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Aécio da Costa Fagundes

EFEITOS ANTINOCICEPTIVOS DO INIBIDOR DA XANTINA OXIDASE ALOPURINOL:

ESTUDOS EXPERIMENTAIS E CLÍNICOS.

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EFEITOS ANTINOCICEPTIVOS DO INIBIDOR DA XANTINA OXIDASE ALOPURINOL: ESTUDOS EXPERIMENTAIS E CLÍNICOS.

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Orientador: Prof. Dr. André Prato Schmidt

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RESUMO

(FAGUNDES AC – EFEITOS ANTINOCICEPTIVOS DO INIBIDOR DA XANTINA OXIDASE ALOPURINOL: ESTUDOS EXPERIMENTAIS E CLÍNICOS)

O alopurinol é um potente inibidor da enzima xantina oxidase, usado principalmente no tratamento de hiperuricemia e gota. Os efeitos antinociceptivos do alopurinol têm sido demonstrados em modelos de dor em roedores. O objetivo da presente tese de doutorado foi investigar os efeitos do alopurinol nos níveis de dor e ansiedade através de estudos experimentais e clínicos. Os resultados estão apresentados sob forma de artigos científicos. O primeiro artigo descreve um estudo experimental onde alopurinol, administrado via intraperitoneal, produziu efeitos antinociceptivos contra a hiperalgesia térmica e mecânica em um modelo tradicional de dor neuropática em camundongos. Neste trabalho, demonstramos que o antagonista seletivo do receptor de adenosina A1 DPCPX preveniu parcialmente a antinocicepção induzida por alopurinol. O alopurinol também causou um aumento nos níveis de algumas purinas no líquido cefalorraquidiano (LCR) de camundongos, incluindo os nucleosídeos inosina e guanosina, e diminuiu a concentração liquórica de ácido úrico. Em estudo clínico em humanos (artigo 2), a administração pré-operatória de alopurinol foi eficaz na redução nos escores de dor 2 horas após a cirurgia de histerectomia abdominal total quando comparados ao grupo placebo. Houve uma mudança significativa nas concentrações de xantina e ácido úrico no líquido cefalorraquidiano antes da cirurgia ($p < 0,01$), sem diferença observada nos níveis de outras purinas. Também foi investigado os efeitos do alopurinol na dor e ansiedade em mulheres com fibromialgia refratária à terapia convencional. Inicialmente, foi feito um estudo piloto (artigo 3) onde uma série de casos com 12 mulheres portadoras de fibromialgia receberam alopurinol oral 300 mg duas vezes ao dia por 30 dias. Como resultado deste estudo, a administração oral de alopurinol causou uma redução significativa da dor até 30 dias de tratamento. Nenhum efeito foi observado em relação aos escores de ansiedade. A seguir, frente ao resultado promissor deste estudo piloto, foi feito o ensaio clínico (artigo 4) com uma amostra de 60 mulheres, comparando a eficácia analgésica do alopurinol oral versus placebo como terapia adjuvante em pacientes com fibromialgia. Neste estudo, a administração oral de alopurinol 300 mg duas vezes ao dia foi ineficaz em melhorar os escores de dor medidos por várias ferramentas até 30 dias de tratamento. Além disso, não foi observado outro benefício da administração de alopurinol sobre a ansiedade, sintomas depressivos e estado funcional nestes pacientes. Nenhum efeito adverso significativo foi observado nos estudos clínicos (artigos 2, 3 e 4). Embora este estudo não tenha apresentado benefício do uso do alopurinol como tratamento adjuvante em paciente com fibromialgia, é válida a realização de novos ensaios clínicos, abrangendo amostras maiores com acompanhamento em longo prazo para esclarecer o seu papel em diferentes condições dolorosas agudas e crônicas, visto o seu conhecido perfil de segurança e os resultados promissores em estudos prévios.

ABSTRACT

(FAGUNDES AC – ANTINOCICEPTIVE EFFECTS OF THE XANTHINE OXIDASE INHIBITOR ALLOPURINOL: EXPERIMENTAL AND CLINICAL STUDIES)

Allopurinol is a potent inhibitor of the enzyme xanthine oxidase, used mainly in the treatment of hyperuricemia and gout. The antinociceptive effects of allopurinol have been demonstrated in rodent pain models. The aim of this doctoral thesis was to investigate the effects of allopurinol on pain and anxiety scores through experimental and clinical studies. The results are presented in the form of scientific manuscripts. The first article describes an experimental study in which allopurinol, administered intraperitoneally, produced antinociceptive effects against thermal and mechanical hyperalgesia in a traditional model of neuropathic pain in mice. In this work, we demonstrated that the selective adenosine A1 receptor antagonist DPCPX partially prevented allopurinol-induced antinociception. Allopurinol also caused an increase in the levels of some purines in the cerebrospinal fluid (CSF) of mice, including the nucleosides inosine and guanosine, and decreased the CSF concentration of uric acid. In a clinical study in humans (article 2), the preoperative administration of allopurinol was effective in reducing pain scores 2 hours after total abdominal hysterectomy surgery when compared to the placebo group. There was a significant change in the concentrations of xanthine and uric acid in the cerebrospinal fluid before surgery ($p < 0.01$), with no difference observed in the levels of other purines. The effects of allopurinol on pain and anxiety in women with fibromyalgia refractory to conventional therapy were also investigated. Initially, a pilot study (article 3) was carried out in which a series of cases with 12 women with fibromyalgia received oral allopurinol 300 mg twice daily for 30 days. As a result of this study, oral administration of allopurinol caused a significant reduction in pain up to 30 days of treatment. No effect was observed in relation to anxiety scores. Next, in view of the promising result of this pilot study, a clinical trial (article 4) was carried out with a sample of 60 women, comparing the analgesic efficacy of oral allopurinol versus placebo as an adjunctive therapy in patients with fibromyalgia. In this study, oral administration of allopurinol 300 mg twice daily was ineffective in improving pain scores measured by various tools up to 30 days of treatment. In addition, there was no other benefit of allopurinol administration on anxiety, depressive symptoms and functional status in these patients. No significant adverse effects were observed in clinical studies (articles 2, 3 and 4). Although this study has not shown the benefit of using allopurinol as an adjuvant treatment in a patient with fibromyalgia, new clinical trials are valid, covering larger samples with long-term follow-up to clarify their role in different acute and chronic painful conditions, as its well-known safety profile and the promising results in previous studies.

LISTA DE ABREVIATURAS

AMPA – ácido α-amino-3-hidroxi-5-metil-4-isoxazolepropionato

ADP – adenosina-5’-difosfato

AMP – adenosina-5’-monofosfato

AMPc – adenosina-3’,5’-monofosfato-cíclico

ATP – adenosina-5’-trifosfato

CGRP – peptídeo relacionado ao gene da calcitonina

GABA – ácido gama-aminobutírico

GMP – guanosina-5’-monofosfato

GMPc – guanosina 3’,5’-monofosfato-cíclico

GDP – guanosina-5’-difosfato

GTP – guanosina-5’-trifosfato

i.c.v. – intracerebroventricular

i.p. – intraperitoneal

i.t. – intratecal

LCR – líquido cefalorraquidiano

MAPK – proteína cinase ativada por mitógeno

NMDA – N-metil-D-aspartato

NGF – fator de crescimento neural

PAF – fator de ativação plaquetário

PKA – proteína cinase A

PKC – proteína cinase C

SNA – sistema nervoso autônomo

SNC – sistema nervoso central

SNP – sistema nervoso periférico

TNF-α – fator de necrose tumoral alfa

APRESENTAÇÃO

Esta tese de doutorado está organizada em três partes: parte I (introdução e objetivos), parte II (quatro artigos científicos) e parte III (discussão, considerações finais e perspectivas). A introdução apresenta o embasamento teórico que motivou a formulação da proposta do trabalho. A seguir, estão dispostos o objetivo geral e os objetivos específicos da tese.

Na parte II encontram-se os resultados obtidos através de quatro artigos científicos. Os tópicos Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas são descritas de forma detalhada em cada artigo científico.

A parte III contém a Discussão, Considerações Finais e Perspectivas que representam um compilado geral acerca dos resultados obtidos nos diferentes trabalhos.

Por fim, as Referências Bibliográficas referem-se somente às citações correspondentes à Introdução e Discussão desta tese.

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PARTE I

Introdução e Objetivos

I.1. INTRODUÇÃO

I.1.a. Dor

Dor é definida como uma experiência sensorial e emocional desagradável associada com lesão tecidual real ou potencial, ou descrita em termos de tal dano (IASP, 1986). Apesar de representar uma sensação incômoda, a dor é um componente fundamental da homeostase e essencial para a sobrevivência visto que tem o propósito de indicar a presença de estímulos nocivos ou potencialmente nocivos que podem apresentar riscos de dano tecidual. Assim, a dor funciona como um "sinal de alerta" permitindo que mecanismos de defesa sejam adotados pelo organismo [Millan, 1999; Julius e Basbaum, 2001]. Entretanto, a dor pode perder seu propósito inicial de alerta e tornar-se persistente ou crônica. Este processo ocorre quando o organismo não consegue corrigir a lesão ou quando são estabelecidos mecanismos adaptativos inadequados [D'Mello e Dickenson, 2008]. Nestes casos, ocorrem alterações no processamento sensorial e o quadro de dor é frequentemente associado à hiperalgesia - percepção exagerada de estímulos dolorosos - e alodinia - estímulos inócuos gerando resposta dolorosa [Millan, 1999]. Este tipo de dor é de difícil tratamento e ocasiona intenso sofrimento, diminuição da capacidade funcional e queda da qualidade de vida [Zimmermann, 2001].

A dor pode ser classificada quanto ao tipo (eventual, aguda e crônica) e à origem (nociceptiva, neurogênica, neuropática e psicogênica). A dor eventual ou transitória não está relacionada a um estímulo nocivo ou lesão significativa (exemplo: vacina). Por outro lado, a dor aguda está relacionada a um estímulo nocivo com lesão tecidual (exemplo: queimadura) e envolve, além de nociceptores periféricos, o sistema nervoso central (SNC) e sistema nervoso autônomo (SNA). A dor crônica trata-se da dor persistente após processo de reparo do organismo ou, em muitos casos, nem se relaciona a processo nocivo detectado, como é o exemplo da fibromialgia [Millan, 1999]. Em relação à origem, a dor pode ser desencadeada por: estimulação excessiva de nociceptores (dor nociceptiva), dano do tecido neuronal na periferia ou no SNC (dor neurogênica), disfunção ou lesão nervosa (dor neuropática) ou, até mesmo, não ser causada por uma fonte somática detectável (dor psicogênica) [Millan, 1999]. Não é apenas a duração que caracteriza o tipo de dor, mas também a capacidade de reparo da lesão pelo organismo e o processamento neural responsável pela transmissão dolorosa [Hill, 2001].

Algumas evidências indicam que nem sempre há associação direta entre dor e lesão tecidual, uma vez que diversas síndromes dolorosas não são detectáveis por métodos de diagnóstico na clínica atual. Esse fato favorece a hipótese de que alterações neurofuncionais podem ser restritas ao âmbito biomolecular [Millan, 1999]. A dor tem caráter multifatorial e sua etiologia pode ser complexa, envolvendo componentes sensoriais, cognitivos e emocionais [Julius e Basbaum, 2001]. A transmissão dolorosa envolve diversos eventos, desde a sensibilização periférica pelo estímulo nocivo até alterações neuroplásticas adaptativas, tais como sensibilização central, alterações na capacidade modulatória de neurônios inibitórios na medula espinhal, entre outras. [Millan, 1999; Hill, 2001; Zimmermann, 2001]. Todos esses processos envolvidos na transmissão de dor geram quadros dolorosos manifestados das mais diversas formas [Apkarian et al., 2008]. Nesse sentido, modelos experimentais de dor têm sido utilizados a fim de aprofundar o entendimento a respeito dos mecanismos de transmissão de dor e para o desenvolvimento de novas alternativas terapêuticas. A avaliação da dor em modelos animais é feita indiretamente por meio de respostas comportamentais, constituindo-se em modelos de nocicepção [Millan, 1999]. A dor abrange a percepção e interpretação de estímulos nocivos. Em contrapartida, a nocicepção é a sensação determinada pela estimulação de receptores presentes nas fibras aferentes primárias, ou seja, é o componente sensorial da dor e corresponde às manifestações neurofisiológicas e neuroquímicas geradas pelo estímulo nocivo [Millan, 1999].

A ocorrência de dor é crescente, sendo que 10% a 50% dos indivíduos no Brasil e em outros países procuram clínicas médicas devido ao sintoma dor [Rocha et al., 2007; Nampiaparampil, 2008; Tunks et al., 2008]. De acordo com estudo realizado pela Sociedade Brasileira para Estudo da Dor em 2017, aproximadamente 37% da população brasileira convive com algum quadro de dor crônica. A dor persistente está constantemente associada com depressão, o que afeta a qualidade de vida [WHO, 2006]. Além disso, vários destes quadros dolorosos são refratários aos tratamentos disponíveis na atualidade. Neste contexto, o entendimento de neurofarmacologia e fisiopatologia da dor, assim como o estudo dos mecanismos centrais e periféricos envolvidos na sua transmissão, tornam-se necessários para a busca incessante de novas terapêuticas adjuvantes.

I.1.b. Mecanismos de transmissão dolorosa

A dor é desencadeada a partir da transformação de estímulos nocivos em potenciais de ação que são transferidos das fibras nervosas periféricas para o sistema nervoso central através da medula espinhal [Zhang e Bao, 2006]. A detecção destes estímulos e sua respectiva transmissão, sob a forma de impulso elétrico, ocorre por intermédio de receptores específicos para a dor (nociceptores), presentes nas terminações de fibras nervosas do tipo Aδ e C [Fitzgerald, 2005]. A presença da bainha de mielina nas fibras nervosas reflete na velocidade de condução dos potenciais de ação: as fibras Aδ (2 a 6 mm de diâmetro), envoltas por bainha de mielina, são responsáveis pela transmissão rápida da dor, enquanto as fibras C (0,4 a 1,2 mm de diâmetro) são amielínicas e transmitem o estímulo doloroso de forma mais lenta (0,5 a 2 $m.s^{-1}$). Outro tipo de fibra, as fibras Aβ (30 a 100 $m.s^{-1}$), são de condução rápida e respondem predominantemente a estímulos inócuos, como propriocepção e tato. Contudo, na presença de alterações neuroplásticas, estas fibras podem participar da amplificação da dor em nível medular [Schaible, 2007; Millan, 1999; D'Mello e Dickenson, 2008].

A sensibilização dos nociceptores ocorre por diferentes estímulos como, por exemplo, lesão tecidual, mudança de temperatura, hipóxia e isquemia. No entanto, há nociceptores "silenciosos" que não são sensibilizados a estímulos de menor intensidade, sendo ativados somente em situações específicas, como na presença de mediadores inflamatórios, resultando na amplificação da resposta à dor [Julius e Basbaum, 2001]. A grande maioria das fibras do tipo C são consideradas polimodais, ou seja, respondem a estímulos mecânicos, térmicos e químicos. Essas fibras são classificadas, por critérios morfológicos, em peptidérgicas e não peptidérgicas. As fibras C peptidérgicas produzem e liberam peptídeos, como a substância P e o peptídeo relacionado ao gene da calcitonina (CGRP) e expressam receptores para o fator de crescimento neural (NGF), apresentando terminais sinápticos em regiões mais externas no corno dorsal da medula espinhal (lâmina I) [Millan, 1999]. As fibras C não – peptidérgicas expressam receptores para o fator neurotrófico derivado da glia (GDNF) e o receptor purinérgico do tipo P_2X_3 e apresentam seus terminais sinápticos mais internamente na substância gelatinosa da medula espinhal (lâmina II). Já as fibras Ad respondem a estímulos mecânicos e térmicos e são subdivididas conforme o limiar de temperatura para a transmissão do estímulo [Millan, 1999].

I.1.c. Transmissão da dor - Mecanismos periféricos

Quando o estímulo nocivo provoca lesão tecidual, inicia-se um processo inflamatório seguido de reparação, que desencadeia uma cascata de sensibilização periférica. A liberação local e difusa de substâncias químicas, denominadas algogênicas, são responsáveis pela sensibilização dos nociceptores. Dentre as substâncias algogênicas destacam-se a acetilcolina, prostaglandinas, histamina, bradicinina, serotonina, leucotrieno, substância P, fator de ativação plaquetário (PAF), íons potássio e hidrogênio, tromboxanos, interleucinas, fator de necrose tumoral (TNF-α), fator de crescimento neural (NGF) e monofosfato cíclico de adenosina (AMPc) [Wood, 2004]. As enzimas liberadas do interior de células lesadas degradam ácidos graxos e formam cininas, como a bradicinina, responsável por promover dilatação arteriolar e aumento da permeabilidade capilar, o que favorece a propagação do processo inflamatório [Millan, 1999; Huang et al., 2006]. A ação da enzima fosfolipase A na membrana celular desencadeia a liberação de ácido araquidônico. Este é metabolizado por 3 sistemas enzimáticos principais - cicloxigenase, lipoxigenase e citocromo P-450- e produz, respectivamente: prostaglandinas, tromboxanos, e prostaciclinas; leucotrienos e lipoxinas; produtos da via da epoxigenase. Todas essas substâncias causam a redução global dos limiares de excitabilidade dos nociceptores [Huang et al., 2006]. A substância P e a neurocinina A facilitam a geração e manutenção do processo inflamatório visto que provocam vasodilatação e aumento da permeabilidade vascular.

A bradicinina, a prostaglandina E_2, o NGF e as interleucinas também apresentam papel importante no processo de sensibilização periférica à dor. A bradicinina e a prostaglandina provocam alterações em receptores específicos ($TRPV_1$) acoplados a canais iônicos ligante-dependente via ativação do AMPc, e das proteínas cinases A (PKA) e C (PKC), o que reduz o tempo pós-hiperpolarização da membrana neural, e consequentemente, reduz o limiar para disparo da fibra nervosa. O NGF e outras neurotrofinas provocam aumento da síntese, do transporte axonal anterógrado e da quantidade de substância P e CGRP nas fibras C, além de reduzirem a atividade do ácido gama-aminobutírico (GABA) em terminações periféricas e centrais [Hill, 2001; Julius e Basbaum, 2001].

A agressão persistente provoca sensibilização de fibras nervosas e alterações no sistema nervoso periférico, promovendo a ativação de nociceptores silentes, hiperalgesia e aumento dos níveis de AMPc e cálcio nos nociceptores [Huang et al., 2006]. Dessa maneira, os nociceptores

passam a desenvolver descargas espontâneas, tornando-se capazes de responder de maneira intensa a estímulos nociceptivos e não-nociceptivos [Huang et al., 2006].

Em suma, a agressão tecidual resulta na liberação de mediadores químicos que provocam alterações no fluxo sanguíneo local, na permeabilidade vascular e produção de sinais inflamatórios como, dor, calor, rubor, tumor e impotência funcional. Inicia-se o processo de sensibilização periférica que vai exacerbar a resposta ao estímulo doloroso. Ocorre também o aumento da condutividade dos canais de cálcio e sódio e redução do influxo de potássio e cloro para o meio intracelular devido à despolarização da membrana neural por tempo prolongado pela ação dos mediadores periféricos. Neste momento, caso este processo seja prolongado, podem ocorrer alterações neuroplásticas em nível central e, consequentemente, o desencadeamento de mecanismos de sensibilização central [Apkarian, 2008].

I.1.d. Processamento central da dor

A liberação das substâncias algogênicas na lesão tecidual periférica desencadeia o processo de transmissão dos estímulos nocivos até os níveis mais centrais através da medula espinhal [Millan, 1999]. Essa transmissão não ocorre de forma passiva visto que os circuitos intramedulares possuem a capacidade de alterar o estímulo e, consequentemente, determinarão de que forma a resposta dolorosa chegará ao córtex cerebral [Zhang e Bao, 2006].

Os estímulos nocivos persistentes nos nociceptores provocam a dor espontânea, redução do limiar de sensibilidade, hiperalgesia – resposta realçada ao estímulo doloroso - e alodinia - dor resultante de um estímulo normalmente inócuo. A hiperalgesia pode ocorrer no local da lesão (hiperalgesia primária) ou se estender para áreas adjacentes (hiperalgesia secundária). A presença destes elementos sugere que, além da sensibilização de nociceptores periféricos, o SNC também tem envolvimento nestas mudanças [Zhuo, 2007].

As fibras aferentes conduzem impulsos nociceptivos até o corno dorsal da medula, área primária de recebimento da maioria das informações sensoriais, que se divide em seis lâminas, caracterizadas pelo recebimento de diferentes tipos de informações [Millan, 1999]. As fibras aferentes do tipo Aδ e C realizam suas sinapses primárias nas lâminas mais superficiais (lâmina I e lâmina II). Após a integração dos impulsos nociceptivos no corno dorsal da medula, ocorrem

projeções através de vias ascendentes para centros superiores, como estruturas subcorticais e corticais [Fitzgerald, 2005]. As vias ascendentes de condução de dor até o cérebro podem ser monossinápticas e polissinápticas [Millan, 1999]. As vias monossinápticas projetam-se diretamente a estruturas superiores e incluem feixes, como o espinotalâmico e espinorreticular. Em contrapartida, as vias polissinápticas tem uma estação de retransmissão a neurônios de segunda ordem até atingirem estruturas superiores, formando feixes como o paleoespinotalâmico e o espinocervical. Diversas dessas vias apresentam conexões com neurônios de terceira ordem em nível talâmico, projetando-se até estruturas corticais [Fitzgerald, 2005].

Além das vias ascendentes, a percepção de dor pode ser modulada pelas vias descendentes, provenientes de estruturas do SNC, como tálamo, tronco cerebral, hipotálamo, córtex, núcleo magno da rafe e substância cinzenta periaquedutal [Zhang e Bao, 2006; Yoshimura e Furue, 2006].

A modulação da dor pelas vias descendentes é caracterizada por ações diretas sobre as vias aferentes primárias e por inibição ou estimulação de interneurônios, sendo predominantemente de caráter noradrenérgico e serotoninérgico [Yoshimura e Furue, 2006]. Algumas características inibitórias das vias noradrenérgicas e serotoninérgicas foram demonstradas em estudos eletrofisiológicos, como: hiperpolarização direta dos neurônios situados no corno dorsal da medula; inibição da liberação de glutamato de fibras aferentes Aδ e C; aumento da liberação de GABA e glicina proveniente de interneurônios inibitórios na medula espinhal. Apesar de efeitos excitatórios serem descritos, o papel fundamental destas vias é o controle endógeno da dor, ou seja, seu efeito predominante é antinociceptivo [Yoshimura e Furue, 2006].

Porém, caso ocorra uma disfunção intrínseca das vias de transmissão dolorosa ou o estímulo periférico seja sustentado ou intenso, fenômenos neuroplásticos podem ocorrer em nível central [Zhuo, 2007]. A partir disso, os mecanismos de sensibilização central podem desencadear síndromes dolorosas crônicas altamente refratárias [Tunks et al., 2008].

I.1.e. Mecanismos de sensibilização central

Na sensibilização central ocorrem mudanças nos impulsos periféricos (com adaptações positivas ou negativas), redução do limiar ou aumento da resposta aos impulsos aferentes, descargas persistentes após estímulos repetidos e ampliação dos campos receptivos de neurônios

do corno dorsal [Zhuo, 2007]. A liberação de neurotransmissores, como substância P, somatostatina, CGRP, neurocinina A, glutamato e aspartato após agressão tecidual estão relacionados com a ativação de potenciais pós-sinápticos excitatórios [Millan, 1999]. Ocorre a soma dos potenciais de ação e despolarização pós-sináptica cumulativa. Por sua vez, o aumento do cálcio promove a ativação da enzima óxido nítrico sintase e a estimulação da transcrição de proto-oncogenes, genes localizados no SNC e envolvidos na formação de encefalinas e dinorfinas.

As encefalinas apresentam ação antinociceptiva e são relacionadas com a redução da neuroplasticidade e hiperalgesia. Já as dinorfinas tem ação algogênica e antinociceptiva, dependendo da situação. [Delander et al., 1997; Jongen et al., 2005]. As neurotrofinas tem como receptores as tirosinases tipo A e B (trkA, trkB), enquanto que a substância P e o CGRP ligam-se aos receptores para neurocininas do tipo NK_1 e NK_2 [Hill e Oliver, 2007]. Com a liberação de aminoácidos excitatórios, peptídeos e neurotrofinas e a interação com receptores específicos, ocorre a ativação de segundos mensageiros (do tipo AMPc, PKA, PKC, fosfatidilinositol, fosfolipase C, fosfolipase A_2). A ativação de receptores NMDA ocorre após uma ativação intensa e persistente dos receptores glutamatérgicos do tipo AMPA. Consequentemente, ocorre a abertura de canais de cálcio, o que contribui para o aumento do influxo de íons cálcio e a produção de prostaglandinas e óxido nítrico. Estes migram em direção à fenda sináptica e estimulam a liberação de glutamato, aspartato, substância P e CGRP, favorecendo a ampliação do processo álgico [Birklein e Schmelz, 2008].

Os mecanismos que favorecem o aumento da eficácia da transmissão sináptica podem ser decorrentes da formação e do transporte de substâncias excitatórias do interior da célula para a fenda sináptica ou da fosforilação dos receptores de membrana e das alterações no tempo de abertura dos canais iônicos. Além disso, as proteínas cinases ativadas por mitógenos (MAPK) modulam a fosforilação dos receptores NMDA e AMPA, amplificando a resposta nociceptiva.

Durante o estabelecimento da sensibilização central na dor neuropática ocorre morte de neurônios inibitórios por meio da excitotoxicidade - toxicidade mediada pelo glutamato - processo que vai contribuir significativamente para a manutenção do quadro doloroso a longo prazo [Zhuo, 2007]. Estudos têm demonstrado o papel das células gliais nos mecanismos de transmissão e manutenção da dor, principalmente quando trata-se de dor crônica de origem neuropática [Gao e Ji, 2010; Gosselin et al., 2010]. As células gliais, predominantemente os astrócitos espinhais, estão

relacionadas ao estímulo doloroso prolongado devido a sua associação com os sistemas purinérgico e glutamatérgico [Ohara et al., 2009; Sweitzer e De Leo, 2011].

I.1.f. Mediadores químicos envolvidos na dor

Os principais mediadores químicos envolvidos nos eventos que ocorrem durante a transmissão da dor periférica e central são: prostaglandinas, leucotrienos, aminoácidos excitatórios (glutamato e aspartato), purinas (ATP), noradrenalina, serotonina, dopamina, óxido nítrico, cininas, taquicininas, substância P, CGRP, galanina, colecistocinina, peptídeo vasoativo intestinal, citocinas, fatores tróficos neurais, entre outros [Fürst, 1999; Millan, 1999]. Dentre estes mediadores químicos, serão abordados apenas os mais envolvidos diretamente com a presente tese: glutamato e purinas (em item à parte).

O glutamato é conhecido como o principal neurotransmissor excitatório no SNC de humanos e mamíferos em geral [McLennan e Liu, 1982]. Desde o início da década de 1960, seu efeito excitatório vem sendo descrito, onde é relacionado em processo de excitação neural rápida, em plasticidade neural, em processos de aprendizado e memória e em processos de neurotoxicidade (excitotoxicidade) [Danbolt, 2001; Carozzi et al., 2008].

Além disso, o glutamato tem papel importante na transmissão de estímulos dolorosos [Bleakman et al., 2006]. A hipótese de que os receptores glutamatérgicos têm papel fundamental nas vias de dor e que estão criticamente envolvidos na transmissão nociceptiva aferente primária, tanto no desenvolvimento quanto na manutenção da nocicepção, tem sido embasada por estudos farmacológicos, eletrofisiológicos e comportamentais. Os receptores glutamatérgicos são classificados em dois grupos: ionotrópicos e metabotrópicos. Os receptores chamados ionotrópicos são canais iônicos modulados por agonistas (NMDA, AMPA, cainato) e os metabotrópicos são acoplados a sistemas de segundos mensageiros através de proteínas G [Danbolt, 2001; Bleakman et al., 2006]. A ativação destes receptores - por glutamato, aspartato ou agonistas dos receptores - modulam a atividade de enzimas (adenilato ciclase, guanilato ciclase, fosfolipase C) e fluxos iônicos transmembrana.

Nesse sentido, a modulação dos receptores glutamatérgicos ionotrópicos e metabotrópicos pode ser uma terapêutica efetiva para tratamento de quadros dolorosos crônicos [Coggeshall e

Carlton, 1997; Bleakman et al., 2006]. Algumas substâncias capazes de bloquear estes receptores apresentam importante efeito antinociceptivo em diferentes espécies de mamíferos, inclusive em humanos [Wiech et al., 2004]. Antagonistas dos receptores de glutamato, como cetamina e MK-801, podem atenuar quadros dolorosos relacionados à reação inflamatória, dano tecidual agudo, isquemia ou dano nervoso [Wiech et al., 2004].

Contudo, o glutamato também pode ser um agente neurotóxico, promovendo graves alterações estruturais, neuroquímicas e comportamentais, quando liberado de forma não modulada para o espaço extracelular, principalmente em algumas condições de insultos agudos ao SNC ou doenças crônicas neurodegenerativas [Parsons et al., 2005]. Este fato representa uma grande limitação clínica, visto que seu uso pode acarretar em efeitos adversos intoleráveis como sedação, disforia, alucinações, distúrbios motores, entre outros [Gardoni e Di Luca, 2006]. Neste contexto, torna-se constante a busca por novos fármacos que sejam capazes de modular a atividade glutamatérgica anormal e apresentem um maior perfil de segurança.

I.1.g. Dor patológica: dor crônica neuropática

Dor neuropática é definida como a dor resultante de uma disfunção ou lesão do nervo devido à doença ou lesão do sistema somestésico [Zimmermann, 2001]. Esse tipo de dor apresenta etiologia heterogênea, com envolvimento de diversos mediadores químicos e imunes [Campbell e Meyer, 2006]. O tratamento da dor neuropática ainda é um desafio clínico uma vez que se trata de uma síndrome complexa, com mecanismos desencadeadores pouco esclarecidos. No entanto, sabe-se que alterações medulares, tais como excitabilidade aumentada, vias inibitórias com atividade reduzida e neuroplasticidade, promovem o desenvolvimento da dor crônica após uma lesão nervosa. É necessária a participação de diversos mediadores químicos para o desencadeamento de uma cascata de eventos que promovem a manutenção do potencial de ação, de forma semelhante ao que ocorre na dor inflamatória. Por tratar-se de um processo crônico, uma das principais caraterísticas desta patologia é um cenário de eventos neuroplásticos relacionados a alterações gênicas de receptores, canais iônicos, neurotransmissores e neuromoduladores, dentre outros [Woolf e Thompson, 1999; Willis, 2001; Binns et al., 2005; Zhuo, 2007].

Após a ativação intensa e persistente de receptores AMPA, a ativação de receptores NMDA é iniciada com aumento do influxo de íons cálcio. Quando se inicia o processo de sensibilização central, com a liberação dos neurotransmissores GABA e glicina, os interneurônios inibitórios ainda ficam ativos e modulam negativamente a dor. No entanto, durante a excitotoxicidade - toxicidade mediada pelo glutamato – ocorre a morte desses neurônios, o que contribui para a manutenção do quadro doloroso a longo prazo. Juntamente a este processo, sinapses aberrantes na medula vão desencadear a amplificação do processo doloroso que se torna, muitas vezes, espontânea [Gold, 2000; Zhuo, 2007]. Dessa forma, a dor resultante é de difícil manejo e muitos casos tornam-se refratários aos tratamentos. A maioria dos modelos animais de dor neuropática foi desenvolvida em ratos a partir de lesões periféricas traumáticas, metabólicas ou tóxicas como, por exemplo, ligadura parcial do nervo ciático, ligadura do nervo espinhal, lesão constritiva crônica do nervo ciático, dentre outros métodos. [Le Bars et al., 2001]. Estes modelos visam provocar hiperalgesia mecânica e térmica, apresentando correlação com as alterações fenotípicas desenvolvidas em humanos [Le Bars et al., 2001; Campbell e Meyer, 2006]. Estudos que promovam uma melhor compreensão da neurobiologia da dor neuropática contribuem para a elaboração de novos fármacos que atuem de maneira mais eficaz neste tipo de dor.

I.1.h. O sistema purinérgico e seu papel na transmissão dolorosa

O sistema purinérgico é composto por bases purínicas - como adenina e guanina - e seus derivados nucleotídeos e nucleosídeos. Estas moléculas são distribuídas dentro e fora das células de organismos vivos e estão envolvidas em diversas funções biológicas, como: construção do DNA e RNA (adenina e guanina), nas vias bioquímicas envolvidas no metabolismo energético celular (ATP) ou como mensageiros secundários nos mecanismos intracelulares de transdução de sinal (AMPc e GMPc) [Bourne et al., 1990; Barnstable et al, 2004]. Diversos estudos demonstraram o papel destas moléculas sobre a homeostase [Burnstock, 2007]. As purinas são classificadas em: derivados da adenina (ATP, ADP, AMP, adenosina, adenina) e derivados da guanina (GTP, GDP, GMP, guanosina e guanina). Também compõem as purinas os metabólitos diretos destes derivados, como a inosina, xantina, hipoxantina e ácido úrico. O ATP e a adenosina são considerados como principais efetores do sistema purinérgico em nível extracelular [Ralevic e Burnstock, 1998].

O ATP é um neurotransmissor clássico em nível central e periférico, armazenado e liberado de terminais pré-sinápticos, mas também é liberado por células não-neuronais e tecido lesado [Ralevic e Burnstock, 1998; Burnstock, 2007]. A adenosina e seus substratos apresentam efeitos neuromodulatórios [Brundege e Dunwiddie, 1997]. Além da ação como neurotransmissores e neuromoduladores, ambos derivados de adenina também agem como fatores tróficos em processos plásticos desenvolvidos no SNP e SNC [Rathbone et al., 1999; Ciccarelli et al., 2001]. As purinas, principalmente a adenosina, apresentam papel importante na modulação da atividade sináptica no SNC, interagindo com sistemas, como glutamatérgico, dopaminérgico, serotoninérgico e colinérgico [Schmidt et al., 2007; Rathbone et al., 2008].

Na transmissão de dor, o ATP e a adenosina exercem influências em sítios periféricos e centrais [Sawynok, 1998; Sawynok et al., 1999; Sawynok e Liu, 2003]. A adenosina tem efeitos antinociceptivos relacionados à inibição pré-sináptica dos terminais nervosos sensoriais (com a diminuição da liberação de substância P e glutamato) e à inibição intrínseca de neurônios pelo aumento da condutância ao K^+ [Sawynok e Liu, 2003]. Por meio da sua ação agonista em receptores A_1, também diminui a produção de óxido nítrico (mediado pelo receptor NMDA) e está relacionada diretamente a analgesia opioide [Sawynok e Liu, 2003].

O ATP interage com receptores purinérgicos específicos do tipo P_2. Estes receptores podem ser subdivididos em P_2X e P_2Y que são acoplados, respectivamente, aos canais iônicos e à proteína G [Burnstock, 2007]. Estudos com modelos experimentais de dor neuropática demonstraram redução ou aumento de receptores P_2X_3. Entretanto, há aumento da sensibilidade desses receptores mesmo na redução [Jarvis et al., 2002].

O bloqueio de receptores P_2X_3 atenua a alodinia mecânica e térmica em ratos [Jarvis et al., 2002]. Os receptores P_2X_4 aumentam sua expressão na microglia após lesão do nervo e o bloqueio farmacológico do P_2X_4 reverte a alodinia [Tsuda et al., 2003]. Ratos que não expressam os receptores P_2X_7, presentes nas células T e macrófagos, são resistentes ao desenvolvimento de dor neuropática [Chessell et al., 2005]. Em contrapartida, receptores P_2Y_1 aumentam em 70% após lesão do nervo ciático em ratos, e também podem estar relacionados ao desenvolvimento de quadros dolorosos [Xiao et al., 2002]. Entretanto, ainda não estão disponíveis para uso clínico fármacos que tenham ação direta sobre os receptores purinérgicos. Uma opção interessante é o uso de fármacos que modulam a atividade da adenosina e que já se encontram disponíveis para uso clínico, como o alopurinol.

O mecanismo de ação do alopurinol é a inibição da xantina oxidase, enzima responsável pela conversão de xantina em ácido úrico, passo final na degradação de purinas [Day et al., 2007]. Dessa forma, a hipoxantina e xantina e seus substratos (adenosina e guanosina, respectivamente) podem ficar acumulados e, consequentemente, há redução do produto final ácido úrico [Day et al., 2007]. O alopurinol é um fármaco amplamente utilizado em humanos para tratamento de hiperuricemia e gota e, além de ter comprovado perfil de segurança, pode ser um novo alvo de tratamento adjuvante de dor crônica, visto que provoca aumento nos níveis plasmáticos e liquóricos de guanosina e adenosina, ambos neuromoduladores com potencial antinociceptivo [Marro et al., 2006]. Em vista disso, o alopurinol foi o fármaco de escolha para avaliar o potencial do sistema purinérgico na modulação da dor através de estudos experimentais e clínicos na presente tese.

I.2. OBJETIVOS

I.2.a. Trabalho experimental com modelo animal

Objetivo geral: investigar os potenciais efeitos antinociceptivos do alopurinol em modelos animais de dor.

Objetivos específicos:

- Avaliar o efeito antinociceptivo do alopurinol em um modelo de dor neuropática em camundongos.
- Investigar o mecanismo de ação antinociceptiva do alopurinol em um modelo de dor neuropática em camundongos.
- Mensurar os níveis liquóricos de purinas em camundongos submetidos à administração intraperitoneal de alopurinol em um modelo de dor neuropática.
- Avaliar potenciais efeitos adversos e alterações locomotoras induzidas pela administração intraperitoneal de alopurinol em camundongos.

I.2.b. Trabalhos experimentais com modelos humanos

Objetivo geral: investigar os efeitos do alopurinol, inibidor da xantina oxidase, em pacientes do sexo feminino, submetidas à histerectomia abdominal total ou em pacientes portadoras de fibromialgia.

Objetivos específicos:

- Avaliar o efeito analgésico do alopurinol em pacientes submetidas a histerectomia abdominal através de escalas de dor.
- Avaliar o efeito ansiolítico do alopurinol em pacientes submetidas ao procedimento cirúrgico.
- Avaliar o efeito analgésico do alopurinol em pacientes portadoras de fibromialgia refratárias à terapia convencional através de escala de dor.
- Avaliar o efeito ansiolítico do alopurinol em pacientes portadoras de fibromialgia refratárias à terapia convencional.

PARTE II

Metodologia e Resultados apresentados na forma de artigos científicos

II.1. ARTIGO 1

The xanthine oxidase inhibitor allopurinol prevents thermal and mechanical hyperalgesia in a mouse model of peripheral mononeuropathy.

(A ser submetido)

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Running title: Allopurinol-induced analgesia in mice.

Abstract:

Background and purpose: Allopurinol is a potent inhibitor of the enzyme xanthine oxidase used primarily in the treatment of hyperuricemia and gout. It is well known that purines exert multiple roles on pain mechanisms. We hypothesized that the inhibition of xanthine oxidase by allopurinol could be a valid strategy to enhance endogenous purinergic activity. The aim of this study was to investigate the antinociceptive profile of allopurinol on a neuropathic pain model in mice.

Experimental approach: Mice received an intraperitoneal injection of vehicle (Tween 10%) or allopurinol (50 to 200 $mg.kg^{-1}$) and submitted to a neuropathic pain model. Additionally, investigation of the mechanism of action of allopurinol, allopurinol-induced locomotor effects and measurements of CSF purine levels were also evaluated.

Key results: Allopurinol produced dose-dependent antinociceptive effects against thermal and mechanical hyperalgesia in a neuropathic pain model. The A_1 adenosine-receptor antagonist DPCPX, but not the A_{2A} adenosine-receptor antagonist SCH58261, partially prevented allopurinol-induced antinociception. No obvious motor deficits were produced by allopurinol at doses up to 200 $mg.kg^{-1}$. Allopurinol also caused an increase in CSF levels of purines, including xanthine, inosine and guanosine, and decreased CSF concentration of uric acid.

Conclusions and implications: This study has demonstrated antinociceptive effects of systemic administration of allopurinol in a neuropathic pain model, complementing previous data on thermal and chemical pain models. These findings indicate that allopurinol, and possibly other xanthine oxidase inhibitors, could be useful for managing chronic neuropathic pain in humans. Allopurinol-induced antinociception may be related to the accumulation of endogenous purines.

Keywords: allopurinol; purines; pain; adenosine; guanosine; xanthine oxidase; antinociception.

Abbreviations: P_1, purinergic receptor type 1; P_2, purinergic receptor type 2; A_1, adenosine receptor type 1; A_{2A}, adenosine receptor type 2A; A_{2B}, adenosine receptor type 2B; A_3, adenosine receptor type 3; DMSO, dimethyl sulfoxide; CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; CNS, central nervous system; ADA, adenosine deaminase; ROS, reactive oxygen species; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; SCH58261, 5-amino-2-(2-furyl)-7-phenylethyl-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5c]pyrimidine.

Introduction:

Allopurinol, or 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, is a structural analogue of hypoxanthine and a potent inhibitor of the enzyme xanthine oxidase that catalyzes the transformation of hypoxanthine to xanthine and uric acid, reducing both uric acid formation and purine degradation [Day et al., 2007; Pacher et al., 2006]. Allopurinol is used primarily in the treatment of hyperuricemia and gout [Rundles, 1992]. Besides its hypouricemic effects, allopurinol has been studied for several other indications including treatment of seizures, psychiatric disorders, and ischemia-reperfusion injury [Day et al., 1994; Garcia Garcia et al., 1990; Akhondzadeh et al., 2005; Paniz et al., 2012].

Both healthy and hyperuricemic patients present reduction of uric acid levels by allopurinol, probably leading to increase in hypoxanthine and xanthine, which are converted to closely related purines, including the inhibitory neuromodulator adenosine. Those events, by enhancing adenosinergic activity, may explain some of the effects of allopurinol on the central nervous system (CNS) [Tada et al., 1991; Wada et al., 1992; Zagnoni et al., 1994; Lara et al., 2000; 2001; 2003; Machado-Vieira et al., 2001; Brunstein et al., 2005].

The purinergic system involves adenosine and ATP as major endogenous effectors, acting on P_1 and P_2 receptors, respectively [Zarrinmayeh and Territo, 2020]. Adenosine is mainly an inhibitory neuromodulator, regulating synaptic activity and causing the release of several neurotransmitters [Sawynok and Liu 2003]. Adenosine modulates pain in the spinal cord and periphery and induces antinociceptive effects in several pain paradigms [Sawynok and Liu 2003; McGaraughty and Jarvis, 2005]. Adenosine mediates its effects mainly through its interaction with four G protein-coupled receptors: A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors [McGaraughty and Jarvis, 2005; Zarrinmayeh and Territo, 2020]. The presence of adenosine receptors has been demonstrated in several cells and tissues throughout the body, including the CNS [Borea et al., 2018].

Previous studies have demonstrated that allopurinol produces dose-dependent antinociceptive effects against several chemical and thermal pain models in rodents [Schmidt et al., 2009; Essawy and Elbaz, 2013]. In the present study, we hypothesized that the inhibition of xanthine oxidase by allopurinol could be a valid strategy to enhance purinergic activity in the management of chronic neuropathic pain. The aims of the present study were: (a) to investigate the antinociceptive activity induced by allopurinol in a neuropathic pain model in mice; (b) to identify,

by means of pharmacological as well as neurochemical approaches, possible mechanisms involved in the allopurinol-induced antinociception; (c) to evaluate acute toxicity induced by allopurinol using a locomotion paradigm.

Methods:

Animals: Male adult Swiss albino mice (30–40 g) were kept on a 12 h light/dark cycle (light on at 7:00 am) at temperature of 22 ± 1 °C housed in plastic cages (five per cage) with tap water and commercial food ad libitum. In all nociceptive behavioral experiments, the animals were acclimatized to the laboratory for at least 1 h before testing. The ethical guidelines for investigations of experimental pain in conscious animals [Zimmermann, 1983] and ARRIVE guidelines [Kilkenny et al., 2010] were followed throughout. The number of animals and the number of intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

Drugs administration: Allopurinol was purchased from Sigma Chemicals (St Louis, MO, USA). DPCPX (8-cyclopentyl-1,3-dipropylxanthine) was purchased from Tocris (Northpoint, UK). SCH58261 (5-amino-2-(2-furyl)-7-phenylethyl-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5c]pyrimidine) was provided by S. Weiss (Vernalis, UK). The anesthetic sodium thiopental was obtained from Cristália (SP, Brazil). Allopurinol was dissolved in a 10% Tween solution. The dose of Tween (10%) did not cause any detectable effect. DPCPX and SCH58261 were diluted in 10% DMSO (dimethyl sulfoxide). All other solutions were dissolved in saline (NaCl 0.9%) and buffered with 0.1 N NaOH or 0.1 N HCl to pH 7.4 when necessary. All other chemicals were purchased from local suppliers. Drug and molecular target nomenclature used in this manuscript conforms with the Guide to Receptors and Channels [Alexander et al., 2008]. Experiments were performed according to the method described by Schmidt et al. [2009]: 20 minutes before the experiment, animals were placed individually in acrylic boxes, which also served as observation chambers. After this adaptation period, treatments were performed. Animals were treated with an intraperitoneal (i.p.) injection (10 $ml.kg^{-1}$) of vehicle (saline or 10% Tween) or allopurinol (50 to 200 $mg.kg^{-1}$). In order to investigate the mechanism of action of allopurinol, some animals were also pretreated (15 minutes in advance) with an i.p. injection of the selective A_1 adenosine-receptor antagonist DPCPX (0.1 $mg.kg^{-1}$) or the selective A_{2A} adenosine-receptor

antagonist SCH58261 (0.5 $mg.kg^{-1}$). DPCPX and SCH58261 doses were adapted from a previous study [Schmidt et al., 2009].

Partial sciatic nerve ligation model (PSNL): Mice were anesthetized intraperitoneally (i.p.) with sodium thiopental (40 $mg.kg^{-1}$, 10 $mL.kg^{-1}$, supplemented as necessary). A partial ligation of the right sciatic nerve was performed by tying the distal 1/3 to 1/2 of the dorsal portion of the sciatic nerve, according to the procedure described in mice by Malmberg and Basbaum [1998]. The left sciatic nerve was dissected and exposed without ligation. The wound was closed and covered with iodine solution. The operated mice received allopurinol (50, 100 or 200 $mg{\cdot}kg^{-1}$, i.p.) or vehicle (Tween 10%) 14 days after surgery and after baseline measurements for the plantar test (thermal hyperalgesia) or von Frey test (mechanical hyperalgesia). The thermal and mechanical hypernociceptive responses were recorded immediately before (0) and after (30 min, 60 min, 120 min, 360 min, 12 h and 24 h) treatment.

Plantar Test (Thermal Hyperalgesia): Thermal hyperalgesia was evaluated by the paw withdrawal test [Hargreaves et al., 1988]. Operated animals were maintained in the animal facility for at least 2 weeks before the experiments, with food and water ad libitum. On the day of the experiment, animals were placed in transparent plastic chambers on an elevated glass floor of the testing apparatus (7370 Plantar Test, Ugo Basile, Varese, Italy) and allowed to acclimate to their surroundings for 20 minutes. After acclimation, a radiant heat source (50-W halogen reflector bulb with intensity controlled by a constant voltage source) was aimed at the plantar surface of 1 of the hind paws through the glass floor. A photoelectric cell automatically turns the heat source off when the reflected light beam is interrupted (ie, when the animal withdraws the paw) and records the paw withdrawal latency at the nearest 0.1 second. A time limit of 30 seconds was used to prevent tissue damage. Both paws were tested at random, and a 1-minute interval between consecutive stimulations of the same hind paw was used. Testing was performed 5 times on each side, and the latencies to each side were averaged. Average values were used for statistical analysis. A score was computed by subtracting the average latency of the sham-operated side (control side) from the average latency of the ligated side (experimental side). Negative difference scores indicated a lower threshold on the ligated side. Only animals displaying negative scores <2 standard deviations were considered neuropathic and were included in the tests. Although there is strong evidence that the experimental neuropathy produces allodynia, heat-induced hyperalgesia is considered the most accurate method to evaluate neuropathy in this context [Bennett and Xie, 1988 and Hargreaves et

al, 1988]. Consequently, in the present study, thermal hyperalgesia was considered as the main parameter to evaluate neuropathic pain.

Measurement of mechanical allodynia: For the evaluation of mechanical allodynia, mice were placed individually in clear Plexiglas boxes (9 X 7 X 11 cm) on elevated wire mesh platforms to allow access to the ventral surface of the right hindpaw. The withdrawal response frequency was measured following 10 applications (duration of 1 s each) of 0.6 g von Frey hair (VFH, Stoelting, Chicago, IL, USA) [Bortalanza et al., 2002]. Stimuli were delivered from below to the plantar surface of the right hindpaw. Animals were acclimatized for 30 min before behavioral testing and mechanical allodynia was evaluated at several time points. The frequency of withdrawal was determined before all experiments.

Measurement of locomotor activity: In order to evaluate non-specific muscle relaxant or neurotoxic effects, we evaluated the effects of allopurinol in spontaneous locomotor activity test. The method to evaluate spontaneous locomotor activity was adapted from Tort et al. [2006]. Mice were randomly allocated to individual triangular boxes (50cm × 30cm × 30cm, 50cm high) with rounded corners, placed on the floor of a soundproof and diffusely illuminated room. Locomotor activities of eight mice were recorded simultaneously by a video-computerized system, with image analysis at four frames per second. The software tracked the animals by distinguishing their white color from the black background of the floor, registering *X* and *Y* horizontal coordinates. The method was set to examine horizontal locomotor activity, ignoring small movements, such as breathing, head and tail actions, and tremors. Animals were individually habituated to the activity cage for 10 min before receiving the i.p. treatments. Animals were placed again in the activity cages 30 min after treatments, and the locomotor activity was recorded for 180 min. The data on locomotor activity is divided in 10 min blocks and presented as a function of time.

Cerebrospinal fluid (CSF) sampling: Groups of operated mice were treated similarly with i.p. administration of allopurinol (200 $mg.kg^{-1}$) or vehicle. After 30 min, mice were anesthetized with sodium thiopental (60 $mg.kg^{-1}$, 10 $mL.kg^{-1}$, i.p.) and placed in a stereotaxic apparatus, where the CSF was drawn (10 - 20 μL per mouse) by direct puncture of the *cisterna magna* with an insulin syringe (27 gauge x 1/2 in length), with the help of a magnifying glass. All samples were centrifuged at 10,000g in an Eppendorf centrifuge during 5 min to obtain cell-free supernatants and stored in separate tubes in -70^{o}C until analysis.

HPLC procedure: High-performance liquid chromatography (HPLC) was performed with aliquots obtained from the CSF cell-free supernatants. The following purines were measured according to Domanski et al. [2006]: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine, guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), guanosine, inosine monophosphate (IMP), inosine, hypoxanthine, xanthine, and uric acid. Analyses were performed with Shimadzu Class-VP chromatography system consisting of a quaternary gradient pump with vacuum degassing and piston desalting modules, Shimadzu SIL-10AF auto injector valve with 50 μL loop, and an UV detector. Separations were achieved on a Supelco C18 250 mm x 4.6 mm, 5 μm particle size column. The mobile phase flowed at a rate of 1.2 $mL.min^{-1}$ and column temperature was 24 ^{o}C. Buffer composition remained unchanged (A: 150 $mmol.L^{-1}$ phosphate buffer, pH 6.0, containing 150 $mmol.L^{-1}$ potassium chloride; B: 15% acetonitrile in buffer A). The gradient profile was modified to the following content of buffer B in the mobile phase: 0% at 0.00 min, 2% at 0.05 min, 7% at 2.45 min, 50% at 10.00 min, 100% at 11.00 min, 100% at 12.30 min, and 0% at 12.40 min. Samples of 10 μL were injected every 18 min into the injection valve loop. Absorbance was read at 254 nm.

Statistical analysis: Data are expressed as mean ± standard deviation (SD). Data were submitted to Kolmogorov-Smirnov test for normality evaluation. Statistical analysis between groups was performed using one-way analysis of variance (ANOVA) plus the *post-hoc* Bonferroni multiple comparisons test when necessary. All results with $p < 0.05$ were considered statistically significant.

Results:

In order to evaluate the effect caused by allopurinol on the neuropathic pain, we performed a chronic sciatic nerve constriction (PSNL) in mice. This injury produced marked allodynia and hyperalgesia on the ipsilateral side two weeks after the nerve injury procedure compared with sham animals (data not shown). The acute systemic treatment with allopurinol (200 $mg.kg^{-1}$) reduced thermal hyperalgesia on the ipsilateral side of the sciatic nerve constriction injury 30 and 60 minutes after drug administration ($p < 0.05$, Figure 1). Administration of vehicle (10% Tween) did not affect nociception as compared with control animals (data not shown). Pretreatment with allopurinol (200 $mg.kg^{-1}$) induced a marked and more sustained inhibition of response frequency

to the von Frey hair application (mechanical hyperalgesia) in the PSNL model up to 2 hours following treatment (Figure 2).

Figure 3 shows that the selective A_1 adenosine-receptor antagonist DPCPX (0.1 $mg.kg^{-1}$), but not the selective A_{2A} adenosine-receptor antagonist SCH58261 (0.5 $mg.kg^{-1}$) partially prevented antinociception induced by allopurinol (200 $mg.kg^{-1}$) in the plantar test.

As depicted in Figure 4, allopurinol (50 to 200 $mg.kg^{-1}$) did not induce significant motor deficits as evaluated by the performance in the locomotor activity test.

Table 1 shows that the CSF concentration of uric acid was significantly reduced 30 minutes after treatment with allopurinol 200 $mg.kg^{-1}$. Conversely, the CSF concentrations of xanthine, guanosine, inosine and GDP were significantly increased in mice treated with allopurinol compared to mice receiving vehicle. The most significant changes were observed for uric acid (approximately 3-fold decrease) and guanosine (approximately 6-fold increase). Intraperitoneal administration of allopurinol did not affect CSF levels of ATP, ADP, AMP, GTP, GMP, IMP, and adenosine as compared to vehicle. CSF allopurinol concentration was estimated in 60.7 ± 15.5 μM, 30 minutes after a single i.p. allopurinol 200 $mg.kg^{-1}$ administration. Allopurinol was not detected in the CSF of control animals.

Discussion:

In this study, i.p. administration of the xanthine oxidase inhibitor allopurinol produced dose-dependent antinociceptive effects against thermal and mechanical hyperalgesia in a neuropathic pain model in mice. However, the selective A_1 adenosine-receptor antagonist DPCPX, but not the selective A_{2A} adenosine-receptor antagonist SCH58261, partially prevented allopurinol-induced antinociception. No obvious locomotor effects were produced by allopurinol at doses up to 200 $mg.kg^{-1}$. This study also demonstrated that i.p. administration of allopurinol significantly increased CSF levels of purines, including the nucleosides inosine and guanosine, and decreased CSF concentration of uric acid.

Although allopurinol has been traditionally used in the treatment of gout and its related symptoms (including pain), only anecdotal reports investigating the effects of allopurinol *per se* on pain are found in the literature [Pinelli et al., 1991; Daskalopoulou et al., 2005; Inkster et al., 2007; Hacimuftuoglu et al., 2006]. The rationale to administer allopurinol for pain is derived from evidence in basic and clinical research on the purinergic system. Purines, especially adenosine and

ATP, and their receptors have been considered important targets for the development of new drugs for pain management [Sawynok and Liu, 2003]. The nucleoside adenosine and its analogs present antinociceptive effects at central and peripheral sites and purinergic receptors (P_1 and P_2) are closely involved in the mechanisms of pain transmission [Sawynok and Liu, 2003]. Endogenous adenosine can be released in the CNS and peripheral tissues, and the regulation of its levels by various pharmacological agents can alter pain processing through activation of adenosine A_1 receptors on neurons, and perhaps other receptors on adjacent structures [Sawynok and Liu, 2003]. Antinociception induced by adenine-based purines seems to be related to adenosine receptors, probably A_1 receptors, since adenosine antagonists such as caffeine and theophylline block their effect and adenosine uptake blockers and adenosine deaminase (ADA) inhibitors enhance antinociception [McGaraughty et al., 2001; Donnelly-Roberts et al., 2008]. Additional effects on inflammatory cells at peripheral sites [Fredholm et al., 1997] and on glia in the CNS [Ogata and Schubert, 1996; Gebicke-Haerter et al., 1996] mediated by adenosine A_{2A}, A_{2B} and A_3 receptors also occur, and these potentially can produce indirect effects on pain transmission. Finally, we have previously demonstrated that allopurinol produced antinociception in four different animal pain models [Schmidt et al., 2009]. Therefore, it is tempting to propose that the xanthine oxidase inhibitor allopurinol could produce accumulation of other purines, which may account, at least partially, for its antinociceptive properties.

The basic mechanism of action of allopurinol and its metabolite oxypurinol is inhibition of xanthine oxidase (they bind strongly to the reduced form of xanthine oxidase and inhibit the enzyme). This leads to a decrease in the systemic concentration of uric acid and an increase in the concentration of the precursors, hypoxanthine and xanthine [Day et al., 2007]. Thus, the primary effect of both allopurinol and oxypurinol is inhibition of uric acid production, and the overall result is the inhibition of the metabolism of xanthine and hypoxanthine leading to greater salvage of these purines by their conversion to inosine, adenosine and guanosine. These findings have been extensively demonstrated after systemic administration of allopurinol both in CNS and periphery [Kim et al., 1987; Ceballos et al., 1994; Marro et al., 2006]. In fact, a significant concentration of allopurinol has been demonstrated in CSF after its systemic administration and a remarkable suppression of CSF uric acid levels has been observed [Kim et al., 1987; Enrico et al., 1997; Akdemir et al., 2001]. Accordingly, in the present study, we demonstrated an approximately 3-fold decrease in the CSF levels of uric acid and a marked increase in the CSF concentrations of

allopurinol (approximately 60 μM), xanthine, guanosine, inosine and GDP 30 minutes after a systemic administration of allopurinol. Consequently, the modulation of CSF concentrations of purines might play a role in the antinociceptive action of allopurinol. Surprisingly, CSF levels of adenosine and hypoxanthine were not affected by the i.p. administration of allopurinol, an opposing finding as compared to previous evidence in sham animals [Schmidt et al., 2009]. These conflicting data may be related to animal characteristics, including the induction of a model of chronic neuropathic pain, or other additional factors that should be addressed in future studies.

In the present study, DPCPX, but not SCH58261, partially prevented allopurinol-induced antinociception in a neuropathic pain model. Altogether, these findings indicate that A_1 adenosine-receptor may be involved in these effects, similarly to previous evidence in acute pain models in mice [Schmidt et al., 2009]. Of note, previous evidence has indicated that the activation of the opioid naloxone-sensitive pathway is unlikely to be involved in the antinociception caused by allopurinol, as naloxone, under conditions where it fully reversed morphine-induced antinociception, had no effect against allopurinol-induced analgesia [Schmidt et al., 2009]. Additionally, there is no evidence that allopurinol presents any direct action on adenosinergic receptors [Day et al., 2007].

Although some of our findings indicate a role for adenosine in allopurinol-induced antinociception, we can not rule out the influence of other purines. This study also demonstrated a significant increase in the CSF concentration of the nucleoside guanosine. Our group and others [Cunha, 2005; Oses et al., 2004] have demonstrated that the nucleosides guanosine and adenosine closely interact in the CNS. A guanine-based purinergic system with relevant physiological and pathological implications to the CNS has been proposed [Schmidt et al., 2000; Lara et al., 2001; Vinadé et al., 2003; Schmidt et al., 2007]. Of note, we have demonstrated that guanosine, as well as adenosine, may modulate pain transmission [Schmidt et al., 2008] and prevents thermal hyperalgesia in rats [Schmidt et al., 2010]. Additionally, spinal administration of inosine or guanine has produced analgesia in animal models of pain [de Oliveira et al., 2016]. Therefore, it is not possible to exclude that the nucleoside guanosine, and perhaps other purines such as guanine, xanthine or inosine, may also influence allopurinol-induced antinociception.

Allopurinol was developed and has been extensively used as an inhibitor of the enzyme xanthine oxidase [Day et al., 2007]. Xanthine oxidase is a highly versatile flavoprotein enzyme that catalyses the oxidative hydroxylation of purine substrates and generates reactive oxygen species

(ROS) [Borges et al., 2002]. ROS have been proposed to contribute to and/or maintain conditions of chronic pain [Kim et al., 2006]. More recently, some data indicated that ROS may also mediate acute pain transmission [Hacimuftuoglu, 2006]. Notably, there is overwhelming acceptance that xanthine oxidase activity is significantly increased in various pathological states, including some pain states [Khalil and Khodr, 2001]. Therefore, the inhibition of this enzymatic pathway may be beneficial for treating pain [Lee et al., 2007]. The administration of allopurinol has been shown to decrease tissue injury following ischemia/reperfusion in a variety of in vitro and in vivo models [Garcia Garcia et al., 1990; Reilly et al., 1991]. Recently, Inkster et al. [2007] showed that allopurinol treatment (50 and 250 $mg.kg^{-1}$) had marked beneficial effects on nerve and vascular function in diabetic rats. That same study also demonstrated that allopurinol (150 $mg.kg^{-1}$) attenuated diabetes-induced tactile allodynia, thermal and mechanical hyperalgesia. These effects may be related to a reduction on xanthine oxidase activity and consequently on aspects of reactive oxygen species-mediated nerve dysfunction via adverse vascular effects [Inkster et al., 2007].

Previous evidence has shown that high doses of allopurinol (approximately 400 $mg.kg^{-1}$) could cause some systemic toxicity with animals displaying decreased locomotor activity in the hole-board test and activity cages and impaired motor coordination as observed in the rotarod test [Schmidt et al., 2009]. Central and systemic administration of the nucleoside adenosine has been traditionally related to significant side effects such as hypotension, sedation and impaired motor function [Sawynok and Liu, 2003]. Therefore, if allopurinol induces antinociception by increasing levels of adenosine, the reduction in pain scores could be related to these alterations. However, the findings of the present study show that allopurinol, in doses up to 200 $mg.kg^{-1}$, did not produce any obvious locomotor activity impairment.

In summary, this study has demonstrated additional evidence on the potential antinociceptive profile of allopurinol in a rodent pain model of thermal and mechanical hyperalgesia. By pharmacological and neurochemical means, we have demonstrated that allopurinol-induced antinociception may be related to purines accumulation in the CNS. Although it is early to propose the use of adenine- or guanine-based purines for clinical research, an interesting approach to investigate their role clinically is the investigation of purine derivatives previously used in humans such as allopurinol. Moreover, because allopurinol seems to be well tolerated with no obvious CNS toxic effects in low to moderate doses, this drug may be useful for treat chronic neuropathic pain in humans.

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Legends:

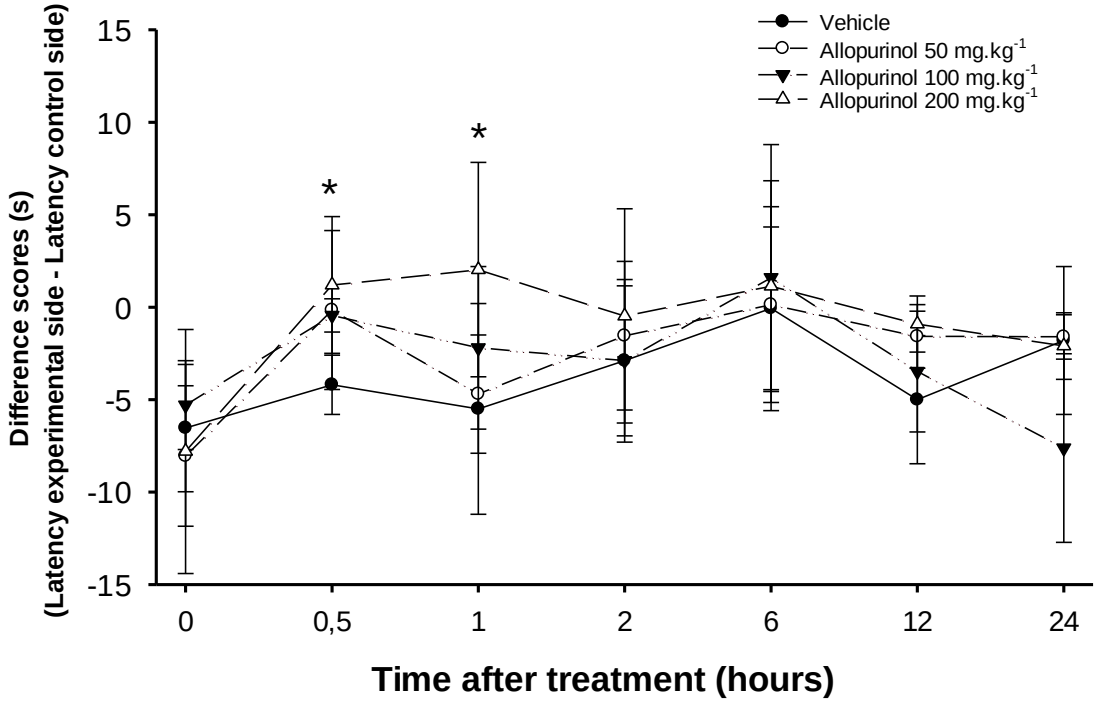
Figure 1: Effects of intraperitoneal (i.p.) administration of allopurinol (50, 100, or 200 $mg.kg^{-1}$) or vehicle (10% tween) on thermal hyperalgesia induced by partial sciatic nerve injury in mice (plantar test). Symbols represent mean difference scores [i.e., latency to right hindpaw withdrawn (experimental side) – latency to left hindpaw withdrawn (control side), in seconds] and vertical bars represent standard deviation (SD). Zero time represents the baseline before treatments. N = 8 animals per group. * = $p < 0.05$ as compared with vehicle, one-way ANOVA followed by Bonferroni's multiple comparison test.

Figure 2: Effects of intraperitoneal (i.p.) administration of allopurinol (50, 100, or 200 $mg.kg^{-1}$) or vehicle (10% tween) on mechanical allodynia induced by partial sciatic nerve injury in mice (von Frey test). Symbols represent response frequency to tactile stimuli and standard deviation (SD). Zero time represents the baseline before treatments. N = 8 animals per group. * = $p < 0.05$ as compared with vehicle, one-way ANOVA followed by Bonferroni's multiple comparison test.

Figure 3: Effects of intraperitoneal (i.p.) administration DPCPX (0.1 $mg.kg^{-1}$) or SCH58261 (0.1 $mg.kg^{-1}$) on antinociceptive effects of allopurinol (200 $mg.kg^{-1}$) or vehicle (10% tween) against thermal hyperalgesia induced by partial sciatic nerve injury in mice (plantar test). Symbols represent mean difference scores [i.e., latency to right hindpaw withdrawn (experimental side) – latency to left hindpaw withdrawn (control side), in seconds] and vertical bars represent standard deviation (SD). Zero time represents the baseline before treatments. N = 8 animals per group. * = $p < 0.05$ as compared with vehicle, one-way ANOVA followed by Bonferroni's multiple comparison test.

Figure 4: Effects of intraperitoneal (i.p.) administration of allopurinol (50, 100, or 200 $mg.kg^{-1}$) or vehicle (10% tween) on the mice spontaneous locomotor activity test. Vehicle (10% tween) or allopurinol was i.p. administered 10 min prior to the behavior measurements. N = 8 animals per group. * = $p < 0.05$ compared with vehicle, one-way ANOVA followed by Bonferroni's multiple comparison test.

Figure 1:



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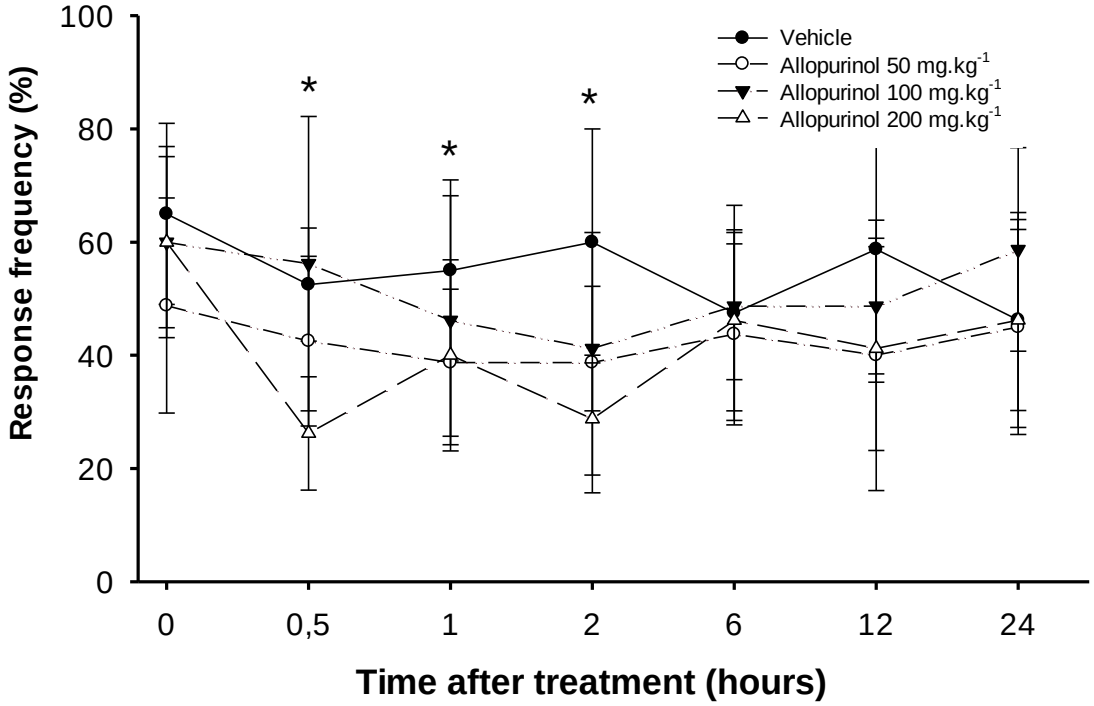

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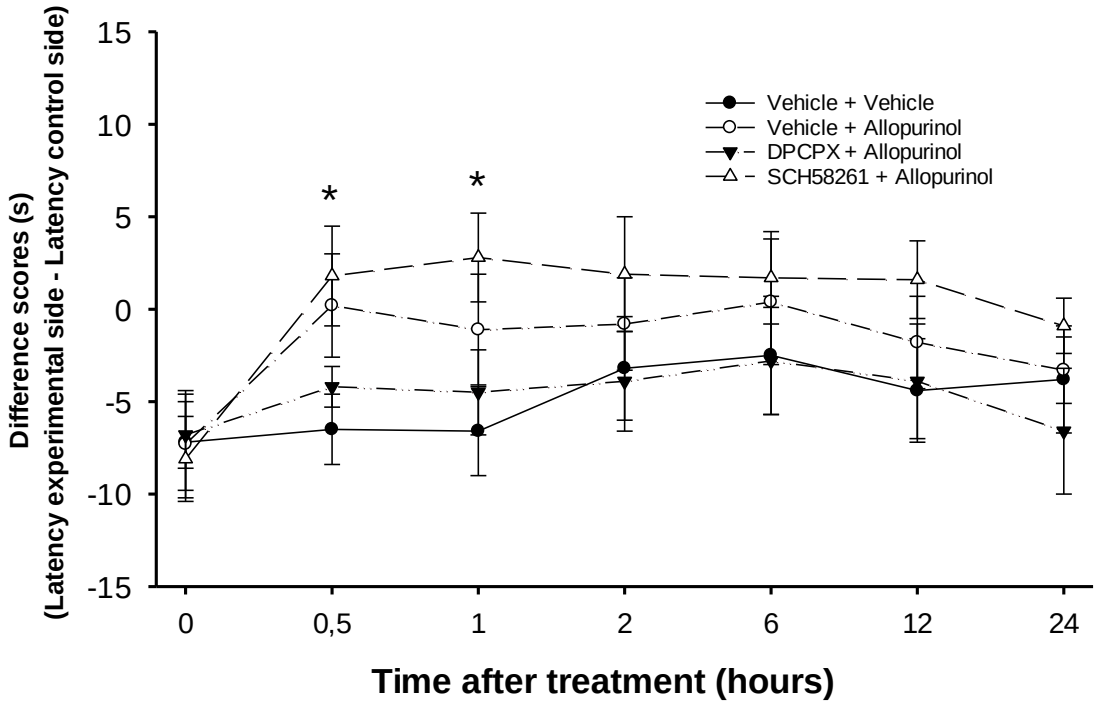

Figure 4:

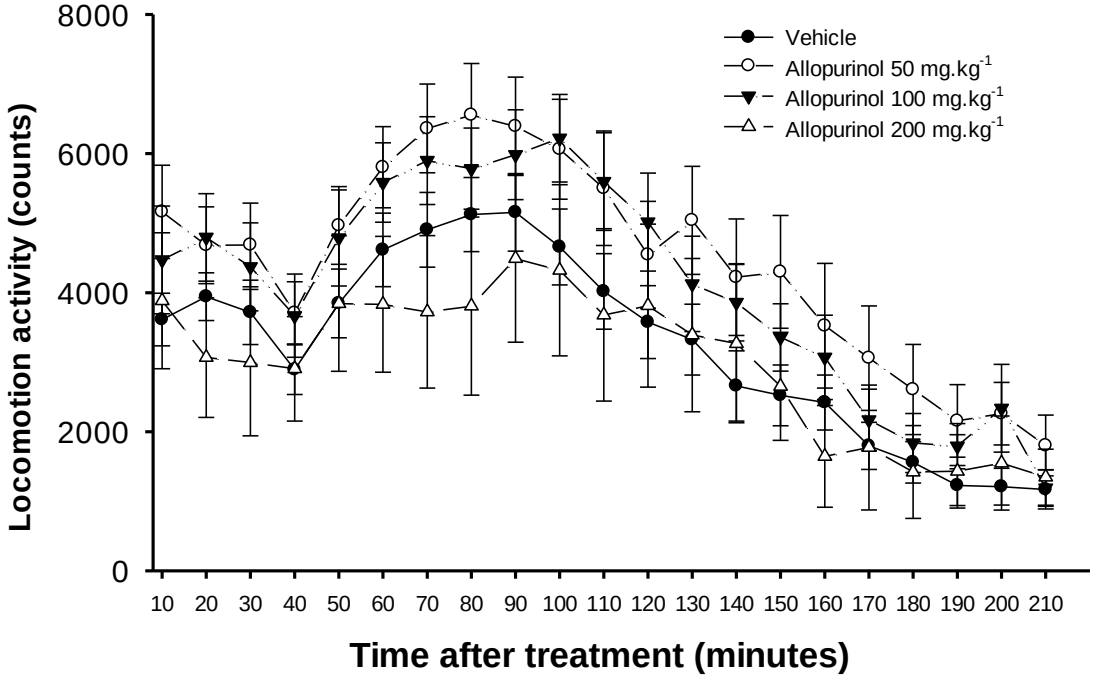

Table 1

CSF purines concentration (µM)

	Groups (treatment)		*p*
	Vehicle	Allopurinol	
GTP	1.14 (0.1)	1.12 (0.1)	0.76
GDP	0.4 (0.2)*	0.75 (0.4)*	0.02
GMP	0.32 (1.0)	0.01 (0.01)	0.41
IMP	1.55 (0.4)	1.39 (0.2)	0.31
ATP	ND	ND	-- / --
ADP	0.48 (0.6)	0.34 (0.2)	0.56
AMP	0.86 (0.7)	1.21 (0.6)	0.31
Inosine	1.62 (0.5) $^{\#}$	4.27 (3.1)$^{\#}$	0.003
Guanosine	0.29 (0.2)$^{\#}$	1.68 (1.8)$^{\#}$	0.007
Adenosine	0.54 (0.2)	0.42 (0.4)	0.39
Hypoxanthine	3.43 (2.4)	4.72 (1.3)	0.18
Xanthine	4.77 (1.0)$^{\#}$	11.97 (9.5)$^{\#}$	0.007
Uric acid	4.68 (2.4)$^{\#}$	1.42 (1.5)$^{\#}$	0.002

Effects of allopurinol (200 mg.kg^{-1}) or vehicle on cerebrospinal fluid (CSF) concentration of purines. Data are shown as mean (± SD). Vehicle was 10% Tween. Vehicle or allopurinol was given intraperitonially 30 min prior to the CSF sampling. $p < 0.05$ was considered statistically significant, Student *t* test for parametric data, Wilcoxon rank-sum test for non-parametric data; *$p < 0.05$; $^{\#}p < 0.01$. (n = 8 animals per group). GTP: guanosine-triphosphate; GDP: guanosine-diphosphate; GMP: guanosine-monophosphate; IMP: inosine-monophosphate; ATP: adenosine-triphosphate; ADP: adenosine-diphosphate; AMP: adenosine-monophosphate; ND: non-detected.

II.2. ARTIGO 2

Allopurinol attenuates postoperative pain and modulates the purinergic system in patients undergoing abdominal hysterectomy: a randomized controlled trial.

(Submetido em "Journal of Anesthesia")

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Running title: Allopurinol-induced analgesia in humans.

Abstract:

Purpose: Allopurinol is a potent inhibitor of the enzyme xanthine oxidase used primarily in the treatment of hyperuricemia and gout. The aim of this study was to compare the analgesic efficacy of preanesthetic allopurinol versus placebo on postoperative pain and anxiety in patients undergoing abdominal hysterectomy.

Methods: This is a prospective, double-blinded, placebo-controlled, randomized clinical trial. We investigated 54 patients scheduled to undergo elective abdominal hysterectomy. Patients were randomly assigned to receive either oral allopurinol 300 mg (n = 27) or placebo (n = 27) the night before and 1 h before surgery. Patients were submitted to evaluation of pain and anxiety before the treatment, for 24 hours postoperatively, 30 and 90 days after surgery. Cerebrospinal fluid was collected at the time of the spinal anesthesia in order to perform the measurement of the central levels of purines.

Results: Preoperative administration of allopurinol was effective in reducing postoperative pain 2 hours after surgery. Allopurinol caused a reduction of approximately 40% in pain scores measured by the visual analogue pain scale after surgery ($p < 0.05$). There was a significant change in the cerebrospinal fluid concentrations of xanthine and uric acid before surgery ($p < 0.01$).

Conclusion: This study showed a weak and short-term benefit of the use of allopurinol as a preanesthetic medication since it was related to a significant reduction on pain scores 2 hours after surgery. The purinergic system is a potential target for new drugs to treat acute or chronic pain. New studies investigating allopurinol and more selective purine derivatives in the management of acute or chronic painful conditions should be performed.

Trial number registration: Brazilian Registry of Clinical Trials – ReBEC #RBR-9pw58p.

Keywords: allopurinol; purines; pain; xanthine oxidase; anxiety; analgesia.

Declarations:

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Conflicts of interest/Competing interests: Authors declare no conflict of interest.

Ethics approval: The protocol was evaluated and approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre (HCPA/UFRGS - CAAE #56000100009; Universal Trial Number U1111-1212-5459; Brazilian Registry of Clinical Trials – ReBEC #RBR-9pw58p – retrospectively registered).

Consent to participate: Participants received a written and an oral explanation of the study and signed an informed consent form.

Consent for publication: All authors have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its publication.

Availability of data and material: Available on reasonable request.

1. Introduction

The purinergic system involves adenosine and ATP as major endogenous effectors, acting on P1 and P2 receptors, respectively [1]. Adenosine and its analogues exert multiple effects on pain transmission at peripheral and central sites [2]. Adenosine mediates its effects mainly through its interaction with four G protein-coupled receptors: A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors. The presence of adenosine receptors has been demonstrated in several cells and tissues throughout the body, including the central nervous system [3]. Adenosine regulates pain transmission in the spinal cord and periphery and induces antinociceptive effects in several pain paradigms [4].

Caffeine and theophylline are the classic P_1 adenosine antagonists currently used in humans, but adenosine agonists for human use are still lacking. Allopurinol, or 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, is a structural analogue of hypoxanthine and a potent inhibitor of the enzyme xanthine oxidase that catalyses the transformation of hypoxanthine to xanthine and uric acid [5,6]. Allopurinol is used primarily in the treatment of hyperuricemia and gout [7]. Besides its hypouricemic effects, allopurinol has been studied for several other indications including treatment of seizures, psychiatric disorders, ischemia-reperfusion injury, protozoal diseases, and as a measure of liver impairment [8-12]. Allopurinol may cause a reduction of the serum levels of uric acid, probably leading to accumulation of purines (for instance, adenosine), which may explain its beneficial anticonvulsant and antipsychotic effects [13-18]. Of note, positive effects of allopurinol in refractory epilepsy [13], aggressive behaviour [16,17], mania [18], and schizophrenia [19,20] have been suggested to be secondary to its inhibitory effect on purine degradation, enhancing adenosinergic activity, despite the lack of direct data to support this hypothesis. Notably, previous studies have shown that allopurinol produces dose-dependent antinociceptive effects against several chemical and thermal pain models in rodents [21,22].

We hypothesized that the inhibition of xanthine oxidase by allopurinol, thereby reducing purine degradation, could be a valid strategy to enhance purinergic activity and cause analgesic

and anxiolytic effects. Given the pivotal role of pain and anxiety as a stress factor in the postoperative period and based on the considerations above, the present study aimed to evaluate the influence of allopurinol as a premedication on postoperative pain and anxiety in women undergoing abdominal hysterectomy. We also compared pre- and postanaesthesia sedation, hemodynamic status, adverse events during surgery and on emergence, and consumption of anaesthetics.

2. Methods

2.1. Ethics, study design, and population

A prospective, randomized, clinical trial of adult patients was performed in a tertiary care hospital in South Brazil. The protocol was evaluated and approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre (HCPA/UFRGS - CAAE #56000100009; Brazilian Registry of Clinical Trials – ReBEC #RBR-9pw58p; Universal Trial Number U1111-1212-5459). Participants received a written and an oral explanation of the study and signed an informed consent form. All ethical guidelines for the protection of human subjects were followed throughout, including the Declaration of Helsinki and its amendments.

A total of sixty-six female patients were considered eligible and sixty-two were subsequently enrolled into the study, with American Society of Anesthesiologists (ASA) status I–II, and ages ranging from 18 to 65 years old. Exclusion criteria include the illiterate or who does not understand the Portuguese language, those with history of psychiatric or neurological symptoms, those with contra-indications for regional anesthesia, who refused to participate of the study or who had already participated in other studies. In total, fifty-four patients satisfactorily completed the study (27 in the allopurinol group and 27 in the placebo group). Eight patients were excluded, four from the allopurinol group and four from the placebo group. In two cases, surgery was cancelled. In two patients, regional anesthesia was not applied. One patient was excluded after receiving benzodiazepines as a sedative agent during surgery. In three patients, a different anesthetic or surgical procedure was performed. Figure 1 describes a CONSORT flow diagram for the present study.

2.2. Anesthetic management

After admission to the operating room, they were monitored using pulse oximetry, non-invasive arterial pressure monitor, 5-lead electrocardiogram, and continuous ST segment analysis in a multiparametric monitor (Siemens monitor SC 9000 Infinity XL, Germany). Peripheral venous puncture was obtained in the upper limb with a 14 or 16G catheter. The anesthetic procedure was standardized, following the administration of intravenous fentanyl (50 to 100 μg), all patients received a combined spinal injection of hyperbaric bupivacaine 0.5% (15 to 20 mg), fentanyl 20 μg and morphine 100 μg, at the level of L_3/L_4. A continuous infusion of propofol (0.05 to 0.1 $mg.kg^{-1}.min^{-1}$) was administered to maintain sedation during surgery. Intra-operative variables, including the length of surgery, blood loss, anesthetic and surgical complications were noted. At the end of the procedure, propofol sedation was stopped and the patient eventually discharged to the postanesthesia care unit (PACU).

2.3. Outcome and study interventions

The main outcome measure used in this study was postoperative pain as assessed by pain scores. Randomization was performed according to a computer-generated random list. Patients were allocated in a double-blind manner, using random numbers, to receive either oral allopurinol 300 mg or placebo the night before surgery (10 pm) and 1 h prior to the start of spinal anesthesia. No other preoperative analgesic or sedative medication was given. Blinding and randomization were undertaken by one investigator not involved in patient evaluation. Other individuals involved in the patients' care were unaware of which treatment group the patient was in.

2.4. Assessment of outcome

Patients enrolled were asked to report any pain in four self-assessment instruments – a verbal pain scale (VPS), a visual analogue pain scale (VAS), a numerical pain scale (NPS) and the McGill pain questionnaire. In the first one, the reported pain was graded from 1 to 4, according to intensity: (1) none, (2) slight, (3) moderate, or (4) severe. VAS is widely used as a measure of self-reported pain assessment. The scale consists of a 100-mm line that pictorially represents a continuum between two extremes: no pain (score of 0) and extreme pain (score of 100). The use of the VAS was explained to the patient the night before surgery. In order to stratify the data of VAS, cut-off points were established from percentiles 25, 50 and 75 of the measures, corresponding to

0.1, 1.8 and 6.9 cm. Based on these cut-off points and the methodological strategy used by Collins et al. [23], absence of pain corresponded to the range from zero to percentile 25 (0.1 cm); mild pain corresponded to the range from the first percentile to the median (0.2 to 1.8 cm); moderate pain corresponded to the range from the median to percentile 75 (1.9 to 6.9 cm), and intense pain corresponded to the scores above the second percentile (7.0 to 10 cm). For the numerical scale, patients were asked to report their pain in numbers ranging from zero (no pain) to 10 (extreme pain). Finally, The McGill Questionnaire [24], adapted to Brazilian Portuguese [25], was used to measure the multidimensional pain experience (sensory, affective and evaluative dimensions).

The measurement of anxiety levels was performed through the State-Trait Anxiety Inventory for Adults (STAI), adapted to Brazilian Portuguese [26,27], during the pre- and postoperative periods. The questionnaire contains two separate 20-item, self-report rating scales for measuring trait- and state-anxiety. Patients responded on a three or four-point scale. Total scores for situational and baseline questions separately range from 20 to 60 or 80, with higher scores denoting higher levels of anxiety. In the postoperative period, the application of the STAI was restricted to items referring to the state of anxiety. According to previous findings [28,29], the mean of anxiety scale scores was used to determine the cut-off point, so that individuals with scores above the average were classified as the high anxiety group and those with scores equal to or below the average as the low anxiety group.

Patients were randomized for premedication in two groups: group 1- Allopurinol 300 mg p.o. the night before surgery (10 pm) and 1 h prior to the spinal anesthesia; group 2- Placebo p.o. the night before surgery (10 pm) and 1 h prior to the spinal anesthesia. In order to apply the pain and anxiety scales in the postoperative period, the patient had to be fully conscious and oriented as to time and space (two points in the consciousness item of the Aldrete and Kroulik's scale). Patients were submitted to evaluation of pain sensitivity and anxiety before the treatment (baseline), for 24 hours postoperatively (time-points were: 2h, 6h, 12h and 24h after surgery), 30 and 90 days after surgery. The analgesic schedule followed the routine of the PACU, which briefly consisted in a combination of oral paracetamol (750 mg) and intravenous dipyrone (1 g) each 6h after surgery and rescue intravenous morphine as needed.

We also evaluated hemodynamic parameters during and after anesthesia (heart rate, arterial pressure, end tidal CO_2, pulse oximetry), levels of postoperative sedation, consumption of anesthetics and analgesics, adverse effects on emergence or in the PACU (hypoxemia, bradycardia,

nausea, vomiting, hypothermia, urinary retention, shivering or hallucination). The extent of the surgical procedure, length of anesthesia and surgery, analgesic block, doses of opioids, and recovery time were also recorded.

2.5. Cerebrospinal fluid (CSF) sampling

Patients scheduled for elective abdominal hysterectomy received a spinal anesthesia technique. The CSF was collected by experienced anesthesiologists. The CSF samples were inspected visually and discarded if blood contamination was present. A total of 0.5 mL of CSF was collected from the patients after successful subarachnoid puncture and before the intrathecal injection of anesthetics. All samples were centrifuged at 10,000 g in an Eppendorf centrifuge during 5 min to obtain cell-free supernatants, stored at -70 ^{o}C within 30 min of collection, and not thawed until laboratory evaluations.

HPLC procedure: High-performance liquid chromatography (HPLC) was performed with CSF cell-free supernatants aliquots for determination of purines concentration, according to Domanski et al. [30], CSF concentrations of the following purines were determined: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine, guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), guanosine, inosine monophosphate (IMP), inosine, hypoxanthine, xanthine, and uric acid. Analyses were performed with Shimadzu Class-VP chromatography system consisting of a quaternary gradient pump with vacuum degassing and piston desalting modules, Shimadzu SIL-10AF auto injector valve with 50 μL loop, and an UV detector. Separations were achieved on a Supelco C18 250 mm x 4.6 mm, 5 μm particle size column. The mobile phase flowed at a rate of 1.2 mL/min^{-1} and the column temperature was 24 ^{o}C. Buffer composition remained unchanged (A: 150 mmol/L^{-1} phosphate buffer, pH 6.0, containing 150 mmol/L potassium chloride; B: 15% acetonitrile in buffer A). The gradient profile was modified to the following content of buffer B in the mobile phase: 0% at 0.00 min, 2% at 0.05 min, 7% at 2.45 min, 50% at 10.00 min, 100% at 11.00 min, and 0% at 12.40 min. Samples of 10 μL were injected into the injection valve loop. Absorbance was read at 254 nm. CSF concentrations of purines are expressed as mean ± SEM in μM.

2.6. Statistical analysis

A power analysis was performed using postoperative pain as the primary outcome. The sample size was calculated so that a mean difference of 30 mm (VAS) between the groups would permit a type 1 error probability of $\alpha = 0.05$ (two-tailed test) with reduced pain scores in the intervention group (allopurinol), and a null hypothesis of $\beta = 0.10$. This indicated that 20 patients would have to be included in each group. A higher number of patients were included to allow more adequate control of potential confounding variables.

Data were stored in Excel software and analyzed by STATA 12.0. Numerical variables were given as mean ± standard error of the mean (SEM). Non-parametric data were expressed as median and interquartile ranges (25% and 75%). Data were submitted to the Shapiro-Wilk test for normality evaluation. Statistical analysis between groups was performed using an unpaired Student's *t*-test for parametric data. Non-parametric data were analyzed by using a two-sample Wilcoxon rank-sum test. Differences in proportions between studied groups and postoperative state-anxiety and pain scores were tested by means of Pearson's X^2 test with Yates continuity correction in univariate analysis (or by Fisher's exact test when the number of expected observations was five or less in at least one cell). Correlation analyses were performed using Pearson's or Spearman's rank sum correlation. To compare the two experimental groups in terms of pain changes, multivariate analysis of variance for repeated measures was used, with the treatment group as the grouping factor, time as the repeated measure and pre-operative state and trait-anxiety as covariates. Mean differences and 95% confidence intervals (95% CI) are provided whenever appropriate. All statistical tests were two-sided and $p < 0.05$ was considered for statistically significant differences.

3. Results:

The patient details are summarized in Table 1. The average age of patients was 48.5 years (SEM ± 1.7). There was no difference in demographic characteristics between groups. Taken together, the preoperative measurements of anxiety and pain scores before surgery showed no statistically significant difference between groups. Additionally, there was no difference in preoperative levels of sedation between groups (data not shown).

Perioperative anxiety scores and discharge time from PACU were not statistically different between groups (Tables 1 and 2). As depicted in Table 3, preoperative administration of allopurinol

was related to lower pain scores than placebo 2h after surgery in both NPS (mean difference of 2.08, 95% CI 0.48 to 3.68, $p = 0.012$) and VAS (mean difference of 1.89, 95% CI 0.30 to 3.50, $p = 0.021$). The analgesic effect of preoperative allopurinol was also demonstrated by the VPS ($X^2 = 8.85$, $p = 0.031$), but not by the McGill pain questionnaire ($p = 0.22$). Additionally, patients that received premedication with allopurinol requested less opioids postoperatively. The mean consumption of morphine in the first 2h after surgery was 3.4 (± 0.7) mg in the placebo group as compared to 1.8 (± 0.4) mg in the allopurinol group (mean difference of 1.6 mg, 95% CI 0.03 to 3.17, $p = 0.045$). The total mean consumption of morphine in the first 24h after surgery was 4.3 (± 0.8) mg in the placebo group as compared to 2.4 (± 0.5) mg in the allopurinol group (mean difference of 1.9 mg, 95% CI 0.14 to 3.74, $p = 0.035$). Notably, there were no differences between groups on perioperative adverse events, intraoperative consumption of anesthetics, perioperative hemodynamic parameters, incidence of nausea or vomiting, levels of postoperative sedation, and pain scores 30 and 90 days after surgery (data not shown).

Table 4 shows that CSF concentration of uric acid was significantly reduced immediately before surgery and after treatment with two p.o. doses of allopurinol 300 mg (mean difference of 6.43 mM, 95% CI 2.27 to 10.59, $p = 0.0037$). Additionally, we observed a significant increase in xanthine levels following allopurinol treatment as compared to placebo (mean difference of 1.22 mM, 95% CI 0.55 to 1.89, $p = 0.0008$). Conversely, oral administration of allopurinol did not affect CSF concentrations of ATP, ADP, AMP, GTP, GDP, GMP, IMP, hypoxanthine, guanosine, adenosine, and inosine.

4. Discussion:

In this study, oral administration of allopurinol before surgery was associated with a significant reduction in pain scores 2h after total abdominal hysterectomy in women. This study has also demonstrated that two doses of allopurinol (300 mg) in the last 12 h before surgery caused a significant increase in CSF levels of xanthine but decreased CSF concentration of uric acid. However, no differences were found between groups in anxiety scores after surgery.

Although allopurinol has been traditionally used in the treatment of gout and its related symptoms, only a few studies have investigated the effects of allopurinol *per se* on pain scores [31-34]. More recently, we and others demonstrated that allopurinol produced antinociceptive effects in several thermal and chemical pain models in rodents [21,22]. The rationale to administer

allopurinol for pain is derived from evidence in basic and clinical research on the purinergic system [35]. Purines and their derivatives have been considered important targets for the development of new drugs for pain management since the nucleoside adenosine and its analogues present antinociceptive effects at spinal, supraspinal and peripheral sites [2, 36-39]. Endogenous adenosine can be released in the central nervous system (CNS) and peripheral tissues, and the regulation of its levels by various pharmacological agents can alter pain processing through activation of adenosine A_1 receptors on neurons, and perhaps other receptors on adjacent structures [2]. Effects on inflammatory cells at peripheral sites [40] and on glia in the CNS [41,42] mediated by adenosine P1 receptors have also been reported, potentially producing indirect effects on pain transmission. Additionally, considering that adenine- and guanine-based purines closely interact in modulating several CNS functions [43], we have demonstrated that some guanine-based purines also produced consistent antinociceptive effects in several animal pain models [44,45]. Therefore, allopurinol, by inhibiting xanthine oxidase and production of uric acid, may produce accumulation of other purines (for instance adenosine and other guanine- and adenine-based purines), which may account for its analgesic properties.

The basic mechanism of action of allopurinol and its metabolite oxypurinol is inhibition of xanthine oxidase (they bind strongly to the reduced form of xanthine oxidase and inhibit the enzyme). This leads to a decrease in the systemic concentration of uric acid and an increase in the concentration of the precursors, hypoxanthine and xanthine [5]. In addition, hypoxanthine can be converted to inosine, IMP and consequently, to adenosine and guanosine [5]. Thus, the primary effect of both allopurinol and oxypurinol is inhibition of uric acid production, and the overall result is the inhibition of the metabolism of xanthine and hypoxanthine leading to greater salvage of these purines by their conversion to inosine, adenosine and guanosine. These findings, both in CNS and periphery, have been extensively demonstrated after systemic administration of allopurinol in several studies in animals and humans [21, 46-49]. Accordingly, in the present study, we demonstrated an important increase in the CSF concentrations of xanthine and a significant reduction in the CSF levels of uric acid (a difference of approximately 30% for both purines). In fact, remarkable suppression of CSF uric acid levels after allopurinol treatment has also been observed elsewhere [46,50,51]. Altogether, these findings may indicate that CSF adenosine and/or other purines accumulation might play a role in the antinociceptive action of allopurinol.

Previous data indicated that the activation of the opioid naloxone-sensitive pathway is unlikely to be involved in the antinociception caused by allopurinol, since naloxone did not affect allopurinol-induced analgesia [21,22]. However, the adenosine-receptor antagonists caffeine and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) prevented allopurinol-induced antinociception, indicating that A_1 adenosine-receptor and adenosine may be involved in these effects [21,22]. Importantly, there is no evidence that allopurinol presents any direct agonist or antagonist effect on adenosinergic receptors [5].

Although previous findings indicate a role for adenosine in allopurinol-induced antinociception [21,22], we cannot rule out the influence of other purines. This study also demonstrated a significant increase in the CSF concentration of xanthine. Our group and others [43, 52-54] have demonstrated that the nucleosides guanosine and adenosine closely interact in the CNS. Additionally, we have proposed a specific guanine-based purinergic system with relevant physiological and pathological implications to the CNS, in addition to the well-characterized adenine-based purinergic system [45]. Of note, we have demonstrated that guanosine, as well as adenosine, may modulate pain transmission [44]. Therefore, it is not possible to exclude at this time that other purines may also influence allopurinol-induced antinociception.

Allopurinol has been extensively used as an inhibitor of the enzyme xanthine oxidase [5]. Xanthine oxidase is a highly versatile flavoprotein enzyme that catalyzes the oxidative hydroxylation of purine substrates and generates reactive oxygen species (ROS) [55]. ROS have been proposed to contribute to and/or maintain conditions of chronic pain [56]. Some data have indicated that ROS may also modulate acute pain transmission [33]. Notably, there is overwhelming acceptance that xanthine oxidase activity is significantly increased in various pathological states, including some pain states [57]. Therefore, the inhibition of this enzymatic pathway may be beneficial for treating pain [58]. Additionally, the administration of allopurinol has been shown to decrease tissue injury following ischemia/reperfusion in a variety of *in vitro* and *in vivo* models [10,59]. Inkster et al. [34] showed that allopurinol treatment had marked beneficial effects on nerve and vascular function in diabetic rats. That same study also demonstrated that allopurinol attenuated diabetes-induced tactile allodynia, thermal and mechanical hyperalgesia. Therefore, it is tempting to propose that the reduction on xanthine oxidase activity and consequently on aspects of ROS might play a role in the postoperative analgesia induced by allopurinol.

Importantly, there were no differences between groups in the incidence of adverse events during the procedure, on emergence from anesthesia and after surgery in PACU. Additionally, no adverse effects over hemodynamic parameters were observed perioperatively. These findings confirm the well-known safety profile of allopurinol and points to a safe administration in the perioperative setting as a premedication in adult patients.

Notably, there are major limitations in the present study. This is a single center study with a small sample size, short-term and low-dose allopurinol treatment, and short follow-up after surgery. Additionally, the analgesic effect induced by premedication with allopurinol is somewhat weak and limited to the immediate postoperative period. New clinical trials are still necessary to determine if allopurinol and other xanthine oxidase inhibitors are effective in reducing pain scores in other clinical settings. These studies should include larger samples and longer follow-up to better determine the impact of premedication with purine derivatives on pivotal postoperative outcomes such as pain and anxiety.

5. Conclusions:

In summary, this study has demonstrated that premedication with allopurinol is associated with mild and short-term analgesic effects after surgery. Allopurinol-induced analgesia may be related to a CNS accumulation of endogenous adenine- or guanine-based purines. Although it is early to propose the use of purines in clinical research, an interesting approach to investigate their role clinically is the investigation of purine derivatives already used in humans such as allopurinol or more selective xanthine-oxidase inhibitors. Allopurinol is an old and extensively used compound and seems to be well tolerated with no obvious CNS toxic effects even in high doses. Therefore, this drug and other more selective xanthine-oxidase inhibitors may be useful in the management of acute and chronic pain in humans.

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Table 1: Demographic data

Characteristics	Groups (treatment)		*p*
	Placebo	Allopurinol	
Age (years)	49.9(1.7)	50.6(1.7)	0.76
Weight (kg)	66.8(2.2)	70.3(2.7)	0.35
BMI (kg/m^2)	27(1.2)	28.5(1)	0.34
Hypertension (n - %)	9(33)	12(44)	0.40
Diabetes type 2 (n - %)	3(11)	1(4)	0.30
Active Smoking (n - %)	4(15)	6(22)	0.73
ASA status (n - %)			
I	6(22)	3(11)	
II	21(78)	24(89)	0.27
Duration of surgery (min)	145(7.2)	149(6.9)	0.71
Time to discharge from PACU (min)	210(13)	240(15)	0.25
Hospitalization after surgery (days)	3.1(0.2)	3.4(0.2)	0.23

Data are shown as mean (± SEM) or absolute values (percentiles). $p < 0.05$ was considered statistically significant; Student *t* test for parametric data; Wilcoxon rank-sum test for non-parametric data; Pearson's X^2 test or Fisher's exact test for categorical data (n = 27 per group). BMI: Body mass index.

Table 2: Comparison of main outcomes between groups – anxiety scores

Time point	Groups (treatment – STAI scores)		*p*
	Placebo	Allopurinol	
Baseline (trait)	37.9 (2.5)	39.8 (2.4)	0.61
Baseline (state)	38.1 (1.7)	37.6 (1.6)	0.85
2h after surgery	38.0 (1.5)	37.8 (1.7)	0.93
6h after surgery	36.0 (1.4)	36.1 (1.3)	0.99
12h after surgery	34.1 (1.5)	34.3 (0.9)	0.87
24h after surgery	34.8 (1.5)	34.0 (0.7)	0.72

Data are shown as mean (± SEM). $p < 0.05$ was considered statistically significant, Student *t* test for parametric data, Wilcoxon rank-sum test for non-parametric data; (n = 27 per group). STAI: State-trait anxiety inventory.

Table 3: Comparison of main outcomes between groups – pain scores

Pain scale	Groups (treatment)					
	Placebo			Allopurinol		
	Baseline	2 h	6 h	Baseline	2 h	6 h
VPS – n (%)						
No pain	21(78)	4(15)*	9(33)	22(81)	8(30)*	14(52)
Mild pain	6(22)	8(30)*	10(37)	5(23)	14(52)*	8(30)
Moderate pain	0(0)	12(44)*	6(22)	0(0)	5(18)*	5(18)
Severe pain	0(0)	3(11)*	2(8)	0(0)	0(0)*	0(0)
VAS – mean (±SEM)	0.95(0.33)	4.3(0.63)*	2.5(0.59)	1.1(0.29)	2.4(0.43)*	2.24(0.5)
NPS – mean (±SEM)	0.62(0.32)	4.84(0.62)*	3.1(0.6)	0.5(0.25)	2.8(0.46)*	2.1(0.47)
McGill – mean (±SEM)	4.25(2.1)	18.8(2.7)	12.2(2.7)	5.0(2.4)	14.6(2.9)	11.6(2.7)

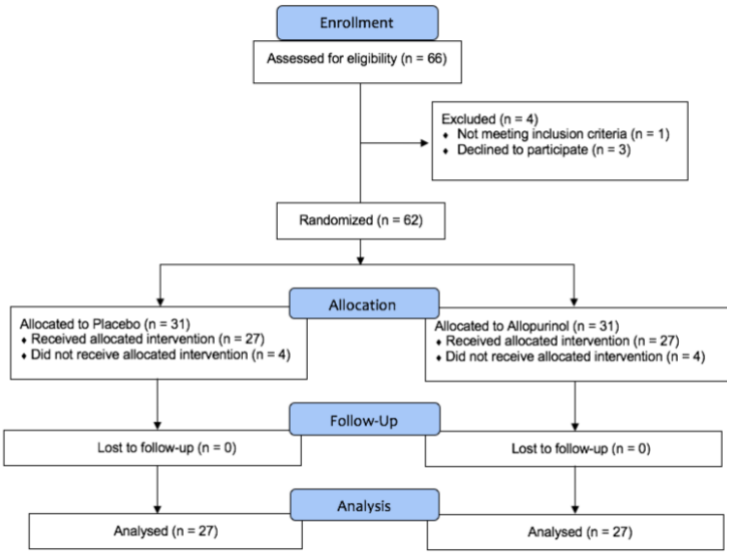
Data are shown as mean (± SEM) for VPS or absolute values (percentiles) for VAS, NPS, and McGill. $p < 0.05$ was considered statistically significant, Student *t* test for parametric data, Wilcoxon rank-sum test for non-parametric data, X^2 test for categorical data; (n = 27 per group). *$p < 0.05$ between groups. VPS: Verbal pain scale; VAS: Visual-analogue pain scale; NPS: Numerical pain scale; McGill: Modified McGill pain questionnaire.

Table 4: Effects of p.o. allopurinol (600 mg) or placebo on CSF purines concentration.

CSF purines concentration (µM)	Groups (treatment)	
	Placebo	Allopurinol
GTP	ND	ND
GDP	ND	ND
GMP	ND	ND
IMP	0.007 (0.004)	0.009 (0.006)
ATP	ND	ND
ADP	0.15 (0.02)	0.18 (0.02)
AMP	0.45 (0.27)	0.39 (0.22)
Inosine	0.8 (0.09)	0.77 (0.1)
Guanosine	0.004 (0.001)	0.006 (0.004)
Adenosine	0.43 (0.07)	0.29 (0.04)
Hypoxanthine	4.05 (0.22)	4.5 (0.34)
Xanthine	3.08 (0.2)*	4.3 (0.23)*
Uric acid	20.7 (1.49)*	14.2 (1.28)*

Data are shown as mean (± SEM). $p < 0.05$ was considered statistically significant, Student *t* test for parametric data, Wilcoxon rank-sum test for non-parametric data; *$p < 0.01$; (n = 27 patients per group). GTP: guanosine-triphosphate; GDP: guanosine-diphosphate; GMP: guanosine-monophosphate; IMP: inosine-monophosphate; ATP: adenosine-triphosphate; ADP: adenosine-diphosphate; AMP: adenosine-monophosphate; ND: non-detected.

Figure 1 (CONSORT flow diagram for the study):



II.3. ARTIGO 3

Effects of allopurinol on pain and anxiety in fibromyalgia patients: a pilot study.

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Abstract

Allopurinol is a potent inhibitor of the enzyme xanthine oxidase used in the treatment of hyperuricemia and gout. The aim of this pilot study was to investigate the effects of allopurinol on pain and anxiety in women displaying fibromyalgia refractory to conventional therapy. This prospective case series enrolled 12 women with previous diagnosis of fibromyalgia refractory to conventional therapy. Patients received an add-on therapy with oral allopurinol 300 mg twice daily for 30 days. Patients were submitted to evaluation for pain and anxiety scores before treatment, 15 and 30 days thereafter. This pilot study has demonstrated that oral administration of allopurinol 300 mg twice daily caused a significant reduction on pain scores up to 30 days of treatment in women with fibromyalgia. No effect was observed regarding anxiety scores. Randomized clinical trials are warranted and should further investigate allopurinol and more selective purine derivatives in the management of acute or chronic pain conditions.

Keywords: allopurinol; chronic pain; fibromyalgia; anxiety; purines.

Introduction

Fibromyalgia is a chronic syndrome characterized by widespread pain and tenderness, accompanied by disturbed sleep, chronic fatigue, cognitive dysfunction, depressive symptoms, and multiple additional functional disturbances. Fibromyalgia usually requires a multimodal therapeutic approach to optimize treatment efficacy, and its management is still challenging since many patients fail to achieve sufficient relief from conventional treatments.[1]

It is well known that purines exert multiple effects on pain transmission. Adenosine and its analogs are involved in multiple biological effects, including modulation of pain transmission at peripheral and central sites.[2] The purine derivative allopurinol is a potent inhibitor of the enzyme xanthine oxidase used primarily in the treatment of hyperuricemia and gout. Notably, previous studies have shown that allopurinol produces dose-dependent antinociceptive effects against several chemical and thermal pain models in rodents.[3] We hypothesized that the inhibition of xanthine oxidase by allopurinol, thereby reducing purine degradation, could be a valid strategy to enhance purinergic activity and treat pain in humans. The aim of this pilot study was to investigate an add-on therapy with oral allopurinol on pain and anxiety in women displaying fibromyalgia refractory to conventional therapy.

Material and methods

A prospective case series was performed in a tertiary care hospital in South Brazil. The protocol was designed to be a pilot study for a larger clinical trial and was approved by the Institution's Research and Ethics Committee (HCPA/UFRGS – CAAE #229000100007; Brazilian Registry of Clinical Trials – ReBEC #RBR-8h7dmq). A total of twelve female patients were enrolled into the study, with American Society of Anesthesiologists (ASA) physical status I–II, and ages ranging from 18 to 65 years old, being excluded the illiterate or who does not understand Portuguese language, those who refused to participate of the study or who had already participated in other studies. Participants received a written and oral explanation of the study and signed an informed consent form. Patients were allocated to receive oral allopurinol 300 mg twice daily for 30 days following initial evaluation. No other medication was added during the follow-up. All patients were maintained on their current medications protocol and no additional changes in the dosage regimen were allowed during follow-up, except for the use of rescue analgesics. Patients

were evaluated for primary and secondary outcomes at baseline, 15 and 30 days after enrollment. The description of the present study was based on CARE guidelines.

Patients were asked to report any pain in four self-assessment instruments – a verbal scale (VPS), a visual analogue scale (VAS), a numerical scale (NPS) and the McGill modified questionnaire, described in detail elsewhere.[4] In the first one, the reported pain was graded from 1 to 4, according to intensity: (1) none, (2) mild, (3) moderate, or (4) severe. VAS is widely used as a measure of self-reported pain assessment. The scale consists of a 100-mm line that pictorially represents a continuum between two extremes: no pain (score of 0) and extreme pain (score of 100). In order to stratify the data of VAS, cutoff points were established considering previous literature,[4] with moderate to severe pain corresponding to scores above 30 mm.[4] For NPS, patients were asked to report their pain in numbers ranging from zero (no pain) to 10 (extreme pain). Finally, The McGill questionnaire was used to measure the multidimensional pain experience (sensory, affective, and evaluative dimensions).[4]

The measurement of anxiety levels was performed through the State-Trait Anxiety Inventory for Adults (STAI).[4] The questionnaire contains two separate 20-item, self-report rating scales for measuring trait- and state-anxiety. Total scores for situational and baseline questions separately range from 20 to 60 or 80, with higher scores denoting higher levels of anxiety. Mean of anxiety scores at baseline was used to determine the cutoff point, so that individuals with scores above the average were classified as the high anxiety group and those with scores equal to or below the average as the low anxiety group.[4]

Data were stored in Excel software and analyzed by STATA 12.0. Numerical variables were given as mean ± standard deviation (SD). Data were submitted to Shapiro-Wilk test for normality evaluation. Statistical analysis between time points was performed using one-way ANOVA followed by Bonferroni's multiple comparison test for numerical data and Pearson's X^2 test for categorical data; $p < 0.05$ was considered for statistically significant differences.

Results

As depicted in Table 1, allopurinol caused a significant reduction in pain scores measured by verbal, numerical and visual analogue pain scales both 15 and 30 days after treatment ($p < 0.05$). No significant effects were observed on pain scores measured by the McGill questionnaire (Table 1). As shown in Table 2, we did not detect any significant difference in both trait and state anxiety

scores after 15 and 30 days of treatment ($p > 0.05$). Notably, no significant adverse events were observed following a 30-day trial of allopurinol in the present population and there were no dropouts.

Discussion

The rationale to administer allopurinol for pain and anxiety is derived from evidence in basic and clinical research on the purinergic system. Adenine-based purines have been considered important targets for the development of new drugs for pain management and the treatment of several neuropsychological disorders.[2,3] Endogenous adenosine can be released in the central nervous system and peripheral tissues, and the regulation of its levels by various pharmacological agents can alter pain processing through activation of adenosine A_1 receptors on neurons, and perhaps other receptors on adjacent structures.[2] We also have demonstrated that some guanine-based purines, especially the nucleoside guanosine, produced consistent antinociceptive and anxiolytic effects in several animal models.[5] Although adenine- or guanine-based purines have been related to some antinociceptive effects in both animals and humans,[5] it is relatively early to propose the use of most purine derivatives for clinical research and practice.

The main contribution of the present case series is to propose an alternative approach to investigate the clinical role of purines, focusing on the investigation of purine derivatives previously used in humans such as the xanthine-oxidase inhibitor allopurinol. Therefore, allopurinol, by inhibiting xanthine oxidase and production of uric acid, may cause accumulation of other purines in the central nervous system and in the periphery (for instance, adenosine and other nucleosides and nucleotides), which may account for potential analgesic and other neuromodulatory properties. The primary effect of allopurinol is inhibition of uric acid production, and the overall result is the inhibition of the metabolism of xanthine and hypoxanthine, leading to greater salvage of these purines by their conversion to inosine, adenosine, and guanosine. These findings, both in the central nervous system and periphery, have been extensively demonstrated after systemic administration of allopurinol in several studies in animals and humans.[3,5]

There are major limitations in the present study. First, this is a pilot study for a larger clinical trial and only few cases were investigated. Notably, there was no control group and patients were solely evaluated as compared to their baseline. Therefore, a placebo effect could have played a major role in the present findings and a randomized clinical trial is pivotal to further evaluate an

intrinsic analgesic effect of allopurinol in humans displaying chronic pain syndromes. We are currently carrying out a single-center, randomized, double-blinded, placebo-controlled clinical trial investigating allopurinol as an adjuvant therapy in women with refractory fibromyalgia pain.

In summary, this study has demonstrated that oral administration of the xanthine oxidase inhibitor allopurinol 300 mg twice daily caused a significant reduction on pain scores after 15 and 30 days of treatment in women with fibromyalgia. No benefit was observed against both trait and state anxiety scores. Considering that previous studies have shown some beneficial effects of some purines against pain in animals and humans, new clinical trials are still warranted to determine if allopurinol is effective in reducing pain in other clinical settings. These studies should include larger samples and longer follow-up to better determine the impact of allopurinol and perhaps more selective xanthine-oxidase inhibitors on pain scores in humans displaying acute or chronic pain.

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Table 1: Comparison among main outcomes between time-points – pain scores.

Variables	Treatment time-points			*p**
	Baseline	15 days	30 days	
VPS (n – %)				0.039
None	0 (0%)	2 (17%)	3 (25%)	
Mild	0 (0%)	3 (25%)	4 (33%)	
Moderate	2 (17%)	3 (25%)	3 (25%)	
Severe	10 (83%)	4 (33%)	2 (17%)	
NPS (mean ± SD)	7.7 (1.4)	5.0 (2.8)[#]	5.2 (2.8)[#]	0.016
VAS (mean ± SD)	7.5 (1.5)	4.6 (2.8)[#]	4.7 (2.9)[#]	0.010
VAS categories (n – %)				0.011
None–mild	0 (0%)	6 (50%)	6 (50%)	
Moderate–severe	12 (100%)	6 (50%)	6 (50%)	
McGill (mean ± SD)	41.5 (14)	34.3 (17)	34.6 (19)	0.508

Data are shown as absolute values (percentiles) or as mean scores (standard deviation – SD). One-way ANOVA followed by Bonferroni's multiple comparison test for numerical data and Pearson's X^2 test for categorical data. *$p < 0.05$ was considered significant. [#]$p < 0.01$ as compared with baseline scores. (n = 12 patients). VPS, Verbal Pain Scale; NPS, Numerical Pain Scale; VAS, Visual Analogue Pain Scale; McGill, McGill modified pain questionnaire.

Table 2: Comparison among main outcomes between time-points – anxiety scores.

Variables	Treatment time-points			*p**
	Baseline	15 days	30 days	
STAI trait anxiety (n - %)				0.89
Low levels	5 (42%)	5 (42%)	4 (33%)	
High levels	7 (58%)	7 (58%)	8 (67%)	
Mean scores (mean ± SD)	55.7 (11)	53.8 (10)	55.1 (9)	0.82
STAI state anxiety (n - %)				0.46
Low levels	6 (50%)	5 (42%)	8 (67%)	
High levels	6 (50%)	7 (58%)	4 (33%)	
Mean scores (mean ± SD)	52.8 (13)	52.7 (12)	50.3 (12)	0.90

Data are shown as absolute values (percentiles) or as mean (standard deviation – SD). One-way ANOVA followed by Bonferroni's multiple comparison test for numerical data and Pearson's X^2 test for categorical data. *$p < 0.05$ was considered significant. (n = 12 patients). STAIC, State-Trait Anxiety Inventory.

II.4. ARTIGO 4

Allopurinol for fibromyalgia pain in adults: a randomized controlled trial.

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Significance: Previous evidence has indicated that allopurinol could display some intrinsic analgesic effects and could be an antinociceptive drug against acute and chronic pain syndromes. However, our randomized controlled clinical trial has shown that allopurinol is not effective in the management of fibromyalgia pain and other fibromyalgia-related outcomes such as anxiety and depression.

Abstract:

Background: Allopurinol is a potent inhibitor of the enzyme xanthine oxidase used in the treatment of hyperuricemia and gout. Since it is well known that purines exert multiple effects on pain transmission, we hypothesized that the inhibition of xanthine oxidase by allopurinol could be a valid strategy to treat pain in humans. This study aimed to compare the analgesic efficacy of oral allopurinol versus placebo as an adjuvant therapy in patients displaying fibromyalgia.

Methods: This randomized, double-blinded, placebo-controlled study included 60 women with diagnosis of fibromyalgia. Patients were randomly assigned to receive either oral allopurinol 300 mg (n = 31) or placebo (n = 29) twice daily during 30 days. The patients were submitted to evaluation for pain sensitivity, anxiety, depression and functional status before treatment, 15 and 30 days thereafter.

Results: Oral administration of allopurinol 300 mg twice daily was ineffective in improving pain scores measured by several tools up to 30 days of treatment ($p > 0.05$). Additionally, no significant effects of allopurinol over anxiety, depressive symptoms, and functional status of fibromyalgia patients were observed in the present study.

Conclusions: Although previous findings indicated that allopurinol could present intrinsic analgesic effects in both animals and humans, this study showed no benefit of the use of oral allopurinol as an adjuvant strategy during 30 days in women displaying fibromyalgia. However, considering previous promising results, new prospective studies are still valid to further investigate allopurinol and more selective purine derivatives in the management of pain syndromes.

Keywords: allopurinol; purines; pain; fibromyalgia; xanthine oxidase; anxiety; depression.

Introduction

Fibromyalgia is a chronic syndrome characterized by widespread pain and tenderness, accompanied by disturbed sleep, chronic fatigue, cognitive dysfunction, depressive symptoms and multiple additional functional disturbances (1,2). Fibromyalgia usually requires a multimodal therapeutic approach to optimize treatment efficacy, since no single medication seems to be capable of offering substantial efficacy against the characteristic symptoms of the disease (3). Unfortunately, few improvements in the overall health of fibromyalgia patients treated with drugs targeting a range of underlying mechanisms have been observed (4,5). Treatment of fibromyalgia is still challenging and continues to warrant new pharmacological and non-pharmacological strategies since many patients fail to achieve sufficient relief from conventional treatments (6).

The purinergic system involves adenosine and ATP as major endogenous effectors, acting on P1 and P2 receptors, respectively (7,8). It is well known that adenosine is involved in multiple biological effects, including modulation of pain at peripheral and central sites (9). Adenosine regulates pain processing at the spinal cord and periphery and displays complex effects in pain, being either pro- or antinociceptive, depending on the site of administration and the receptor subtype activated (10). Most studies indicate that endogenous adenosine causes analgesia because of the combination of an A_1 receptor-mediated antinociception and an A_{2A} receptor-mediated anti-inflammatory activity (11,12). Interestingly, adenosine has been related to antinociceptive effects in several pain paradigms (13) and intravenous or spinal administration of adenosine has shown to be analgesic in humans (15-17).

Allopurinol, or 1,5-dihydro-4*H*-pyrazolo(3,4-*d*)pyrimidin-4-one, is a structural analogue of hypoxanthine and a potent inhibitor of the enzyme xanthine oxidase that catalyzes the transformation of hypoxanthine to xanthine and uric acid, reducing both uric acid formation and purine degradation (18,19). Allopurinol is used primarily in the treatment of hyperuricemia and gout (20). Besides its hypouricemic effects, allopurinol has been studied for several other indications including treatment of seizures, psychiatric disorders and ischemia-reperfusion injury (21-25). Both healthy and hyperuricemic patients present reduction of uric acid levels following oral administration of allopurinol, probably leading to accumulation of purines, including the neuromodulator adenosine, which may explain its beneficial anticonvulsant and antipsychotic effects (26-33). Notably, previous studies have shown that allopurinol produces dose-dependent antinociceptive effects against several chemical and thermal pain models in rodents (34,35).

We hypothesized that the inhibition of xanthine oxidase by allopurinol, thereby reducing purine degradation, could be a valid strategy to enhance purinergic activity and cause analgesic effects in patients displaying chronic pain, which is in line with the anticonvulsant and neuropsychiatric effects observed with allopurinol treatment. The aims of the present study were to evaluate the influence of oral allopurinol compared to placebo as an add-on therapy in women displaying fibromyalgia pain. We also compared the effects of allopurinol on anxiety scores, depressive symptoms and functional status of fibromyalgia patients.

Methods

Ethics, study design and population

A prospective, randomized, clinical trial of adult women was performed in a tertiary care hospital in South Brazil. The protocol was evaluated and approved by the Institutional Review Board and Ethical Committee of Hospital de Clínicas de Porto Alegre (HCPA/UFRGS - CAAE #229000100007; Universal Trial Number U1111-1212-5540; Brazilian Registry of Clinical Trials – ReBEC #RBR-8h7dmq). Participants received a written and an oral explanation of the study and signed an informed consent form.

A total of seventy-eight female patients were initially considered eligible and sixty-six women subsequently enrolled into the study, with American Society of Anesthesiologists (ASA) status I–II, and ages ranging from 18 to 65 years old, being excluded the illiterate or who does not understand Portuguese language, those who refused to participate of the study or who had already participated in other studies. Patients were considered eligible to the study according to the American College of Rheumatology criteria for fibromyalgia diagnosis (36), relying on a Symptom Severity Scale which was used to quantify the fibromyalgia-related symptom severity before enrollment. All patients were maintained at their current medications protocol during the entire study, which consisted essentially in a combination of pharmacological (gabapentinoid drugs associated with tricyclic antidepressants or serotonin-noradrenaline reuptake inhibitors) and non-pharmacological treatment (acupuncture). No changes in current medications dosage or additional therapies were allowed during the follow-up of 30 days, except for the use of rescue analgesics. The analgesic schedule followed the institutional routine of institutional ambulatory and all rescue medications were registered for further analysis.

In total, sixty patients satisfactorily completed the study (31 in the allopurinol group and 29

in the placebo group). Six patients were excluded, two from the allopurinol group and four from the placebo group. In three patients, fibromyalgia treatment was initially modified not following the study's protocol. Three patients in both groups missed follow-up consultations and were ultimately excluded from final analysis. Figure 1 describes a CONSORT flow diagram for the present study.

Outcome and study interventions

The main outcome measure used in this study was pain as assessed by visual analogue scale. Randomization was performed according to a computer-generated random list. Patients were allocated in a double-blind manner, using random numbers, to receive either oral allopurinol 300 mg or placebo twice daily for 30 days following initial evaluation. No other medication was added during the follow-up. Blinding and randomization were undertaken by one investigator not involved in patient evaluation. Other individuals involved in the patients' care were unaware of which treatment group the patient was in.

Assessment of outcome

Patients enrolled were asked to report any pain in four self-assessment instruments – a verbal scale (VPS), a visual analogue scale (VAS), a numerical scale (NPS) and the McGill modified questionnaire. In the first one, the reported pain was graded from 1 to 4, according to intensity: (1) none, (2) slight, (3) moderate, or (4) severe. The Visual Analogue Scale (VAS) is widely used as a measure of self-reported pain assessment. The scale consists of a 100-mm line that pictorially represents a continuum between two extremes: no pain (score of 0) and extreme pain (score of 100). The use of the VAS was explained to the patient at the baseline evaluation. In order to stratify the data of VAS, cut-off points were established from percentiles 25, 50 and 75 of the measures, corresponding to 0.1, 1.8 and 6.9 cm. Based on these cut-off points, the clinical significance of the values and the methodological strategy used by Collins et al. (37), absence of pain corresponded to the range from zero to percentile 25 (0.1 cm); mild pain corresponded to the range from the first percentile to the median (0.2 to 1.8 cm); moderate pain corresponded to the range from the median to percentile 75 (1.9 to 6.9 cm), and intense pain corresponded to the scores above the second percentile (7.0 to 10 cm). For the numerical scale, patients were asked to report their pain in numbers ranging from zero (no pain) to 10 (extreme pain). Finally, The McGill

Questionnaire (38), adapted to Brazilian Portuguese (39), was used to measure the multidimensional pain experience (sensory, affective and evaluative dimensions).

The measurement of anxiety levels was performed through the State-Trait Anxiety Inventory for Adults (STAI), adapted to Brazilian Portuguese (40), during all evaluation time points. The questionnaire contains two separate 20-item, self-report rating scales for measuring trait- and state-anxiety. Patients responded on a three or four-point scale. Total scores for situational and baseline questions separately range from 20 to 60 or 80, with higher scores denoting higher levels of anxiety. In all time points, the application of the STAI questions referred to both trait- and state-anxiety. According to previous findings (41), the mean of anxiety scale scores at baseline was used to determine the cut-off point, so that individuals with scores above the average were classified as the high anxiety group and those with scores equal to or below the average as the low anxiety group.

Additionally, we applied the Fibromyalgia Impact Questionnaire (FIQ), adapted to Portuguese (42), in order to evaluate the current health and functional status of women with the fibromyalgia syndrome. The Fibromyalgia Impact Questionnaire (FIQ) is a brief 10-item, self-administered instrument that measures physical functioning, work status, depression, anxiety, sleep, pain, stiffness, fatigue, and well-being (43).

The Montgomery-Åsberg Depression Rating Scale (MADRS), adapted to Brazilian Portuguese (44), was used to measure depressive symptoms. An intraclass correlation of 0.80 was observed for the agreement between different evaluators.

The Positive and Negative Affect Schedule (PANAS) questionnaire, adapted to Brazilian Portuguese (45), consists of 10 positive affects (PA), (interested, excited, strong, enthusiastic, proud, alert, inspired, determined, attentive and active) and 10 negative affects (NA), (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery and afraid). Participants are asked to rate items on a scale from 1 to 5, on the basis of the strength of emotion where “1 = very slightly or not at all” and “5 = extremely”. For both of the PANAS domains, scores can range between 10 and 50. A higher score on the positive domain indicates greater PA (e.g. they are happier). However, a higher score on the negative domain indicates greater NA (e.g. they are more depressed) (46).

In the first evaluation, each patient was submitted to the application of all tools before the treatment initiation. This stage allowed patients to get used to the pain and psychological scales

application technique. During this period the patient answered pain scales, psychological tools and a structured questionnaire to obtain data about demographic characteristics and chronic diseases. A visual analogue scale for patient satisfaction with treatment was also applied in all evaluation time-points. In order to minimize a possible influence of the word ‘‘pain’’ upon anxiety and depression scores, all tools were applied in different pseudo-random sequences.

Before evaluation, patients were randomized for treatment in two groups: group 1- Allopurinol 300 mg p.o. twice daily during 30 days; 2- Placebo p.o. twice daily during 30 days. Additional evaluations regarding pain and psychological parameters were performed 15 and 30 days after treatment initiation.

Statistical analysis

A power analysis was performed using post-treatment pain as the primary outcome measure. The sample size was calculated so that a mean difference of 30 mm (VAS) between the groups would permit a type 1 error probability of $\alpha = 0.05$ (two-tailed test) with reduced pain scores in the intervention group (allopurinol), and a null hypothesis of $\beta = 0.10$. This indicated that 20 patients would have to be included in each group. A higher number of patients were included to allow more adequate control of the potential confounding bias.

Data were stored in Excel software and analyzed by STATA 12.0. Numerical variables were given as mean ± standard deviation (SD). Data were submitted to Shapiro-Wilk test for normality evaluation. Statistical analysis between groups was performed using unpaired Student’s *t* test. Differences in proportions between studied groups and anxiety or pain scores were tested by means of Pearson’s X^2 test with Yates continuity correction in univariate analysis. To compare the two experimental groups (allopurinol and placebo) in terms of pain changes, two-way analysis of variance for repeated measures was used, with the treatment group as the grouping factor and time as the repeated measure. $p < 0.05$ was considered for statistically significant differences.

Results:

The patient details are summarized in Table 1. The average age of patients was 51.9 years (SD ± 8.2). There was no difference in demographic characteristics between groups. Taken together, the baseline measurements of anxiety, depression and pain scores before treatment showed no statistically significant difference between groups. Additionally, there was also no

difference in baseline score of functional status between groups (data not shown). Notably, there was a tendency for increased baseline pain scores in the allopurinol group as represented by VAS ($p = 0.05$), VPS ($p = 0.15$) and NPS ($p = 0.06$) pre-treatment evaluations.

As depicted in Table 2, we did not find a significant difference between groups in pain scores measured by verbal, numerical, visual analogue and McGill pain scales both 15 and 30 days after treatment ($p > 0.05$). Additionally, patients that received allopurinol did not request lower doses of rescue analgesics at all time points (data not shown). Figure 2 shows a comparison of pain scores measured by VAS and McGill scales considering the evolution of pain scores through time. Following a two-way analysis of variance for repeated measures, no statistically significant difference was found between treatments ($p = 0.31$ for VAS scores, $p = 0.45$ for NPS scores, and $p = 0.19$ for McGill scores). Stratification of VAS scores according to cut-off points in two main categories of pain (none to mild and moderate to severe pain) indicated similar results between groups ($p = 0.24$, $p = 0.57$ and $p = 0.50$ for baseline, 15 and 30 days, respectively).

As depicted in Table 3, we did not find a significant difference between groups in both trait and state anxiety levels after 30 days of treatment ($p > 0.05$). Stratification of STAI scores according to cut-off points for high and low levels of anxiety also indicated no difference between groups ($p = 0.09$, $p = 0.58$ and $p = 0.13$ for trait anxiety in baseline, 15 and 30 days, respectively; $p = 0.78$, $p = 0.07$ and $p = 0.40$ for state anxiety in baseline, 15 and 30 days, respectively). Additionally, Table 4 demonstrates scores for current health and functional status (FIQ), depressive symptoms (MADRS) and positive and negative affects (PANAS). Again, no significant difference was observed between groups after all variable comparisons ($p > 0.05$).

The index of satisfaction with allocated treatment was similar following allopurinol (VASS mean scores = 6.4 ± 5.8 and 6.2 ± 2.5, 15 and 30 days after treatment, respectively) and placebo (VASS mean scores = 4.5 ± 2.8 and 5.2 ± 2.5, 15 and 30 days after treatment, respectively) ($p = 0.11$ and $p = 0.12$ for VASS scores, 15 and 30 days, respectively). Notably, there were no differences between groups on the incidence of adverse events, consumption of other medications, incidence of nausea or vomiting, and levels of somnolence after 30 days of treatment (data not shown).

Discussion:

In this study, oral administration of the xanthine oxidase inhibitor allopurinol 300 mg twice daily did not affect pain scores measured by several tools after 15 and 30 days of treatment in women with fibromyalgia. Additionally, allopurinol, as compared to placebo, did not improve anxiety scores, affective parameters, depressive symptoms, overall health and functional status of patients with fibromyalgia. No significant adverse events after 30 days of treatment were observed in the present study.

The population sample studied was relatively homogenous as baseline characteristics such as gender, age, weight, psychological parameters and functional status were quite similar between both groups. Although not statistically significant, we observed a trend for higher baseline pain scores in the allopurinol group in three out of four pain scales (VAS, VPS and NPS). This surprising finding may have significantly affected results, since patients who are more refractory to treatment might have been allocated to the allopurinol group. Probably, the relatively small sample size was an important factor for this minor imbalance between groups. Of note, although statistically insignificant, repeated measures ANOVA indicated a trend of improvement in pain scores (measured by VAS and McGill) in the allopurinol group, something that deserves to be further clarified in a larger study in the future.

As high levels of state- or trait-anxiety may be a risk factor for increased pain levels (47,48), it is essential to evaluate anxiety scores before and after treatment. In this study, allopurinol did not significantly affect both trait and state anxiety. However, despite the fact that STAI is widely used (41) and has been validated for evaluating the level of anxiety in different situations and the response to anxiolytic drugs (40), to date there is no gold standard to measure anxiety in adults. It is possible that STAI is not able to accurately detect potential anxiolytic effects of allopurinol. Therefore, the actual effects of oral treatment with allopurinol on anxiety levels may be further clarified with additional tools, more prolonged treatment and larger follow-up in the future.

The rationale to administer allopurinol for pain is derived from evidence in basic and clinical research on the purinergic system. Adenine-based purines have been considered important targets for the development of new drugs for pain management, since the nucleoside adenosine and its analogues have demonstrated antinociceptive effects following both systemic and central administration (9,49). Endogenous adenosine can be released in the CNS and peripheral tissues,

and the regulation of its levels by various pharmacological agents can alter pain processing through activation of adenosine A_1 receptors on neurons, and perhaps other receptors on adjacent structures (9). Additional effects on inflammatory cells at peripheral sites (50) and on glia in the central nervous system (CNS) (51,52) mediated by adenosine P1 receptors have also been reported, potentially producing indirect effects on pain mechanisms. We also have demonstrated that some guanine-based purines, especially the nucleoside guanosine, produced consistent antinociceptive effects in several animal pain models (53). Although adenine- or guanine-based purines have been related to some antinociceptive effects in both animals and humans (54), it is relatively early to propose the use of most purine derivatives for clinical research and practice. Therefore, an interesting approach to investigate the role of purines clinically is the investigation of purine derivatives already used in humans such as the xanthine-oxidase inhibitor allopurinol (55).

Although allopurinol has been traditionally used in the treatment of gout and its related symptoms, there were only few reports investigating the effects of allopurinol *per se* on pain scores (56-59). We have demonstrated that allopurinol produced antinociceptive effects in several thermal and chemical pain models in rodents (34), findings confirmed by another group (35). In a prospective case series, an add-on therapy with oral allopurinol 300 mg twice daily caused a significant reduction on pain scores in women with fibromyalgia (60). More recently, we have shown that oral administration of two doses of allopurinol (300 mg) before surgery was associated with a significant reduction in pain scores 2h after total abdominal hysterectomy in women (data not published). Additionally, our previous findings indicated that only two doses of allopurinol (300 mg), in the last 12h before surgery, caused a significant increase in the cerebrospinal fluid (CSF) levels of xanthine and decreased CSF concentration of uric acid. Therefore, allopurinol, by inhibiting xanthine oxidase and production of uric acid, may produce accumulation of other purines in the CNS and in the periphery (for instance adenosine and other nucleosides and nucleotides), which may account for potential analgesic and other neuromodulatory properties.

The basic mechanism of action of allopurinol and its metabolite oxypurinol is inhibition of xanthine oxidase (they bind strongly to the reduced form of xanthine oxidase and inhibit the enzyme). This leads to a decrease in the systemic concentration of uric acid and an increase in the concentration of the precursors, hypoxanthine and xanthine (18). In addition, hypoxanthine can be converted to inosine, and consequently, to adenosine and guanosine (18). Thus, the primary effect of allopurinol is inhibition of uric acid production, and the overall result is the inhibition of the

metabolism of xanthine and hypoxanthine leading to greater salvage of these purines by their conversion to inosine, adenosine and guanosine. These findings, both in CNS and periphery, have been extensively demonstrated after systemic administration of allopurinol in several studies in animals and humans (34,61-64). However, in the present study, we could not properly measure systemic levels of purines after allopurinol treatment, since our method for chromatographic quantification is not accurate for blood sample analysis. Furthermore, in order to avoid venous puncture and a potential increase in dropouts during follow-up, we did not collect blood samples for uric acid measurement to further evaluate for adherence to the protocol. Certainly, future studies should address measurement of systemic purines to both confirm adherence to treatment and investigate underlying mechanisms for xanthine oxidase inhibitors.

Although previous findings indicate a role for adenosine and A1 adenosine-receptor in allopurinol-induced antinociception (34,35), other mechanisms have been proposed: i. effects involving guanine-based purines (34,53); ii. involvement of other purine derivatives (for instance, xanthine) (54); iii. role of the emerging A3 adenosine-receptor subtype in pain (65); iv. direct inhibition of xanthine oxidase pathway thereby reducing the production of reactive oxygen species (ROS) and attenuating pathological pain (66-69). However, in our population of women with fibromyalgia, we were not able to detect any benefit from the administration of allopurinol, even after 30 days of treatment. The lack of analgesic effects of allopurinol may be related to intrinsic characteristics of the fibromyalgia population. Fibromyalgia patients usually present a wide variety of signs and symptoms and the underlying mechanisms are much more complex and largely unclear (1). It is essential to emphasize that most traditional analgesics, very effective in other pain syndromes, have failed to improve quality of life, analgesia and functional status of patients displaying fibromyalgia (1,6,70). Hence, remains unclear if allopurinol could be a valid strategy for the treatment of other chronic pain syndromes, including neuropathic pain, since previous studies indicated that adenosine may be effective in the management of neuropathic pain (9,12,15,16).

Importantly, there were no differences between groups in the incidence of adverse events after 30 days of treatment, even in combination with several other medications traditionally used for managing fibromyalgia. These findings confirm the well-known safety profile of allopurinol and points to a safe administration in this population for future trials in other pain syndromes.

This study has some limitations. Firstly, this is a small clinical trial with a short treatment follow-up. Therefore, this study seems to be somewhat underpowered to detect more subtle differences between groups and it is unable to evaluate long-term benefits of allopurinol in those patients. Additionally, the analgesic effects of allopurinol have been demonstrated in animal models of acute and neuropathic pain. This study, conversely, evaluated the efficacy of allopurinol in patients displaying nociplastic pain. It is possible that allopurinol may prove more efficacious in other pain models or there could be a sub-group of patients who respond favourably to the treatment. Finally, the failure of the present study to measure blood levels of purines, including uric acid, is an important limitation both for investigating the mechanism of action and for assessing adherence to the treatment protocol.

In summary, this study has demonstrated that oral treatment with allopurinol during 30 days did not reduce pain scores in patients with fibromyalgia. Additionally, no other benefits of allopurinol over anxiety, depressive symptoms and functional status in fibromyalgia patients were observed following 30 days of treatment. Considering that previous studies have shown some beneficial effects against pain in animals and humans, new clinical trials are still warranted to determine if allopurinol is effective in reducing pain scores in other clinical settings. These studies should include larger samples and longer follow-up to better determine the impact of allopurinol and perhaps more selective xanthine-oxidase inhibitors on pain scores in humans displaying acute or chronic pain.

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Figure 1 (CONSORT flow diagram for the study):

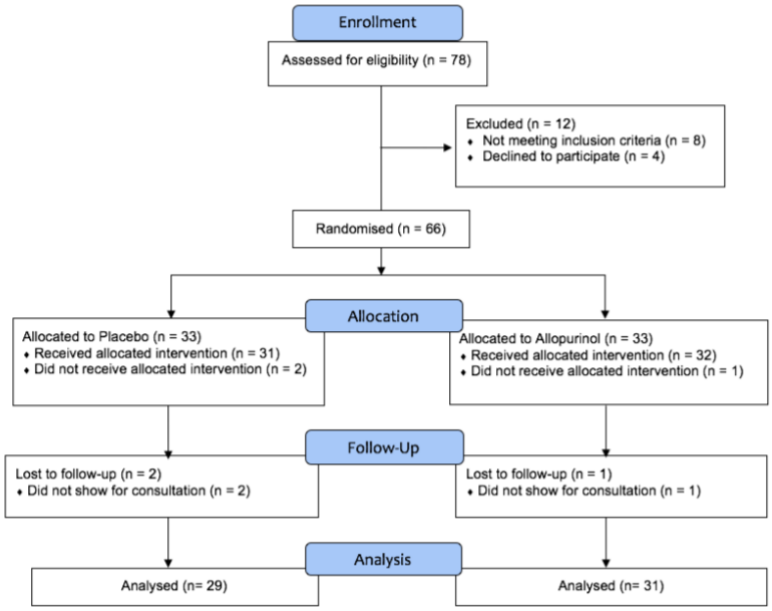
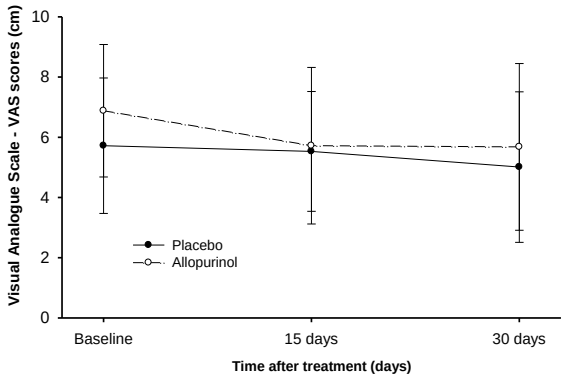


Figure 2:

A



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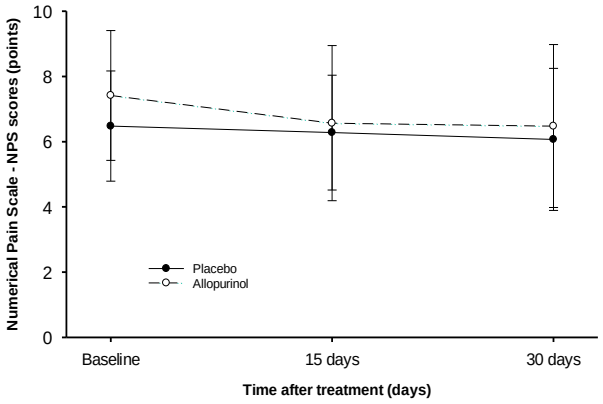

C

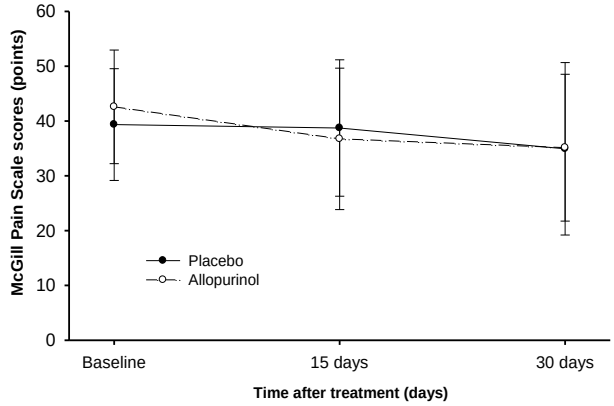

Figure 2: Comparison of pain scores measured by VAS (A), NPS (B), and McGill (C) pain scales considering time. Data are shown as mean (±SD). Two-way analysis of variance (two-way ANOVA) for repeated measures was used with the treatment group as the grouping factor and time as the repeated measure. No statistically significant difference was found between treatments (p = 0.31 for VAS scores, p = 0.45 for NPS scores and p = 0.19 for McGill scores). n = 29 for placebo and n = 31 for allopurinol. VAS: Visual analogue pain scale; NPS: Numerical pain scale; McGill: Modified McGill pain scale.

Table 1: Demographic data

Characteristics	Groups (treatment)		*p*
	Placebo	Allopurinol	
Age (years)	52.5(7.9)	51.5(10)	0.68
Weight (kg)	74.1(14)	72.5(15)	0.67
BMI (kg/m^2)	28.9(5.8)	28.3(4.9)	0.65
Hypertension (n - %)	15(52)	14(45)	0.61
Diabetes type 2 (n - %)	4(14)	5(16)	0.80
Active Smoking (n - %)	3(10)	3(9.7)	0.93
ASA status (n - %)			
I	2(7)	7(23)	
II	27(93)	24(77)	0.15

Table 1 legend: Data are shown as mean (±SD) or absolute values (percentiles). $p < 0.05$ was considered statistically significant; Statistical analysis was performed using unpaired Student's *t* test for numerical data and Pearson's X^2 test for categorical data (n = 29 for placebo and n = 31 for allopurinol). BMI: Body mass index; ASA: American Society of Anesthesiologists.

Table 2: Comparison of main outcomes between groups – pain scores

Pain scale	Groups (treatment)					
	Placebo			Allopurinol		
	Baseline	15 days	30 days	Baseline	15 days	30 days
VPS – n (%)						
No pain	0(0)	0(0)	2(7)	0(0)	2(7)	2(7)
Mild pain	6(21)	8(27)	9(31)	3(10)	5(16)	6(19)
Moderate pain	14(48)	15(52)	12(41)	11(35)	14(45)	12(39)
Severe pain	9(31)	6(21)	6(21)	17(55)	10(32)	11(35)
VAS – mean (±SD)	5.7(2.2)	5.5(2.0)	5.0(2.5)	6.9(2.2)	5.7(2.5)	5.7(2.7)
NPS – mean (±SD)	6.5(1.7)	6.3(1.8)	6.1(2.2)	7.4(2.0)	6.6(2.4)	6.5(2.5)
McGill – mean (±SD)	39.4(10)	38.7(12)	34.9(16)	42.6(10)	36.7(13)	35.1(13)

Table 2 legend: Data are shown as mean (±SD) or absolute values (percentiles); No statistically significant difference was found between treatments; $p < 0.05$ was considered statistically significant; Statistical analysis was performed using unpaired Student's *t* test for numerical data and Pearson's X^2 test for categorical data; (n = 29 for placebo and n = 31 for allopurinol); VPS: Verbal pain scale; VAS: Visual-analogue pain scale; NPS: Numerical pain scale; McGill: Modified McGill pain scale.

Table 3: Comparison of secondary outcomes between groups – anxiety scores

Time point	Groups (treatment – STAI scores)		
	Placebo	Allopurinol	*p*
Trait-anxiety:			
Baseline	56.2 (13)	55.0 (12)	0.71
15 days of treatment	52.0 (15)	55.6 (11)	0.28
30 days of treatment	50.9 (14)	54.0 (13)	0.37
State-anxiety:			
Baseline	55.1 (12)	54.4 (12)	0.83
15 days of treatment	51.6 (13)	55.4 (10)	0.20
30 days of treatment	50.8 (12)	51.4 (11)	0.83

Table 3 legend: Data are shown as mean (±SD); $p < 0.05$ was considered statistically significant; Statistical analysis was performed using unpaired Student's *t* test; (n = 29 for placebo and n = 31 for allopurinol); STAI: State-trait anxiety inventory.

Table 4: Comparison of secondary outcomes between groups – MADRS, PANAS and FIQ scores

Time point	Groups (treatment – scores)		
	Placebo	Allopurinol	*p*
MADRS:			
Baseline	22.4 (10)	24.2 (10)	0.51
15 days of treatment	19.7 (11)	19.7 (10)	0.99
30 days of treatment	18.3 (11)	20.8 (12)	0.41
PANAS - PA:			
Baseline	24.9 (8)	23.1 (7)	0.37
15 days of treatment	23.7 (9)	21.6 (7)	0.32
30 days of treatment	23.6 (8)	24.9 (8)	0.55
PANAS - NA:			
Baseline	24.3 (8)	24.5 (8)	0.93
15 days of treatment	23.6 (9)	23.8 (7)	0.93
30 days of treatment	22.3 (8)	23.1 (8)	0.72
FIQ overall scores:			
Baseline	75.9 (15)	78.2 (18)	0.62
15 days of treatment	70.5 (18)	76.7 (17)	0.19
30 days of treatment	72.0 (17)	75.2 (15)	0.46

Table 4 legend: Data are shown as mean (±SD); $p < 0.05$ was considered statistically significant; Statistical analysis was performed using unpaired Student's *t* test; (n = 29 for placebo and n = 31 for allopurinol); MADRS: Montgomery-Åsberg Depression Rating Scale; PANAS: Positive and Negative Affect Schedule; PA: Positive Affects; NA: Negative Affects; FIQ: Fibromyalgia Impact Questionnaire.

PARTE III

Discussão, considerações finais e perspectivas

III.1. DISCUSSÃO

Esta tese apresentou como proposta de trabalho a investigação acerca da administração do alopurinol para a redução de escores de dor e ansiedade, seguindo uma linha de pesquisa já desenvolvida há alguns anos por este grupo na Universidade Federal do Rio Grande do Sul. A ideia de utilizar derivados de purinas foi embasada nos resultados de pesquisas relacionadas ao sistema purinérgico. Purinas derivadas de adenina têm sido consideradas alvos importantes para o desenvolvimento de novos fármacos para o controle da dor, uma vez que o nucleosídeo adenosina e seus análogos demonstraram efeitos antinociceptivos após administração sistêmica e central [Sawynok, 1998; Sawynok e Liu, 2003]. A adenosina endógena pode ser liberada no SNC e nos tecidos periféricos, e a regulação de seus níveis por vários agentes farmacológicos pode alterar o processamento da dor por meio da ativação dos receptores A1 da adenosina nos neurônios, e talvez outros receptores nas estruturas adjacentes [Sawynok e Liu, 2003]. Efeitos adicionais em células inflamatórias em locais periféricos [Fredholm et al., 1997] e da glia no sistema nervoso central (SNC) [Ogata e Schubert, 1996; Gebicke-Haerter et al., 1996] mediada por receptores P1 de adenosina também foram relatados, potencialmente produzindo efeitos indiretos na transmissão da dor. Anteriormente, foi demonstrado que algumas purinas derivadas de guanina, especialmente o nucleosídeo guanosina, produziram efeitos antinociceptivos consistentes em vários modelos de dor em animais [Schmidt et al., 2010]. Embora purinas derivadas de adenina ou guanina tenham sido relacionadas a alguns efeitos antinociceptivos em animais e humanos [Schmidt et al., 2007a], é relativamente cedo para propor o uso da maioria dos derivados de purina para pesquisa clínica e prática. Portanto, a escolha do inibidor da xantina-oxidase alopurinol justifica-se por ser um derivado de purinas já com experiência prévia de uso em humanos [Connor, 2009], visto que é amplamente utilizado no tratamento da gota e seus sintomas relacionados. Embora existam poucos relatos sobre o uso do alopurinol na redução de escores de dor [Pinelli et al., 1991; Daskalopoulou et al., 2005; Inkster et al., 2007; Hacimuftuoglu et al., 2006], seus efeitos antinociceptivos foram evidenciados em vários modelos de dor térmica e química em roedores [Schmidt et al., 2009; Essawy e Elbaz, 2013].

No primeiro artigo desta tese, o alopurinol (200 $mg.kg^{-1}$) produziu efeitos antinociceptivos contra a hiperalgesia térmica e mecânica em um modelo tradicional de dor neuropática em

camundongos. Neste trabalho, demonstramos que o antagonista seletivo do receptor de adenosina A1 DPCPX preveniu parcialmente a antinocicepção induzida por alopurinol. Nenhum déficit motor óbvio foi produzido pelo alopurinol em doses de até 200 $mg.kg^{-1}$. O alopurinol também causou um aumento nos níveis de algumas purinas no líquido cefalorraquidiano (LCR) de camundongos, incluindo os nucleosídeos inosina e guanosina, e diminuiu a concentração liquórica de ácido úrico. Este estudo experimental inicial demonstrou a existência de potenciais efeitos antinociceptivos da administração sistêmica de alopurinol em um modelo de dor neuropática, complementando dados anteriores em modelos de dor térmica e química. Essas descobertas indicam que o alopurinol, e possivelmente outros inibidores da xantina oxidase, podem ser úteis para o controle da dor neuropática crônica em humanos.

O segundo protocolo de pesquisa desta tese demonstra que a administração oral de alopurinol antes da cirurgia foi associada à redução significativa nos escores de dor 2h após a histerectomia abdominal total em mulheres. A amostra populacional estudada foi homogênea, pois as características pré-operatórias (sexo, idade, peso, ansiedade pré-operatória e estado de dor) dos pacientes eram semelhantes. O efeito primário do alopurinol é a inibição da produção de ácido úrico, e o resultado geral é a inibição do metabolismo da xantina e da hipoxantina, levando a um maior resgate dessas purinas por sua conversão em inosina, adenosina e guanosina. Esses achados, tanto no sistema nervoso central quanto na periferia, foram amplamente demonstrados após a administração sistêmica de alopurinol em vários estudos em animais e humanos [Kim et al., 1987a, b; Ceballos et al., 1994; Marro et al., 2006; Schmidt et al., 2009]. No presente estudo, a administração de duas doses de alopurinol (300 mg) nas últimas 12 horas antes da cirurgia causou um aumento importante nas concentrações de xantina no líquido cefalorraquidiano (LCR) (aproximadamente 40%) e uma redução significativa nos níveis de ácido úrico no LCR (aproximadamente 11%). Na verdade, uma supressão significativa nos níveis de ácido úrico no LCR após o tratamento com alopurinol também foi observada em outros estudos [Kim et al., 1987a; Enrico et al., 1997; Akdemir et al., 2001]. Os achados deste estudo clínico indicam que o acúmulo de adenosina e de outras purinas no LCR pode desempenhar um papel importante na ação antinociceptiva do alopurinol.

No terceiro trabalho desta tese, realizamos um estudo piloto em pacientes com dor crônica, portadores de fibromialgia, submetidos a um tratamento de 30 dias com alopurinol associado às demais medicações analgésicas já utilizadas pelos pacientes incluídos. A administração oral do

alopurinol, 300 mg duas vezes ao dia, causou uma redução significativa nos escores de dor após 15 e 30 dias de tratamento em mulheres com fibromialgia. Dentre as principais limitações deste estudo, pode-se citar o número de casos investigados (12 pacientes) e a ausência de um grupo controle. Apesar dos resultados promissores encontrados neste estudo piloto, um ensaio clínico realizado posteriormente (artigo 4 desta tese) demonstrou que a mesma dosagem de alopurinol não reduziu os escores de dor em pacientes com fibromialgia. Além disso, nenhum outro benefício do alopurinol sobre a ansiedade, sintomas depressivos e estado funcional em pacientes com fibromialgia foi observado após 30 dias de tratamento. A amostra populacional estudada foi relativamente homogênea, visto que características basais como sexo, idade, peso, parâmetros psicológicos e estado funcional foram similares entre o grupo de estudo e o grupo placebo. Além disso, os pacientes alocados nos dois grupos de estudo foram cegados para o tratamento, assim como os pesquisadores envolvidos no protocolo de pesquisa, conferindo maior acurácia para os dados obtidos no presente estudo. Os escores de dor foram medidos por diferentes instrumentos e, embora não seja estatisticamente significativo, observou-se uma tendência para maiores escores basais de dor no grupo alopurinol em três das quatro escalas de dor (VAS, VPS e NPS). Esse achado pode ter afetado significativamente os resultados, uma vez que os pacientes mais refratários ao tratamento podem ter sido alocados para o grupo do alopurinol. Provavelmente, o tamanho relativamente pequeno da amostra foi um fator importante para esse pequeno desequilíbrio entre os grupos. Digno de nota, embora estatisticamente insignificante, a realização de ANOVA de medidas repetidas indicou uma tendência de melhora nos escores de dor (medidos por VAS e McGill) no grupo alopurinol, algo que merece ser esclarecido em uma população maior e em novos ensaios clínicos no futuro.

Em relação ao mecanismo de ação do alopurinol, estudos anteriores indicaram que a ativação da via opioide sensível à naloxona provavelmente não está envolvida na antinocicepção causada pelo alopurinol, uma vez que a naloxona não teve efeito contra a analgesia induzida por alopurinol [Schmidt et al., 2009; Essawy e Elbaz, 2013]. No entanto, os antagonistas do receptor de adenosina cafeína e DPCPX impediram a antinocicepção induzida por alopurinol, indicando que o receptor de adenosina A1 e a adenosina podem estar envolvidos nesses efeitos [Schmidt et al., 2009; Essawy e Elbaz, 2013]. É importante ressaltar que não há evidências de que o alopurinol apresente qualquer efeito agonista ou antagonista direto sobre os receptores adenosinérgicos [Day et al., 2007].

Embora descobertas anteriores indiquem um papel da adenosina na antinocicepção induzida por alopurinol [Schmidt et al., 2009; Essawy e Elbaz, 2013], não podemos descartar a influência de outras purinas. Este estudo também demonstrou um aumento significativo na concentração de xantina e inosina no LCR. Nosso grupo e outros [Cunha, 2005; Dobolyi et al., 2000; Oses et al., 2004, 2007] demonstraram que os nucleosídeos guanosina e adenosina interagem intimamente no SNC. Além disso, propusemos um sistema purinérgico específico derivado de guanina com implicações fisiológicas e patológicas relevantes para o SNC, além do sistema purinérgico derivado de adenina bem descrito [Schmidt et al., 2007a]. É importante ressaltar que demonstramos que a guanosina, assim como a adenosina, podem modular a transmissão da dor [Schmidt et al., 2010]. Portanto, não é possível desconsiderar neste momento que outras purinas também possam influenciar a antinocicepção induzida por alopurinol.

O alopurinol tem sido amplamente utilizado como inibidor da enzima xantina oxidase [Day et al., 2007]. A xantina oxidase é uma enzima flavoproteína altamente versátil que catalisa a hidroxilação oxidativa de substratos de purina e gera espécies reativas de oxigênio (ROS) [Borges et al., 2002]. ROS foram propostos para contribuir e / ou manter condições de dor crônica [Kim et al., 2006]. Alguns dados indicaram que as ROS também podem modular a transmissão da dor aguda [Hacimuftuoglu, 2006]. Notavelmente, há uma aceitação de que a atividade da xantina oxidase é significativamente aumentada em vários estados patológicos, incluindo alguns estados de dor [Khalil e Khodr, 2001]. Portanto, a inibição dessa via enzimática pode ser benéfica para o tratamento da dor [Lee et al., 2007]. Além disso, a administração de alopurinol demonstrou diminuir a lesão do tecido após isquemia / reperfusão em uma variedade de modelos *in vitro* e *in vivo* [Garcia Garcia et al., 1990; Reilly et al., 1991]. Inkster et al. [2007] mostraram que o tratamento com alopurinol teve efeitos benéficos marcantes sobre a função nervosa e vascular em ratos diabéticos. Esse mesmo estudo também demonstrou que o alopurinol atenuou a alodinia tátil induzida pelo diabetes e a hiperalgesia térmica e mecânica. Portanto, é possível propor que a redução da atividade da xantina oxidase e, consequentemente, de aspectos das ROS, possa ter um papel na analgesia pós-operatória induzida pelo alopurinol.

No ensaio clínico com resultados negativos em pacientes portadores de fibromialgia, a falta de efeitos analgésicos do alopurinol pode estar relacionada a características intrínsecas desta população de pacientes, e não, necessariamente, reflete a realidade de outros tipos de dor crônica. A dor pós-operatória parece ser mais previsível e geralmente está fortemente correlacionada à

quantidade de lesão tecidual [Caumo et al., 2002]. Por outro lado, os pacientes com fibromialgia geralmente apresentam uma ampla variedade de sinais e sintomas e os mecanismos subjacentes são muito mais complexos e amplamente obscuros [Cohen H, 2017]. É essencial enfatizar que a maioria dos analgésicos tradicionais, muito eficazes em outras síndromes dolorosas, não tem conseguido melhorar a qualidade de vida, analgesia e estado funcional de pacientes com fibromialgia [Atzeni et al., 2017; Schmidt-Wilcke e Diers, 2017; Cohen H, 2017]. Portanto, não está claro se o alopurinol poderia ser uma estratégia válida para o tratamento de outras síndromes de dor crônica, incluindo dor neuropática (estudos anteriores indicaram que a adenosina pode ser eficaz no tratamento da dor neuropática [Rauck et al., 2015; Burnstock G, 2016; Sollevi A, 1997; Sawynok e Liu, 2003]).

Diferentemente do estudo 2, no protocolo com pacientes fibromiálgicos não foi possível medir adequadamente os níveis sistêmicos de purinas após o tratamento com alopurinol, uma vez que nosso método de quantificação cromatográfica não é preciso para análise de amostras de sangue. Além disso, a fim de evitar a punção venosa e um potencial aumento de abandono durante o acompanhamento, não coletamos amostras de sangue para dosagem simples de ácido úrico para avaliar melhor a adesão ao protocolo. Certamente, estudos futuros devem abordar a medição de purinas sistêmicas para confirmar a adesão ao tratamento e investigar os mecanismos subjacentes para os inibidores da xantina oxidase.

Em relação aos escores de ansiedade, nenhum benefício foi observado com a administração de 300 mg de alopurinol em ambos os estudos clínicos. Como altos níveis de ansiedade-traço ou estado podem ser um fator de risco para níveis aumentados de dor [Caumo et al., 2002; Schmidt et al., 2007b], é essencial avaliar os escores de ansiedade antes e após o tratamento. No entanto, nestes estudos foi utilizado o STAI (Inventário de Ansiedade Traço-Estado) que, apesar de ser uma ferramenta amplamente utilizada [Spielberger et al., 1973] e ter sido validado para avaliar o nível de ansiedade em diferentes situações e a resposta aos medicamentos ansiolíticos [Biaggio AM, 1980], até o momento não há padrão-ouro para medir a ansiedade em adultos. É possível que o STAI não seja capaz de detectar com precisão os potenciais efeitos ansiolíticos do alopurinol. Portanto, os efeitos reais do tratamento oral com alopurinol sobre os níveis de ansiedade podem ser melhor esclarecidos com ferramentas adicionais, tratamento mais prolongado e maior acompanhamento no futuro. Além disso, em estudos que comparem condição pré e pós-operatória,

é importante a presença de uma população homogênea em relação aos escores de ansiedade pré-operatória para prevenir um potencial viés de confusão.

A administração do alopurinol parece ser bem tolerada, sem efeitos tóxicos óbvios no SNC, mesmo em altas doses. É importante ressaltar que, nos estudos presentes nesta tese de doutorado, também não houve ocorrência de efeitos adversos em ambos os contextos clínicos, mesmo em combinação com outros medicamentos em pós-operatório ou em combinação com vários outros medicamentos tradicionalmente usados para controlar a fibromialgia, o que confirma o perfil de segurança do alopurinol, sendo uma alternativa para uso seguro em pesquisas clínicas. Diante do que foi exposto, considerando a segurança e os efeitos benéficos da administração do alopurinol contra dor em animais e humanos em estudos anteriores, é válida a realização de novos ensaios clínicos com amostras maiores e acompanhamento a longo prazo para melhor compreensão do seu impacto na redução dos escores de dor e ansiedade em outras síndromes dolorosas agudas ou crônicas.

III.2. CONSIDERAÇÕES FINAIS E PERSPECTIVAS

A presente tese de doutorado demonstrou alguns resultados experimentais obtidos das investigações do potencial efeito antinociceptivo do inibidor de xantina oxidase alopurinol em um modelo animal de dor crônica neuropática, bem como apresentou algumas abordagens clínicas para avaliar o potencial analgésico do alopurinol em quadros dolorosos tradicionais em humanos, incluindo pacientes com dor pós-operatória e fibromialgia.

Durante a formulação desta tese, podemos observar que houve uma evolução no conhecimento sobre potenciais benefícios e funções dos derivados purinérgicos na transmissão dolorosa. Uma nova utilização para o alopurinol foi proposta e investigada, sendo certamente a maior contribuição do presente trabalho. Os resultados da presente tese indicam que fármacos que sejam capazes de modular o sistema purinérgico podem apresentar importantes efeitos biológicos, incluindo efeitos antinociceptivos em animais e humanos. Portanto, esses achados podem indicar que outros derivados do sistema purinérgico, incluindo antagonistas mais seletivos da xantina oxidase, devem ser investigados em futuros estudos experimentais e clínicos.

Neste contexto, considerando os achados experimentais e clínicos da presente tese de doutorado, podemos afirmar que a investigação dos diferentes componentes do sistema purinérgico como alvo para desenvolvimentos de novos fármacos analgésicos deve seguir em frente, sendo uma linha de pesquisa contínua na área de dor nesta instituição.

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