UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

EFICÁCIA DA ESCADA ANALGÉSICA DA ORGANIZAÇÃO MUNDIAL DA SAÚDE (OMS) EM UM MODELO DE SÍNDROME DOLOROSA INDUZIDA POR PACLITAXEL EM RATOS

DISSERTAÇÃO DE MESTRADO

Kelly de Vargas Pinheiro

Santa Maria, RS, Brasil, 2014

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Por

Kelly de Vargas Pinheiro

Dissertação apresentada ao curso de mestrado do Programa de Pós-Graduação em Farmacologia da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de
Mestre em Farmacologia.

Orientador: Prof. Dr. Juliano Ferreira

Santa Maria, RS, Brasil

2014

Universidade Federal de Santa Maria
Centro de Ciências da Saúde
Programa de Pós-Graduação em Farmacologia

A comissão examinadora, abaixo assinada,
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elaborada por

Kelly de Vargas Pinheiro

como requisito parcial para obtenção do grau de

Mestre em Farmacologia

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Santa Maria, 09 de abril de 2014

AGRADECIMENTOS

Ao meu orientador, professor Juliano Ferreira, pela excelente orientação, pela oportunidade de crescimento profissional e pessoal, pelos inúmeros ensinamentos, pela paciência e compreensão e, acima de tudo, pelo exemplo de ética e profissionalismo.

Á Deus, pela vida e saúde para encarar com tranquilidade as dificuldades do cotidiano.

Aos meus pais Juarez e Nair pelos momentos de plenitude e apoio familiar incondicionais. Agradeço pela paciência, confiança e cuidado sempre presentes. Obrigada por me ensinaram a perseguir meu ideal com dedicação e coragem. Minhas referências! À vocês, meu amor e minha eterna gratidão.

Á minha querida e amada irmã Fran, por ser sempre o melhor exemplo que alguém pode ter. "...Ter um irmão é a arte de fazer um laço, pois o nó entre as duas pontas de uma mesma fita permanecerá atado por toda vida."...Obrigada pelos conselhos, pela amizade, por tudo! À você meu carinho e admiração.

Ao meu namorado, Beto, pelo amor, incentivo e compreensão durante esta trajetória. Sem o seu apoio e companheirismo certamente eu não teria chegado até aqui. Obrigado por me fazer sentir capaz de realizar mais um sonho..."te amo pra sempre, e pra sempre não tem fim! "

Aos meus colegas do LABNEURO pelos diversos aprendizados, pela convivência diária e pelos momentos inesquecíveis que vivenciamos. Agradeço em especial a Flávia e a Sara, cuja ajuda foi imprescindível para a realização deste trabalho.

Às minhas queridas colegas do curso de Farmácia onde juntas construímos mais que um "Sexteto Fantástico". Bruna, Carol, Fê, Maíra,Tássia obrigada pela amizade, incentivo, por escutarem minhas angústias e dividirem sorrisos.Sentirei saudades...

Um agradecimento especial aos animais que contribuíram para que este trabalho fosse possível.

Ao Programa de Pós-Graduação em Farmacologia da Universidade Federal de Santa Maria, pela disponibilização de recursos para a realização deste trabalho.

À Universidade Federal de Santa Maria pela oportunidade de realização deste curso, bem como a CAPES pelo apoio financeiro.

Enfim, a todos aqueles que de alguma forma contribuíram para a elaboração e o desenvolvimento deste trabalho. MUITO OBRIGADA!

"As pessoas sempre põem a culpa nas circunstâncias por serem quem são. Não acredito em circunstância: os indivíduos de sucesso são aqueles que saem e procuram as condições que desejam; e, se não as encontram, criam-nas."
(George Bernard Shaw **)**

“...O tempo esperado é o agora
Sua consciência lhe direciona
Seus sentidos lhe alertam
E suas emoções não
mais são desprezadas
Antes que tudo acabe
É preciso fazer iniciar
Mesmo com dor e sofrimento
Antes arriscar do que apenas sonhar.”

(Cecília Meireles)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria, RS, Brasil

EFICÁCIA DA ESCADA ANALGÉSICA DA ORGANIZAÇÃO MUNDIAL DA SAÚDE (OMS) EM UM MODELO DE SÍNDROME DOLOROSA INDUZIDA POR PACLITAXEL EM RATOS

Autora: Kelly de Vargas Pinheiro
Orientador: Juliano Ferreira

Local e data da Defesa: Santa Maria, 9 de abril de 2014.

O uso do paclitaxel no câncer é limitado por uma síndrome dolorosa caracterizada por uma fase aguda e crônica, e também, pela falta de terapias eficazes para o seu tratamento. Assim, avaliou-se a eficácia dos analgésicos usados na escada da organização mundial da saúde (OMS), utilizada para o alívio da dor do câncer, em um modelo de síndrome dolorosa induzida por paclitaxel (SDIP). A hiperalgesia foi avaliada através de filamentos de von Frey. A síndrome dolorosa foi induzida por quatro injeções de paclitaxel em dias alternados. As fases agudas e crônicas foram avaliadas 24 h e 15 dias após a primeira administração, respectivamente. Os ratos foram tratados por via oral com veículo, paracetamol (degrau 1 da escada), codeína sozinha ou em combinação com paracetamol (degrau 2) e morfina (degrau 3), após a avaliação das fases aguda ou crônica. Paracetamol, codeína e morfina foram equi-eficazes na reversão da fase aguda da SDIP, mas os opióides, foram mais potentes quando comparados ao paracetamol. Codeína mais paracetamol teve eficácia e potência semelhante, quando administrados em conjunto, mas produziu um efeito mais duradouro. A repetição do tratamento com paclitaxel também levou a uma marcada hiperalgesia na fase crônica da síndrome dolorosa. O paracetamol, a codeína e a morfina revertearam parcialmente a

hiperalgesia induzida por paclitaxel, perdendo a sua eficácia e, no caso de codeína, a potência quando comparados à fase aguda. No entanto, a administração de codeína com paracetamol aumentou a potência e a eficácia do opióide, produzindo um efeito anti-hiperalgésico mais prolongado.Juntos, os analgésicos da escada são capazes de reverter ambas as fases aguda e crônica da SDIP, sendo que a codeína mais paracetamol apresentou-se mais potente, eficaz promovendo um efeito de longa duração. Assim, os analgésicos escada da OMS podem ser úteis para o tratamento da SDIP.

Palavras-chave: Quimioterapia, opióides, neuropatia, dor aguda.

ABSTRACT

Dissertation of Master's Degree
Graduating Program in Pharmacology
Federal University of Santa Maria, RS, Brazil

EFFICACY OF WORLD HEALTH ORGANIZATION ANALGESIC LADDER IN A MODEL OF PACLITAXEL-INDUCED PAIN SYNDROME

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Place and date: Santa Maria, April, 9th, 2014.

Paclitaxel use in cancer is limited by a painful syndrome characterized by acute and chronic phases and by the lack of efficacious therapies. Thus, we assessed the efficacy of analgesics used in the World Health Organization (WHO) ladder for a cancer pain relief in a model of paclitaxel-induced pain syndrome (P-IPS). Hyperalgesia was measured with von Frey filaments. P-IPS was induced in rats by four injections of paclitaxel on alternate days. The acute and chronic phases were assessed 24 h and 15 days after the first injection, respectively. Rats were treated orally with vehicle, acetaminophen (step 1 of the ladder), codeine alone or plus acetaminophen (step 2) and morphine (step 3) after acute or chronic phases assessment. Acetaminophen, codeine and morphine were equi-efficacious in reversing the acute phase of the P-IPS, but opioids were more potent than acetaminophen. Codeine plus acetaminophen had similar efficacy and potency when administered together, but produced longer-lasting effect. The repeated treatment with paclitaxel also led to a marked hyperalgesia in the chronic phase of the painful

syndrome. Acetaminophen, codeine and morphine partially reversed chronic phase of P-IPS, losing their efficacy and, in the case of codeine, potency when compared to acute phase. However, the administration acetaminophen with codeine increased the potency and the efficacy of the opioid, producing a long-lasting anti-hyperalgesic effect. Together, analgesics of WHO ladder are capable of reverting both acute and chronic phases of P-IPS, with codeine plus acetaminophen presenting more potent, efficacious and long-lasting effect. Thus, WHO analgesics ladder could also be useful to treat P-IPS.

Key words: chemotherapy; opioids; neuropathy; acute pain.

LISTA DE FIGURAS

REVISÃO BIBLIOGRÁFICA

MANUSCRITO

LISTA DE ABREVIATURAS

AINES	Anti-inflamatórios não esteroidais
ANOVA	Análise de variância
ED_{50}	Dose efetiva 50
E_{max}	Efeito máximo
g	Grama
h	Horas
i.p.	Intraperitoneal
kg	Quilograma
mg	Miligrama
min	Minutos
mL	Mililitro
p.o.	Via oral (do latim *per os*)
SDIP	Síndrome dolorosa induzida por paclitaxel

SUMÁRIO

APRESENTAÇÃO

No item **INTRODUÇÃO** está descrita uma breve revisão sobre os temas abordados nesta dissertação.

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigo, o qual se encontra no item **ARTIGO**. As seções Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no próprio artigo e representam a íntegra deste estudo.

O item **CONCLUSÃO,** encontrado no final desta dissertação, apresenta interpretações e comentários gerais sobre o artigo científico contido neste trabalho.

O item **REFERÊNCIAS BIBLIOGRÁFICAS** refere-se somente às citações que aparecem nos itens **INTRODUÇÃO**, **REVISÃO BIBLIOGRÁFICA** e **DISCUSSÃO** desta dissertação.

1. INTRODUÇÃO

A dor é um fenômeno complexo e difícil de ser avaliado. Sua origem e seu duplo papel como uma função fisiológica fundamental e por outro lado, como uma doença debilitante têm fascinado os cientistas durante séculos (KUNER, 2010). A dor pode ser denominada como uma experiência sensorial e emocional desagradável associada a dano tecidual real ou potencial ou descrita em termos de tal lesão (LOESER & TREEDE, 2008). Quanto à dor no câncer, esta, somada às incapacidades primariamente relacionadas à neoplasia pode ser a causa da redução das atividades normais do paciente (PORTENOY et al.,1999; MANTYH et al., 2002).

A etiologia da dor oncológica pode ser multifatorial, podendo ser relacionada ao tumor, ao tratamento ou, ainda, devido aos métodos de diagnóstico. A terapia do câncer é responsável pela dor em aproximadamente 15-25% dos pacientes que recebem quimioterapia, radioterapia ou procedimento cirúrgico (HIGGINSON, 1997). O paclitaxel é um agente antineoplásico altamente eficaz contra a proliferação de células cancerígenas, amplamente utilizado, sozinho ou em combinação com outros agentes quimioterápicos, no tratamento dos mais variados tipos de tumores sólidos, incluindo os cânceres de mama, ovário, pulmão e de cabeça e pescoço. Seu mecanismo de ação consiste na sua ligação ao longo dos microtúbulos estabilizando-os e suprimindo a sua dinâmica, levando à interrupção do processo mitótico e apoptose das células em divisão (GORNSTEIN & SCHWARZ, 2014).

Paradoxalmente, embora não estejam dividindo as células, os neurônios são igualmente susceptíveis ao paclitaxel e isto provoca complicações graves para a sua utilização como um agente terapêutico. Como o paclitaxel não é capaz de atravessar a barreira hematoencefálica (BHE), acaba afetando especificamente o sistema periférico, e leva a uma neuropatia axonal predominantemente sensorial (PARK et.al., 2011). A neuropatia periférica induzida por quimioterapia (NPIQ) é

clinicamente caracterizada como uma neuropatia sensorial, os sintomas mais comuns incluem dormência, formigamento e dor em queimação. Esses sintomas sensoriais, geralmente, começam simetricamente nos pés e nas mãos, e são observados em pacientes recebendo tal tratamento e caracterizam a dor crônica presente na síndrome dolorosa induzida por paclitaxel (WOLF et al., 2008). A incidência e a gravidade da neuropatia aumentam com doses únicas e cumulativas mais elevadas, e os sintomas neurológicos podem chegar a tal gravidade que exija a cessação ou redução do tratamento (LEE & SWAIN, 2006; CARLSON & OCEAN, 2011).

Ainda que a dor crônica esteja bem estabelecida, muitos pacientes relatam experiência dolorosa nos primeiros dias de tratamento com o paclitaxel, caracterizando a fase aguda da síndrome dolorosa induzida por esse agente quimioterápico (LOPRINZI et al., 2007). Moulder e colaboradores (2010) demonstraram em um estudo randomizado incidência de dor aguda após 3 horas de infusão com paclitaxel em pacientes com câncer de mama metastático. Além disso, estudos recentes sugerem que a dor aguda presente na fase aguda da síndrome dolorosa induzida por paclitaxel (SDIP) parece de alguma forma estar relacionada com a intensidade da dor na fase crônica desta síndrome (LOPRINZI et al., 2011).

É notável que muito frequentemente, medidas de alívio da dor são exigidas em vários estágios do câncer e, apesar do considerável progresso científico e farmacológico, a dor continua sendo substancialmente subtratada. O alívio adequado da dor em pacientes oncológicos, pode ser obtido através de protocolos simples de administração oral de analgésicos, como sugerido pela escada analgésica da Organização Mundial da Saúde (OMS) (ZECH et al., 1995). Além do uso de medidas não-farmacológicas, a OMS recomenda que a farmacoterapia

consista em um tratamento de três degraus, a partir de não-opióides (como por exemplo, antiinflamatórios não esteroidais - AINES) para opióides fracos e por último, opióides fortes, com ou sem combinações de analgésicos, de acordo com a necessidade, sendo que drogas adjuvantes podem ser adicionadas a cada passo (WHO, 1986).

Atualmente ainda não existem protocolos terapêuticos validados para o tratamento da síndrome dolorosa induzida por paclitaxel (ROWINSKI et al., 1993b; WASSERHEIT et al., 1996; GORDON et al., 1997; LOPRINZI et al., 2011). Portanto, é indiscutível a necessidade de pesquisas que investiguem terapias adequadas para o alívio desse tipo de dor, por isso o presente estudo pretende avaliar o efeito anti-hiperalgésico da escada analgésica proposta pela OMS em um modelo pré-clínico da síndrome dolorosa induzida por paclitaxel.

Além disso, as evidências clínicas sugerem a necessidade de um tratamento profilático, associado ao tratamento da síndrome dolorosa já estabelecida, ou seja, um tratamento efetivo para as fases aguda e crônica desta síndrome.

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar a eficácia da escada analgésica da organização mundial da saúde em um modelo de síndrome dolorosa induzida por paclitaxel em ratos.

2.2. Objetivos Específicos

2.2.1. Avaliar o efeito do paracetamol, codeína, morfina isolados e a combinação de codeína e paracetamol sobre a alodínia mecânica em um modelo agudo e crônico da síndrome dolorosa induzida por paclitaxel em ratos;

2.2.2. Investigar a provável associação entre a fase aguda e o desenvolvimento da fase crônica da síndrome dolorosa induzida por paclitaxel;

2.2.3. Verificar o efeito do tratamento durante a fase aguda e o desenvolvimento da fase crônica da síndrome dolorosa;

2.2.4. Avaliar os possíveis efeitos adversos hepáticos, renais e na coordenação motora causados pela administração de paracetamol, codeína e morfina isolados e da combinação de codeína mais paracetamol.

3. REVISÃO BIBLIOGRÁFICA

3.1. Dor

3.1.1 Dor considerações gerais

O termo dor, conforme a Associação Internacional para o Estudo da Dor ("IASP"-International Association for the Study of Pain) é definido como uma experiência sensorial e emocional desagradável, associada a uma lesão tecidual atual ou potencial ou descrita em termos de tal lesão (LOESER & TREEDE, 2008).

A dor aguda tem função biológica de preservação da integridade e da defesa, como consequência de uma lesão ou iminência de lesão tecidual. Por outro lado, a dor muitas vezes evolui de um sistema de alerta para uma dor crônica e debilitante. A dor crônica é um dos principais fatores que levam à incapacidade e afastamento das atividades cotidianas, perda de funcionalidade e da qualidade de vida. Apesar dos muitos estudos e avanços em áreas de conhecimento relacionadas à dor, como epidemiologia, fisiopatologia e terapêuticas, os resultados dos tratamentos preventivos das recorrências ainda não são satisfatórios (JULIUS & BASBAUM 2001).

O componente sensorial da dor (nocicepção) é formado por várias vias que ligam diversos componentes do sistema nervoso de maneira hierárquica. Os estímulos nocivos tais como calor, frio, compressão intensa ou algumas substâncias químicas, ativam as terminações nervosas livres e periféricas de fibras aferentes sensoriais primárias do tipo C e Aδ, chamadas de nociceptores. As fibras C são de pequeno diâmetro e possuem baixa velocidade de condução, pois são amielinizados e são ativados por estímulos mecânicos, térmicos e químicos. Estas fibras apresentam percepção lenta e resposta de longa duração (dor lenta), as fibras C podem também ser classificadas como peptidérgicas e não-peptidérgicas. Enquanto as fibras nociceptivas Aδ possuem médio diâmetro e são pouco mielinizadas e, por

isso, conduzem mais rapidamente os estímulos periféricos, sendo ativadas por estímulos mecânicos e térmicos (dor rápida) (LOESER, 2001, MEYER et al, 2008, BASBAUM et al 2009).

Um terceiro grupo de fibras aferentes mielinizadas são as fibras de grande diâmetro Aβ, as quais são responsáveis por mediar a transmissão rápida de estímulos sensoriais, esses estímulos são caracterizados como inócuos ou não nocivos (estímulos proprioceptivos), e assim diferem consideravelmente das fibras Aδ. Apesar disso, em algumas condições patológicas, após lesão, as fibras Aβ sofrem alteração de função e passam a transmitir impulsos nociceptivos. Um exemplo disso ocorre durante o tratamento do câncer, no qual alguns quimioterápicos conseguem lesionar preferencialmente este tipo de fibra. Devido a isso, os pacientes em tratamento passam a apresentar distúrbios sensoriais periféricos (POSTMA et al., 1995; DOUGHERTY et al., 2004; BASBAUM et al., 2009).

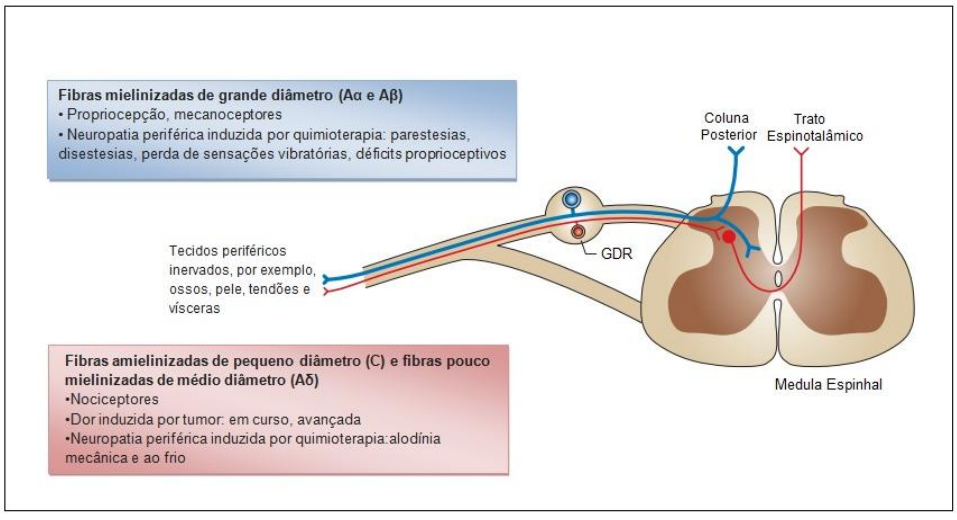

Figura 1: Fibras aferentes nervosas sensoriais primárias envolvidas na geração da dor e/ou neuropatia induzida por tumores e terapias antitumorais (Adaptado de Manthy, 2006).

As fibras são formadas por neurônios cujos corpos celulares encontram-se nos gânglios da raiz dorsal e trigeminal e que conduzem as informações nociceptivas até o corno dorsal da medula espinhal e ao núcleo trigeminal na ponte, respectivamente (WOOLF & MA, 2007). Imediatamente, um reflexo de retirada mediado pela medula espinhal é desencadeado no intuito de remover a região do corpo ameaçada (WATKINS & MAIER, 2002). Nas lâminas superficiais do corno dorsal da medula espinhal, as terminações dos nociceptores liberam vários neurotransmissores que estimulam neurônios de segunda ordem. Estes neurônios formam vias que irão distribuir informações para circuitos cerebrais responsáveis pela produção das dimensões sensoriais e afetivo-motivacionais da dor (CRAIG, 2003; HUNT & MANTYH, 2001).

Um segundo propósito da dor é desencadear comportamentos de recuperação, em resposta à dor originada por lesões no próprio organismo. Neste caso, a lesão tecidual já ocorreu e a área lesionada está inflamada ou infectada, e os reflexos espinhais não são tão importantes, pois não existe uma fonte externa de estímulo para ser evitada. Os estímulos provenientes da área lesionada chegam a centros cerebrais superiores (p. ex. tálamo e córtex) que organizam comportamentos apropriados de recuperação para proteger e facilitar a resolução da lesão (WATKINS & MAIER, 2002).

Ao contrário destes propósitos claramente protetores, a dor pode se tornar crônica quando o organismo não é capaz de produzir a resolução da lesão ou quando a plasticidade neuronal que ocorre durante a doença mantém a dor mesmo após a resolução da lesão. É o que acontece, por exemplo, em doenças inflamatórias ou após a lesão nervosa (neuropatias). As dores crônicas mais comuns incluem a neuralgia do trigêmeo, a fibromialgia, as síndromes dolorosas complexas

regionais, a dor associada com a artrite, a dor do membro fantasma e as síndromes dolorosas centrais (ASHBURN & STAATS, 1999). Durante estas síndromes, o processamento sensorial é anormal. Estímulos ambientais que normalmente são inócuos, tais como leve toque ou pequenas alterações na temperatura ambiente, produzem a sensação de dor, isto é, alodínia. Estímulos que normalmente são percebidos como dolorosos produzem percepção exagerada de dor, isto é, hiperalgesia. Esses fenômenos são frequentemente apresentados por indivíduos acometidos por doenças inflamatórias, tais como artrite, por dores neuropáticas, como as originadas por terapia antineoplásica, ou ainda por diferentes tipos de câncer (DOUGHERTY et al., 2004; MANTYH et al., 2002, 2006).

Finalmente, a dor pode ainda aparecer espontaneamente, sem a necessidade de estimulação externa, podendo ser descrita como dor em queimação ou em choque. A dor crônica difere substancialmente da dor aguda não somente em relação ao seu caráter persistente, mas está principalmente associada com alterações adaptativas, tais como à neuroplasticidade em vários níveis do sistema nervoso, sendo de difícil tratamento (COSTIGAN et al., 2009; WOOLF & MA, 2007; WOOLF & SALTER, 2000).

Em vista disso, se tem claramente a necessidade de busca por fármacos que podem ser úteis para o desenvolvimento de estratégias terapêuticas mais eficazes no tratamento de síndromes dolorosas irresponsivas, principalmente aquelas relacionadas à fisiopatologia oncológica.

3.1.2 Dor associada ao câncer

Para a grande parte dos pacientes oncológicos a dor caracteriza o primeiro sinal da neoplasia e acarreta em diminuição significativa da qualidade de vida (PORTENOY et al.,1999; MANTYH et al., 2002).

A dor do câncer pode estar relacionada ao tumor primário ou suas metástases, à terapia anticancerosa e aos métodos de investigação; em alguns pacientes pode, também, não estar relacionada à neoplasia (FOLEY, 1993; MANTYH et al., 2006). Para pacientes e familiares a falta de tratamento adequado para a dor é um dos fatores mais preocupantes, uma vez que é um dos sintomas mais comuns e angustiantes descritos por pacientes com câncer. Além disso, não é puramente uma experiência física, mas envolve vários outros componentes do funcionamento humano, incluindo o humor, personalidade,comportamento e as relações sociais (SAUNDERS, 1978).

A dor relacionada ao câncer, quando inadequadamente controlada, pode ocasionar um impacto profundo na vida desses pacientes. É importante observar que o objetivo da terapêutica na dor oncológica é o de proporcionar suficiente alívio para que pacientes possam tolerar o diagnóstico e abordagens terapêuticas necessárias para tratar o câncer, e mais do que isso é aumentar a qualidade de vida dos mesmos (TAY & HO, 2009).

Porém, ao que se poderia esperar grande parte dos estudos que envolvem pacientes com dor e câncer não caracteriza o fenômeno álgico nos seus diversos elementos. Isto acarreta lacuna na compreensão das síndromes dolorosas, no diagnóstico etiológico da dor, na programação terapêutica e na avaliação da resposta obtida. Essa incompreensão dos sintomas dolorosos no câncer está, principalmente, relacionada à origem multifatorial dessa dor, que frequentemente

pode ser resultado do tratamento empregado no combate do câncer. A quimioterapia é a terapia antitumoral mais amplamente utilizada, e comuns são as experiências dolorosas relatadas pelos pacientes submetidos a esse tipo de tratamento.

3.1.3 Dor associada ao tratamento quimioterápico

Para os pacientes com câncer, o recebimento de um regime quimioterapêutico é um dos fatores mais importantes na determinação da sobrevivência e uma melhor qualidade de vida. No entanto, a neurotoxicidade e a dor são efeitos secundários de muitos dos mais usados agentes anti-neoplásicos (QUASTHOFF & HARTUNG, 2002).

O paclitaxel é um dos agentes antineoplásicos mais efetivo e comumente utilizado no tratamento de uma série de tumores sólidos tais como o de mama, ovário, pulmão, cabeça e pescoço. Porém, a este fármaco está associado uma síndrome peculiar de dores agudas, que tem sido referida como “artralgias e mialgias induzidas por paclitaxel” e atualmente associada a fase aguda da síndrome dolorosa induzida por paclitaxel (SDIP) descrita em até 58% dos pacientes, geralmente se desenvolve dentro de 1-3 dias de administração de paclitaxel; e os sintomas desaparecem em grande parte dentro de uma semana (ROWINSKY et al., 1993; GARRISON et al., 2003; LOPRINZI et al., 2007; LOPRINZI et al., 2011).

A dor presente na fase aguda pode resultar da sensibilização de nociceptores com base em descrições de pacientes sobre a dor e, além disso, estudos realizados em animais mostram o desenvolvimento de lesão no nervo 24 horas após a administração de paclitaxel. Esses dados estão de acordo com o observado na clínica, uma vez que os pacientes oncológicos, geralmente, relatam sintomas dolorosos já nos primeiros dias de tratamento com paclitaxel (LOPRINZI et al.,2007).

Outros dois graves efeitos colaterais decorrentes do uso deste quimioterápico são a mielossupressão e a neurotoxicidade periférica. O fator estimulante de colônias de granulócitos eficazmente neutraliza a neutropenia, na maioria dos pacientes. Mas por outro lado, não existem terapias aceitáveis para prevenir ou minimizar danos nos nervos, fazendo da neurotoxicidade um significativo efeito colateral limitante de dose (ROWINSKY et al., 1993a,b; WASSERHEIT et al., 1996; GORDON et al., 1997).

A neuropatia periférica induzida por quimioterapia pode ser extremamente dolorosa e/ou incapacitante, e está relacionada à fase crônica da SDIP, causando perda significativa de habilidades funcionais e diminuindo a qualidade de vida. Agentes quimioterapêuticos neurotóxicos podem provocar danos estruturais nos nervos periféricos, resultando em processamento somatossensorial aberrante do sistema nervoso periférico e/ou central (WINDEBANK, 1999). Esta neuropatia periférica resultante pode afetar ambos os axônios de fibras sensoriais pequenas e grandes, porém são as fibras mielinizadas Aβ as preferencialmente lesionadas por administração de alguns quimioterápicos incluindo o paclitaxel. Um curso clínico comum começa com parestesias (formigamento) e disestesias, comumente localizadas nos dedos dos pés e das mãos. Estes sintomas se espalham proximalmente e afetam ambos os membros inferiores e superiores com uma característica de distribuição em “luva e meia” (LOMONACO et al., 1992).

Assim, apesar das fases aguda e crônica da SDIP serem classificadas como entidades clínicas distintas, um recente estudo demonstrou, que ambas podem ser manifestações de um patologia nervosa. Além disso, a elevada incidência da dor aguda naqueles paciente tratados com paclitaxel, além de um efeito bastante incômodo pode anunciar o início da neuropatia periférica (REEVES et al., 2012).

3.1.4 Tratamento da dor oncológica

Muito frequentemente, medidas de alívio da dor são exigidas em vários estágios do câncer. Embora menos de 15% dos pacientes com a doença não metastática relatem dor, 80% ou mais de pacientes terminais com câncer amplamente disseminado experimentam dor que exige tratamento (FOLEY, 1997). A maioria dos pacientes referidos para controle de sintoma relacionado ao câncer tem pelo menos dois locais anatomicamente distintos de dor, e mais de 40% têm quatro ou mais locais (TWYCROSS & FAIRFIELD, 1982).

Os pacientes com câncer podem apresentar diferentes tipos de dor, desde somática visceral à neuropática. A dor pode ser bem controlada, em 80% a 90% dos pacientes com câncer com a utilização de analgésicos e adjuvantes convencionais de acordo com os princípios da escada analgésica para alívio da dor do câncer proposta pela Organização Mundial de Saúde (OMS) (WALKER et al., 1988; GROND et al., 1991; ZECH et al., 1995). O paracetamol ou as drogas antiinflamatórias não-esteroidais (AINES) são analgésicos eficazes para pacientes com dor leve e podem ser combinados com opiáceos nos pacientes com dor moderada a grave. A experiência com o uso da escada da OMS mostrou que o princípio simples de subir de não-opiáceos a analgésicos opiáceos fortes é seguro e eficaz. Em grande parte dos pacientes, os efeitos secundários associados com o uso dos opiáceos podem facilmente ser controlados com uma combinação de instrução ao paciente e confiança sobre a natureza transitória da sedação e vômito, a de seleção cuidadosa da dose e via do opiáceo, e do uso de drogas adicionais tais como os antieméticos e os laxantes (BRUERA & NEUMANN, 1999). As drogas adjuvantes são usadas para síndromes dolorosas de difíceis tratamentos tais como

dor neuropática e dor óssea e também juntamente com as demais classes de fármacos nos três degraus da escada. Entre os agentes usados frequentemente para o tratamento da dor neuropática estão os antidepressivos tricíclicos como amitriptilina e imipramina, os anticonvulsivantes tais como a gabapentina, e os inibidores seletivos da recaptação de serotonina e noradrenalina como duloxetina (TURK et al., 2011; MIKA et al., 2013).

Infelizmente, mesmo como os inúmeros pacientes que desencadeiam sintomas de dor em decorrência do tratamento quimioterapêutico ainda não existem medicamentos regulamentados para o tratamento da síndrome dolorosa induzida por paclitaxel (ROWINSKI et al., 1993; WASSERHEIT et al., 1996; GORDON et al., 1997; LOPRINZI et al., 2011). Por isso a necessidade de pesquisas que investiguem as terapias mais adequadas para o alívio desse tipo de dor em um modelo experimental de síndrome dolorosa induzida por paclitaxel em ratos, abrangendo ambas as fases aguda e crônica desta síndrome (RIGO et al., 2013). Além disso, as evidências clínicas disponíveis a respeito da SDIP são limitadas, assim, estudos pré-clínicos podem trazer novas evidências favorecendo o entendimento e de fato o alívio efetivo para os sintomas dolorosos presentes nesta condição.

Dessa forma, a utilização da escada analgésica proposta pela OMS (ver figura 2) parece ser uma alternativa simples para o tratamento da dor em decorrência da terapia antitumoral, caracterizada neste estudo como a síndrome dolorosa induzida por paclitaxel (SDIP), uma vez que os analgésicos que a compõe já fazem parte da terapêutica utilizada prática clínica.

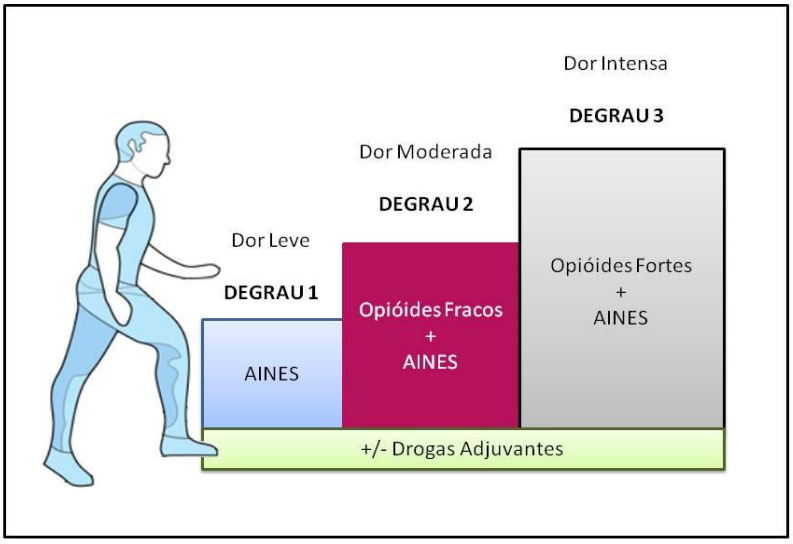

Figura 2: Escada Analgésica da Organização Mundial da Saúde (Adaptado de WHO, 1986).

4. MANUSCRITO

Artigo submetido à revista Cancer Chemotherapy and Pharmacology

Efficacy of the World Health Organization analgesic ladder in a model of paclitaxel-induced pain syndrome

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Abstract

Purpose

Paclitaxel use in cancer treatment is limited by a painful syndrome characterized by acute and chronic phases and by the lack of efficacious therapies. Thus, we assessed the efficacy of analgesics used in the World Health Organization (WHO) ladder for a cancer pain relief in a model of paclitaxel-induced pain syndrome (P-IPS).

Methods

Hyperalgesia was measured with von Frey filaments. P-IPS was induced in rats by four injections of paclitaxel on alternate days. The acute and chronic phases were assessed 24 h and 15 days after the first injection, respectively. Rats were treated orally with vehicle, acetaminophen (step 1 of the ladder), codeine alone or plus acetaminophen (step 2), and morphine (step 3) after acute or chronic phases assessment.

Results

Acetaminophen, codeine and morphine were equi-efficacious in reversing the acute phase of the P-IPS, but opioids were more potent than acetaminophen. Codeine plus acetaminophen had similar efficacy and potency when administered together, but produced longer-lasting effect. The repeated treatment with paclitaxel also led to a marked hyperalgesia in the chronic phase of the painful syndrome. Acetaminophen, codeine and morphine partially reversed chronic phase of P-IPS, losing their efficacy and, in the case of codeine, potency when compared to acute phase. However, the administration acetaminophen with codeine increased the potency and the efficacy of the opioid, producing a long-lasting anti-hyperalgesic effect.

Conclusion

Together, analgesics of WHO ladder are capable of reverting both acute and chronic phases of P-IPS, with codeine plus acetaminophen presenting more potent, efficacious and long-lasting effect. Thus, WHO analgesics ladder could also be useful to treat P-IPS.

Key words: chemotherapy; opioids; neuropathy; acute pain;

1. Introduction

In 2012, cancer was responsible for 8.2 million deaths worldwide [1]. Furthermore, pain is the first sign of cancer for many patients, which in most cases, is associated with a significant decrease in their quality of life [2-3]. Cancer pain may be originated from different processes, such as direct infiltration/involvement of the tumor (tumor-induced pain) or even as a side effect toxicity of the cancer therapy (e.g., chemotherapy-induced pain) [4-6]. Pain is one of the most common symptoms in patients receiving cancer chemotherapy. It may be predominantly spontaneous, e.g., with a burning or pricking, or characterized by evoked pain such as mechanical allodynia (pain evoked normally not noxious stimuli) and hyperalgesia (an exacerbated response to a noxious stimulus) [7-9].

The antineoplastic agent paclitaxel (Taxol®), originally derived from the bark of the western yew Taxus brevifolia, has been widely used therapeutically based on their activity against a variety of solid tumors. However, paclitaxel treatment is associated with several side effects, such as a painful syndrome with acute and chronic phases [10, 11]. The acute phase of the paclitaxel-induced pain syndrome (P-IPS) is developed in the first days of treatment and affects a large proportion of patients [12, 13]. Besides acute pain, long-term use of paclitaxel may also induce a chronic peripheral neuropathy, which is the neurotoxic effect of paclitaxel most commonly reported by patients, limiting the antineoplasic treatment [14]. Furthermore, the acute pain induced by paclitaxel appears to be somehow related to the severity of the later neuropathic pain [13, 15]. Unfortunately, there are no current standard therapies to prevent or minimize both phases of pain related to paclitaxel [13, 15-18].

Cancer pain can be adequately treated in 80% to 90% of patients through the use of analgesics and adjuvants in accordance with the principles determined by the analgesic ladder for cancer pain relief proposed by the World Health Organization (WHO) [19-21]. Besides WHO ladder is extensively used to treat tumor-related pain, clinical and pre-clinical studies investigating its efficacy in chemotherapy-related pain are limited. Thus, the aim of our study was to investigate the effects of

using the WHO analgesic ladder in an experimental model of paclitaxel-induced pain syndrome in rats covering both acute and chronic painful phases.

2. Materials and methods

2.1 Animals

Experiments were conducted using male adult Wistar rats weighing 180–250 g. Rats were maintained in polycarbonate cages, with free access to food and water, on a 12-h alternating light-dark schedule in a temperature-controlled (22 ± 3 ºC) room. Animals were allowed to adapt to the test environment for 1 h before testing. Rats were kept and used in accordance to the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA), and the National Institutes of Health guide for the care and use of Laboratory Animals (Publication No. 85-23, revised 1985). All experiments of this study were approved by the Ethics Committee of the Federal University of Santa Catarina (process number PP00872). The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of drug treatments.

2.2 Drugs and reagents

Paclitaxel (6 mg/ml paclitaxel solution in Cremophor® EL and ethanol dihydrate, Accord, São Paulo, Brazil) was dissolved in saline (0.9% NaCl) on the days of execution of the experiments. Morphine sulfate (Dimorf® (10 mg/mL) or codeine phosphate (Codein®, 30 mg/mL) were obtained from Cristália (São Paulo, Brazil) and acetaminophen was obtained from Anqiu Lu`an Pharmaceutical (Shandong Anqiu, China). Morphine or codeine was dissolved in saline solution (NaCl, 0.9%) and acetaminophen was dissolved in vehicle solution (5% Tween 80, 20% polyethyleneglycol and 75% saline).

2.3 Administration of drugs

The injections of the paclitaxel were performed by intraperitoneal (i.p.) route, as described below. Administrations of acetaminophen, codeine, codeine plus acetaminophen or morphine were carried out orally (p.o.). In both procedures, the volume of 1 ml per 1 kg was used.

2.4 Induction of paclitaxel-induced pain syndrome (P-IPS)

Paclitaxel-induced pain syndrome was carried out as previously described [10, 22]. The chronic pain associated with painful syndrome was induced by four injections of paclitaxel (1 mg/kg, i.p.) on alternate days (days 1, 3, 5, and 7), as previously described. The chronic phase of the painful syndrome was measured 15 days after the first injection, while the acute phase of P-IPS was assessed within 24 h after a single injection of paclitaxel (1 mg/kg, i.p.).

2.5. Behavioral tests

2.5.1 Evaluation of nociception – von Frey test

The mechanical threshold 50% was determined before (baseline) and several times after treatments. The measurement of threshold 50% with a series of flexible nylon von Frey filaments of increasing stiffness (6–100 g) using the Up-and-Down method [23] was performed as previously described by Rigo et al. (2013) [22]. The paw withdrawal threshold 50% response was then calculated from the resulting scores as described previously by Dixon (1980) [24] and was expressed in grams (g). The animals showing a 50% reduction in the threshold 50% compared to baseline values were considered hyperalgesic.

2.5.2 Evaluation of locomotor activity - Open field test

The spontaneous locomotor activity was assessed using the open-field test as previously reported by Archer (1973) [25]. The locomotor activity after acetaminophen (100 mg/kg, p.o.), codeine (30 mg/kg, p.o.), codeine plus acetaminophen (3 and 30 mg/kg, p.o.) or morphine (10 mg/kg, p.o.) treatment was compared to the vehicle-treated group. The apparatus was a round arena (57 cm in diameter) with the floor divided into 21 equal areas. The number of areas crossed with all paws and number of rearings was recorded for 5 min.

2.6 Biochemical markers of toxicity

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the urea and creatinine levels are sensitive indicators of liver and kidney injury, respectively. To biochemically evaluate the occurrence of liver or kidney toxicity, vehicle, acetaminophen (100 mg/kg,

p.o.), codeine (30 mg/kg, p.o.), codeine plus acetaminophen (3 and 30 mg/kg, p.o.) or morphine (10 mg/kg, p.o.) were administered. The animals were euthanized at different time points after treatments (2 h for acetaminophen or codeine plus acetaminophen, 1 h for codeine alone and 0.5 h for morphine). The activities of ALT, AST and urea and creatinine serum levels were assessed according to the standard procedures provided, in automatized system Cobas Mira ® with the commercially available diagnostic kits (BioClin diagnostics - Quibasa Química Básica Ltd., Belo Horizonte, Brazil).

2.7 Experimental protocol

Firstly, we evaluated the baseline mechanical threshold (threshold 50%) of all animals. After, a group of rats received a single administration of paclitaxel (1 mg/kg, i.p.) and another group received four alternate injections of paclitaxel (1 mg/kg, i.p.). Next, the animals had their mechanical threshold evaluated 24 h (acute phase) and 15 days (chronic phase) after the first administration of paclitaxel (1 mg/kg, i.p.), respectively. In both groups, the animals that had a reduction in, at least, 50% in the mechanical threshold (compared with baseline value) were considered hyperalgesic and followed the experimental protocol.

Then, the time-course and the dose-response curve of antihyperalgesic effect caused by p.o. treatment with acetaminophen (3-100 mg/kg, p.o.), codeine (0.3-10 mg/kg, p.o.), morphine (0.3-10 mg/kg, p.o.) or codeine (0.3-3 mg/kg, p.o.) plus acetaminophen (3-30 mg/kg, p.o.) were performed in the acute phase of P-IPS.

Likewise, in the chronic phase of P-IPS, the time-course and the dose-response curve of antihyperalgesic effect caused by p.o. treatment with acetaminophen (3-100 mg/kg, p.o.), codeine (0.3-30 mg/kg, p.o.), morphine (1-10 mg/kg, p.o.) or codeine (0.3-3 mg/kg, p.o.) plus acetaminophen (3-30 mg/kg) were also performed.

The next step was to investigate whether the mechanical hyperalgesia, in the acute phase, was involved with the development of the mechanical hyperalgesia in the chronic phase of P-IPS. For this, another group of animals had their mechanical threshold evaluated before and 24 h after the first injection of paclitaxel (1mg/kg, i.p.); the animals were then separated into two other groups called

acute pain affected group or acute pain non-affected group. Both groups followed receiving 3 injections of paclitaxel (1mg/kg, i.p.), and 15 days after the first injection of paclitaxel they had their mechanical threshold also evaluated.

In order to assess whether treatment with codeine plus acetaminophen was able to reverse hyperalgesia in the acute phase and/or prevent the hyperalgesia in the chronic phase, in another group, once the mechanical hyperalgesia was established, acute pain affected group or non-affected group were treated with codeine plus acetaminophen (3+30 mg/kg, p.o.) or vehicle solution (5% Tween 80, 20% polyethyleneglycol and 75% saline). However only the acute pain affected group was evaluated, in the von Frey test, 120 min post-treatment. Then, both acute pain affected and acute pain non-affected groups continued receiving the three paclitaxel injections in alternate days, and had their mechanical sensibility re-evaluated 15 days after the first injection, at the chronic phase of P-IPS.

An independent group of animals was used to evaluate possible adverse effects induced by the treatment. The animals, without injection with paclitaxel, were administered with acetaminophen (100 mg/kg, p.o.), codeine (30 mg/kg, p.o.), morphine (10 mg/kg, p.o.), or codeine plus acetaminophen (3+30 mg/kg, p.o.) and the spontaneous (open-field test) locomotor activity and biochemical parameters were evaluated in the time point where the anti-hyperalgesic effect was maximum.

In all experiments, the rats were assigned to individual experimental groups and the behavioral tests and biochemical parameters were performed by an experimenter blind to the treatment conditions. Each experiment was performed at least two batches.

2.8 *Statistical analyses*

Results were expressed as means ± SEM. Statistical analyses were carried out using GraphPad Prism 4.0 software. Significance of differences between groups was evaluated with unpaired t-test, one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls' test or two-way ANOVA followed by Bonferroni's test when appropriate. F values demonstrated in the text were obtained from one-way or two-way ANOVA analysis. Where two-way ANOVA was used, the F

values indicate the interaction between time and treatment factors. Significance was considered when $p<0.05$. The effective dose 50 (ED_{50}) values were obtained by non-linear regression using sigmoidal dose–response with a variable slope equation. The percentages of maximum effect (E_{max}) were calculated for the maximal developed responses in comparison with vehicle-treated animals.

3. Results

3.1 Effect of acetaminophen, codeine or morphine on mechanical hyperalgesia in acute stage of paclitaxel pain syndrome

Animals submitted to an injection of paclitaxel (1 mg/kg, i.p.) presented mechanical hyperalgesia 24 h after its administration [$F(6,35)=15.90$, $p<0.001$ for Fig. 1A; $F(5,30=22.00)$, $p<0.001$ for Fig. 1C; and $F(5,30)=14.15$, $p<0.001$ for Fig. 1E]. In our experimental conditions, 83±5% of all animals treated with paclitaxel presented acute pain hyperalgesia.

When compared with animals that received vehicle, acetaminophen (100 mg/kg, p.o.) was able to reverse paclitaxel-induced acute hyperalgesia from 60 up to 240 min after its treatment, with a maximum (peak) effect at 120 min [$F(6,66)= 5.51$, $p<0.001$; Fig. 1A]. The anti-hyperalgesic effect also occurred at doses of 10 and 100 mg/kg (Fig. 1B). Similarly, the administration of codeine (3 mg/kg, p.o.) was also able to revert the hyperalgesia induced by paclitaxel from 30 up to 120 min after its treatment and the peak effect was observed 60 min after its administration [$F(5,50)=28.32$, $p<0.001$; Fig. 1C]. The anti-hyperalgesic effect occurred at doses of 1, 3 and 10 mg/kg (Fig. 1D). Additionally, the treatment with morphine (3 and 10 mg/kg, p.o.) was able to reverse the hyperalgesia induced by paclitaxel only 30 min after its treatment [$F(5,50)=2.97$, $p< 0.05$; Fig. 1E and F].

The calculated parameters of potency (effective dose 50) and efficacy (maximal effect) for all treatments are demonstrated in Table 1. Treatments were equi-efficacius in reducing paclitaxel-induced acute hyperalgesia, almost abolishing the nociceptive response. The order of potency was codeine ≈ morphine > acetaminophen.

3.2 Effect of acetaminophen, codeine or morphine on mechanical hyperalgesia in the chronic stage of paclitaxel pain syndrome

Repeated treatment with paclitaxel (1 mg/kg, i.p.) for four alternated days led to a reduction in the mechanical threshold in the paw of rats 15 days after the first injection [$F(6, 35)=28.36$, $p<0.001$ for Fig. 2A; $F(5,24)=20.89$, $p<0.001$ for Fig. 2C; and $F(5,30)=28.36$, $p<0.001$ for Fig. 2E]. In our experiments, we observed 82±6% of all animals treated with paclitaxel presented hyperalgesia in the chronic phase. The treatment with acetaminophen (10 mg/kg, p.o.) was able to reverse paclitaxel-induced chronic hyperalgesia when compared with animals that received vehicle solution from 30 up to 240 min after its administration, with a maximum (peak) effect at 120 min [$F(6,54)= 0.53$, $p<0.001$; Fig. 2A]. This effect occurred at doses of 10 and 100 mg/kg (Fig. 2B).

In addition, the oral administration of 10 mg/kg codeine also produced a reduction of nociceptive response from 30 up to 120 min after treatment with paclitaxel [$F(5,45)=3.48$, $p<0.001$; Fig. 2C]. This effect occurred at doses of 3, 10 and 30 mg/kg with a maximum effect observed at 60 min after its administration (Fig. 2D). Moreover, the administration of morphine (3 mg/kg, p.o.) was able to revert the hyperalgesia induced by paclitaxel only 60 min after its treatment [$F(5,65)=2.36$, $p<0.05$; Fig. 2E]. The anti-hyperalgesic effect occurred at doses of 1, 3 and 10 mg/kg (Fig. 2F).

The calculated potency (ED_{50}) and efficacy (I_{max}) for all treatments are demonstrated in Table 1. The potency and efficacy of treatments with acetaminophen, codeine or morphine were not statistically different in reducing paclitaxel-induced chronic hyperalgesia. When compared to the acute phase, codeine (but not acetaminophen and morphine) had a significant loss in its potency. Moreover, treatments had a trend to be less efficacious in the chronic than in the acute phase of hyperalgesia.

3.3 Effect of the combination of codeine and acetaminophen on mechanical hyperalgesia in the acute and chronic stages of paclitaxel pain syndrome

Based on their ED_{50} values on the acute phase, we investigated the combination of codeine and acetaminophen (dose relation of 1:10). The acute hyperalgesia produced by a single administration of

paclitaxel [$F(7,40)=28.41$ $p<0.001$; Fig. 3A] was fully reversed by the combination of codeine (3 mg/kg, p.o.) plus acetaminophen (30 mg/kg, p.o.) from 60 up to 360 min after its treatment, when compared with animals that received vehicle solution [$F(7,70)=16.17$, $p<0.001$; (Fig. 3A). The anti-hyperalgesic effect occurred at all doses tested and the peak inhibition at 120 min after its administration (Fig. 3B). The treatment with codeine plus acetaminophen was also able to abolish paclitaxel-induced chronic hyperalgesia from 60 up to 240 min after its administration [$F(7,40)=50.67$, $p<0.001$; (Fig. 3C)] and with all tested doses (Fig. 3D). Acetaminophen co-administration was capable of increasing both the potency and the efficacy of codeine in the chronic, but not in the acute phase of paclitaxel-induced hyperalgesia (Table 1).

3.4. Relation between acute and chronic phases of paclitaxel-induced pain syndrome

Different group of rats were repeatedly treated with paclitaxel (4 injections on alternate days) and hyperalgesia was assessed 24 h and 15 days after the first injection. As described before, just a fraction of paclitaxel injected rats presented acute hyperalgesia. Of note, rats that presented acute hyperalgesia (acute-pain affected group) had a significant greater pain in the chronic phase when compared to animals that did not present acute hyperalgesia (acute pain non-affected group) (Fig. 4A). Since the combination of codeine plus acetaminophen was more potent and effective than the other treatments, next we investigated the effect of this combination on the relationship between acute and chronic phases of paclitaxel-induced hyperalgesia. The administration of codeine plus acetaminophen (3-30 mg/kg, p.o.) either in the acute pain affected (at doses that fully revese acute hyperalgesia) or acute pain non-affected group (data not shown) was not capable of preventing the exacerbation of chronic hyperalgesia observed in rats with acute hyperalgesia (Fig. 4B).

3.5. Assessment of the side effects of drugs on locomotor and biochemical parameters

Treatment with effective doses of acetaminophen, codeine, codeine plus acetaminophen or morphine did not alter the locomotor activity compared with the vehicle treated animals, as evaluated

by both the total number of crossings and rearings in the open-field test (Table 2). Treatment with acetaminophen, codeine, codeine plus acetaminophen or morphine caused no changes in serum AST or ALT enzyme activities, or in creatinine or urea concentrations when compared with the vehicle treated animals (Table 2).

4. Discussion

Paclitaxel is a chemotherapeutic agent, with activity against several tumors. However, most patients under its treatment have reported pain as a very common adverse effect. The pain appears after a single or cumulative dose of paclitaxel, known as painful syndrome [26-28]. Despite advances in the treatment of pain, it still remains undertreated, due to its multifactorial etiology and therefore new therapeutic approaches are essential [29]. In this study, we investigated the efficacy of World Health Organization (WHO) analgesic ladder in a preclinical model of paclitaxel- induced pain syndrome.

Apart acute phase of paclitaxel-induced pain syndrome (P-IPS) causes significant morbidity resulting in a significant reduction in quality of life of patients and maybe due to the impact of other common paclitaxel-associated side effects (i.e. hair loss, anaphylaxis and neuropathy), there has been limited discussion, controlled studies and experimental models in the literature regarding acute P-IPS [13, 30]. Thus, we firstly have developed an animal model of acute P-IPS. Similarly to what was observed previously by Rigo and colleagues [22], we found a reduction in the mechanical threshold 24 h after a single injection of paclitaxel in 83±5% of the treated rats. Our results are in accordance with patients with paclitaxel-associated acute pain syndrome situations, which are developed in up to 70% of patients, usually 1-3 days after the administration of paclitaxel and may be described as increased pain with tactile contact [12, 13].

The treatment of the acute phase of P-IPS is often unsatisfactory and there is a paucity of data on its prevention and treatment. To date, just one randomized, controlled trial for the management of this important adverse effect has been published. Such study has shown no superiority of glutamine versus placebo to prevent P-IPS [31].The effectiveness of pharmacologic therapies for the prevention and

treatment of acute phase P-IPS has been gleaned from case series or toxicity results of phase I-III clinical trials, with the majority of the published data being reported qualitatively also been reported [32]. Several studies have reported, but not proven, that nonsteroidal anti-inflammatory drugs (NSAIDs) are able to supply partial or complete relief of pain in the majority of patients. Opioid treatment has been reported to treat symptoms refractory to NSAIDs, although data of their effectiveness are limited. Despite studies, they have not yielded enough evidence to establish a standard practice [32, 33]. Here we observed that the treatment with acetaminophen, codeine or morphine was very efficacious in reversing the mechanical hyperalgesia observed in acute stage of paclitaxel-induced pain syndrome, reinforcing the clinical reports that NSAIDs and opioids may be useful to treat acute phase of P-IPS.

We have also investigated the effect of NSAIDs and opioids on the mechanical hyperalgesia induced by repeated administration of paclitaxel. As previously demonstrated [10, 22] this chemotherapic has also led to a marked hyperalgesia 15 days after the first injection indicating similarity to the chronic and neuropathic phase of P-IPS. Apart to be also able to reverse chronic phase of P-PIS in rats, acetaminophen, codeine or morphine exhibited a trend to be less efficacious when compared to that observed in the acute phase. Corroborating our results, a study published by Xiao and colleagues (2008) indicated that, differently of other NSAIDs, high doses of acetaminophen were able to reverse hyperalgesia in vincristine-evoked painful neuropathies in rats [34]. Similar to acetaminophen, only high doses of morphine produce a partial relief when given to established chemotherapy-evoked neuropathic hyperalgesia in rats. Thus, these data are in accordance the findings of Flatters & Bennett (2004), who showed that similar to other neuropathies, paclitaxel-induced neuropathy is also relatively resistant to opioid therapy [34-37]. Of note, we have also observed that codeine had almost ten times loss of potency in the chronic phase of P-PIS, compared to the acute phase. Since the potency of morphine was not significantly changed in the chronic phase, we may suggest that the loss of codeine potency is not due to the reduced interaction with opioid receptor, but it seems to related to be pharmacokinetics issues that must be further investigated.

Since, in our findings, codeine had greater efficacy to reverse hyperalgesia in acute and chronic phases of P-IPS when compared to morphine, we have also analyzed the effect of the combination of codeine plus acetaminophen in P-IPS. In the acute phase, combination of codeine plus acetaminophen reverses the hyperalgesia without changes the drugs potency, but producing a longer lasting anti-hyperalgesic effect. On the other hand, the combination of codeine plus acetaminophen inhibited the hyperalgesia also present in the chronic phase, with increased potency, but similar efficacy when compared with the acute process. Thus, a combination therapy may lead to improved pain relief in the P-IPS. Accordingly, combinations of (NSAIDs), such as acetaminophen, with opioids, such as codeine, are currently used in clinical practice to reduce opioid requirements [38-40]. In addition, the WHO guidelines emphasize that the oral administration is preferred over parenteral routes as well as the around the clock dosing to prevent pain. With a more lasting effect, the oral use of the combination of codeine plus acetaminophen decreases constant interventions in patients and making possible treatment of pain in the chronic stage, a peripheral neuropathy, characterized as refractory most protocols for the treatment pain. Thus, access to pain relief is a crucial concern for patients with cancer. For this, the use of a treatment that can cover both acute and chronic painful phases is fundamental. Studies suggest that pain can be adequately treated in the majority of oncology patients (over 70%) by existing therapies and by following the WHO model [21, 29, 41].

In a recent study [13], patients treated with paclitaxel who experienced intense pain following the first administration of this drug developed a more severe peripheral neuropathy in a next stage, suggesting that the acute phase induced by paclitaxel is related to the degree of pain associated to a posterior nerve injury. In the present study, we have also found the same relation between the mechanical hyperalgesia found in the acute phase and the degree of chronic phase of the P-IPS in rats. The acute pain affected group developed a greater hyperalgesia in the chronic stage when compared with the acute pain non-affected group. In accordance with our results, Rigo and colleagues (2013) demonstrated that the acute pain is related to the severity of the chronic pain symptoms [22]. However, we have observed that a single dose treatment with codeine plus acetaminophen in the acute phase of

the P-IPS was not able to alter hyperalgesia induced by paclitaxel in the chronic phase syndrome. This probably occurs because a single administration of the combination is not enough to reverse hyperalgesic symptoms in the chronic phase of P-IPS. So, this fact may limit the use of this protocol (codeine plus acetaminophen in a single dose) in clinical practice. Once paclitaxel is usually used in several protocols in cancer therapy (with more than one administration), a repeated treatment with codeine plus acetaminophen could prevent the onset of painful symptoms of the chronic pain syndrome.

Besides their beneficial effect to be clinically efficacious for relieving cancer-related pain, opioids and NSAID may have their use limited due to their side effects, such as increased locomotor activity and hepato-nephrotoxicity, respectively [42, 43]. We have observed the oral treatment with acetaminophen, codeine, codeine plus acetaminophen or morphine neither induced changes in the motor function nor caused any alterations in the activity of enzymes ALT/AST or in the creatinine/urea levels that would indicate liver or renal injury, respectively. These data indicate that the analgesics tested have low toxicity for the doses, route and parameters used.

Together, analgesics of WHO ladder are capable of reverting both acute and chronic phases of P-IPS, with codeine plus acetaminophen presenting more potent, efficacious and long-lasting effect. Therefore, WHO analgesics ladder could also be useful to treat P-IPS and clinical controlled studies assessing the therapeutic potential of codeine plus acetaminophen are encouraged.

Acknowledgments

This study was supported by the Conselho Nacional de Desenvolvimento Científico (CNPq; Brasília, Distrito Federal, Brazil) and the Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES; Brasília, Distrito Federal, Brazil). We also acknowledge the receipt of fellowships from CNPq and CAPES.

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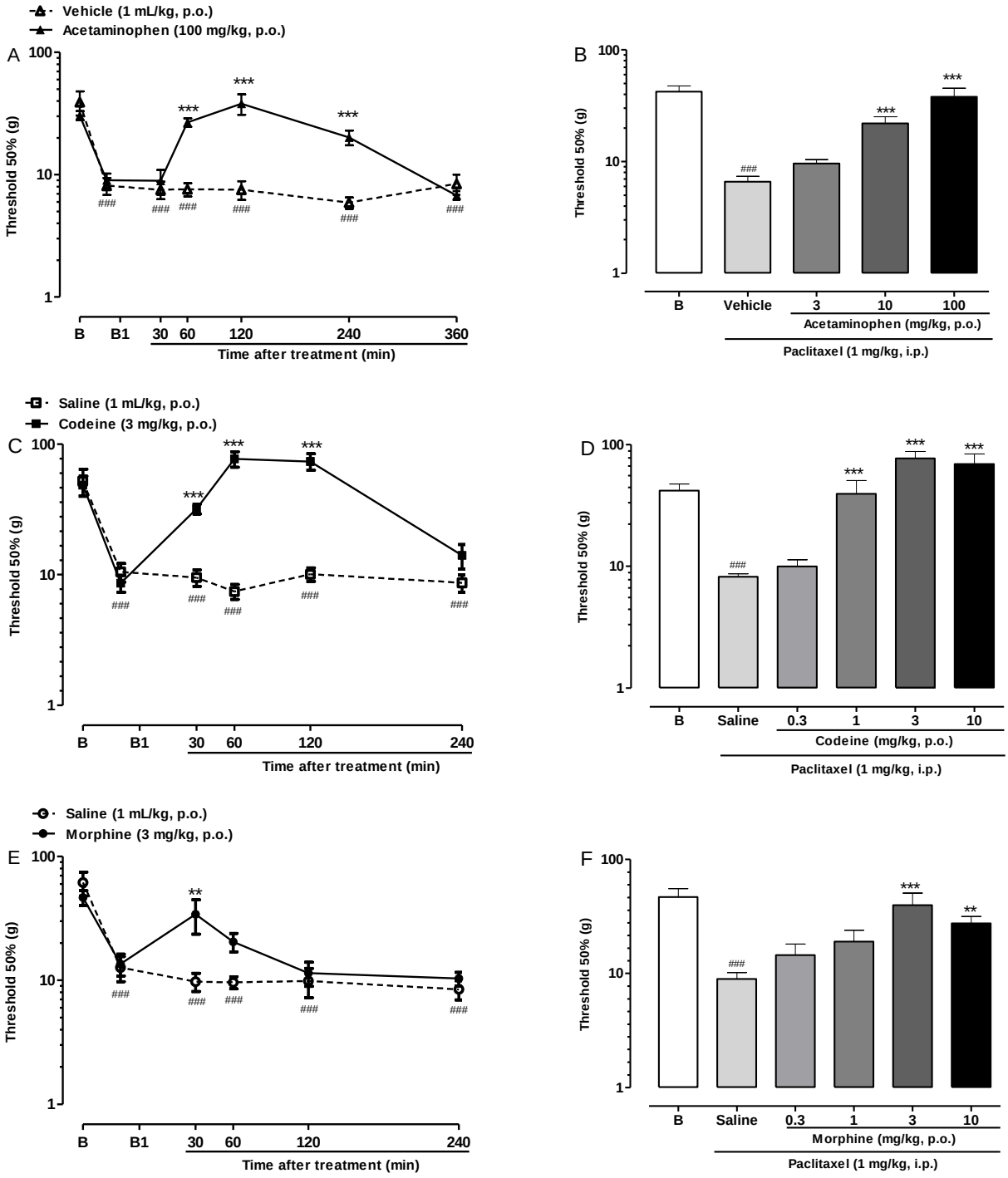

Figure 1. Effect of acetaminophen, codeine or morphine on mechanical hyperalgesia induced by a single injection of paclitaxel (1 mg/kg, i.p.) in rats. Time course (A, C, E) and dose–response (B, D, F) curves after the administration of acetaminophen (3-100 mg/kg, p.o.; A and B) -, codeine (0.3-10 mg/kg, p.o.; C and D) - or morphine (0.3-10 mg/kg, p.o.; E and F), respectively, in rats. B in the x axis denotes the baseline threshold 50% before paclitaxel treatment and B1 denotes the baseline threshold 50% after paclitaxel and before analgesics. Each point or bar represents the mean of 6-7 rats, and vertical lines show the SEM. ###p<0.001 compared with baseline (B), one-way ANOVA followed by Dunnett's test. **p<0.01 and ***p<0.001 compared with the control groups (saline 0.9% or vehicle solution), two-way ANOVA followed by Bonferroni's test (A, C and E). **P<0.01 and ***p<0.001 compared with the control groups (saline 0.9% or vehicle solution) group, one-way ANOVA followed by Dunnett's test (B, D and F).

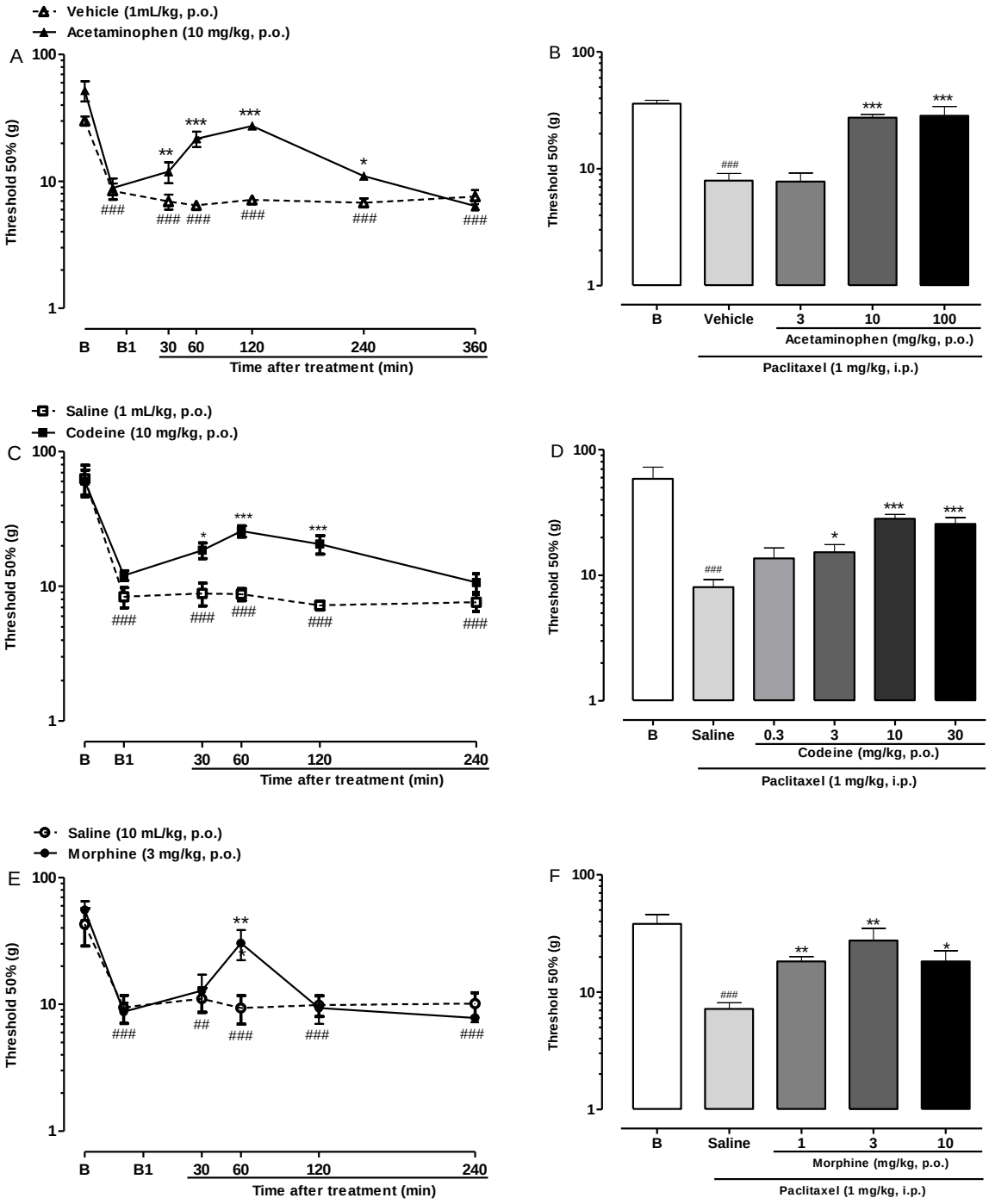

Figure 2. Effect of acetaminophen, codeine or morphine on mechanical hyperalgesia induced by the continuous administration of paclitaxel (1 mg/kg, i.p.) in rats. Time course and dose–response curves after the administration of acetaminophen (3-100 mg/kg, p.o.; A and B) -, codeine (0.3-30 mg/kg, p.o.; C and D) – or morphine (1-10 mg/kg, p.o.; E and F), respectively, in rats. B in the x axis denotes the baseline threshold 50% before paclitaxel treatment and B1 denotes the baseline threshold 50% after paclitaxel and before analgesics Each point or bar represents the mean of 6-7 rats, and vertical lines show the SEM. ###p<0.001 compared with baseline (B), one-way ANOVA followed by Dunnett's test. *p<0.05, **p<0.01 and ***p<0.001 compared with the control groups (saline 0.9% or vehicle solution), two-way ANOVA followed by Bonferroni's test (A, C and E). **p<0.01 and ***p<0.001 compared with the control groups (saline 0.9% or vehicle solution) group, one-way ANOVA followed by Dunnett's test (B, D and F).

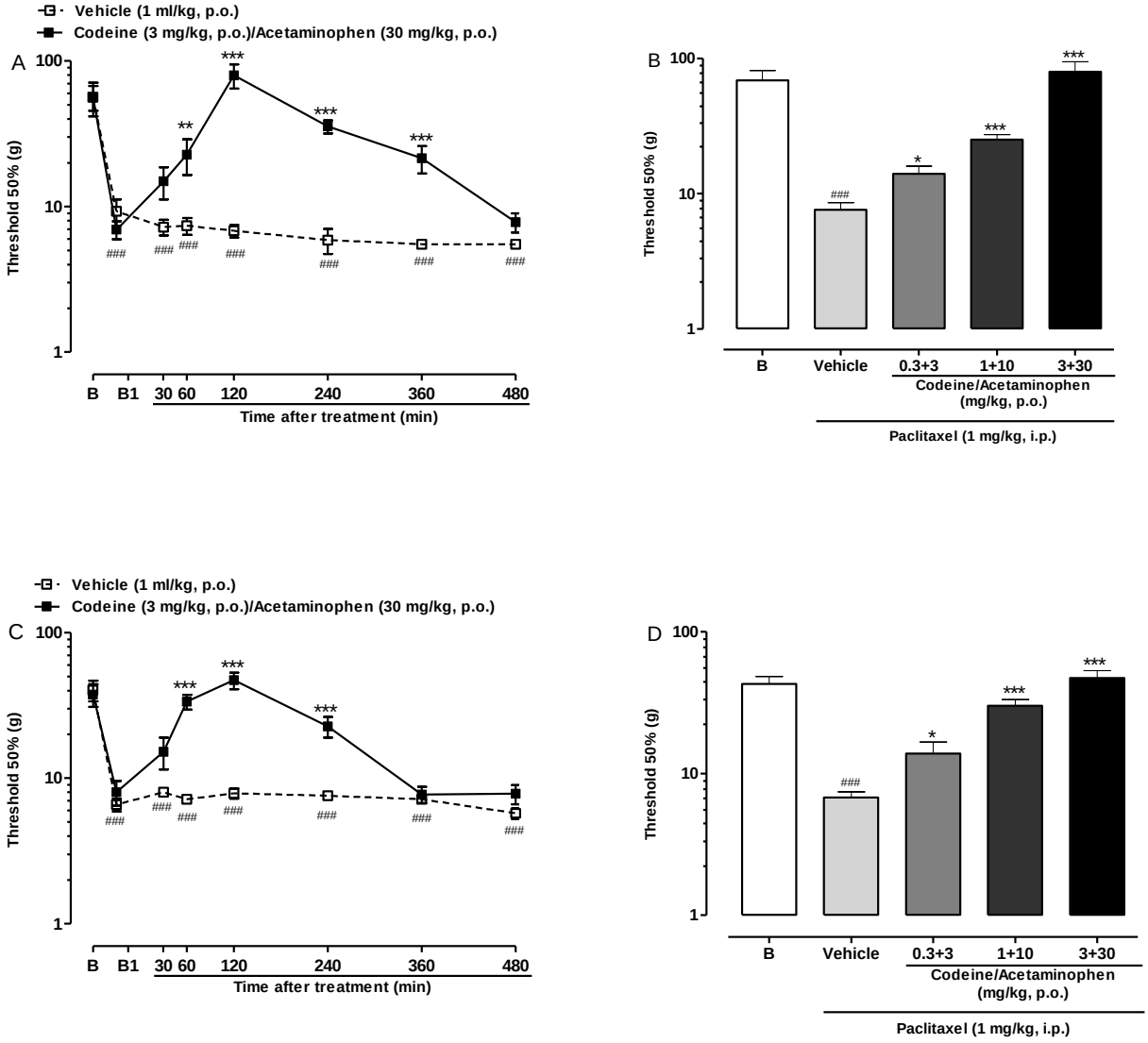

Figure 3. Effect of the combination of codeine plus acetaminophen on mechanical hyperalgesia induced by a single and continuous injection of paclitaxel (1 mg/kg, i.p.) in rats. Time course and dose–response curves, respectively, after the administration of codeine plus acetaminophen (3-30 mg/kg, p.o.) - (A and B) -, in the P-APS. And codeine plus acetaminophen (3-30 mg/kg, p.o.) - (C and D), in the CIPN. B in the x axis denotes the baseline threshold 50% before paclitaxel treatment and B1 denotes the baseline threshold 50% after paclitaxel and before analgesics. Each point or bar represents the mean of 6-8 rats, and vertical lines show the SEM. ###p<0.001 compared with baseline (B), one-way ANOVA followed by Dunnett's test. **p<0.01 and ***p<0.001 compared with control (vehicle solution) group, two-way ANOVA followed by Bonferroni's test (A and C). **P<0.01 and ***p<0.001 compared with control (vehicle solution) group, one-way ANOVA followed by Dunnett's test (B and D).

Table 1. The effective dose 50 (ED_{50}), maximal effect (E_{max}) and time to anti-hyperalgesia start (S), peak (P) and last (L) of acetaminophen, codeine, codeine plus acetaminophen or morphine on acute and chronic both phases of paclitaxel-induced hyperalgesia in rats.

Treatment	Acute			Chronic		
	ED_{50} (mg/kg)	**E_{max} (%)**	**Time S/P/L (h)**	**ED_{50} (mg/kg)**	**E_{max} (%)**	**Time S/P/L (h)**
Acetaminophen	7 (5-10)	91±7	1/2/4	10 (3-30)	82±4	0.5/2/4
Codeine	0.7 (0.4-1.2)	100	0.5/1/2	6 (3-13)	75±6	0.5/1/2
Codeine + Acetaminophen	0.7 (0.5-1.0) + 7 (5-10)	100	2/2/6	0.5 (0.3-0.6) + 5 (4-6)	100	1/2/4
Morphine	1.4 (0.4-5.3)	86±13	0.5/0.5/0.5	2 (1-5)	72±17	1/1/1

Data are expressed as geometric means accompanied by their respective 95% confidence intervals for ED_{50} and as mean± SEM for E_{max} values.

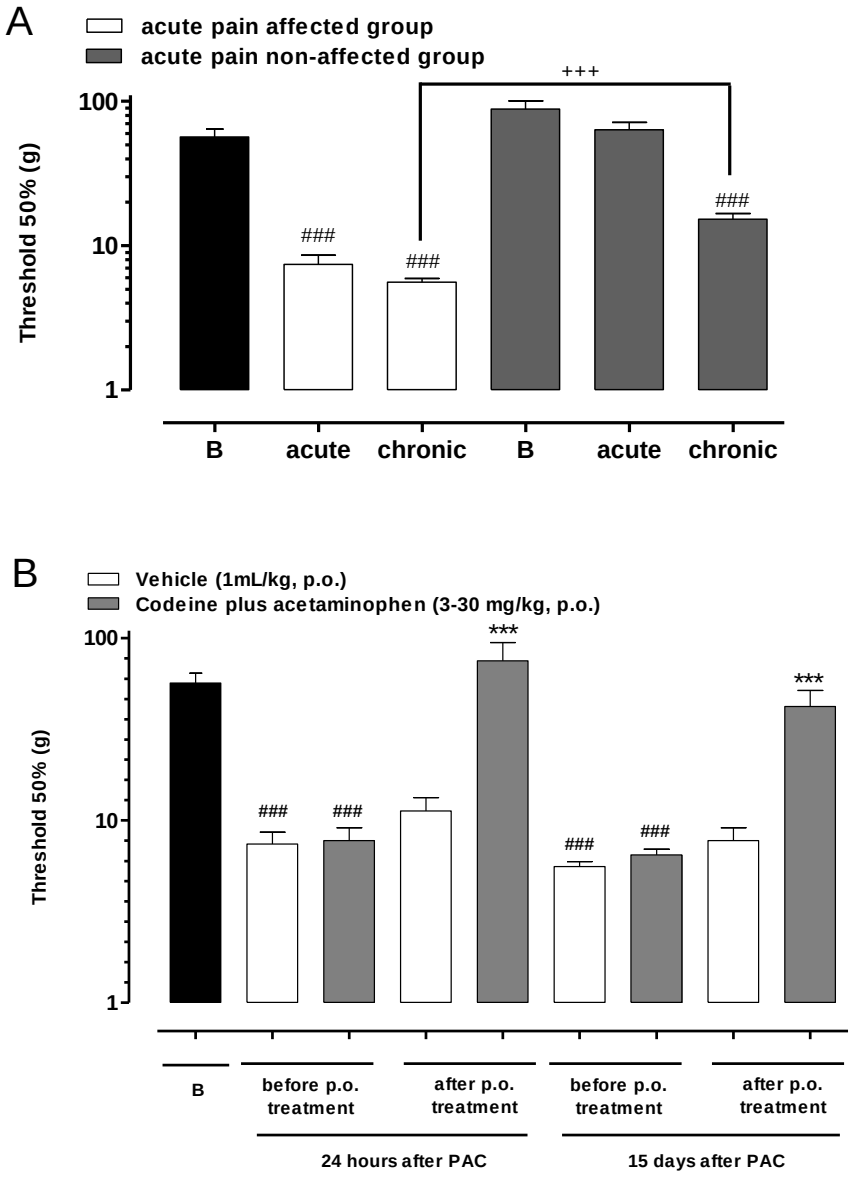

Figure 4. Relation between the acute mechanical hyperalgesia and the degree of chronic mechanical hyperalgesia induced by paclitaxel (1 mg/kg, i.p.) in rats. Comparison of chronic mechanical hyperalgesia between acute pain-affected group and acute pain non-affected group (A). Effect of the pre-treatment on acute pain induced by paclitaxel with codeine plus acetaminophen (3+30 mg/kg, p.o.) in acute-pain affected rats (B). Acute and chronic mechanical hyperalgesia were evaluated 24 h or 15 days after the first paclitaxel injection. B in the x axis denotes the baseline threshold 50% before paclitaxel treatment. Each point or bar represents the mean of 6 rats, and vertical lines show the SEM. Statistical analysis was performed using unpaired t-test or one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test or Dunnett test; $^{###}p<0.001$ denotes significance level in comparison with the B (baseline) group (animals with acute or chronic pain) ; $^{+++}p<0.001$ denotes the significance levels when compared chronic pain in the acute pain affected and non-affected group; ***$p<0.001$ denotes the significance level in comparison with vehicle treated animals.

Table 2. The effect of acetaminophen, codeine, morphine, codeine plus acetaminophen or vehicle on spontaneous (open-field test) locomotor activity in rats and biochemical parameters after this treatment.

Treatment	Dose (mg/kg)	Motor function		Biochemical parameters			
		Crossing (n.)	Rearing (n.)	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Vehicle	---	49±3	25±3	114±5	148±7	0.481±0,029	54±3
Acetaminophen	100	35±6	16±3	94±3	173±10	0.475±0.016	49±2
Codeine	30	41±4	20±2	128±6	186±11	0.490±0.028	50±5
Codeine + Acetaminophen	3 + 30	47±4	22±3	71±13	162±7	0.491±0.024	48±2
Morphine	10	47±9	25±2	132±22	138±7	0,521±0.032	44±2

Significant differences were not observed between most groups (one-way ANOVA followed by Dunnett's test). Data represent the mean ± S.E.M of 4-6 animals.

Carta referente à submissão do artigo

Submission Confirmation

Thank you for submitting your manuscript to *Cancer Chemotherapy and Pharmacology*.

Manuscript ID:	CCP-14-0196
Title:	Efficacy of the World Health Organization analgesic ladder in a model of paclitaxel-induced pain syndrome
Authors:	Pinheiro, Kelly Rigo, Flávia Oliveira, Sara Hausen, Bruna Moresco, Rafael Ferreira, Juliano
Date Submitted:	31-Mar-2014

5. CONCLUSÕES

Tendo em vista os resultados obtidos no presente estudo, pode-se concluir que:

- Juntos, os analgésicos da escada proposta pela OMS são capazes de reverter ambas as fases aguda e crônica da síndrome dolorosa induzida por paclitaxel. E a combinação de codeína mais paracetamol apresentou-se mais potente, eficaz promovendo um efeito de longa duração;

- A presença da fase aguda está relacionada com a gravidade dos sintomas neuropáticos encontrados na fase crônica da síndrome dolorosa induzida por paclitaxel;

- A administração da combinação de codeína mais paracetamol (3-30 mg / kg, v.o.) na fase aguda da síndrome dolorosa induzida por paclitaxel não foi capaz de prevenir a exacerbação da hiperalgesia na fase crônica da síndrome;

- Os analgésicos testados possuem baixa toxicidade para a dose, via e parâmetros utilizados.

Consideração final

O uso da escada analgésica parece ser uma opção simples, segura e eficaz para o tratamento da síndrome dolorosa induzida por paclitaxel.

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