA_4 was administrated every two days at the dose of 10 ng/animal, and analyzes were performed on the 2^{nd} and 30^{th} days after stimulus. Time points were selected based on the analgesic effect of LXA_4 and also in order to elucidate the potential of this lipid mediator in the early and late stages of TiO_2-induced pain and inflammation. In order to detect any toxic effects of

chronic LXA_4 treatment in tissues, at 30th day post-treatment stomach samples were collected to assess myeloperoxidase (MPO) activity and blood samples to assess serum levels of aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine. The knee joint was washed for leukocytes recruitment analysis. The entire knee joint was used to determine the levels of cytokine, by enzyme-linked immunosorbent assay (ELISA) (TNF-α, IL-1β, IL-6 e IL-10); mRNA expression by reverse transcriptase-quantitative real time polymerase chain reaction (RT-qPCR); oxidative stress by the measurement of reduced glutathione (GSH) concentration and free-radical scavenging ability (ABTS) assay, and, mRNA expression of Nrf2. In addition, receptor activator of nuclear factor kappa B (RANK) mRNA expression assay was used to evaluate the mRNA expression of molecule involved in bone resorption.

Animals

Male Swiss mice weighing between 20 and 25 g from Universidade Estadual de Londrina (Londrina, Paraná, Brazil) were used in this study. All mice were housed in standard clear plastic cages with free access to water and food, light/dark cycle of 12/12h and controlled temperature (21°±1°C). Mice were acclimatized to the testing room at least 1 h before the experiments and all behavioral testing was performed between 9 a.m. and 5 p.m. Animal care and handling procedures were in accordance with the International Association for Study of Pain (IASP) guidelines and were approved by the Universidade Estadual de Londrina Ethics Committee on Animal Research and Welfare (process number 11147.2016.40). All efforts were made to minimize the number of animals used and their suffering.

Chemical compounds

Materials were obtained from the following sources: saline solution (NaCl 0.9%; Frenesius Kabi Brasil Ltda, Aquiraz, CE, Brazil), Lipoxin A_4 (LXA_4), ≥ 95% purity, was purchased from Cayman Chemical (Denver, CO, USA) and Titanium dioxide (TiO_2; pure, MW 79,90; purchased from Synth, Diadema, SP, Brazil).

Evaluation of articular mechanical hyperalgesia

The knee joint mechanical hyperalgesia was evaluated. Mice were placed in acrylic cages with a wire grid floor, and the stimulations were performed only when

the animals were quiet and with the four paws on the grid floor. This method consists of an electronic pressure-meter, with force transducer fitted with polypropylene tip (Eletronic von frey Analgesimeter; Insight instruments, Ribeirao Preto, SP, Brazil). To evaluate knee joint pain it was used a large tip (4.15 mm^2), to exclude subcutaneous effect [1]. An increase perpendicular force was applied to the central area of the plantar surface of the hind paw to induce flexion of tibiofemoral joint followed by hind paw withdrawal when joint was injured. A digital analgesimeter recorded the intensity of the force applied (in grams) when the paw was withdrawal. The test was performed at the times 1, 3, 5, 7 and 24h in the first day after LXA_4 treatment and every two days from 2nd to the 30th days. The investigators were blinded to the treatment groups. The results were expressed as withdrawal mechanical threshold in grams (g).

Articular edema measurements

Articular edema of the tibiofemoral joint was assessed through measurements of the transverse diameters using a caliper (Digmatic Caliper, Mitutoyo Corporation, Kanagawa, Japan). The edema was determined for each mouse knee joint by the difference indicated times post-stimulus and zero time. The test was performed at the times 1, 3, 5, 7 and 24h in the first day after LXA_4 treatment and every two days from 2nd to the 30th days. The results were expressed as Δ mm/joint.

Liver and kidney toxicity assays

Blood samples were collected at 30th day post TiO_2 stimulus, centrifuged (0.4 *rcf*, 20 min, 4 °C), and the serum was separated to assess the safety of treatment with LXA_4. AST and ALT were used as markers of hepatotoxicity, and acetaminophen was used as a positive drug control (650 mg/kg, intraperitoneal (i.p.), diluted in sterile saline, once). Urea and creatinine levels were used to evaluate nephrotoxicity, and diclofenac was a positive drug control (200 mg/kg, orally, diluted in sterile saline, once). The samples were processed according to the manufacturer's instructions (Labtest Diagnóstico S. A., Brazil). Results were presented as U/mL (AST and ALT) or mg/dL (urea and creatinine) of serum.

Myeloperoxidase activity

On the 30th day, samples of the stomach were harvested in 50 mM K_2HPO_4 buffer (pH 6.0) containing 0.5% hexadecyl trimethylammonium bromide (HTAB) and kept at − 80 °C until use. Frozen samples were homogenized using a tissue turrax (Tissue-Tearor 985370, BioSpec Products, Bartlesville, OK, USA) and centrifuged (2 min, 16,000g, 4 °C), and the resulting supernatant was assayed using a spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Vantaa, Finland) for MPO activity determination at 450 nm. Briefly, 15 µL of sample was mixed with 200 µL of 50 mM phosphate buffer (pH 6.0), containing 0.167 mg/mL *O*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The MPO activity of samples was compared to a standard curve of neutrophils. Indomethacin (2.5 mg/kg, i.p., diluted in tris/HCl buffer, for 7 days) was used as positive drug control for stomach damage. The results were presented as MPO activity (number of neutrophils × 10^6/ mg of tissue).

Evolution of leukocyte migration

The total and differential counts of recruited leukocytes to the knee joint cavity were determined on 2nd and 30th as previously described [11]. Briefly, knee joint cavities were washed with saline containing EDTA, which was recovered to evaluate total and differential cell counts. Total cell counts were performed in Neubauer chamber using Turk solution, and differential cell counts (100 cells per slide) were performed in slices stained with the panoptic kit (Laborclin, Pinhais,PR, Brazil) under a light microscope (Olympus CX31RTSF, Tokyo, Japan). Results were expressed as total leukocytes, polymorphonuclear, and mononuclear cells (cells × 10^3/ synovial cavity).

Cytokine measurement

Knee joint samples collection on the 2nd day were homogenized in 500 µL of buffer containing protease inhibitors. Samples were centrifuged (3000 rpm × 15 min × 4°C). TNF-α, IL-1β, IL-10 and IL-6 levels were determined from the supernatant by ELISA, using eBioscience kits (Thermo Fisher Scientific, Vienna, Austria). The results were expressed as pictograms (pg) of cytokine/mg of protein.

GSH assay

The levels of synovial GSH were determined using a spectrophotometric method on 2nd day [26; 27; 28]. Frozen samples of knee joints were homogenized in cold 0.02 M EDTA. The homogenate was treated with 50% trichloroacetic acid and centrifuged (15 min x 1,500 g). The resulting supernatant received 0.4 M Tris-HCl, pH 8.9, and next, samples were vortex-mixed, and 10 mM dithiobisnitrobenzoic acid was added, followed by votex-mixing. After these procedures, samples were allowed to stand for 5 minutes before being read at 412 nm. The preparation of standard curves requested in the test were made using different concentrations of GSH. The results were presented as nmols of GSH/mg of protein.

ABTS assays

After the knee joint collection at 2nd day, the samples were homogenized with 500 µL of 1.15% KCl, centrifuged (10 min x 200 g x 4°C) and the supernatants were used. The free-radical scavenging ability to the sample was determined using the ABTS assay [27; 28]. For the ABTS assay, ABTS reagent was diluted in phosphate buffer saline at pH 7.4 to an absorbance of 0.80 at 730 nm. Subsequently, 1.0 mL of diluted ABTS solution was mixed in 20 µL of the supernatant. After 6 min, the absorbance was measured at 730 nm. The results were equated against a standard Trolox curve (1.5-30 µmol/L, final concentrations). The results are expressed as Trolox equivalents per milligram of protein.

RT-qPCR

To assess the mRNA expression of genes, the knee joint was dissected on 2nd and 30th days after TiO_2. Samples were homogenized in TRIzol reagent®. Total RNA was extracted by using the SV Total RNA Isolation System (Promega). The purity of total RNA was measured using a spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Vantaa, Finland) and the wavelength absorption relationship (260/280) was between 1.8 and 2.0 for all preparations. Reverse transcription of total RNA to cDNA and qPCR was carried out using GoTaq® 2-Step RT-qPCR System (Promega) and specific primers, A no-reverse transcription control was applied for cDNA production (running the samples without adding reverse transcriptase enzyme) and a no-template control (NTC) was carried out for qPCR (running the qPCR reaction without cDNA). The qPCR reaction was performed in a StepOnePlus™ Real-Time PCR System (Applied Biosystems®). The

relative gene expression was measured using the comparative 2−(ΔΔCq) method. The primers sequences are presented on Table 1. The expressions of glyceraldehyde 3-phosphate dehydrogenase (Gapdh) and β-actin mRNA were used as the reference gene, and the results were expressed as mRNA expression (normalized to GAPDH or β-actin).

Statistical analysis

Data were analyzed using GraphPad Prism statistical software (GraphPad Software, Inc., USA-500.288, version 7.0). The results were presented as means ± SEM of measurements made on six mice in each group per experiment and are representative of two separate experiments. Two-way repeated measures analysis of variance (ANOVA) followed by Tukey's post test was used to compare all groups and doses at all times when responses were measured at different times after the stimulus injection. Differences between responses were evaluated by one-way ANOVA followed by Tukey's post test for data of single time point. $P<0.05$ was considered significant.

RESULTS

LXA_4 reduces TiO_2-induced articular mechanical hyperalgesia and joint edema in a dose-dependent manner

Firstly, it was verified rather or not LXA_4 reduce articular mechanical hyperalgesia and edema induced by intra-articular stimulus with TiO_2. The injection of 3 mg/joint of TiO_2 induced mechanical hyperalgesia (Fig. 1A) and edema (Fig. 1B) at all evaluated time points. Mice were treated with LXA_4 (0.1, 1 or 10 ng/ animal, 100µl i.p.) or vehicle (ethanol plus saline). Animals were treated 24h after TiO_2 i.a. injection and subsequently on alternate days, once it was observed that LXA_4 analgesia effect decreased 2 days after the treatment. Therefore, on day 3 after TiO_2 injection the second treatment was carried (Fig. 1A). LXA_4 reduces hyperalgesia in a dose-dependent manner, where the dose of 0.1 ng/animal did not reduce TiO_2-induced articular mechanical hyperalgesia, except in the 18th day, demonstrating that at this dose LXA_4 has no analgesic effect. The doses of 1 and 10 ng/animal reduced TiO_2-

induced articular mechanical hiperalgesia in 24 h and from the 2nd to 30th days. The dose of 1 and 10 ng/animal were statistically different compared to the other doses and the stimulated group. Therefore, LXA_4 presented a dose–response with maximal effect with the dose of 10 ng/animal, which was chosen for the next experiments. The dose of 10 ng/animal of LXA_4 reduced TiO_2-induced articular edema 24h after TiO_2 i.a. injection at day 1 until 30th day. The reduction of edema by a dose of 10 ng/animal of LXA_4 was statistically different compared to the stimulus (Fig. 1B). The saline group did not demonstrate mechanical hyperalgesia (Fig. 1A) or edema (Fig. 1B). The vehicle did not change any of the evaluated parameters (Fig. 1A and 1B).

LXA_4 does not induce liver or kidney damage, or stomach lesions

At 30th day the serum samples and stomach were collected to evaluated if a chronic treatment with LXA4 would induce gastric, hepatic and renal damage, by assessing the concentrations of AST, ALT, urea, creatinine and MPO activity, (Supplementary Fig. 1). The treatment with LXA_4 did not modify the serum concentration of AST (Supplementary Fig. 1A), ALT (Supplementary Fig. 1B), creatinine (Supplementary Fig. 1C), ureia (Supplementary Fig. 1D) and MPO activity in stomach (Supplementary Fig. 1E). Thus, chronic 30 days treatment with 10 ng/animal of LXA_4 did not induce detectable gastric, hepatic or renal lesion/damage. Positive control groups demonstrates that the assays used may serve as selective markers of tissue injury [8; 27].

LXA_4 reduces TiO_2-induced recruitment of total, polymorphonuclear and mononuclear leukocytes to the knee joint

Leukocytes recruitment to the knee joint is a hallmark of rheumatic diseases [29]. To investigate the effect of LXA_4 on leukocyte recruitment on 2nd and 30th days post-TiO_2 stimulus, knee joints washes were collected from the knee joints to evaluate the total number of leukocytes, polymorphonuclear cells and mononuclear cells (Fig. 2). Importantly, there was a significant increase in the number of total of leukocytes recruited to the joint in stimulated group when compared to the saline group in 2nd (Fig. 2A) and 30th days after stimulus (Fig. 2D). Importantly, the treatment with LXA_4 at 10 ng/animal reduced TiO_2-induced recruitment of total leucocyte (Fig. 2A and 2D),

mononuclear cells (Fig. 2B and Fig. 2E) and polymorphonuclear (Fig. 2C and Fig. 2F) at all evaluated time points.

LXA_4 modulates TiO_2-induced cytokine production

The potential of LXA_4 in the modulation of cytokines (TNF-α, IL-1β, IL-6 and IL-10) were analyzed in the joint tissue at the 2nd and 30th days post TiO_2 stimulus (Fig. 3). At 2nd day, the i.a. injection of TiO_2 induced a significant increase in the levels of IL-1β (Fig. 3B), TNF-α (Fig. 3C) and IL-6 release (Fig. 3D) in the joint compared to the saline group. Only one treatment with 10 ng/animal of LXA_4 was sufficient to reduce the levels of the cytokines induced by TiO_2 (Fig. 3B-D) at this time point. In addition, IL-10 production was also increased by LXA_4 (Fig. 3E), evidencing the anti-inflammatory capacity since of this mediator within only one treatment.

In order to evaluate the expression of pro-IL-1β (2nd and 30th days) and IL-10 (30th day) mRNA, knee joint samples were collected on 2nd and 30th days. Importantly, the results demonstrated that TiO_2 induced a significant increase in the expression of pro-IL-1β on 2nd day (Fig. 3A) which was kept for 28 days (30 days post TiO_2 stimulus) (Fig. 3F), when compared to the saline groups. Conversely, the treatment with LXA_4 significantly inhibited the expression of pro-IL-1β at the 2nd and 30th days (Fig. 3A and 3F). Thus, LXA_4 reduced the expression of an important cytokine involved in the mechanisms of pain, edema and recruitment of leukocytes [30; 31]. Furthermore, the levels of IL-10 mRNA were upregulated on the 30th day, when compared to the other groups (Fig. 3G). Thus, LXA_4 modulates the TiO_2-induced cytokines in a dependent manner of IL-10 release.

LXA_4 inhibits TiO_2-induced decrease of antioxidant capacity

It has already been demonstrated in other models that the TiO_2 nanoparticles induce the production of ROS and consequently oxidative stress in various organs [32; 33; 34; 35]. To verify this issue, knee joints samples were collected on the 2nd day and the parameters of the antioxidant capacity of LXA_4 were measured by the GSH and ABTS assay (Fig. 4). TiO_2 stimulus reduced levels of endogenous antioxidant as observed in ABTS free radical scavenging ability (Fig. 4A) and GSH levels (Fig. 4B) in tissues when compared with saline group. A single treatment with 10 ng/animal of

LXA_4 significantly restored the levels of ABTS (Fig. 4A) and GSH (Fig. 4B) in the joint tissue, demonstrating that treatment with LXA_4 reestablished the ability to eliminate the ABTS radical and positively regulated GSH levels. In agreement, the treatment with LXA_4 induced the expression of Nrf2 mRNA, compared with TiO_2 group (Fig. 4C). Thus, the antioxidant effect of LXA4 has already been demonstrated [45].

LXA_4 reduces TiO_2-induced RANK mRNA expression

The levels of RANK, RANKL and OPG also regulate bone resorption. The interactions of RANKL and RANK induce bone resorption mediated by osteoclasts [36]. Considering the progressive effects of TiO_2, knee joint samples were collected on the 30th day to evaluate RANK mRNA expression. TiO_2 induced an increase of RANK mRNA expression, which was reduced after LXA_4 treatments (Fig. 5A). Therefore, the role of LXA4 in the modulation od osteoclastogenesis has been described previously [63].

DISCUSSION

Arthroplasty is an effective procedure in improving the quality of life of patients, relieving discomfort and reestablishing joint mobility [2; 37]. Although this procedure is considered successful, in about 10-15% of the cases, metallic nanoparticles, including TiO_2, are released and generate a pro-inflammatory microenvironment, which, most of the times culminates in implant-induced inflammatory arthritis, prosthetic rejection and the need of surgical revision [5; 9; 10].

In this study, we evaluated the early and late stages of TiO_2-induced inflammation and pain in a mice model of inflammatory chronic arthritis. Thus, we had chosen the 2nd and 30th days after TiO_2 stimulus for all analysis. In particular, the second day was chosen due to the observed analgesic effect of LXA_4 after a single treatment. This period of analysis is important to demonstrate the mechanisms involved in the LXA_4 action for reduction of the initial inflammatory parameters and the importance of the resolving process at the initial stage. Moreover, the effects and mechanisms of LXA_4 treatments were also evaluated in the chronic arthritis model.

Therefore, we showed that the SPRLM LXA_4 ameliorates chronic arthritis induced by TiO_2 in mice. In addition, we have shown that a single treatment with LXA_4 reduced joint pain, edema and leukocytes recruitment to the knee joint induced by TiO_2. Moreover, these effects are related to the reduction of oxidative stress and production of cytokines in the knee joint tissue.

Among the available therapies for patients with prosthesis-induced arthritis are corticosteroids, opioids and non-steroidal anti-inflammatory drugs (NSAIDs) [10]. The dose and chronicity of these drugs may cause side effects such as respiratory failure, dependence, gastrointestinal disorders, hepatotoxicity and nephrotoxicity [38]. In this sense, the search for new compounds to save as therapeutic alternative in important.

The present results demonstrated that LXA_4 at the dose of 10 ng/animal has analgesic effect and reduces edema in a dose-dependent manner, our data corroborate with studies in which LXA_4 attenuates carrageenan-induced mechanical hyperalgesia [17] and edema [18]. Furthermore, we demonstrated that LXA_4 is a safe drug for the treatment of prosthesis-induced arthritis since long-term treatments does not induce gastric, hepatic or renal damage, unlike the mentioned current available therapies.

The anti-inflammatory and resolving action of LXA_4 in this TiO_2-induced arthritis model was demonstrated by the reduction of leukocytes in the joint at the early and late stages of this chronic arthritis model. The total number of leukocytes in the synovial cavity was higher at the early stage (2^{nd} day) than at the 30^{th} day, mainly with mononuclear cells at this late stage. The decrease of inflammatory infiltrate was associated to the decrease in the local cytokines levels. In fact, LXA_4 treatment reduced TNF-α, IL-1β and IL-6 levels in the early stage of TiO_2-induced arthritis, in addition to the increased expression of IL-10 at 2^{nd} and 30^{th} days after stimulus. Macrophages have a well-described role in the pathophysiology of arthritis, contributing to oxidative stress at the site and cytokines production (IL-6, IL-1β and TNF-α). These inflammatory microenvironment promote the recruitment of new cells, activation of fibroblasts, followed by that production RANKL and macrophage colony of stimulating factor 1 (M-CSF1), which chronically activate osteoclasts and leading to bone erosion [39]. In this sense, the LXs act as agonists of resolutive macrophages, stimulating clearance apoptotic PMN; important process in the resolution of inflammation [15].

Cytokines such as IL-1β and TNF-α directly activate nociceptive neurons [30; 40] and amplify immune response. LXA_4 has a well-described function in the reduction of cell migration by mechanisms such inhibition of integrins agglomeration and neutrophil motility [41], which corroborates to damping of leukocyte-endothelium interactions and reduction of leukocyte diapedesis [42; 43]. These evidences demonstrate that the reduction of these pro-inflammatory cytokines, which orchestrate the inflammatory response, may contributed to the inhibition of leukocyte recruitment to the knee joint and, in consequence, confer analgesic effect and edema reduction.

The role of LXA_4 in NF-κB inhibition is an important anti-inflammatory mechanism that was reported in other studies in different cell types [16; 23; 24; 26]. A previous study reported the action of LXA_4 on pain relief in non-compressible lumbar disc herniation model through inhibition of the production of pro-inflammatory cytokines such TNF-α and IL-1β and, regulation of IL-10 and TGF-β [44]. Furthermore, the treatment with LXA_4 was also demonstrated ameliorating the levels of anti-inflammatory cytokines (TGF-β and IL-10) after exposition to UV light [45]. It is known that IL-10 restricts polarization of M1 macrophages and blocks the IL-33/ST2 axis during arthritis [46], inhibits neutrophil recruitment, matrix metalloproteinases (MMP) activity and edema [47; 48], common features in inflammatory joint diseases, where LXA_4 shown an important action [49].

Oxidative stress is present in arthritis and plays an important role in the development of inflammatory pain [50]. The reactive oxygen and nitrogen species produced at the site of inflammation contribute to the manifestation of pain and direct activation of nociceptive neurons [16; 51]. The TiO_2 stimulus triggers a pro-inflammatory cascade of cytokines and mediators, which culminated in lipid peroxidation, DNA damage and protein breakdown [52]. There is evidences demonstrating the action of LXA_4 on the inhibition of oxidative stress by improving diabetes-induced erectile dysfunction [53]. In addition, LXA_4 treatment increased antioxidant capacity and Nrf2 expression on the skin after UV exposure [45]. In a model of intestinal ischemia-reperfusion, LXA_4 treatments showed to mediate Keap1-Nrf2 signaling, by promoting the dissociation of Keap1 from Nrf2 and its translocation to the nucleus, culminating in the increase HO-1 gene expression [20] as already demonstrated in others animal models [54; 55].

The ABTS assay represent the overall antioxidant ability of a tissue, and GSH levels directly access the levels of this endogenous antioxidant [56]. In this study, we demonstrate that the LXA_4 treatment reestablished the levels of antioxidant parameters (ABTS and GSH) in the model of arthritis induced by TiO_2 in the initial period, assisting in the resolution process, by modulating Nrf2 expression in the knee joint. Higher expression of Nrf2 induces GSH expression, inhibit the production of pro-inflammatory cytokines [57] and increases the production of IL-10 and TGF-β [57; 58]. Previous studies with different experimental conditions indicate that Nrf2 is important to protect tissues from damage induced by TiO_2 [59; 60].The induction of Nrf2 elucidates, in part, the maintenance of endogenous antioxidants and the reduction of inflammation, a mechanism that protects the joint against the effects of TiO_2 and reliefs pain.

Joint destruction due to matrix degradation and excessive bone loss characterize inflammatory bone diseases such as arthritis [61]. TiO_2-induced cytokines (IL-1β and TNF-α) increase the release of MMP that activates chondrocytes and osteoclasts [61]. The differentiation of the osteoclasts itself requires the activation of RANK / RANKL, which results in osteoclastogenesis [62]. It has already been shown that LXA_4 suppresses osteoclastogenesis *in vitro* by modulating signaling pathways such as the reduction of RANK [63]. LXA_4 was shown to inhibit the release of MMP by human synovial fibroblasts [49]. Our data also have shown that LXA_4 reduces TiO_2-induced RANK mRNA expression in the joint, which explains in part the action of LXA_4 in decreasing bone degradation. These findings suggest that LXA_4 represents a promising therapeutic approach in the reduction of prosthesis-induced arthritis pain.

CONCLUSION

Concluding, the present study demonstrated the analgesic, anti-inflammatory and antioxidant effects of LXA_4 in a chronic model of TiO_2-induced arthritis in mice. LXA_4 attenuates TiO_2-induced inflammatory pain, joint edema, recruitment of leukocytes, inhibition of oxidative stress and cytokines modulation. Thus, this study demonstrated that an isolated SPRLM, such as LXA_4, is a promising approach to the treatment of complications related to implant-induced inflammation for its efficacy and safety.

Conflict of interest

Authors declare no conflict of interest.

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References

[1] A.T. Guerrero, W.A. Verri, Jr., T.M. Cunha, T.A. Silva, F.A. Rocha, S.H. Ferreira, F.Q. Cunha, and C.A. Parada, Hypernociception elicited by tibio-tarsal joint flexion in mice: a novel experimental arthritis model for pharmacological screening. Pharmacology, biochemistry, and behavior 84 (2006) 244-51.

[2] C.M. Callahan, B.G. Drake, D.A. Heck, and R.S. Dittus, Patient outcomes following tricompartmental total knee replacement. A meta-analysis. Jama 271 (1994) 1349-57.

[3] M.M. Siddiqui, S.J. Yeo, P. Sivaiah, S.L. Chia, P.L. Chin, and N.N. Lo, Function and quality of life in patients with recurvatum deformity after primary total knee arthroplasty: a review of our joint registry. The Journal of arthroplasty 27 (2012) 1106-10.

[4] H. Maradit Kremers, D.R. Larson, C.S. Crowson, W.K. Kremers, R.E. Washington, C.A. Steiner, W.A. Jiranek, and D.J. Berry, Prevalence of Total Hip and Knee Replacement in the United States. The Journal of bone and joint surgery. American volume 97 (2015) 1386-97.

[5] W.H. Harris, Wear and periprosthetic osteolysis: the problem. Clinical orthopaedics and related research (2001) 66-70.

[6] J.R. Gurr, A.S. Wang, C.H. Chen, and K.Y. Jan, Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213 (2005) 66-73.

[7] T. Dorner, J. Haas, C. Loddenkemper, V. von Baehr, and A. Salama, Implant-related inflammatory arthritis. Nature clinical practice. Rheumatology 2 (2006) 53-6; quiz 57.

[8] S.M. Borghi, S.S. Mizokami, F.A. Pinho-Ribeiro, V. Fattori, J. Crespigio, J.T. Clemente-Napimoga, M.H. Napimoga, D.L. Pitol, J.P.M. Issa, S.Y. Fukada, R. Casagrande, and W.A. Verri, Jr., The flavonoid quercetin inhibits titanium dioxide (TiO2)-induced chronic arthritis in mice. The Journal of nutritional biochemistry 53 (2018) 81-95.

[9] P.H. Wooley, R. Morren, J. Andary, S. Sud, S.Y. Yang, L. Mayton, D. Markel, A. Sieving, and S. Nasser, Inflammatory responses to orthopaedic biomaterials in the murine air pouch. Biomaterials 23 (2002) 517-26.

[10] N. Cobelli, B. Scharf, G.M. Crisi, J. Hardin, and L. Santambrogio, Mediators of the inflammatory response to joint replacement devices. Nature reviews. Rheumatology 7 (2011) 600-8.

[11] W.A. Verri, Jr., F.O. Souto, S.M. Vieira, S.C. Almeida, S.Y. Fukada, D. Xu, J.C. Alves-Filho, T.M. Cunha, A.T. Guerrero, R.B. Mattos-Guimaraes, F.R. Oliveira, M.M. Teixeira, J.S. Silva, I.B. McInnes, S.H. Ferreira, P. Louzada-Junior, F.Y. Liew, and F.Q. Cunha, IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. Annals of the rheumatic diseases 69 (2010) 1697-703.

[12] C.C. Ferreira, V.P. Ricci, L.L.d. Sousa, N.A. Mariano, and M.G.N. Campos, Improvement of Titanium Corrosion Resistance by Coating with Poly-Caprolactone and Poly-Caprolactone/Titanium Dioxide: Potential Application in Heart Valves. Materials Research 20 (2017) 126-133.

[13] E.P. Brennan, K.A. Nolan, E. Borgeson, O.S. Gough, C.M. McEvoy, N.G. Docherty, D.F. Higgins, M. Murphy, D.M. Sadlier, S.T. Ali-Shah, P.J. Guiry, D.A. Savage, A.P. Maxwell, F. Martin, C. Godson, and G. Consortium, Lipoxins attenuate renal fibrosis by inducing let-7c and suppressing TGFbetaR1. Journal of the American Society of Nephrology : JASN 24 (2013) 627-37.

[14] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology. Nature 510 (2014) 92-101.

[15] C.N. Serhan, Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. Prostaglandins, leukotrienes, and essential fatty acids 73 (2005) 141-62.

[16] X. Jiang, Z. Li, S. Jiang, X. Tong, X. Zou, W. Wang, Z. Zhang, L. Wu, and D. Tian, Lipoxin A4 exerts protective effects against experimental acute liver failure by inhibiting the NF-kappaB pathway. International journal of molecular medicine 37 (2016) 773-80.

[17] S. Abdelmoaty, G. Wigerblad, D.B. Bas, S. Codeluppi, T. Fernandez-Zafra, S. El-Awady el, Y. Moustafa, D. Abdelhamid Ael, E. Brodin, and C.I. Svensson, Spinal

actions of lipoxin A4 and 17(R)-resolvin D1 attenuate inflammation-induced mechanical hypersensitivity and spinal TNF release. PloS one 8 (2013) e75543.

[18] C.I. Svensson, M. Zattoni, and C.N. Serhan, Lipoxins and aspirin-triggered lipoxin inhibit inflammatory pain processing. The Journal of experimental medicine 204 (2007) 245-52.

[19] V. Nascimento-Silva, M.A. Arruda, C. Barja-Fidalgo, and I.M. Fierro, Aspirin-triggered lipoxin A4 blocks reactive oxygen species generation in endothelial cells: a novel antioxidative mechanism. Thrombosis and haemostasis 97 (2007) 88-98.

[20] X. Han, W. Yao, Z. Liu, H. Li, Z.J. Zhang, Z. Hei, and Z. Xia, Lipoxin A4 Preconditioning Attenuates Intestinal Ischemia Reperfusion Injury through Keap1/Nrf2 Pathway in a Lipoxin A4 Receptor Independent Manner. Oxidative medicine and cellular longevity 2016 (2016) 9303606.

[21] C. Godson, S. Mitchell, K. Harvey, N.A. Petasis, N. Hogg, and H.R. Brady, Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. Journal of immunology 164 (2000) 1663-7.

[22] J.F. Parkinson, Lipoxin and synthetic lipoxin analogs: an overview of anti-inflammatory functions and new concepts in immunomodulation. Inflammation & allergy drug targets 5 (2006) 91-106.

[23] S.H. Wu, P.Y. Liao, L. Dong, and Z.Q. Chen, Signal pathway involved in inhibition by lipoxin A(4) of production of interleukins induced in endothelial cells by lipopolysaccharide. Inflammation research : official journal of the European Histamine Research Society ... [et al.] 57 (2008) 430-7.

[24] J. Walker, E. Dichter, G. Lacorte, D. Kerner, B. Spur, A. Rodriguez, and K. Yin, Lipoxin a4 increases survival by decreasing systemic inflammation and bacterial load in sepsis. Shock 36 (2011) 410-6.

[25] T. Sun, E. Yu, L. Yu, J. Luo, H. Li, and Z. Fu, LipoxinA(4) induced antinociception and decreased expression of NF-kappaB and pro-inflammatory cytokines after chronic dorsal root ganglia compression in rats. European journal of pain 16 (2012) 18-27.

[26] R. Casagrande, S.R. Georgetti, W.A. Verri, Jr., D.J. Dorta, A.C. dos Santos, and M.J. Fonseca, Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. Journal of photochemistry and photobiology. B, Biology 84 (2006) 21-7.

[27] M.S. Hohmann, R.D. Cardoso, F.A. Pinho-Ribeiro, J. Crespigio, T.M. Cunha, J.C. Alves-Filho, R.V. da Silva, P. Pinge-Filho, S.H. Ferreira, F.Q. Cunha, R. Casagrande, and W.A. Verri, Jr., 5-lipoxygenase deficiency reduces acetaminophen-induced hepatotoxicity and lethality. BioMed research international 2013 (2013) 627046.

[28] S.M. Borghi, T.T. Carvalho, L. Staurengo-Ferrari, M.S. Hohmann, P. Pinge-Filho, R. Casagrande, and W.A. Verri, Jr., Vitexin inhibits inflammatory pain in mice by targeting TRPV1, oxidative stress, and cytokines. Journal of natural products 76 (2013) 1141-9.

[29] V. Fattori, F.A. Amaral, and W.A. Verri, Jr., Neutrophils and arthritis: Role in disease and pharmacological perspectives. Pharmacological research 112 (2016) 84-98.

[30] A.M. Binshtok, H. Wang, K. Zimmermann, F. Amaya, D. Vardeh, L. Shi, G.J. Brenner, R.R. Ji, B.P. Bean, C.J. Woolf, and T.A. Samad, Nociceptors are interleukin-1beta sensors. The Journal of neuroscience : the official journal of the Society for Neuroscience 28 (2008) 14062-73.

[31] D.P. Mascarenhas, M.S. Pereira, G.Z. Manin, J.I. Hori, and D.S. Zamboni, Interleukin 1 receptor-driven neutrophil recruitment accounts to MyD88-dependent pulmonary clearance of legionella pneumophila infection in vivo. The Journal of infectious diseases 211 (2015) 322-30.

[32] X. Sang, L. Zheng, Q. Sun, N. Li, Y. Cui, R. Hu, G. Gao, Z. Cheng, J. Cheng, S. Gui, H. Liu, Z. Zhang, and F. Hong, The chronic spleen injury of mice following long-term exposure to titanium dioxide nanoparticles. Journal of biomedical materials research. Part A 100 (2012) 894-902.

[33] H. Shi, R. Magaye, V. Castranova, and J. Zhao, Titanium dioxide nanoparticles: a review of current toxicological data. Particle and fibre toxicology 10 (2013) 15.

[34] K.T. Huang, C.T. Wu, K.H. Huang, W.C. Lin, C.M. Chen, S.S. Guan, C.K. Chiang, and S.H. Liu, Titanium nanoparticle inhalation induces renal fibrosis in mice via an oxidative stress upregulated transforming growth factor-beta pathway. Chemical research in toxicology 28 (2015) 354-64.

[35] Y.J. Hwang, Y.S. Jeung, M.H. Seo, J.Y. Yoon, D.Y. Kim, J.W. Park, J.H. Han, and S.H. Jeong, Asian dust and titanium dioxide particles-induced inflammation and oxidative DNA damage in C57BL/6 mice. Inhalation toxicology 22 (2010) 1127-33.

[36] M.H. Napimoga, J.T. Clemente-Napimoga, C.G. Macedo, F.F. Freitas, R.N. Stipp, F.A. Pinho-Ribeiro, R. Casagrande, and W.A. Verri, Jr., Quercetin inhibits inflammatory bone resorption in a mouse periodontitis model. Journal of natural products 76 (2013) 2316-21.

[37] A.J. Carr, O. Robertsson, S. Graves, A.J. Price, N.K. Arden, A. Judge, and D.J. Beard, Knee replacement. Lancet 379 (2012) 1331-40.

[38] D.L. Scott, F. Wolfe, and T.W. Huizinga, Rheumatoid arthritis. Lancet 376 (2010) 1094-108.

[39] I.A. Udalova, A. Mantovani, and M. Feldmann, Macrophage heterogeneity in the context of rheumatoid arthritis. Nature reviews. Rheumatology 12 (2016) 472-85.

[40] X. Jin, and R.W.t. Gereau, Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. The Journal of neuroscience : the official journal of the Society for Neuroscience 26 (2006) 246-55.

[41] V. Patcha, J. Wigren, M.E. Winberg, B. Rasmusson, J. Li, and E. Sarndahl, Differential inside-out activation of beta2-integrins by leukotriene B4 and fMLP in human neutrophils. Experimental cell research 300 (2004) 308-19.

[42] J.G. Filep, C. Zouki, N.A. Petasis, M. Hachicha, and C.N. Serhan, Lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 modulate adhesion molecule expression on human leukocytes in whole blood and inhibit neutrophil-endothelial cell adhesion. Advances in experimental medicine and biology 507 (2002) 223-8.

[43] J.G. Filep, C. Zouki, N.A. Petasis, M. Hachicha, and C.N. Serhan, Anti-inflammatory actions of lipoxin A(4) stable analogs are demonstrable in human whole blood: modulation of leukocyte adhesion molecules and inhibition of neutrophil-endothelial interactions. Blood 94 (1999) 4132-42.

[44] G.S. Miao, Z.H. Liu, S.X. Wei, J.G. Luo, Z.J. Fu, and T. Sun, Lipoxin A4 attenuates radicular pain possibly by inhibiting spinal ERK, JNK and NF-kappaB/p65 and cytokine signals, but not p38, in a rat model of non-compressive lumbar disc herniation. Neuroscience 300 (2015) 10-8.

[45] R.M. Martinez, V. Fattori, P. Saito, C.B.P. Melo, S.M. Borghi, I.C. Pinto, A.J.C. Bussmann, M.M. Baracat, S.R. Georgetti, W.A. Verri, Jr., and R. Casagrande, Lipoxin A4 inhibits UV radiation-induced skin inflammation and oxidative stress in mice. Journal of dermatological science (2018).

[46] S. Chen, B. Chen, Z. Wen, Z. Huang, and L. Ye, IL-33/ST2-mediated inflammation in macrophages is directly abrogated by IL-10 during rheumatoid arthritis. Oncotarget 8 (2017) 32407-32418.

[47] A.T. Fujimura, R.M. Martinez, F.A. Pinho-Ribeiro, A.M. Lopes Dias da Silva, M.M. Baracat, S.R. Georgetti, W.A. Verri, Jr., M. Chorilli, and R. Casagrande, Resveratrol-Loaded Liquid-Crystalline System Inhibits UVB-Induced Skin Inflammation and Oxidative Stress in Mice. Journal of natural products 79 (2016) 1329-38.

[48] H.F. Penaloza, P.A. Nieto, N. Munoz-Durango, F.J. Salazar-Echegarai, J. Torres, M.J. Parga, M. Alvarez-Lobos, C.A. Riedel, A.M. Kalergis, and S.M. Bueno, Interleukin-10 plays a key role in the modulation of neutrophils recruitment and lung inflammation during infection by Streptococcus pneumoniae. Immunology 146 (2015) 100-12.

[49] S. Sodin-Semrl, B. Taddeo, D. Tseng, J. Varga, and S. Fiore, Lipoxin A4 inhibits IL-1 beta-induced IL-6, IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances synthesis of tissue inhibitors of metalloproteinases. Journal of immunology 164 (2000) 2660-6.

[50] C.M. Quinonez-Flores, S.A. Gonzalez-Chavez, D. Del Rio Najera, and C. Pacheco-Tena, Oxidative Stress Relevance in the Pathogenesis of the Rheumatoid Arthritis: A Systematic Review. BioMed research international 2016 (2016) 6097417.

[51] D. Salvemini, J.W. Little, T. Doyle, and W.L. Neumann, Roles of reactive oxygen and nitrogen species in pain. Free radical biology & medicine 51 (2011) 951-66.

[52] E. Ventura, R. Durant, A. Jaussent, M.C. Picot, M. Morena, S. Badiou, A.M. Dupuy, C. Jeandel, and J.P. Cristol, Homocysteine and inflammation as main determinants of oxidative stress in the elderly. Free radical biology & medicine 46 (2009) 737-44.

[53] K. Cui, Z. Tang, C.C. Li, T. Wang, K. Rao, S.G. Wang, J.H. Liu, and Z. Chen, Lipoxin A4 improves erectile dysfunction in rats with type I diabetes by inhibiting oxidative stress and corporal fibrosis. Asian journal of andrology 20 (2018) 166-172.

[54] H. Zong, X. Li, H. Lin, C. Hou, and F. Ma, Lipoxin A4 pretreatment mitigates skeletal muscle ischemia-reperfusion injury in rats. American journal of translational research 9 (2017) 1139-1150.

[55] L. Wu, H.H. Li, Q. Wu, S. Miao, Z.J. Liu, P. Wu, and D.Y. Ye, Lipoxin A4 Activates Nrf2 Pathway and Ameliorates Cell Damage in Cultured Cortical Astrocytes Exposed to Oxygen-Glucose Deprivation/Reperfusion Insults. Journal of molecular neuroscience : MN 56 (2015) 848-857.

[56] 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, Jr., and R. Casagrande, Naringenin Inhibits UVB Irradiation-Induced Inflammation and Oxidative Stress in the Skin of Hairless Mice. Journal of natural products 78 (2015) 1647-55.

[57] J. Kim, Y.N. Cha, and Y.J. Surh, A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. Mutation research 690 (2010) 12-23.

[58] Z. Sun, S.Y. Park, E. Hwang, B. Park, S.A. Seo, J.G. Cho, M. Zhang, and T.H. Yi, Dietary Foeniculum vulgare Mill extract attenuated UVB irradiation-induced skin photoaging by activating of Nrf2 and inhibiting MAPK pathways. Phytomedicine : international journal of phytotherapy and phytopharmacology 23 (2016) 1273-1284.

[59] M.I. Azevedo, A.F. Pereira, R.B. Nogueira, F.E. Rolim, G.A. Brito, D.V. Wong, R.C. Lima-Junior, R. de Albuquerque Ribeiro, and M.L. Vale, The antioxidant effects of the flavonoids rutin and quercetin inhibit oxaliplatin-induced chronic painful peripheral neuropathy. Molecular pain 9 (2013) 53.

[60] S. Gui, B. Li, X. Zhao, L. Sheng, J. Hong, X. Yu, X. Sang, Q. Sun, Y. Ze, L. Wang, and F. Hong, Renal injury and Nrf2 modulation in mouse kidney following chronic exposure to TiO(2) nanoparticles. Journal of agricultural and food chemistry 61 (2013) 8959-68.

[61] C.C. Wang, L. Guo, F.D. Tian, N. An, L. Luo, R.H. Hao, B. Wang, and Z.H. Zhou, Naringenin regulates production of matrix metalloproteinases in the knee-joint and

primary cultured articular chondrocytes and alleviates pain in rat osteoarthritis model. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 50 (2017) e5714.

[62] Y.H. Zhang, A. Heulsmann, M.M. Tondravi, A. Mukherjee, and Y. Abu-Amer, Tumor necrosis factor-alpha (TNF) stimulates RANKL-induced osteoclastogenesis via coupling of TNF type 1 receptor and RANK signaling pathways. The Journal of biological chemistry 276 (2001) 563-8.

[63] C. Liu, H. Guan, C. Cai, F. Li, and J. Xiao, Lipoxin A4 suppresses osteoclastogenesis in RAW264.7 cells and prevents ovariectomy-induced bone loss. Experimental cell research 352 (2017) 293-303.

Table 1: Primers sequences for RT-qPCR

Gene	***Sense***	***Antisense***
pro-IL-1β	5-′GAAATGCCACCTTTTGACAGTG-3′	5′-TGGATGCTCTCATCAGGACAG-3′
IL-10	5’-GCTGGACAACATACTGCTAACC-3’	5’-CTGGGGCATCACTTCTACCA-3’
Nrf2	5´- TCACACGAGATGACGTTAGGGCAA-3´	5´-TACAGTTCTGGGCGGCGGACTTTAT-3´
RANK	5´-CTAATCCAGCAGGGA AGCAAAT-3´	5´-GACACGGGCATAGAGTCAGTTC-3´
Gapdh	5′-CATACCAGGAAATGAGCT TG-3′	5′-ATGACATCAAGAAGGTGGTG-3′
β-actin	5′-AGCTGC GTTTTACACCCTTT-3′	5′-AAGCCATGCCAATGTTGTCT-3′

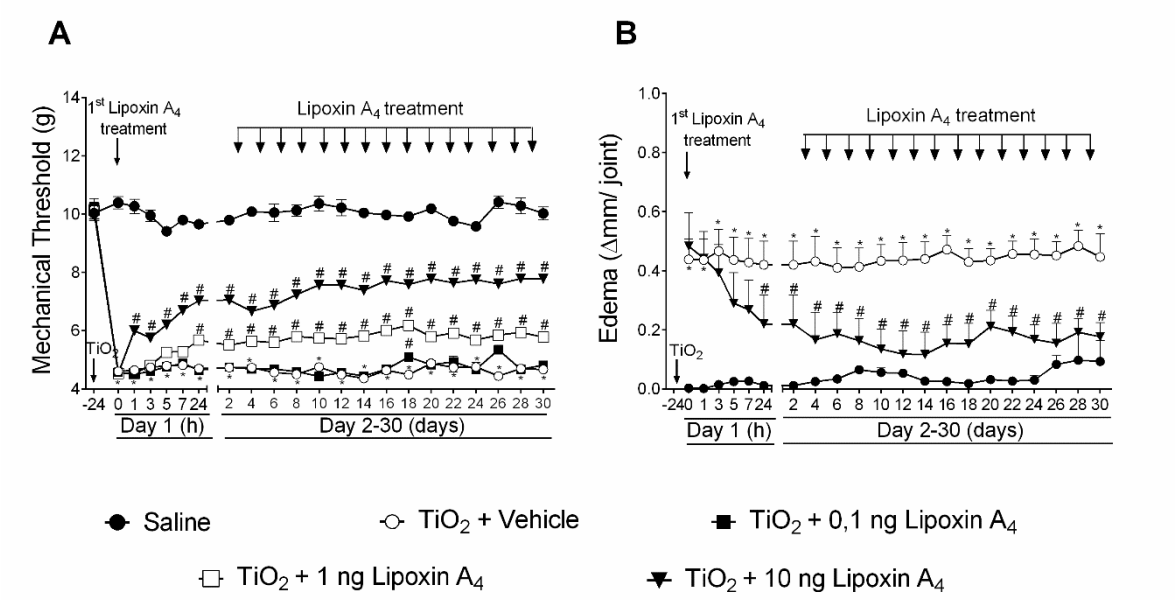


Figure 1. LXA4 reduces TiO_2-induced articular mechanical hyperalgesia and joint edema in a dose-dependent manner. Mice were treated for 30 days with LXA_4 (0,1, 1, 10ng/ animal, i.p.) or vehicle (ethanol) starting 24h after intra-articular injection of TiO_2 (3mg/ joint) and mechanical hyperalgesia (A) and edema (B) was evaluated 1, 3, 5, 7, 24 h (day 1) and subsequently and every two days until 30th day. Results are expressed as mean ± SEM, n= 6 mice per group per experiment and are representative of two separate experiments (*p< 0.05 compared to the saline group; #p<0.05 compared to the TiO_2 and LXA_4 groups), repeated measures two-way ANOVA followed by Tukey's post test.

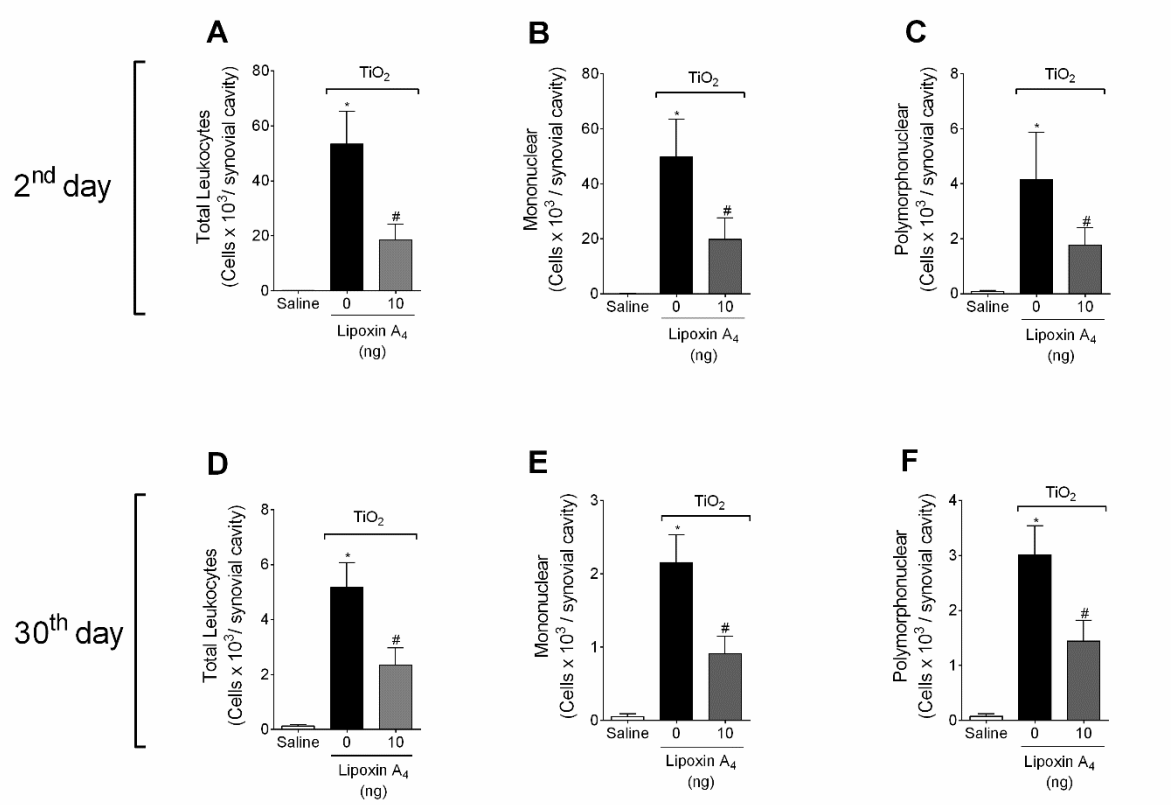


Figure 2. LXA4 reduces TiO_2-induced recruitment of total, polymorphonuclear and mononuclear leukocytes to the knee joint. Mice were treated with a single treatment (2nd day) and for 30 days with LXA_4 (10ng/ animal, i.p.) starting 24h after intra-articular injection of TiO_2 (3mg/ joint) and knee joint wash was collected for count total leucocytes (A and D), mononuclear (C and E) and polymorphonuclear cells (C and F). Results are expressed as mean ± SEM, n=6 mice per group per experiment, two independent experiments (*p<0.05 compared to the saline group; #p<0.05 compared to the TiO_2 group, one-way ANOVA followed by Tukey's post-test).

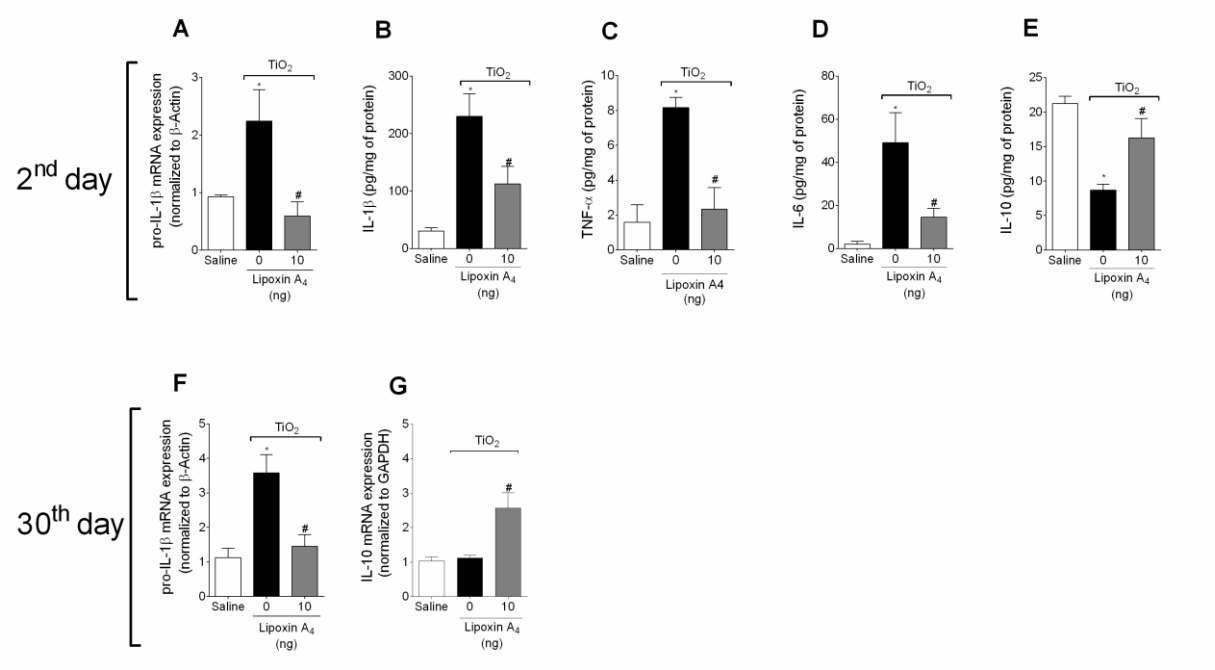


Figure 3. **LXA4 modulates TiO_2-induced cytokine production.** Mice were treated with a single treatment (2nd day) and for 30 days with LXA_4 (10ng/ animal, i.p.) starting 24h after intra-articular injection of TiO_2 (3mg/ joint) and knee joint was collected. Pro-IL-1β mRNA expression (A and F), IL-1β measurement (B) TNF-α measurement (C), IL-6 measurement (D), IL-10 measurement and IL-10 mRNA expression (E and G). Results are expressed as mean ± SEM, n=6 mice per group per experiment, two independent experiments (*$p<0.05$ compared to the saline group; #$p<0.05$ compared to the TiO_2 group, one-way ANOVA followed by Tukey's post-test).

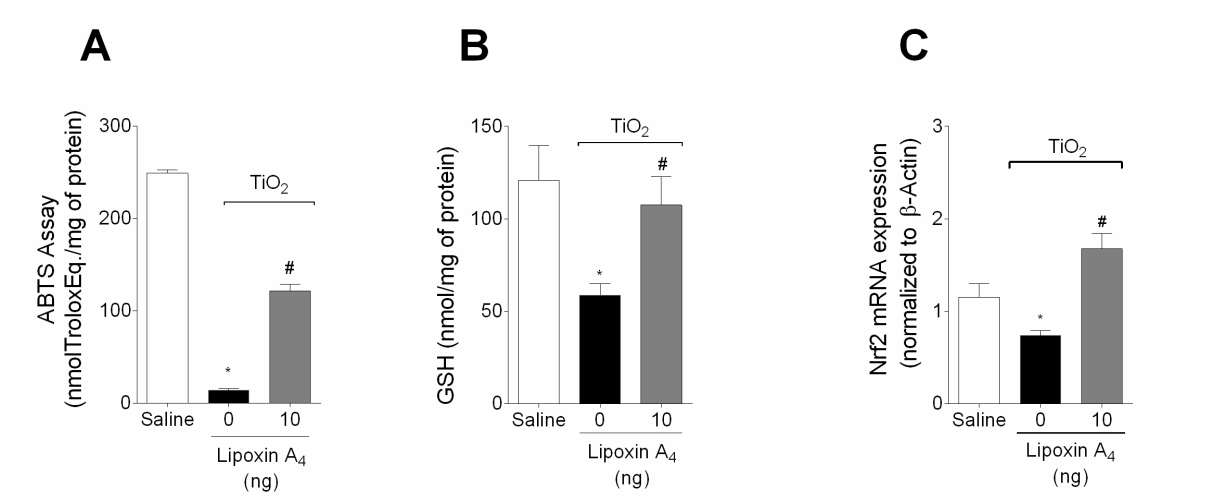


Figure 4. LXA4 inhibits TiO_2-induced decrease of antioxidant capacity. Mice were treated with a single treatment (2nd day) with LXA_4 (10ng/ animal, i.p.) starting 24h after intra-articular injection of TiO_2 (3mg/ joint) and knee joint was collected. Free-radical scavenging ability (ABTS) assay (A), reduced glutathione (GSH) concentration (B) and Nrf2 mRNA expression (C). Results are expressed as mean ± SEM, n=6 mice per group per experiment, two independent experiments (*$p<0.05$ compared to the saline group; #$p<0.05$ compared to the TiO_2 group, one-way ANOVA followed by Tukey's post-test).

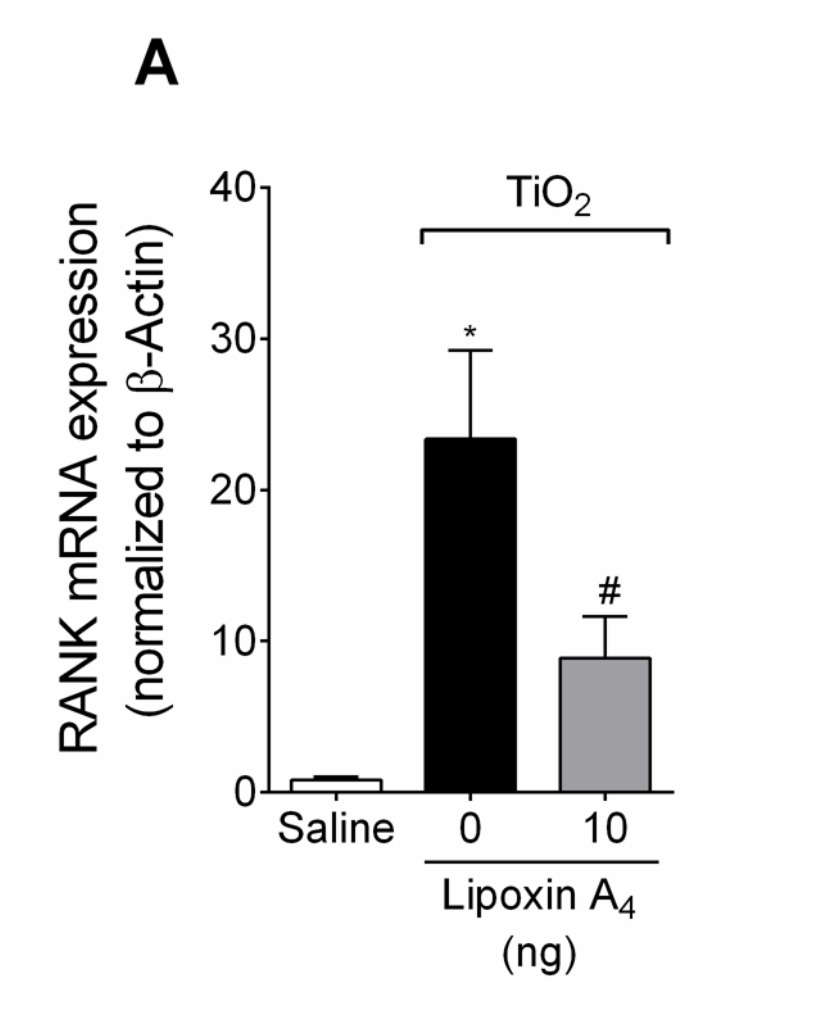
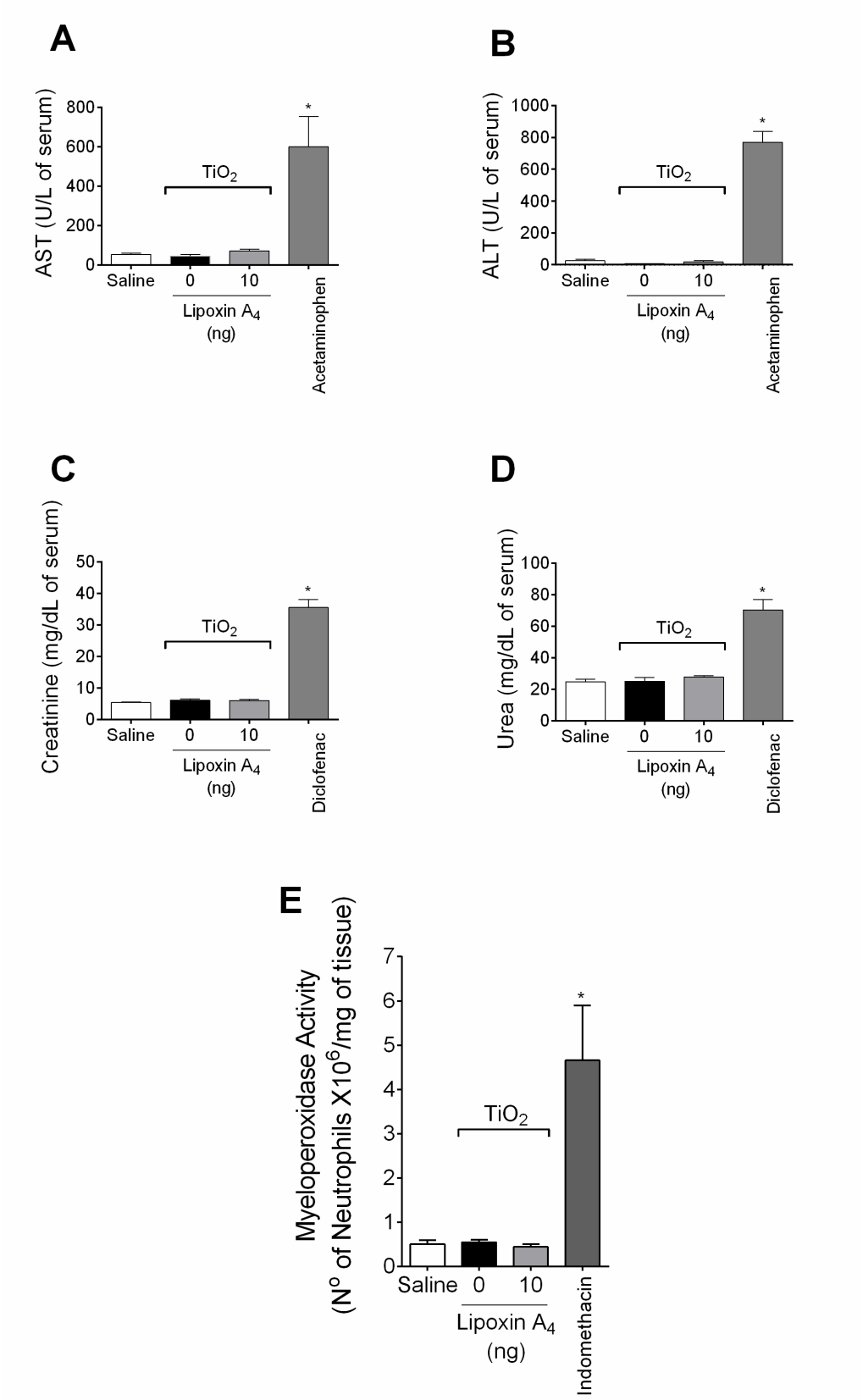


Figure 5. LXA4 reduces TiO_2-induced RANK mRNA expression. Mice were treated for 30 days with LXA_4 (10ng/ animal, i.p.) starting 24h after intra-articular injection of TiO_2 (3 mg/joint) and knee joint was collected and RANK mRNA expression was evaluated (A). Results are expressed as mean ± SEM, n=6 mice per group per experiment, two independent experiments (*$p<0.05$ compared to the saline group; #$p<0.05$ compared to the TiO_2 group, one-way ANOVA followed by Tukey's post-test).

1



Supplementary Figure 1. LXA4 does not induce liver or kidney damage, or stomach lesions. Mice were treated for 30 days with LXA_4 (10ng/ animal, i.p.) starting 24h after intra-articular injection of TiO_2 (3mg/ joint) and serum and stomach was collected. AST (A), ALT (B), creatinine (C) and urea (D), serum levels and MPO activity in the stomach (E) were determined to evaluate treatment toxicity.

As positive drug control for gastric, hepatic, and renal toxicity, indomethacin (2.5 mg/kg, i.p., diluted in tris/HCl buffer, during 7 days), acetaminophen (650 mg/kg, i.p., diluted in saline), and diclofenac (200 mg/kg, p.o., diluted in saline, once) were used, respectively.Results are expressed as mean ± SEM, n=6 mice per group per experiment, two independent experiments (*p<0.05 compared to the all groups, one-way ANOVA followed by Tukey's post-test).

5. CONCLUSÃO

Em resumo, o presente estudo demonstrou o efeito analgésico, anti-inflamatório e efeitos antioxidantes da LXA_4 em modelo crônico de artrite induzida por TiO_2 em camundongo. LXA_4 atenua a dor inflamatória, edema articular, recrutamento de leucócitos induzido por TiO_2, pela inibição do estresse oxidativo, bem como a expressão e produção de citocinas. Desta forma, é possível que um MLPR isolado, como a LXA_4 seja uma promissora abordagem para atenuar as complicações relacionadas à inflamação induzida por implantes.