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Stéfano Leite Dau

EFEITO ANALGÉSICO DO TRATAMENTO COM CLORETO DE AMÔNIO 2% E ACETAMINOFENO ASSOCIADO À FENILBUTAZONA EM EQUINOS

Santa Maria, RS
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Tese apresentada ao curso de doutorado do Programa de Pós-Graduação em Medicina Veterinária, área de concentração em Cirurgia e Clínica Veterinária, da Universidade Federal de Santa Maria (UFSM,RS), como requisito parcial para obtenção do título de **Doutor em Medicina Veterinária.**

Orientador: Prof. PhD Flávio Desessards de La Côrte

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Aprovado em 04 de setembro de 2020:

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RESUMO

EFEITO ANALGÉSICO DO TRATAMENTO COM CLORETO DE AMÔNIO 2% E ACETAMINOFENO ASSOCIADO À FENILBUTAZONA EM EQUINOS

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ORIENTADOR: Flávio Desessards de La Côrte

O manejo da dor crônica é um fator de grande importância dentro da medicina veterinária por estar relacionado com o bem-estar dos animais. A claudicação crônica de origem no casco é um problema frequente dentro da clínica de equinos e o seu tratamento apresenta diferentes índices de sucesso. Os compostos neurolíticos possuem ação similar à neurectomia, porém permitem que os animais mantenham seu sistema fisiológico de defesa com menos efeitos colaterais. Contudo, estes fármacos perderam credibilidade quanto ao seu emprego pelo uso incorreto. Acreditava-se, equivocadamente, que estes produtos atuavam de forma similar aos anestésicos locais. Desta forma, a primeira parte deste trabalho foi dividido em duas etapa. A primeira etapa consisitu da avaliação objetiva do efeito analgésico do cloreto de amônio 2 % (CA2%) em diferentes casos de claudicações crônicas ligadas ao casco (aparelho podotroclear, n=5; aparelho podotroclear associado à articulação interfalangena distal, n=6; articulação interfalangeana distal, n= 2; laminite, n=1; e dor solear, n=1) por um período de 62 dias. A injeção perineural do CA2% no nervo digital palmar foi capaz de induzir uma melhora parcial a completa na claudicação, sendo observados melhores resultados nos dias 12 e 19 após o tratamento. Observou-se que lesões radiográficas de moderada a severa do osso navicular e articulação interfalangeana distal podem interferir negativamente na eficácia do CA2%. Posteriormente, a segunda etapa, avaliou-se a neurotoxicidade do CA2% para melhor entendimento do mecanismo de ação deste composto. Assim, 18 e 6 nervos digitais palmares foram tratados com CA2% e com solução fisiológica respectivamente, para posterior coleta por neurectomia seguida da avaliação histológica. O CA2% induziu degeneração Walleriana de moderada à severa até 62 dias após o tratamento, similar ao observado nos nervos tratados com solução fisiológica. A injeção perineural com CA2% não apresentou interferência em neurectomias futuras. Os resultados reforçam que o CA2% demonstra ser uma opção terapêutica útil e segura para o manejo da dor crônica ligada ao casco em equinos. A analgesia multimodal, por sua vez, é uma importante técnica de manejo da dor crônica e apresenta menores índices de efeitos colaterais quando comparada com a utilização de um medicamento para a mesma finalidade. Dessa forma, a terceira parte deste estudo avaliou o efeito analgésico e a toxicidade do acetaminofeno (AC, 20 mg Kg^{-1}) associado à fenilbutazona (FBZ, 2.2 mg Kg^{-1}) sobre a claudicação com origem no casco induzida por cintas metálicas. Pôde-se observar, baseado na diferença da intensidade da claudicação, que a associação de AC com FBZ apresentou um potencial analgésico total superior ao tratamento com AC ($p=0.008$), FBZ ($p= 0.0117$) e o grupo controle ($p<0.0001$). A associação de AC com FBZ, duas vezes ao dia, por 14 dias não apresentou alterações significativas dos parâmetros hematológicos e bioquímicos quando comparados aos valores de referência. Estes dados reafirmam o potencial analgésico do acetaminofeno associado com a fenilbutazona para o manejo da dor em equinos. Estudos futuros sobre a ação do acetaminofeno, associado ou não a outros fármacos, em diferentes modelos experimentais ou clínicos, se fazem necessários antes da recomendação na rotina clínica.

Palavras-chave: Anti-inflamatórios não esteroides. Claudicação. Dor Crônica. Neurolíticos.

ABSTRACT

ANALGESIC EFFECT OF THE TREATMENT WITH 2% AMMONIUM CLHORIDE AND ACETAMINOPHEN ASSOCIATED WITH PHENYLBUTAZONE IN HORSES

AUTHOR: Stefano Leite Dau
ADVISOR: Flávio Desessards de La Corte

The management of chronic pain is an important factor within veterinary medicine since it is related to animals' welfare. Chronic hoof lameness is a frequent problem in the practitioners' routine and it presents different success rates. Neurolytic compounds have a mechanism of action similar to the neurectomy, but they allow animals to maintain their physiological defense system. However, these drugs have lost credibility regarding their use due to their misuse. It was mistakenly believed that these have similar mechanism of action than local anesthetics. Thus, the first part of this work aimed to objectively assess the analgesic effect of ammonium chloride 2% (2%AC) in different cases of chronic hoof (podotrochlear apparatus, n= 5; podotrochlear apparatus associated with the distal interphalangeal joint, n= 6; distal interphalangeal joint, n= 2; laminitis, n= 1; and bruised sole, n= 1) for a period of 62 days. Perineural injection of 2%AC into the palmar digital nerve was able to induce partial to complete lameness improvement, with better results being observed on days 12 and 19 after treatment. Moderate to severe radiographic lesions of navicular bone and distal interphalangeal joint can negatively interfere on the 2%AC analgesic effect. Subsequently, in a second study was carried out to assess the neurotoxicity of 2%AC to better understand the mechanism of action of this drug. Thus, 18 and 6 digital palmar nerves were treated with 2%AC and saline solution, respectively, and later nerve samples were collect by neurectomy for histological evaluation. 2%AC induced moderate to severe Wallerian degeneration for up to 62 days after treatment, similar to those observed in nerves treated with saline. Perineural injection with 2%AC did not interfere in future neurectomy. The results of both studies reinforce that 2%AC can be a useful and safe therapeutic option for the management of chronic hoof lameness in horses. In turn, multimodal analgesia is also an important technique for managing chronic pain and has lower rates of side effects when compared to administration of one medication for the same purpose. Therefore, the third part of this study evaluated the analgesic effect and toxicity of acetaminophen (ACET, 20 mg Kg^{-1}) associated with phenylbutazone (PBZ, 2.2 mg Kg^{-1}) on lameness induced by hoof clamps. The association of ACET with PBZ demonstrated an overall analgesic effect, based on the change in the lameness, superior to the treatment with only ACET ($p=0.008$), PBZ ($p=0.0117$) and the control group ($p<0.0001$). The association of ACET with PBZ twice a day for 14 days did not show significant changes in hematological and biochemical parameters when compared to reference values. These data reaffirm the analgesic potential of acetaminophen associated with phenylbutazone for pain management in horses. Future studies to evaluate potential analgesic of different therapeutic protocols with ACET, associate or not with others drugs, in different experimental or clinical models, are necessary before the recommendation of its use in the clinical routine.

Keywords: Non-steroidal anti-inflammatory drugs. Lameness. Chronic pain. Neurolytic compounds.

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LISTA DE ILUSTRAÇÕES

Figure 4 – Mean and *s.d* of serum biochemical paramenters of 12 horses treated orally twice a day for 14 days with acetoaminophen (20 mg Kg^{-1}), acetaminophen (20 mg Kg^{-1}) associated with phenylbutazone (2.2 mg Kg^{-1}) and with phenylbutazone (4.4 mg Kg^{-1}) only. Dot lines along x axis represent laboratory references.* statistical difference ($p<0.05$) between mean of day 0 compared with day 7; @ statistical difference ($p<0.05$) between mean of day 0 compared with day 14; and # statistical difference ($p<0.05$) between mean of day 7 compared with day 14 …… 61

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1. INTRODUÇÃO

Os cavalos das diferentes modalidades esportivas estão sujeitos ao desenvolvimento de patologias no sistema locomotor, as quais se manifestam clinicamente por claudicação e queda de desempenho (ROSS, 2003). A dor com origem no casco é uma importante causa de claudicação, principalmente nos membros torácicos, podendo representar um terço das claudicações em algumas modalidades esportivas (DABAREINER et al., 2005ab; MURRAY et al., 2006; ABREU et al., 2011).

O casco é uma estrutura complexa e as diferentes estruturas abrigadas no seu interior podem ser a origem da dor em um caso de claudicação (DYSON e MARKS, 2003), porém nem sempre o clínico tem à sua disposição técnicas de diagnóstico por imagem de referência para identificar precisamente as lesões nestas estruturas. Logo, após uma resposta positiva utilizando a técnica de bloqueio do nervo palmar digital, muitos casos são classificados genericamente como dor palmar, anteriormente conhecida como síndrome/doença do navicular (MURRAY et al., 2006). Contudo, este diagnóstico permite a inclusão de diferentes alterações podais como a sinovite/osteoartrite da articulação interfalangeana distal (AID), desmite dos ligamentos colaterais da AID e do osso navicular, desmite do ligamento ímpar, edema do osso navicular, tendinite do tendão flexor digital profundo, bursite do osso navicular, osteite da terceira falange e dor solear (BAXTER e STASHAK, 2011).

As doenças ortopédicas crônicas, como a dor palmar, são desafios frequentes na medicina esportiva equina (SANCHEZ e ROBERTSON, 2014). Normalmente, nos casos de dor crônica ligada ao casco, os clínicos optam pela associação de diferentes modalidades terapêuticas a fim de alcançar uma melhor resposta clínica, como o emprego do casqueamento e ferrageamento corretivos, administração de anti-inflamatórios não-esteroidais (AINES), e infiltração intrasinovial com corticosteroides associados ou não ao hialuronato de sódio (GUTIERREZ-NIBEYRO et al., 2010).

A dessensibilização dos nervos periféricos, por meio de cirurgia (neurectomia) ou aplicação de compostos neurolítcos, é um procedimento paliativo que visa controlar a dor e propiciar um melhor bem-estar para o animal. Os agentes neurolíticos são amplamente utilizados na medicina humana para o tratamento de

diferentes doenças crônicas (MANCHIKANTI et al., 2001; ROSEN, 2004; KOYYALAGUNTA e BURTON, 2010). Na medicina veterinária, a má utilização das técnicas de dessensibilização nervosa associada a resultados insatisfatórios, levou a uma descredibilidade da eficácia destes métodos pelos clínicos (HARKINS et al., 1997; CAMPOS et al., 2013). Os agentes neurolíticos apresentam vantagens quando comparados à neurectomia, pois não retiram a percepção da dor aguda importante para a defesa do animal, e ao mesmo tempo permitem a dessensibilização ou diminuição do estimulo doloroso crônico (NICOLETTI et al., 2007; SCHNEIDER et al., 2014). Porém, existem poucos estudos que permitem um melhor entendimento dos efeitos clínicos dos agentes neurolíticos nas diferentes patologias do sistema locomotor, principalmente para aquelas ligadas ao casco dos equinos.

Sabe-se que a terapia multimodal, que compreende a utilização de mais de um fármaco para o mesmo objetivo terapêutico, pode ser uma forma de obter resultados mais satisfatórios e com menores efeitos colaterais quando comparada à utilização de apenas um medicamento (MUIR e WOOLF, 2001; MUIR, 2010). O acetaminofeno, popularmente conhecido como paracetamol, apresenta baixo potencial analgésico, mas é muito utilizado como adjuvante para analgesia pós-cirúrgica em humanos porque possibilita o emprego de doses menores de opióides (WARD e ALEXANDER-WILLIAMS, 1999). A combinação de diferentes AINES com o paracetamol apresenta um efeito sinérgico para analgesia, de forma que possibilita a utilização de menores doses dos fármacos e, consequentemente, uma menor incidência de efeitos colaterais (MIRANDA et al., 2006). Os equinos, diferente dos pequenos animais, apresentam maior tolerância à utilização do acetaminofeno, porém existem poucos estudos sobre a sua aplicabilidade clínica (WEST et al., 2011; MERCER et al., 2019). Primeiramente, West e colaboradores (2011) relataram um possível efeito sinérgico do paracetamol associado à fenilbutazona para o controle da dor em um pônei com laminite crônica. Posteriormente, um estudo com claudicação induzida demonstrou um potencial analgésico similar entre o acetaminofeno e o flunixin meglumine (FOREMAN et al., 2016). Recentemente, comprovou-se que é segura a utilização de múltiplas doses de paracetamol por até 14 dias em equinos (MERCER et al., 2019). Entretanto, não se tem comprovado o efeito sinérgico e a segurança da associação do acetaminofeno com a fenilbutazona no controle da dor em equinos.

Visto a importância e as limitações dos estudos sobre a eficácia e segurança da utilização dos agentes neurolíticos e do acetaminofeno em equinos, ressalta-se a importância de estudos para avaliar o potencial analgésico, segurança e possível efeito sinérgico destes compostos em casos clínicos, ou experimentais, de dor ligada ao casco.

2. REVISÃO BIBLIOGRÁFICA

2.1. Mecanismos da dor

A dor é uma experiência sensorial e emocional que representa a consciência pelo animal de um dano ou ameaça para a integridade de seus tecidos (MOLONY e KENT, 1997). A nocicepção corresponde ao processo de detecção, transmissão, modulação, projeção e interpretação no sistema nervoso central de um estímulo nocivo (MUIR, 2010). Este processo se inicia pelos neurônios nociceptivos que transformam o estímulo nocivo em impulsos elétricos e transmitem este sinal por meio das fibras aferentes aos neurônios de segunda ordem, localizados no corno dorsal da medula espinhal. Estes projetam o sinal para o córtex cerebral que interpreta e emite a resposta ao estímulo (MUIR, 2010).

O processo inflamatório produz substâncias químicas como prostaglandinas, histaminas, citocinas e leucotrienos que modulam os nociceptores de forma que o limiar de dor diminui, tornando o organismo mais sensível a estímulos nocivos (MUIR e WOOLF, 2001). Isto normalmente ocorre na percepção periférica em casos de dor aguda. Já os processos de dor crônica, são caracterizados por um estímulo mais prolongado e severo, podendo gerar uma sensibilização central que culmina com hiperalgesia, alodinia e hipersensibilidade secundária (MUIR, 2010). A sensibilização central pode durar horas ou dias e ocorre principalmente pela remoção do bloqueio de magnésio e ativação dos receptores N-metil-D-aspartato (NMDA) pelo glutamato (LATREMOLIERE e WOOLF, 2009). A inibição noradrenégica geralmente ocorre após estimulação central de receptor alfa-2 (α-2) e liberação de neurotransmissores inibitórios, como norepinefrina, opióides endógenos e o ácido gama-amino-butírico (GABA) (TASAKA, 2006).

Casos de dor crônica em equinos como visto na laminite e osteoartrite podem apresentar quadros de dor contínua por meses a anos em decorrência dos processos de sensibilização e desinibição (MUIR, 2010). Assim, a dor crônica deve ser combatida para evitar que as respostas nervosas e endógenas sejam ativadas e acabem afetando negativamente funções de homeostase importantes para o bem-estar dos animais (CARSTENS e MOBERG, 2000).

2.1.1. Manejo da dor em equinos

A abordagem farmacológica para o controle da dor é muito importante, porém alguns pontos como tecido lesionado, intensidade e localização da dor devem ser considerados (MUIR e WOOLF, 2001; MUIR, 2010). Existem cinco categorias de fármacos que são amplamente utilizadas no manejo da dor em equinos: anti-inflamatórios esteroides, anti-inflamatórios não-esteroides (AINES), opióides, agonistas alfa-2 adrenérgicos e anestésicos locais (SANCHEZ e ROBERTSON, 2014). Cada uma destas categorias possui um mecanismo de ação diferente, logo são utilizadas separadamente ou em conjunto, de forma sinérgica ou aditiva, de acordo com o caso clínico (MIRANDA et al., 2006; TASAKA, 2006). Terapias alternativas como a acupuntura, quiropraxia e o emprego de nutracêuticos são frequentemente utilizadas como adjuvantes nas terapias convencionais a fim de obter uma melhor resposta ao tratamento (FLEMING, 2002).

Os AINES apresentam propriedades anti-inflamatória, analgésica e antipirética, e tem como principal mecanismo de ação o bloqueio da síntese de prostaglandinas e leucotrienos pelas enzimas cicloxigenases (TASAKA, 2006). Alguns desses fármacos são classificados como não seletivos por inibirem tanto a enzima cicloxigenase tipo 1 (COX-1) quanto a tipo 2 (COX-2), e outros são classificados como seletivos por atuarem apenas na enzima COX-2 (TASAKA, 2006). Essa diferenciação é decorrente dos efeitos adversos destes fármacos, uma vez que a COX-1 é uma enzima importante na homeostase celular (MUIR, 2010). Os AINES seletivos COX-2 apresentam maior segurança e potencial analgésico semelhante aos AINES não seletivos, porém também podem causar efeitos colaterais, uma vez que a COX-2 desempenha funções fisiológicas no sistema nervoso central, rins, olhos e órgãos reprodutivos (MUIR, 2010). Os anti-inflamatórios esteroides, também conhecidos como glicocorticoides, atuam na cascata inflamatória em um estágio anterior aos AINES, por meio do bloqueio da enzima fosfolipase-A2, responsável por clivar os fosfolipídios da membrana celular em ácido araquidônico, que posteriormente é utilizado na produção de prostaglandinas e leucotrienos (TASAKA, 2006; MUIR, 2010).

Por sua vez, os opiódes são eficazes como analgésicos para equinos por meio de sua ação sobre receptores muscarínicos e kappa. Porém, estes compostos apresentam efeitos colaterais que dependendo de como são administrados se

sobrepõe ao propósito primário (TASAKA, 2006; MUIR, 2010). Os melhores efeitos analgésicos dos opióides são obtidos quando administrados em conjunto com outros fármacos como os AINES, agonistas α-2 adrenérgicos e cetamina, permitindo o uso de uma dose menor e, desta forma, diminuindo efeitos adversos como ataxia, excitação, cólica e constipação (MUIR, 2010). A administração por via epidural é uma forma segura de administração de opióides, uma vez que permite um bom índice de analgesia tanto nos membros pélvicos quanto nos torácicos e com baixa incidência de efeitos colaterais (FREITAS et al., 2011; CARREGARO et al., 2014; SCHAFFER et al., 2014).

Os agonistas α-2 adrenérgicos produzem um estado de estupor, ataxia, relaxamento muscular e analgesia por meio de ativação dos receptores α-2 tanto no sistema nervoso central quanto no periférico (MUIR, 2010). Seus efeitos normalmente são doses-dependentes e podem causar, além do efeito desejado, bradicardia, depressão respiratória, hipotensão e raramente comportamento violento (MUIR, 2010). Seu principal uso clínico está na contenção dos animais para realização de procedimentos em estação como manejo de feridas, remoção de tumores cutâneos e infiltrações articulares (MUIR, 2010). Outra aplicação destes medicamentos está no controle da dor visceral e somática (MOENS et al., 2003; ELFENBEIN et al., 2009).

A utilização de anestésicos locais no controle da dor é menos frequente quando comparado com os compostos mencionados anteriormente, pois é necessária a utilização de catéteres perineurais ou epidurais para a infusão contínua destes fármacos (DRIESSEN et al., 2008). O efeito analgésico destes compostos ocorre por bloqueio dos canais de sódio impedindo a propagação do estímulo nervoso. O tempo de duração deste bloqueio varia de acordo com o composto utilizado (SILVA et al., 2015). A administração sistêmica destes compostos por meio de uma dose em *bolus* seguido de infusão contínua intravenosa também auxilia no controle da dor, além de apresentar propriedades procinética, antiarritmogência e antiendotóxica (ROBERTSON et al., 2005; MALONE et al., 2006; MUIR, 2010).

2.1.2. Emprego de neurolíticos no manejo da dor crônica em equinos

A síndrome da dor palmar ou plantar, também conhecida com síndrome do navicular, é responsável por grande parte dos casos de claudicação crônica em

equinos (DABAREINER et al., 2005; MURRAY et al., 2006). Esta patologia apresenta diferentes taxas de sucesso, as quais estão correlacionadas a diferentes fatores como: a correta identificação da lesão, terapias empregadas e respeito ao período de reabilitação (DABAREINER et al., 2003; GUTIERREZ-NIBEYRO et al., 2015). Casos refratários ao tratamento clínico, normalmente, levam à retirada precoce do equino da atividade esportiva. A neurectomia do nervo digital palmar é uma das técnicas cirúrgicas mais antigas utilizadas na medicina de equinos, sendo ela uma alternativa para alívio da dor crônica em determinados casos (FURST e LISCHER, 2019). Esta técnica pode apresentar complicações como desensibilização incompleta do casco, formação de neuromas, infecção da muralha do casco e da terceira falange, ruptura do tendão flexor digital profundo e recorrência precoce da claudicação (MAHER et al., 2008; GUTIERREZ-NIBEYRO et al., 2015). A fim de preservar o bem-estar animal e garantir o nível de competição dos atletas, a Federação Equestre Internacional não permite que animais com hipossensibilidade induzida pela neurectomia participem de competições regulamentadas por ela (FEI, 2019).

A neurólise química é um método de dessensibilização temporária, com duração de semanas a meses, que apresenta vantagens em relação à neurectomia por evitar a formação de neuromas e outras complicações relacionadas à secção do nervo (MAHER et al., 2008; SCHNEIDER et al. 2014; GUTIERREZ-NIBEYRO et al., 2015). Os principais agentes neurolíticos utilizados no manejo da dor crônica são compostos à base de sais de amônio (CAMPORS et al., 2013; ROCHA et al., 2016), fenóis (SCHNEIDER et al. 2014; D'SOUZA e WARNER, 2020), álcoois (NOCOLETTI et al, 2007; ESCODRO et al, 2015) e derivados da planta *Sarracina purpurea* (HARKINS et al., 1997; CAMPOS et al., 2013).

O mecanismo de ação dos agentes neurolíticos baseia-se na capacidade de lesionar o nervo bloqueando, assim, a condução do estímulo doloroso (BOESCH, 2019). O nervo periférico é composto por mielina, axônio, endoneuro, fascículo, perineuro e epineuro, sendo as lesões oriundas da utilização dos neurolíticos classificadas de acordo com as estruturas acometidas (CHOI et al., 2016). Seddon (1942) classificou as lesões dos nervos periféricos de acordo com a extensão da lesão do axônio e de suas camadas teciduais em: neuropraxia, lesão leve com perda motora e sensitiva sem alteração estrutural; axonotmese, perda de continuidade axonal e subsequente degeneração Walleriana do segmento distal sem lesão da

célula de Schwann, o que permite a sua regeneração de acordo com o grau de desorganização do nervo; e neurotmese, quando ocorre separação completa do nervo com desorganiação do axônio impedindo o crescimento axonal, dificultando assim a recuperção espontânea. Posteriormente, Sunderland (1951) propos uma modificação na classificação de SEDDON (1942) subdividindo a axonotmese em três categorias baseadas nas estruturas envolvidas e sua regeneração espontânea (Figura 1).

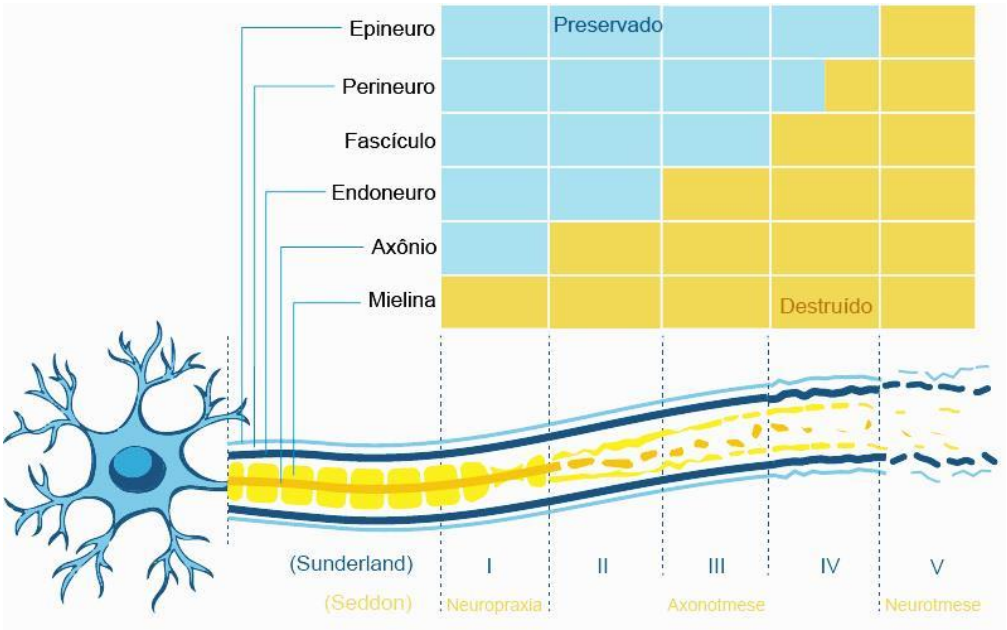

Figura 1 – Ilustração das classificações de SEDDON (1942) e SUDERLAND (1951) quanto à lesão dos nervos periféricos. Fonte: adaptado de MACKAY (2006) por Ana Maria Antonello 2020.

O tipo de neurolítico, sua concentração e a forma como é aplicado (intra ou perineural) são características importantes que influenciam no grau de lesão provocado no nervo e, consequentemente, no potencial analgésico destes compostos (KOBAYASHI et al., 1997; SCHNEIDER et al., 2014; NICOLETTI et al., 2007). Os compostos fenólicos, como o formaldeído, provocam graus mais severos de axonotmese a neurotmese, de acordo com a concentração e volume utilizados, o que está relacionado com uma menor chance de regeneração do nervo (SCHNEIDER et al., 2014; D'SOUZA e WARNER, 2020). Estes compostos são poucos utilizados em decorrência do maior risco de efeitos indesejados como desconforto, inflamação, edema, fibrose e necrose dos tecidos adjacentes ao nervo (SCHNEIDER et al., 2014; D'SOUZA e WARNER, 2020). Por outro lado, a utilização de compostos alcóolicos em diferentes concentrações também promove graus variados de axonotmese, porém sem comprometer a estrutura do nervo facilitando

assim a sua regeneração (SCHNEIDER et al., 2014; CHOI et al., 2016). Existem poucos relatos de reações de desconforto após aplicação perineural (NICOLETTI et al., 2007) e intraneural (SCHNEIDER et al., 2014) destes compostos. Da mesma forma, os sais de amônio são considerados seguros para utilização, pois promovem lesões de neuropraxia a graus moderados de axonotmese (KOBAYASHI et al., 1997; HERTL et al., 1998).

O emprego de neurolíticos para manejo da dor crônica ligada a diferentes patologias em humanos apresenta resultados satisfatórios (MANCHIKANTI et al., 2001; ROSEN, 2004; KOYYALAGUNTA e BURTON, 2010). Dentro da medicina veterinária, estes compostos são empregados principalmente na clinica de equinos com o objetivo de mimetizar o efeito da neurectomia dos nervos digitais palmares em casos de dor crônica ligada ao casco. A utilização incorreta dos agentes neurolíticos com o intuito de promover um bloqueio imediato de longa duração após sua aplicação, e assim possibilitar que os animais participem de eventos esportivos livre de dor, fez com que estas drogas perdessem credibilidade terapêutica entre os médicos veterinários e as entidades reguladoras. Entretanto, diferente da resposta obtida com a injeção intra ou perineural, de substâncias anestésicas como lidocaína e bupivacaína, os neurolíticos não promovem a completa dessensibilização após a sua aplicação (HARKINS et al., 1997; CAMPOS et al., 2013), mesmo após utilização intraneural de formaldeído (SCHNEIDER et al., 2014).

Mesmo a injeção intraneural dos nervos digitais palmares em equinos com álcool etílico 98% e formaldeído não foram capazes de promover a dessensibilização completa dos membros a estímulos sensitivos (SCHNEIDER et al., 2014). Em contraste, há relatos experimentais de dessensibilização substancial a completa, frente a estímulos mecânicos no casco em equinos após a utilização destes compostos neurolíticos (NICOLETTI et al., 2007; SCHNEIDER et al., 2014).

2.1.3. Emprego do acetaminofeno na Medicina Veterinária

O acetaminofeno, conhecido também como paracetamol, é um anti-inflamatório não esteróide que na década de 1950 foi utilizado em grande escala tanto em humanos quanto para animais pelo seu efeito analgésico e antipirético (TASAKA, 2006). Porém, as doses utilizadas em animais foram extrapoladas de

experimentos humanos e resultaram em relatos de muitos casos de intoxicação, principalmente em pequenos animais (TASAKA, 2006).

A sua ação anti-inflamatória é considerada inferior quando comparada a outros fármacos como o flunixin meglumine e a fenilbutazona (TASAKA, 2006). O paracetamol atua de forma mais específica na cicloxigenase 3 (COX-3) presente no cérebro, bloqueando a ação de pirógenos endógenos no centro hipotalâmico regulador da temperatura (TASAKA, 2006). Ele também interfere nos receptores de substância P ou na inibição dos neurônios excitados pela substância P na medula (MIRANDA *et al.*, 2006), além de estimular a liberação de serotonina (MALLET et al., 2010). Este fármaco possui um alto potencial de dissociação (pKa) e baixo grau de ligação às proteínas plasmáticas, o que lhe confere características farmacodinâmicas diferentes das obtidas com a administração dos outros AINES como, por exemplo, a possibilidade de melhor penetrar a barreira hematoencefálica (WARD e ALEXANDER-WILLIAMS, 1999; TASAKA, 2006). Por esta mesma razão ela se torna uma opção se uma associação com outros AINES é cogitada.

A biodisponibilidade do paracetamol após administração oral e retal em humanos é estimada em 63 a 89% e 24 a 98%, respectivamente, sendo que a via retal é a mais utilizada em pacientes pediátricos (WARD e ALEXANDER-WILLIAMS, 1999). Estudos em equinos mostram uma boa biodisponibilidade (91%) após administração oral quando comparada com cães (45%) (NEIRINCKX et al., 2010). A absorção do acetaminofeno ocorre principalmente no intestino delgado, logo a velocidade da abertura gástrica influencia na sua absorção (DOHERTY *et al.*, 1998; LOHMANN *et al.*, 2002). Uma pequena quantidade do fármaco sofre o metabolismo de primeira passagem no fígado (NEIRINCKX *et al.*, 2010). O pico plasmático ocorre próximo de duas horas em humanos, aproximadamente 56 minutos em cães (KUKANICH, 2016) e 31 minutos em equinos (DOHERTY et al., 1998) após administração oral.

Estudos em humanos demonstram a compartimentalização do paracetamol no líquido cérebro-espinhal, sendo que a concentração máxima neste compartimento ocorre 0.72 a 0.78 horas após o pico plasmático (ANDERSON et al., 1998). O tempo de meia vida no homem é de aproximadamente duas horas e a sua metabolização ocorre principalmente por meio de conjugação com o ácido glucurônico (60%) e sulfúrico (35%) no fígado, sendo que após 24 horas, praticamente todo o fármaco é recuperado na urina na forma conjugada

(STEVENTON et al., 1996). O método de metabolização em animais é semelhante, porém cães e equinos apresentam tempo de meia vida de 56,4 minutos e 118 minutos respectivamente (ENGELKING et al., 1987; KUKANICH, 2016). Esta diferença entre espécies é atribuída à maior expressão de enzimas hepáticas para as reações de conjugação em equinos e caninos do que em humanos (NEIRINCKX et al., 2010).

A principal aplicação clínica do paracetamol em humanos tem sido como fármaco adjuvante no tratamento pós-cirúrgico, reduzindo a quantidade e dose de opióides como a morfina (WARD e ALEXANDER-WILLIAMS, 1999). Quando a ação deste composto é comparada com a de outros AINES ele apresenta efeito analgésico inferior em casos de dor leve, moderada e severa em humanos (WARD e ALEXANDER-WILLIAMS, 1999). O efeito analgésico do acetaminofeno melhora quando associado à opióides fracos como a codeína em caso de dores moderadas (ZHANG e PO, 1996). Um estudo de metanálise com casos de dor muscular, dentária e pós-operatória em humanos demonstrou que a combinação de AINES clássicos com paracetamol proporcionou um alívio adicional da dor (ALTMAN, 2004). Miranda e colaboradores (2006) demonstraram um sinergismo entre o paracetamol e diferentes AINES, de maneira que doses mais baixas destes fármacos proporcionaram analgesia visceral satisfatória após a administração de ácido acético intraperitoneal em ratos, além de menor incidência de efeitos adversos.

Estudos sobre o efeito analgésico do acetaminofeno em animais, que não ratos, são escassos, sendo a maioria destes relacionados com relatos de intoxicação acidental em cães e gatos (JONES et al., 1992) e suas formas de tratamento (RUMBEIHA et al., 1995). Em cães, foi demonstrado que a administração de codeína (67,5 mg) e acetaminofeno (600 mg) por via oral não altera o limiar nociceptivo quando avaliado por meio do dispositivo eletrônico de Von Frey (KUKANICH, 2016). Por outro lado, recentemente o acetaminofeno (20 mg/kg) e o flunixin meglumine (1,1 mg/kg) apresentaram analgesia semelhante avaliando-se a intensidade da claudicação induzida por pressão na ranilha e a frequência cardíaca em equinos quando comparados com o grupo sem tratamento (FOREMAN et al., 2016). Anteriormente a este, um relato de caso de laminite aguda tratado com a associação de acetaminofeno (20 mg/kg) como fenilbutazona (4,4mg/kg) promoveu um melhor controle da dor, sem a observação de efeitos colaterais (WEST et al., 2011).

3. ARTIGO 1

Trabalho publicado:

Management of chronic foot lameness with 2% ammonium chloride on the palmar digital nerves

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Management of chronic foot lameness with 2% ammonium chloride on the palmar digital nerves

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Abstract

This case series describes the analgesic effect of 2% ammonium chloride (2% AC) in horses with chronic foot pain. Ten horses with foot pain related to chronic laminitis (n = 1), bruised sole (n = 1), distal interphalangeal joint (DIPJ; n = 1), podotrochlear apparatus (PA; n = 4) and PA associated with DIPJ (PA + DIPJ; n = 3) received perineural injections with 3 mL of 2% AC on the palmar digital nerves. A Five horses with pain related to PA + DIPJ (n = 3), PA (n = 1) and DIPJ (n = 1) were treated with saline as control. The analgesic effect was evaluated as lameness improvement (LI) rate (%) using a body-mounted inertial sensor system, and was assessed at 5, 12, 19, 35, 47 and 62 days after treatment. Horses treated with 2% AC demonstrated a mean LI rate above 50% from Day 12 (63% _ 26) to Day 62 (65% _ 26). Control horses has an overall LI of 28% (_ 23%) and a LI above 50% was evidenced in horses with PA + DIPJ (n = 2) and PA pain (n = 1) at different times. Horses with PA pain presented higher LI rates (72% _ 23) than that presented by horses with PA + DIPJ (51% _ 9) or DIPJ (51% _ 19). Horses with severe radiographic lesions of the navicular bone and DIPJ had the lowest LI rates after treatment. The 2% AC is a useful treatment to be included in the clinical management of chronic foot pain involving the podotrochlear apparatus with mild radiographic lesions.

Introduction

Chronic foot pain is an important cause of poor performance in a variety of equine sport modalities (Dabareiner et al. 2005a; Murray et al. 2006). Chronic foot pain has traditionally been treated by corrective trimming and shoeing; administration of non-steroidal anti-inflammatory drugs; or injection of corticosteroids and/or hyaluronic acid in the distal interphalangeal joint (DIPJ) and navicular bursa (NB) (Dabareiner et al. 2003a; Gutierrez-Nibeyro et al. 2010).

In addition to medical treatment and shoeing practices, surgery can be done to treat chronic foot lameness. Although effective to alleviate foot pain, low pastern neurectomy is the last treatment option for horses with a poor response to conservative therapy (Maher et al. 2008). The structures involved and the surgical technique determine the success rate (Gutierrez-Nibeyro et al. 2010). Depending on the surgical technique, variable post-operative complications can occur (Maher et al. 2008; Gutierrez-Nibeyro et al. 2015a).

The use of chemical substances to mimic neurectomy has been used in human medicine for the management of chronic pain of diverse etiologies (Manchikanti et al. 2001; Rosen 2004); (Koyyalagunta & Burton 2010). In veterinary medicine, equine practitioners usually apply neurolytic agents to provide long-term nerve block (Harkins et al. 1997a). However, unlike the response to injection of local anesthetics, complete limb desensitization does not occur after perineural injection of neurolytic agents (Harkins et al. 1997a; Campos et al. 2013b). Even after intraneural injection of 98% ethyl alcohol or formaldehyde, skin sensitivity and thermal nociception persist (Schneider et al. 2014). In contrast, substantial to complete desensitization of mechanic nociception of the foot has been demonstrated experimentally after injection of neurolytic compounds on the palmar nerves (Nicoletti et al. 2007a; Schneider et al. 2014). Therefore, the present case series aimed to objectively describe the analgesic effect of 2% ammonium chloride[1] (2% AC) injected on the palmar digital nerves (PDN) of horses with chronic foot pain of different etiologies.

Cases Histories

After physical examination, 15 horses were equipped with a body-mounted inertial sensor system[2] to objectively assess lameness intensity, and to compare lameness intensity after diagnostic blocks. A diagnosis of chronic foot lameness was based on the presence of lameness for more than two months and a lameness improvement (LI) rate greater than 80% 10 minutes after the PDN block. Demographic information on the horses is presented in Table 1. Horses were involved in one of four exercise activities: equine assisted-therapy (for

children and adults with health issues); police patrol horses; show jumping horses; and a retired horse. The horses involved in equine assisted-therapy were ridden at a walk or trot, for three to four hours a day, four days a week. Police patrol horses participated in afternoon street patrols three times a week and had two days of mild exercise (walk, trot, and gallop) in an arena. The show jumping horses were ridden five days per week, with one day a week for jumping exercises, and the other days of light riding only. The retired horse was kept in a paddock without an exercise routine. Horses were not treated with any other medication or corrective trimming or shoeing during the evaluation.

Lameness exam

Lameness evaluation was performed with a body-mounted inertial sensor system[2]. Horses were trotted by hand in a straight line on a hard surface (asphalt/concrete/ compacted sand). A minimum of 30 strides was collected for each evaluation. Care was taken to keep the same person and surface during all evaluations. Data obtained by the software used in the present study is presented as mean and standard deviation (± SD) of the maximum head difference (HDmax), minimum head difference (HDmin), and vector sum (VS) obtained by the formula $\sqrt{HDmax^2 + HDmin^2}$. Lameness was identified when mean HDmax, HDmin, and VS were outside the repeated normal range (+/- 6 mm for HDmax and HDmin, and +/- 8.5 mm for VS) and, to promote collection of consistent data, when the mean SD of each measure was less than the absolute value of the mean. (Kramer and Keegan, 2004). The lameness improvement (LI) rate was used to evaluate the effect of blocks and 2% AC analgesia. The LI rate was calculated using the following formula: ($VS_{\text{before treatment}} - VS_{\text{after treatment}}$)/ ($VS_{\text{before treatment}}$).

Digital blocks and analgesia evaluation

The PDN block was performed injecting of 1.5 mL of 2% lidocaine hydrochloride[3] on each branch of the PDN with a 26 G $^1/_2$ needle inserted axially to the neurovascular bundle in a proximal to distal direction, proximal to the ungular cartilage (Baxter & Stashak 2011). To more precisely identify the pain source, blocks of the deep digital flexor tendon sheath (DDFTS), navicular bursa (NB), and distal interphalangeal joint (DIPJ) were performed. The DDFTS block was performed by injecting 5 mL of 2% lidocaine hydrochloride using the palmar approach (Jordana et al., 2014). The NB block was guided by contrast radiography with a 19 G 3.5-inch spine needle using a distal palmar approach to the navicular position (Schramme et al., 2000). To confirm correct deposition of anesthetic, 3 mL of 2% lidocaine

hydrochloride with 0.5 mL of positive contrast media was injected. The DIPJ anesthesia was achieved using 5 mL of 2% lidocaine hydrochloride injected with a 23 G $^{3}/_{4}$ needle using a dorsal lateral approach (Dyson, 1991). All blocks were performed on same day and after the lameness intensity returned to baseline respecting a minimum of two hours between blocks (Silva et al, 2015). The same author (S.L.D.), with seven years of experience as a veterinarian, performed all blocks under supervision of a senior clinician (F.D.C.). Lameness was evaluated before and at five and 10 minutes after each block (Table 2). Horses with evidence of contralateral lameness after the block were classified with bilateral lameness.

Radiographic exam

A radiographic study was performed based on the clinical findings of each horse, and included up to six radiographic views (lateromedial, dorsopalmar, dorso65ºproximal-palmarodistal oblique, dorso45ºlateral-palmaromedial oblique, dorso45ºmedial-dorsolateral oblique, and palmaro45ºproximal-palmarodistal oblique). One author (M.S.A) evaluated the radiographs and graded the navicular bone (NvB) and DIPJ lesions according to a system previously described (Dyson 1991; Dyson 2008) (Supplementary Item 1).

Lameness findings

Impact forelimb lameness was evident in all horses and the mean lameness intensity (VS ± SD) before treatment was 58 mm (± 35.4). Bilateral lameness was identified in nine horses. The duration of lameness was variable, with four horses being lame for 4-6 months, three horses lame for 6-12 months, and eight horses lame for more than 12 months.

Combining the clinical findings, blocks responses, and radiographic images, the identified pain sources were: podotrochlear apparatus (PA; n=5), PA and DIPJ (PA+DIPJ; n=6), bruised sole (n=1), chronic laminitis (n=1), and DIPJ only (n=2). Only one horse with PA pain was diagnosed with a grade IV NvB lesion, characterized by a large cystic-like lesion and moderate bone remodeling on the medial and lateral border of NvB (Fig. 1a). Horses with PA+DIPJ pain (n=6) had more severe lesions when compared to horses with PA pain. Five horses had grade IV NvB lesions, with sclerosis (Fig. 1d) and new bone formation of the flexor cortex (Fig. 1b), associated with moderate calcification of the collateral ligaments. These horses also had mild reactions on the extensor process of P3 classified as grade I DIPJ lesions. A grade II lesion, characterized by severe bone proliferation on the dorsomedial aspect of P2 and P3 with a decrease of the joint space on the medial aspect (Fig. 1c), was

identified in one horse. Mild dorsal rotation of P3 was identified in the horse with chronic laminitis and a reverse P3 palmar angle in the horse with a bruised sole.

Treatment and outcomes

Cases 1-10 were treated injecting each ramus of PDN with 3 mL of 2% AC and cases 11-15 with 3 mL saline solution per ramus of palmar digital nerve, serving as a control to compare the influence of time on lameness intensity. The treatment was applied only to one limb, and in cases of bilateral lameness the most affected limb was injected. One day after the diagnostic blocks, horses were injected with 2% AC, or saline, using 3 mL per ramus of the PDN. The LI rate was evaluated 5, 12, 19, 35, 47, and 62 days after treatment (Table 3). All evaluations were performed at the same time during the morning. No adverse effects, including local irritation, edema, or skin lesions, were observed after treatment. The lowest LI mean after treatment with 2% AC was on day 5 (39% ± 37) and the highest was on day 19 (68% ± 26). Horses treated with 2% AC presented a LI mean above 60% from day 12 (63% ± 26) to 62 (65% ± 26). Horses with pain associated with the PA had higher LI rates (72% ± 23) than those diagnosed with PA+DIPJ (51% ± 9). Treatment responses were better on day 35 (94% ± 11) for horses with PA and day 47 (66% ± 21) for horses with PA+DIPJ pain. The DIJ pain case demonstrated response to treatment similar to PA + DIJ cases, however response to treatment in this case was variable ranging from below 50% on days 12, 19, and 42 to between 60-70% improvement on days 5, 35, and 62. The horse with laminitis had a positive response (71-100%) to treatment from day 12 to 62. The horse with a bruised sole had a LI rate below 50% from day 19 to 62. Horses treated with saline solution had a LI rate below 50% on days 5 (22% ±44), 12 (33% ±38), 19 (8% ±9), 35 (14% ±20), and 47 (6% ±9). Case 11 and 14 had a LI rate of 64% and 73% on day 62. Case 15 presented more variability during study presenting a LI above 50% on days 5, 12 and 62.

Discussion

Two percent ammonium chloride produced partial to complete analgesia for at least 62 days in horses with different causes of chronic forelimb foot lameness. The mean LI of all 2% AC treated horses was 61.2% (± 30.5) and the variability of response was considered acceptable for a clinical study. Possible, complete resolution of pain in some cases could be achieved by associating 2% AC injection with other conventional therapies as previously described (Dabareiner et al. 2003a; Gutierrez-Nibeyro et al. 2010). The best analgesia effect was observed 19 days after the 2% AC injection. On day 19, three horses had mean LI rates of

50-70% (57.1% ± 2.6) and four horses had LI rates above 70% (91.8% ± 11.6). It suggested that the best responses to 2% AC treatment for chronic foot lameness, as assessed by LI, should be expected some days after injection. These results differ from other studies with neurolytic agents where equine practitioners expected an earlier response to treatment (Harkins et al. 1997a).

The overall LI of horses treated with saline solution was 28% (±23%) and a LI above 50% was evidenced in cases 11, 14 and 15. The improvement of case 11 was attributed to a ten-day period of stall rest that the horse was submitted before the last evaluation by the owners. The presence of bilateral forelimb lameness in cases 14 and 15 could explain the variability observed in these cases. No statistical analyses for LI were performed due the small number of horses in both groups.

Variables such as volume and precision on the deposition of local anesthetic agents into the neurovascular fascia may impact the onset and degree of analgesia after perineural block (Schumacher et al. 2013). However, perineural injection of 2% AC produced analgesic improvement rates comparable to those described after intraneural injection of 98% ethyl alcohol (Schneider *et al.*, 2014). Compared with the analgesic effect of 2% AC, a greater and longer analgesic effect was observed after perineural injection of the palmar nerve with absolute ethyl alcohol and 0.75% benzyl alcohol (Nicoletti et al. 2007a).

Since moderate to severe lesions were correlated to the presence of soft tissue injury, including tendinitis of the DDFT and desmitis of the navicular ligaments (Parkes et al. 2015), the severity of radiographic lesions may explain the variability of LI rates observed in cases 1, 4, 8, and 9. Clinical cases involving the DDFT or distal impar and collateral ligaments of navicular bone responded poorly to conservative management (Gutierrez-Nibeyro et al. 2010) and palmar digital neurectomy (Gutierrez-Nibeyro et al. 2015a). Moreover, the lower LI rates observed in cases of PA+DIPJ and DIPJ may be related to non-desensitization of the dorsal branches of palmar digital nerve due to the technique used for the PDN injection. These branches are related to mechanical nociception of the proximal aspect of the foot, specifically the coronary band (Paz et al. 2016). Therefore, the injection of the 2% AC more proximally, at the base of sesamoid bones, may result in better LI rates in cases involving the DIPJ.

The use of neurolytic compounds is considered safe because the perception of acute pain remains after perineural (Harkins et al. 1997a; Nicoletti et al. 2007a; Campos et al. 2013b) and intraneural (Schneider et al. 2014) injection of these drugs. This temporary pain relief is a positive aspect when compared to neurectomy. Neurectomy results in desensitization of most of the foot for a prolonged period of time, and can result in potentially

fatal complications including deep digital flexor tendon rupture, undetected sepsis of third phalanx, and fracture of the navicular bone (Maher et al. 2008; Gutierrez-Nibeyro et al. 2015a). Additional uses for 2% AC could be for pain relief during rehabilitation of chronic diseases like laminitis, PA pain, or recovery from orthopedic surgery, including arthrodesis, when sepsis or implant failure is no longer a concern.

The authors are aware of the limitations of this study. Using routine clinical cases, a small number of horses, and the lack of more representative control group make it difficult to compare the analgesic effect of 2% AC to other causes of chronic foot lameness. However, this information is useful to practitioners who routinely face similar cases to those presented in this case series. A clinical trial with a large number of horses and using multiple imaging modalities, including magnetic resonance imaging, to more precisely identify the pain source, and to evaluate the analgesic effect of neurolytic agents for management of chronic foot pain, is necessary.

Conclusions

Perineural injection of 2% ammonium chloride on the palmar digital nerves can be useful to produce a partial to complete analgesia for at least 62 days in horses with different causes of chronic forelimb foot lameness. Our results suggest that the most improvement in LI is seen on days 12 and 19 after treatment. Moderate to severe radiographic lesions of the navicular bone and distal interphalangeal joint may negatively affect the analgesic effect.

Author's declaration of interests

The authors declare no financial or personal association with the pharmaceutical company that could inappropriately influence or bias the content of the paper.

Ethical animal research

The Ethics Committee on Animal Use of the Federal University of Santa Maria approved this study by protocol number CEUA 9788240815.

Source of founding

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Authorship

S.L. Dau and M.S. Azevedo were responsible for the study design and execution, and preparation of the manuscript. C. Cantareli and F. Ceni assisted in performing the study and preparation of manuscript. K. Brass and F. D. de La Corte were involved in study design and preparation of manuscript.

Manufacturer's address

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[2] Equinosis® Q with Lameness Locator®, St Louis, MO, US.

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Table 1. Background and exercise activity of horses (cases 1-15).

Case	Age (years)	Breed	Gender	Weight (kg)	Lameness duration (months)	Exercise modality
1	16	Standardbred	Mare	380	>12	Equine assisted-therapy
2	12	Criollo	Mare	360	8	Retired
3	15	Criollo	Gelding	420	6	Police patrol
4	16	Standardbred	Gelding	520	>12	Police patrol
5	5	Standardbred	Gelding	470	>12	Police patrol
6	18	Standardbred	Gelding	480	10	Show Jumper
7	16	Standardbred	Gelding	500	8	Show Jumper
8	14	Criollo	Gelding	440	>12	Equine assisted-therapy
9	18	Criollo	Gelding	420	>12	Equine assisted-therapy
10	8	Criollo	Gelding	400	4	Equine assisted-therapy
11	16	Standardbred	Mare	500	8	Show Jumper
12	10	Standardbred	Gelding	520	>12	Show Jumper
13	8	Standardbred	Gelding	500	>12	Police patrol
14	6	Standardbred	Gelding	450	6	Police patrol
15	9	Standardbred	Mare	510	>12	Police patrol

Table 2. Response to diagnostic blocks, radiographic lesions, and pain source identified in horses injected with 2% ammonium chloride (cases 1-10) or saline (cases 11-15) on the palmar digital nerves.

Case	Lameness improvement after diagnostic blocks (%) (minutes)								Radiographic lesion grade		Pain source
	PDN		NB		DIPJ		DDFTS				
	5’	10’	5’	10’	5’	10’	5’	10’	NvB	DIPJ	
1	100	100	100	100	36	44	34	38	III	0	PA
2	-	100	-	-	-	-	-	-	Dorsal displacement/ rotation of P3		Chronic laminitis
3	72	100	100	100	100	100	0	7	I	I	PA + DIPJ
4	90	100	75	88	27	56	23	61	IV	0	PA
5	100	100	13	100	49	55	5	0	I	II	DIPJ
6	100	100	100	100	48	100	36	34	II	0	PA
7	100	100	100	100	0	48	32	27	II	0	PA
8	100	100	3	20	100	100	6	11	IV	I	PA + DIPJ
9	100	100	100	100	54	31	25	47	IV	I	PA + DIPJ
10	-	100	-	-	-	-	-	-	Negative palmar angle of P3		Bruised sole
11	63	80	90	100	70	100	43	46	II	0	PA + DIPJ
12	75	100	0	0	60	100	-	-	0	I	DIPJ
13	100	100	100	100	88	87	-	-	IV	II	PA + DIPJ
14	100	100	-	-	60	100	-	-	II	0	PA
15	100	100	100	100	100	100	50	44	IV	I	PA + DIPJ

Palmar digital nerve (PDN); navicular bursa (NB); distal interphalangeal joint (DIPJ); deep digital flexor tendon sheath (DDFTS); navicular bone (NvB); block not performed (-); podotrochlear apparatus (PA); podotrochlear apparatus and distal interphalangeal joint (PA+DIPJ) pain.

Table 3. Lameness improvement over time after injection with 2% ammonium chloride (cases 1-10) or with saline solution (cases 11-15) on the palmar digital nerves of horses based on the pain source identified in the foot.

Pain source	Case	VS (mm)	Lameness Improvement (%) Days after injection					
			5	12	19	35	47	62
PA	1	92	25	88	83	100	71	63
	4	57	63	67	100	75	32	47
	6	31	8	41	76	100	100	100
	14	35	0	3	0	0	35	73
	7	10	0	100	100	100	100	100
PA+DIPJ	3	83	59	49	59	40	90	33
	8	47	0	22	55	0	39	65
	9	122	100	64	50	70	70	56
	11	48	8	23	21	21	2	64
	13	34	0	18	4	3	*	*
	15	16	100	100	8	0	0	51
DIPJ	5	41	65	41	28	69	32	69
	12	47	0	20	0	47	17	48
Chronic Laminitis	2	125	0	100	100	71	92	90
Bruised sole	10	84	70	59	24	36	42	22

Podotrochlear apparatus and distal interphalangeal joint (PA+DIPJ); podotrochlear apparatus (PA); distal interphalangeal joint (DIPJ) and Vector Sum (VS) before treatment. *Horse was removed due colic complication.

Figure

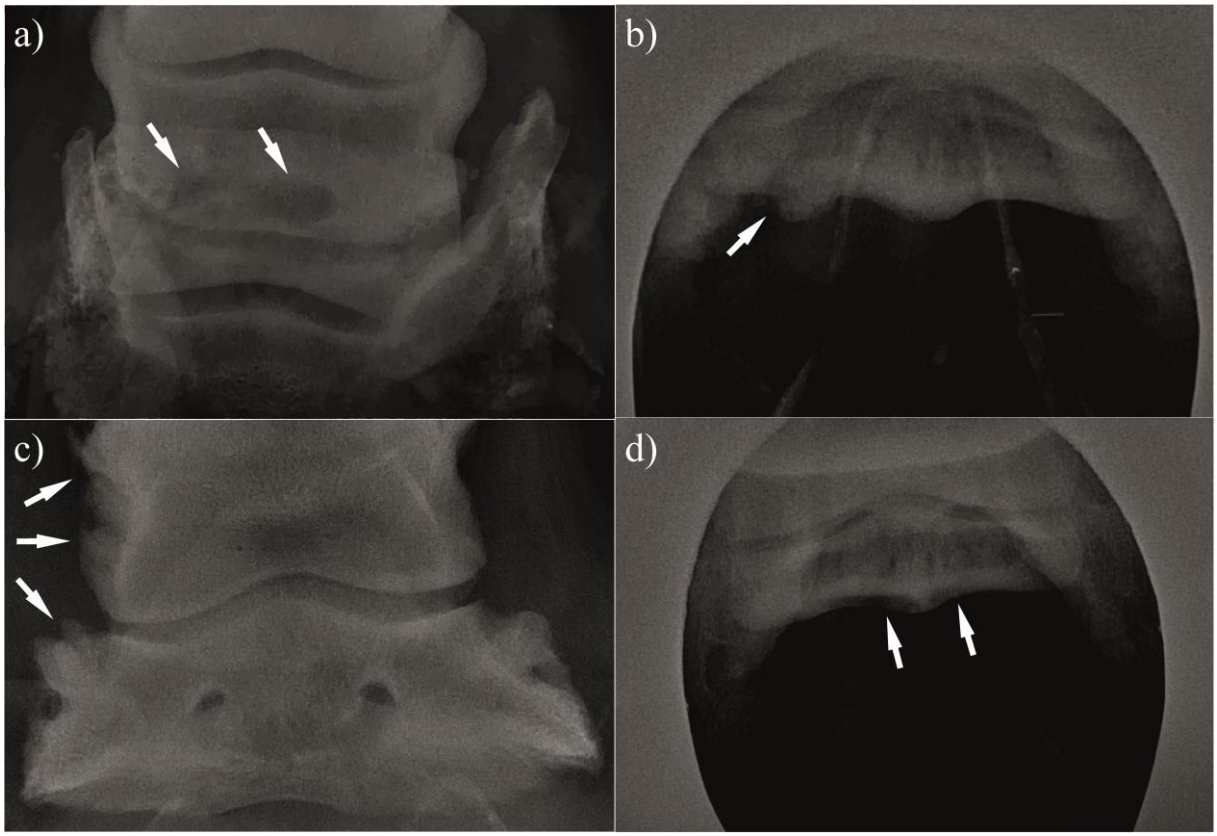

Fig. 1. (a) Dorso65ºproximal-palmarodistal oblique view of a navicular bone (case 4). Medial is to the left. There is a large central circular radiolucent area and two small cystic-like lesions on the medial aspect near the dorsal border (arrow) and a small fragment on the lateral border. (b) Palmaro45ºproximal-palmarodistal oblique view (case 9). Medial is to the right. There is a radiolucent defect associated with bony proliferation on the lateral portion of flexor cortex (arrow). (c) Dorsopalmar view of the distal interphalangeal joint lesion classified as grade II (case 5). Medial is to the left. There is bony proliferation of the second and third phalanx (arrows) and narrowing of the medial aspect of the joint surface. (d) Palmaro45ºproximal-palmarodistal oblique view (case 8). Medial is to the left. There is a radiolucent defect on the medial aspect of flexor cortex associated with bony proliferation (arrow). An increase of spongiosa opacity and a circular radiolucent area on the lateral aspect are evident.

Supporting information

System utilized to classify the navicular bone and distal interphalangeal joint lesions as previously described (Dyson, 1991; Dyson, 2008).

Navicular bone lesions were classified as:

Grade I: good navicular bone condition associated with less than 6 lucent zones along the distal border;

Grade II: slight definition between the palmar cortex and the medulla, up to 8 lucent zones distributed on the distal horizontal border and mild entheseophyte formation on the proximal border of the navicular bone;

Grade III: poor corticomedullar definition, thickening of dorsal and flexor cortices, more than 7 radiolucent zones along distal and proximal borders, large entheseophyte formation on the proximal border and discrete mineralization of the collateral ligament of the navicular bone;

Grade IV: large cyst-like lesion within the medulla, poor corticomedullar definition and new bone formation on the flexor cortex.

Distal interphalangeal lesions were described as:

Grade 0: good distal interphalangeal joint congruity with mild alteration of extensor process of P3

Grade I: fair congruity of joint space; some osteophytes on the distal dorsal or palmar aspect of P2 and on the dorsoproximal aspect of the navicular bone;

Grade II: no congruity of joint space; osteophytes on the distal dorsal or palmar aspect of P2 and on the dorsoproximal aspect of the navicular bone; mineralized or osseous body on the dorsoproximal aspect of extensor process of P3.

4. ARTIGO 2

Trabalho publicado:

Histologic Evaluation of Palmar Digital Nerves after perineural injection of 2% ammonium chloride in miniature horses

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Histologic Evaluation of Palmar Digital Nerves after perineural injection of 2% ammonium chloride in miniature horses

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Authors' contributions: SLD: study design, data acquisition, analysis and interpretation, and manuscript preparation; FDLC: study design, critical revision of the manuscript for important intellectual content; MSA: data acquisition, manuscript preparation; RM: data acquisition, manuscript preparation; and RAF: data acquisition, manuscript preparation. All authors approved the final version of the manuscript.

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Abstract

Neurolytic compounds are widely employed by equine practitioners for the management of lameness, mostly related to the foot. The present study aimed to evaluate the neurotoxicity of 2% ammonium chloride (2% AC) applied adjacent to the palmar digital nerves (PDNs) in six miniature horses. The 2% AC and 0.9% saline solution were randomly injected into three and one palmar digital nerve of each horse, respectively. Nerve samples were collected by neurectomy performed under general anesthesia at 5, 12, 19, 35, 47, and 62 days after treatment, with one horse per day of surgery. The inflammatory reaction to perineural injection was evaluated by an increase of pastern superficial skin temperature through thermography 24 h after treatments. Histological lesions were classified as absent, mild, moderate, and severe Wallerian degeneration. An increase of 2.43 ± 0.79°C and 1.69 ± 0.55°C was observed in the 2% AC and control groups, respectively ($p<0.05$). Moreover, histologic lesions were observed after perineural injection of 2% AC (severe, n=5/18; moderate, n=4/18; mild, n=5/18; absent, n=4/18) and saline solution (moderate, n=3/6; mild, n=1/6; absent, n=2/6) ($p=0.46$). The 2% AC demonstrated to be as safe as 0.9% saline solution, producing mild to severe Wallerian degeneration for up to 62 days after injection with no interference in further neurectomy.

Keywords: chronic foot pain, ethyl alcohol, lameness, neurolytic compound

1. Introduction

Chronic lameness, mostly related to the foot, is frequently diagnosed in sport horses (Dabareiner et al. 2005b; Murray et al. 2006). Currently, there are many therapeutic options for the management of chronic foot pain; however, few studies have been performed to describe which therapy presents better rates according to the diagnosis(Barrett et al. 2017). Palmar digital nerve neurectomy is normally recommended to treat chronic cases with poor response to conservative therapies, but many complications have been reported after surgery (Gutierrez-Nibeyro et al. 2015b). Neurolytic compounds are presented as an alternative to neurectomy associated with fewer complications; however both therapies present regulatory and ethical concerns that should be pointed out before their application, since they can illegally enhance performance (Van hoogmoed & Snyder 2002). Alcohol, phenol, and ammonium salts are neurolytic agents that have different analgesic effects and nerve toxicity (Kobayashi et al. 1997; Hertl et al. 1998; Nicoletti et al. 2007b; Schneider et al. 2014). Intraneural alcohol injection has been reported to present a prolonged analgesic effect with lower soft tissue inflammation than formaldehyde. Fibrosis is a complication related to different alcohol concentrations after intraneural (Schneider et al. 2014) and perineural injections (Escodro et al. 2018).

Ammonium salts have been described to present intermediate analgesic duration of action associated with mild to absent nerve injury similar to those reported for local anesthetic drugs (Kobayashi et al. 1997; Hertl et al. 1998). In a previous study (Campos et al. 2013a), the authors reported that 1% ammonium chloride presented a lack of analgesic effect; however, they proposed that this compound could produce analgesia by different action mechanism in a prolonged time than what is used in this study. A clinical study described an intermediate to satisfactory analgesic effect of 2% AC applied to the palmar digital nerves to treat different cases of chronic foot lameness in horses (Dau et al. 2020). Therefore, the present study aimed to evaluate the neurotoxicity of 2% ammonium chloride (2% AC) after perineural injection of the palmar digital nerves (PDNs) in horses.

2. Materials and methods

2.1 *Animals and Ethics*

Once approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria by protocol number CEUA 9788240815, and after obtaining written

consent from the owners, six adult miniature horses aged 8 ± 3 years and weighing 182 ± 16 kg were selected for this study. During the study, animals were kept in an individual stall with free access to water and fed with alfalfa hay three times per day.

2.2 *Perineural injection*

Perineural injections were performed on the same day in all horses, with three and one ramus of PDNs from both forelimbs of each horse injected with 3 mL of 2% AC (n=18) and 3 mL of 0.9% saline solution (n=6), respectively. The ramus utilized as a control was randomly selected for each horse. Initially, the pastern of both forelimbs was clipped on the palmar aspect a day before the PDNs were injected. The lateral and medial rami of PDNs were identified by palpation of the neurovascular bundle and were dislocated to insert a 26-G needle axially to the bundle followed by injection of treatments. The injection site of each nerve was measured from the coronary band to the point where the needle entered the skin, for future reference during neurectomy.

2.3 *Thermographic evaluation*

Thermographic evaluation was performed with the FLIR ThermaCAM™ E25 (operating temperature 12°C–40°C, emissivity of 1, and focal distance of 2 m) before and at 1, 3, 6, 12, and 24 h after perineural injection. The limbs were brushed to remove any dirt, and a period of 15 min was allowed for acclimatization at room temperature. All images were obtained in the same stall, which was enclosed in wind and solar heat. The superficial skin temperature symmetry between the 2% AC and control groups was compared by average temperature of five vertical equidistant points on the lateral and medial aspects of the pastern using a palmar view with the FLIR Quick Report 1.2 SP2 software (©FLIR Systems, 2009).

2.4 *Neurectomy*

A bilateral neurectomy was performed 5, 12, 19, 35, 47, and 62 days after perineural injection by the same surgeon (FDC), with one horse per day of surgery. Horses were intravenously sedated prior to surgery with xylazine (0.5 mg/kg^{-1}) and butorphanol (0.02 mg/kg^{-1}). A bolus of ketamine (2 mg/kg^{-1}) with diazepam (0.06 mg /kg^{-1}) was intravenously administered to induce anesthesia, which was maintained with isoflurane in a semi-closed circle rebreathing system. Horses were in dorsal recumbent positions with forelimbs maintained in an extended position. The recorded needle insertion site

was marked with a permanent pencil prior limb preparation with povidone-iodine and 70% alcohol. A 2-cm skin incision was performed parallel and axial to the neurovascular bundle and distally to the site marked. Blunt dissection of the subcutaneous tissue with a curved mosquito hemostatic forceps was performed to identify PDNs and to separate them from the palmar digital artery and vein. After exposure, a nerve fragment of 1.5 cm was harvested by pull-through guillotine technique (Matthews et al. 2003) and then identified and stored in a buffered formalin solution for histological processing. The skin was closed using cruciate interrupted sutures with 2-0 nylon after the surgical site was flushed with sterile saline solution. A bandage was placed on each limb from the hoof to the middle of the cannon bone and then changed every 2 days until the sutures were removed 14 days after surgery.

2.5 *Histologic process and classification*

Samples were processed by conventional histochemical techniques, embedded in paraffin, sectioned into a 5-µm slice, deparaffinized, rehydrated and stained with hematoxylin and eosin (HE). Histological lesions were ranked according to the degree of Wallerian degeneration (absent = 0, mild = 1, moderate = 2, and severe = 3) by a pathologist (RAF), who was blinded to the PDN treatment.

2.6 *Statistical analysis*

Thermographic data were evaluated for normality using the Shapiro-Wilk test and then analyzed by two-way ANOVA to compare superficial skin temperature of the nerves from the 2% AC and control groups over time, and the group means of each time point were compared by the Bonferroni's test. PDN lesion means of the 2% AC and control groups were compared by the Student's t test. All tests were performed with a significance of 95% using the GraphPad Prism software (GraphPad Prism, version 8.0.0, San Diego, CA, USA).

3. Results

Perineural injections were performed without any complications, and only one horse demonstrated some discomfort as pawing the ground with the forelimb during the first 15 min after treatment. An increase in superficial skin temperature of 2.43°C (±0.79) and 1.69°C (±0.55) was observed in the 2% AC and control groups, respectively, during

the first 24 h after treatment. No difference in the superficial skin temperature was observed between the groups at any time point ($p>0.05$) (Table 1).

Surgical procedures were performed without any complications, and PDNs were identified and harvested easily with the exception of a horse from day 35, in which the surgeon reported more difficulty in isolating the medial ramus of the PDN from the left forelimb treated with 2% AC. No complications were observed during the postoperative period.

Histological lesions were defined mostly by different degrees of substitution of the axonal wall by homogeneous material, markedly eosinophilic and ellipsoid shape, characterized by the presence of axonal spheroids indicating Wallerian degeneration. A replacement of fascicles by fibrous connective tissue characterizing fibrosis was observed in one sample treated with 2% AC harvested on day 35, which was associated with more difficulty in isolating the nerve during the surgery, as previously described (Figure 1). The classification of histological lesions from PDNs is presented in Table 2. No difference ($p=0.44$) was observed between the mean score lesions of the 2% AC (1.56 ± 1.15) and saline (1.17 ± 0.98) treatments.

4. Discussion

The 2% AC induced mild inflammation since the increase in pastern superficial skin temperature observed (2.43 ± 0.79°C) was similar to that observed in 0.9% saline solution (2.43 ± 0.79°C) during the first 24 h ($p>0.05$). One horse presented some discomfort in the first minutes after 2% AC injection. The perineural injection of 10% ammonium chloride also produced a painful reaction like pawing, elevating the limb, and rolling immediately after PDN injection and also induced and inflammatory response, compared with the control group, for up to 8 days after treatment (Van hoogmoed & Snyder 2002). Painful reactions and prolonged inflammatory responses such as swelling of the cannon bone and exacerbated reaction at the injection site were also reported after perineural injection of 100% ethyl alcohol (Nicoletti et al. 2007b) and 49.75% ethanolic solution (Escodro et al. 2018). An intermittent mild lameness that persisted for more than 1 day was described after intraneural injection of 98% ethyl alcohol and formaldehyde (Schneider et al. 2014). This reinforces that previous, or concomitant, injection of local anesthetic drugs could be of great value to avoid neurolytic adverse effects (Kobayashi et al. 1997; Van hoogmoed & Snyder 2002) and, according to the drug composition and concentration, more severe undesired

complications may occur (Nicoletti et al. 2007b; Schneider et al. 2014; Escodro et al. 2018).

In the present study, the authors treated more nerves with 2% AC (n=18) than with 0.9% saline solution (n=6) to obtain more information about the mechanism of action of 2% AC. Palmar digital nerve lesions varied between treatments, and no difference was detected. However, severe lesions were observed only on the nerves injected with 2% AC. One sample treated with 2% AC was difficult to identify during neurectomy and it presented with fibrosis on histologic examination. The authors attribute the fibrosis formation to an incidental intraneural injection rather than a direct action of the compound applied perineurally (Farber et al. 2013). Intraneural and intrafascicular injections have been described to present more severe lesions using different local anesthetic drugs and even saline solution compared with perineural injection (Kobayashi et al. 1997; Kapur et al. 2007; Farber et al. 2013; Damjanovska et al. 2015). For this reason, intraneural injection aiming at a better response to treatment should not be recommended or desired by practitioners (Farber et al. 2013). The absence of nerve lesions observed in 2% AC samples (n=4/18) could be associated with the failure of neurolytic compound to reach the nerve due to incorrect drug deposition far away from the neurovascular bundle. An increase in the injected volume may be a solution to avoid this failure related to uncorrect needle placement. The nerve injuries observed after saline injection reinforce that any agent may produce nerve lesions when injected into or adjacent to a nerve (Kobayashi et al. 1997; Farber et al. 2013; Damjanovska et al. 2015).

The 2% AC nerve samples presented similar but less severe lesions to those described for 98% ethyl alcohol injection, which induced demyelination and fiber alignment preservation, loss of axons associated with mild degeneration, fibrosis, and inflammation (Schneider et al. 2014). Perineural injection of palmar nerve with a commercial aqueous extract of *Sarracenia purpurea*, which containing 0.75% benzyl alcohol in its composition and 100% ethyl alcohol, induced lesions such as axonotmesis (injury associated with Wallerian degeneration without damage to the endoneurium) and neurotmesis (lesion involving the axon, myelin, edoneurium, and perineurium) (Nicoletti et al. 2007b). The analgesic effect reported for different neurolytic compounds could be related to the severity of nerve lesions induced by them; thus, clinicians should be aware that, according to the diagnosis achieved for chronic foot lameness, some neurolytic may not be efficient in relieving pain (Escodro et al. 2018;

Dau et al. 2020). This reinforces the need of more controlled studies of different neurolytic compounds commercially available associated to gold standard diagnosis protocols to better understand their efficacy in the management of common pathologic conditions of the equine foot (Barrett et al. 2017). Understanding how neurolytic compounds work is important to base their ethical clinical use and also help authorities regulate and control their use in sport horses.

5. Conclusion

The 2% AC produced mild to severe Wallerian degeneration for up to 62 days after treatment. The perineural block of PDNs with 2% AC was demonstrated to be as safe as the injection of 0.9% saline solution and that their use presented no interference in the further neurectomy.

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Table 1 – Pastern superficial skin temperature (°C), mean, and standard deviation (± SD), before and after perineural injection of the palmar digital nerves with 2% ammonium chloride (2% AC) and 0.9% saline solution (control) in six miniature horses

Group	**Time after perineural injection (hours)**					
	Before	**01**	**03**	**06**	**12**	**24**
2% AC (n=18)	30.39 ± 1.61	33.64 ± 0.92	33.04 ± 1.59	32.87 ± 1.53	33.04 ± 1.16	31.51 ± 1.61
Control (n=6)	30.75 ± 1.64	33.16 ± 0.89	32.75 ± 2.28	32.37 ± 2.26	32.26 ± 1.55	31.69 ± 0.9

* No difference was observed between treatments (p>0.05).

Table 2 – Histologic lesion degree (HLD) of the palmar digital nerves injected perineurally with 2% ammonium chloride (2% AC) and 0.9% saline solution (control) at different days after treatment (DAT) in six miniature horses

Horse / DAT		Left forelimb		Right forelimb	
		Lateral	Medial	Lateral	Medial
01 / 05	Treatment	2% AC	2% AC	Control	2% AC
	HLD	+	-	++	+++
02 / 12	Treatment	2% AC	2% AC	2% AC	Control
	HLD	-	+++	+++	+
03 / 17	Treatment	2% AC	2% AC	2% AC	Control
	HLD	++	+	++	-
04 / 35	Treatment	2% AC	2% AC	2% AC	Control
	HLD	+	-	+++	++
05 / 47	Treatment	2% AC	Control	2% AC	2% AC
	HLD	+++	++	++	+
06 / 62	Treatment	Control	2% AC	2% AC	2% AC
	HLD	-	-	++	+

Histologic lesions were classified according to the degree of Wallerian degeneration as absent (-), mild (+), moderate (++), and severe (+++).

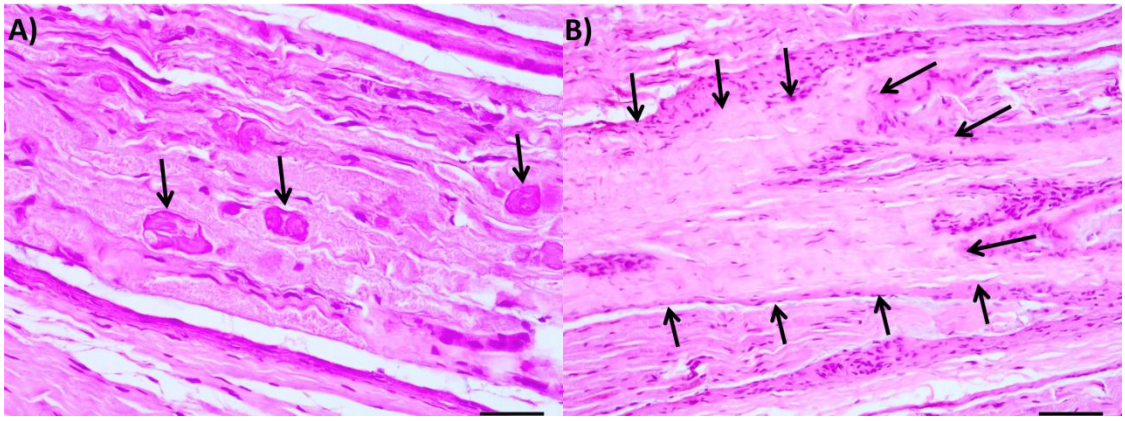

Figure 1 - A) Palmar digital nerve sections harvested on day 12 treated with 2% ammonium chloride presenting axonal spheroids demonstrating severe Wallerian degeneration (HE [10x]). B) Palmar digital nerve sections harvested at day 35 treated with 2% ammonium chloride presenting with intrafascicular fibrosis (HE [20x]).

5. ARTIGO 3

Trabalho a ser submetido para publicação:

ANALGESIC EFFECT AND SAFETY OF ACETAMINOPHEN ADMINISTERED ALONE OR IN COMBINATION WITH PHENYLBUTAZONE IN A REVERSIBLE MODEL OF EQUINE FOOT LAMENESS

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RESEARCH PAPER

Analgesic effect and safety of acetaminophen administered alone or in combination with phenylbutazone in a reversible model of equine foot lameness

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Authors' contributions: SLD: study design, data acquisition, analysis and interpretation, manuscript preparation; FDLC: study design, critical revision of the manuscript for important intellectual content; MSA: data acquisition, manuscript preparation; AABJ: data acquisition, manuscript preparation. All authors approved the final version of the manuscript.

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Conflict of Interest Statement: The authors declare no conflict of interest.

Abstract

Objective: to evaluate the analgesic effect and toxicity of acetaminophen (ACET) associated with phenybutazone (PBZ) using a foot lameness protocol in horses.

Study design: prospective, randomized, crossover.

Animals: twelve mixed-breed horses without lameness.

Methods: Horses received four treatments after lameness induction: control, ACET 20 mg Kg^{-1} orally (PO), ACET 20 mg Kg^{-1} PO associated with PBZ 2.2 mg Kg^{-1} intravenously (IV) (ACET+PBZ) and with PBZ 4.4 mg Kg^{-1} IV (PBZ). Horses were evaluated at 30, 60, 90, 120, 150 and 180 minutes after each treatment. Heart rate (HR) was measured five minutes before lameness evaluations. Analgesic effect was evaluated as change on HR and lameness intensity. For toxicity analysis, horses were distributed in three groups (ACET, ACET+PBZ and PBZ) and treated twice daily for 14 days. Blood samples were collected for complete blood count and serum biochemical analysis before the first dose and at days 7 and 14 of treatment.

Results: A significant reduction on HR was observed comparing induction and after treatment for ACET (at 60 and 120 min), PBZ (at 60 to 120 min) and ACET+PBZ (at 150 min) ($p<0.05$). Overall effect of ACET+PBZ had superior analgesic effect compared with ACET ($p=0.0081$), PBZ ($p=0.0117$) and control ($p<0.0001$), and PBZ was more effective than control ($p=0.0441$). The ACET+PBZ was superior to negative control in change lameness severity only at 120 minutes ($p=0.0314$). The PBZ group demonstrated significant decrease of albumin; ACET+PBZ presented significant increase of urea nitrogen and decrease of creatinine; and significant decrease of erythrocytes and hemoglobin was associated with ACET treatment. Despite the significant differences observed, blood parameters were inside the laboratory reference intervals.

Conclusions and clinical relevance: The association of ACET+PBZ was safe and more effective to relief pain than ACET, PBZ or control. Further investigation using different pain sources and therapeutic protocols should be performed before its recommendation for clinical routine.

Keywords: lameness, non-steroidal anti-inflammatory drugs, phenylbutazone.

Introduction

Phenylbutazone is the most traditional non-steroidal anti-inflammatory drug (NSAID) administrated to treat acute and chronic orthopedic disorders in horses (Sanchez & Robertson 2014). The main action of this drug is by nonselective suppression of cyclooxygenase enzymes (COX-1 and COX-2) activity, and prolonged or administration of high doses were related to gastrointestinal and renal toxicity secondary to inhibition of constitutive COX-1 activity (MacAllister et al. 1993; Doucet et al. 2008; Knych 2017).

The association of drugs with different mechanisms of action to enhance one or more therapeutic purposes, such as analgesia improvement, had proven efficacy associated with decreasing of side effects (Muir 2010; Sanchez & Robertson 2014; Guedes 2017). In human medicine, acetaminophen is usually associated with other drugs, mainly with opioids (Zhang & Li Wan Po 1996) and NSAIDs drugs (Altman 2004; Miranda et al. 2006), aiming to enhance the analgesic action of those drugs using smaller or regular doses (Ward & Alexander-Williams 1999; Miranda et al. 2006). Acetaminophen is a NSAID with analgesic and antipyretic characteristics associated with low anti-inflammatory action (Sharma & Mehta 2014). Despite the long history of acetaminophen administration in humans, the mechanism of action is still under investigation and it is recognized to act in serotonin, cannabinoid, opioid and nitric oxide pathways, and also as an inhibitor of prostaglandin production with apparent selectivity for COX-2 enzymes (Graham et al. 2013; Sharma & Mehta 2014).

In veterinary medicine, there are few studies about the use of acetaminophen as analgesic or antipyretic drug and the reports mainly described cases of poisoning in dogs and cats (Jones et al. 1992; Rumbeiha et al. 1995; McConkey et al. 2009). The acetaminophen presents a bioavailability of 91% when administered orally to horses (Neirinckx et al. 2010). This drug reaches the maximum plasmatic concentration in approximately 30 to 60 minutes after single or multiple oral dose administration and no adverse effects were reported (Doherty et al. 1998; Lohmann et al. 2002; Mercer et al. 2020).

The potential analgesic effect of acetaminophen in horses was first described as an adjunctive therapy for pain management of a laminitic pony (West et al. 2011). Later,

an experimental study using a reversible model of equine foot pain reinforced the analgesic potential of acetaminophen in horses, demonstrating a similar reduction of lameness intensity between acetaminophen (20 mg Kg^{-1}) and flunixin meglumine (1.1 mg Kg^{-1}) (Foreman et al. 2016). Recently, a study described the safety of acetaminophen (20 mg Kg^{-1}) orally administered using single and multiple clinical doses in adult horses over 14 days (Mercer et al. 2020).

There have been no published data about the analgesic effect, nor toxicity, of acetaminophen associated with phenylbutazone in horses. The presented study hypothesized that: 1) association of acetaminophen, at clinical dose 20 mg Kg^{-1}, with phenylbutazone, at half clinical dose 2.2 mg Kg^{-1}, would produce analgesia similar to phenylbutazone, at clinical dose 4.4 mg Kg^{-1}, in a reversible model of foot pain; 2) the association of acetaminophen could be safely administered for a 14 days period.

Material and Methods

The study protocol was approved by Ethic Committee on Animal Use of the Federal University of Santa Maria by protocol number CEUA 7836130617 and written consent obtained by owners before study enrollment.

Animals

Twelve healthy adult crossbred horses aging 12 ± 3 years and weighting 450 ± 125 kg were selected for this study after physical examination. Horses were sound from lameness based on objective assessment asymmetry parameters. Routine trimming and shoeing were performed in all horses before the study.

Instrumentation and objective gait evaluation

Lameness was objectively assessed with a body-mounted inertial sensors system composed by two accelerometers, one attached on the dorsal midline of sacral tuberosities and a second on the poll region, and a gyroscope fixed on the dorsal aspect of right forelimb pastern (Lameness Locator®, Columbia, MO, USA). The magnitude (mm) of forelimb lameness was assessed considering the push-off ($H_{DIFFMAX}$) and the impact ($H_{DIFFMIN}$) components, which represent the maximum and minimum head height difference between right and left limb of each stride respectively. The lameness intensity (VS) was calculated as the quadratic mean of $H_{DIFFMAX}$ and $H_{DIFFMIN}$.

Lameness was considered when the means of $H_{DIFFMAX}$ and $H_{DIFFMIN}$ were above 6 mm and higher than the *s.d* and the VS above 8.5. All gait analysis involved collection of at least 30 strides at trot on a flat compacted sand surface in a straight line.

Lameness induction

The method of reversible lameness with hoof metal clamp was adapted from a previous study (Swaab et al. 2015). Galvanized hoof clamps were randomly applied in one forelimb to limit the hoof expansion and then induce lameness. Two metal plates were attached on the dorsomedial and dorsolateral aspect of the hoof wall to prevent proximal migration of the clamp when tightened. The clamp was gradually tightened until to observe VS values of 40 ± 10 and software's qualitative data of strong evidence of moderate/severe to severe forelimb lameness, which represented, in the visual assessment, a consistent lameness with head bob at trot in a straight line. The bolt length and number of turns of the screw necessary to induce lameness in the first trial were recorded and used as reference to create the same lameness severity in subsequent trials. A gait analysis was performed 30 minutes after clamp removal to assess residual lameness.

Analgesic effect assessment

A prospective, randomized crossover study was performed. Horses were randomly submitted to four treatments, respecting a washout period of 7 days between treatments. Treatments were: control; acetaminophen (ACET) 20 mg Kg^{-1} orally (PO); acetaminophen 20 mg Kg^{-1} PO associated with phenylbutazone 2.2 mg Kg^{-1} intravenously (IV) (ACET+PBZ); and phenylbutazone 4.4 mg Kg^{-1} IV (PBZ). Lameness was objectively assessed before and after lameness induction and at 30, 60, 90, 120, 150, 180 minutes after treatments. The heart rate (HR) was recorded before lameness evaluation, at five minutes after lameness induction and five minutes before subsequent evaluations by indirect stethoscope auscultation. The analgesic effect was evaluated by changes on HR and also calculated as a relative change on lameness intensity (ΔLI) by the formula: $(VS_{timepoint}\text{-}VS_{induction})/ VS_{induction}$. The acetaminophen administered orally was ordered from a well-known compounding pharmacy in 10gr-syringes each, and the phenylbutazone used intravenously was a commercially available compound (Fenilbutazona OF®, Cravinhos, SP, Brazil). The IV injection was

performed by jugular puncture with an 18 G needle attached to a 10 mL syringe after aseptic preparation with 70% ethyl alcohol.

Toxicity Evaluation

In a second stage, horses were randomly distributed according to the treatments previously described in ACET (n=4), ACET+PBZ (n=4) and PBZ (n=4) groups, respecting a 30-day washout period from the end of the analgesic study. Horses were submitted to a 14-day period of treatment, and all the drugs were administered orally twice a day using the doses previous described. Daily examination was performed before each administration for signs of colic or loss of appetite. Blood samples were collected before and at 7 and 14 days after the first treatment for complete blood count (CBC) (RBC, red blood cells; hemoglobin; PCV, packed cell volume; platelets; and WBC, white blood cells) and serum biochemical analysis (albumin; GGT, gamma glutamyl-transferase; AST, aspartate transferase; CK. creatine kinase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; creatinine; and TPP, total plasma protein). The acetaminophen administered was the same as previously described, and the phenylbutazone administered orally was ordered from compounding pharmacy in single syringes of 1 gr and 2 gr each.

Statistical Analysis

The $H_{DIFFMAX}$, $H_{DIFFMIN}$, VS, ΔLI, HR, CBC and serum biochemical parameters presented normal distribution by D'Agostino-Pearson test. The analgesic effect was assessed by comparing the ΔLI and HR between groups using 2-way repeated measures ANOVA followed by post-hoc Tukey's multiple comparison tests to assess significant differences at each time point. The toxicity was evaluated comparing CBC and biochemical parameters means of each group overtime by 2-way repeated measures ANOVA followed by post-hoc Tukey's multiple comparison test to assess significant differences (GraphPad Prism version 8.1.0, Software, La Jolla, California, USA).

Results

The mean (± *s.e.*) values for $H_{DIFFMAX}$, $H_{DIFFMIN}$, VS and HR are presented on Figure 1. The pain generated after lameness induction produced a significant HR elevation in all treatment groups compared with baseline rates ($p<0.05$). A significant reduction on HR was observed comparing lameness induction with after treatment for ACET (at 60 and

120 min), PBZ (at 60 to 120 min) and ACET+PBZ (at 150 min) treatments ($p<0.05$). No difference in HR was observed between treatments over time.

No difference was observed on $H_{DIFFMAX}$, $H_{DIFFMIN}$ and lameness intensity comparing groups at base line, after lameness induction and after hoof clamp removal (Figure 1). The hoof clamp allowed induction of consistent lameness for ACET (VS, 37.35±5.82; HR), ACET+PBZ (VS, 39.68±8.55), PBZ (VS, 34.15±4.7) and control (VS, 37.95±11.82) groups compared with baseline ($p<0.05$) (Figure 1C). Comparing the overall treatment effect over ΔLI of all treatments, ACET+PBZ demonstrated to be more effective than ACET ($p=0.0081$), PBZ ($p=0.0117$) and control ($p<0.0001$) in relieving the induced pain; and PBZ demonstrated more effectiveness compared with control ($p=0.0441$). In turn, the ACET+PBZ group was superior to control in change lameness severity only at 120 minutes ($p=0.0314$). There was no difference in ΔLI between ACET, PBZ and control groups along the time evaluated (Figure 2). A considerable reduction of lameness severity was observed after hoof clamp removal and some horses required more than 30 minutes to return to baseline values.

The ACET group presented difference in erythrocytes ($p=0.0157$), hemoglobin ($p=0.008$) and PCV ($p=0.024$) levels comparing values from day 7 with day 14 after treatment (Figure 3). The PBZ group demonstrated difference on albumin concentrations from before ($\bar{x} = 2.95 \pm 0.13$, $p=0.0124$) and 7 days ($\bar{x} = 2.73 \pm 0.1$, $p=0.0367$) after treatment compared to 14 days of treatment ($\bar{x} = 2.50 \pm 0.08$) (Figure 4). Higher BUN concentrations were observed in the ACET+PBZ group at day 7 after treatment compared with before treatment values ($p= 0.0062$), and a slight difference ($p=0.0462$) was observed in creatinine concentrations from day 7 ($\bar{x} = 1.35 \pm 0.13$) to day 14 ($\bar{x} = 1.23 \pm 0.13$).However, despite the differences observed, all blood parameters evaluated presented values within the laboratory reference intervals. None of the horses showed inappetence or colic symptoms during the multiple-dose administration.

Discussion

Despite the long and traditional use of acetaminophen as analgesic and antipyretic drug in human medicine (Altman 2004; Graham et al. 2013), there still is a lack of information about its clinical efficacy in veterinary medicine. The results of this study

demonstrated that the combination of acetaminophen with 2.2 mg Kg^{-1} clinical dose of PBZ presented significant efficacy in reducing lameness induced by the hoof clamp technique for up to 180 minutes after treatment (Figure 2B). This therapeutic association presented efficacy when compared overall treatment effect with the drugs administered alone (ACET or PBZ alone). The ACET produced similar analgesia compared to PBZ, although only PBZ presented overall efficacy in reducing lameness intensity compared with control.

The method of lameness induction with hoof clamp demonstrated to be efficacious in producing a consistent mechanical stimulus along the evaluated period, since no significant improvement on lameness severity was observed in the control group (Figure 2B). Limitations of this experimental model that may have interfered on HR interpretation could be the length of time and lameness assessment used, whereas studies using other experimental lameness methods were performed with horses in the stall and for up to 12 hours after treatment (Foreman et al. 2010; Foreman & Ruemmler 2011; Foreman et al. 2012; Foreman & Ruemmler 2013; Foreman et al. 2016). Moreover, the lameness severity induced before treatments were initiated promoted a significant increase of HR compared with baseline values and these HR were similar to those described using a heart bar shoe model of lameness induction (Foreman & Ruemmler 2011; Foreman & Ruemmler 2013), which corroborated that our experimental design was efficient to inflict reversible discomfort.

A strength point of this study was the possibility to evaluated analgesic effect data from acetaminophen and phenylbutazone based on objective lameness assessment obtained by wireless inertial sensors system. Although the authors are aware that this system presents few limitations, but using this system the variability of the subjective lameness evaluation was excluded. While objective evaluation does not replace the lameness exam, it should be indeed used as a tool to better understand lameness (Keegan et al. 2012; Keegan et al. 2013). However, this system gives us more reliability to present data about the clinical efficacy of ACET associated or not with PBZ on the experimental design, which reinforces the analgesic potential of ACET as previously described (West et al. 2011; Foreman et al. 2016).

The administration of acetaminophen alone was not able to produce significant changes on lameness severity and HR compared with the control group as previously reported (Foreman et al. 2016). In contrast, the combination of ACET+PBZ induced significant changes on lameness severity compared with control, PBZ and ACET treatments, leading us to accept our first hypothesis. This association may have some advantage compared with administration of PBZ alone to manage chronic pain cases that require a long period of treatment, since using a smaller dose of PBZ the incidence of side effects, such as gastric ulcer, right dorsal colitis and kidney toxicity, should be lower than those observed using clinical or higher doses of PBZ (MacAllister et al. 1993; Doucet et al. 2008). Previous data demonstrated that the association PBZ (2.2 mg Kg^{-1}) with flunixin meglumine (1.1 mg Kg^{-1}) effectively alleviated natural occurring lameness compared with PBZ administered alone at the same dose (Keegan et al. 2008). The meloxicam, a selective/preferential COX- 2, and firocoxib, a selective COX-2, are NSAIDs approved to manage pain in horses in some countries. Despite their advantage associated with lower side effects using label doses, a lower effectiveness (in alleviating mechanical pain induced by heart bar shoe) has been reported when compared with PBZ (Foreman et al. 2014; UCVM et al. 2017).

The second hypothesis was also accepted, since the blood parameters were into the reference ranges and no signs of abdominal pain or inappetence were observed.
The association of ACET+PBZ demonstrated to be as safe as the drugs administered alone for a period of 14 days. Interestingly, despite significant differences observed in some blood and serum biochemical parameters, it is unlikely that these variations would promote undesirable clinical effects because these parameters were within reference range. Similar results were reported in horses during multiple dose administration of ACET only for the same period of time (Mercer et al. 2020). Further experimental and clinical investigations about association of ACET with different NSAIDs, mainly selective COX-2, or opioid drugs using clinical or smaller doses is warranted based on the results of this study.

Conclusion

The combination of acetaminophen with a 2.2 mg Kg^{-1} clinical dose of phenylbutazone could be safely administered orally for a 14 days period. This association of drugs induced equal to superior analgesia than usual clinical administration of phenylbutazone

(4.4 mg Kg^{-1}) and acetaminophen alone (20 mg Kg^{-1}), without production of side effects. Further investigation using different painful clinical situations should be performed to analyze the analgesic potential of ACET alone, or in combination with others NSAIDs, before its recommendation in clinical routine.

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Figures

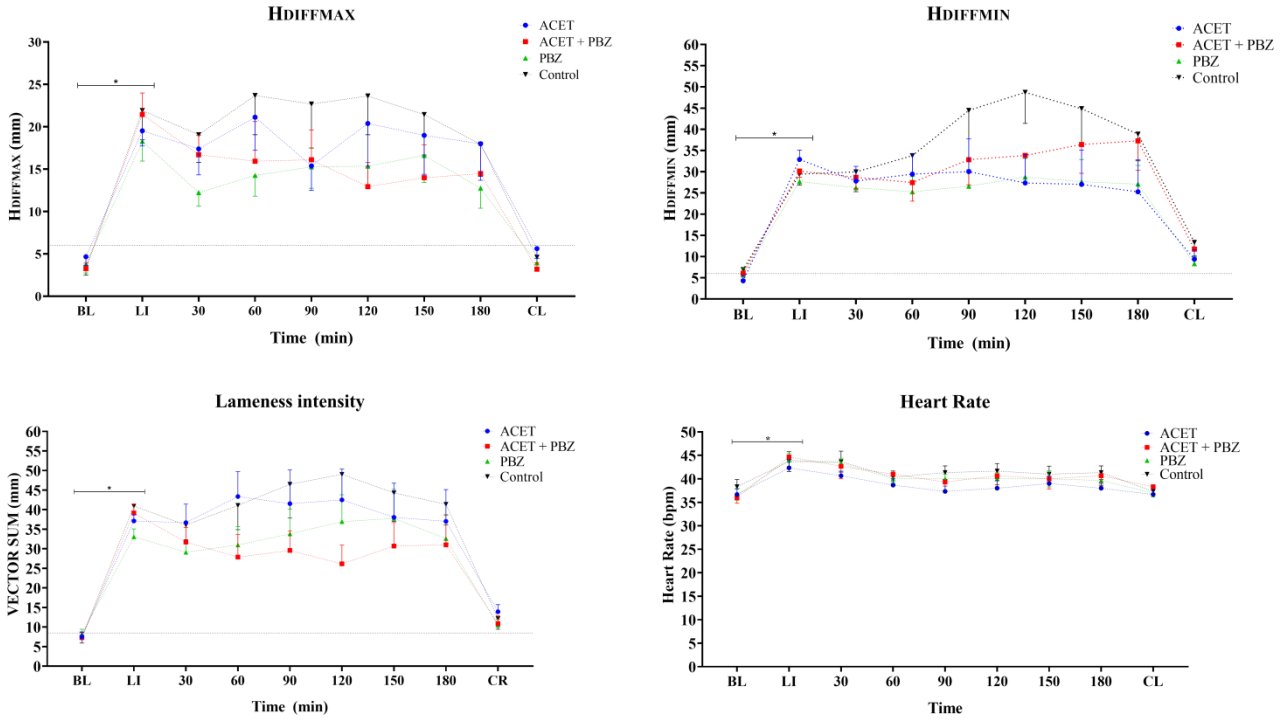

Figure 1 - Mean and standard error (*s.e*) of forelimb lameness and heart rate parameters from 12 horses before and after lameness induction followed by treatment with acetaminophen (20 mg Kg^{-1}), acetaminophen (20 mg Kg^{-1}) associated with phenylbutazone (2.2 mg Kg^{-1}), phenylbutazone (4.4 mg Kg^{-1}) and without any treatment . BL (baseline); LI, lameness induction; and CR (clamp removal). * Statistical difference ($p<0.05$) between mean of BL compared with LI.

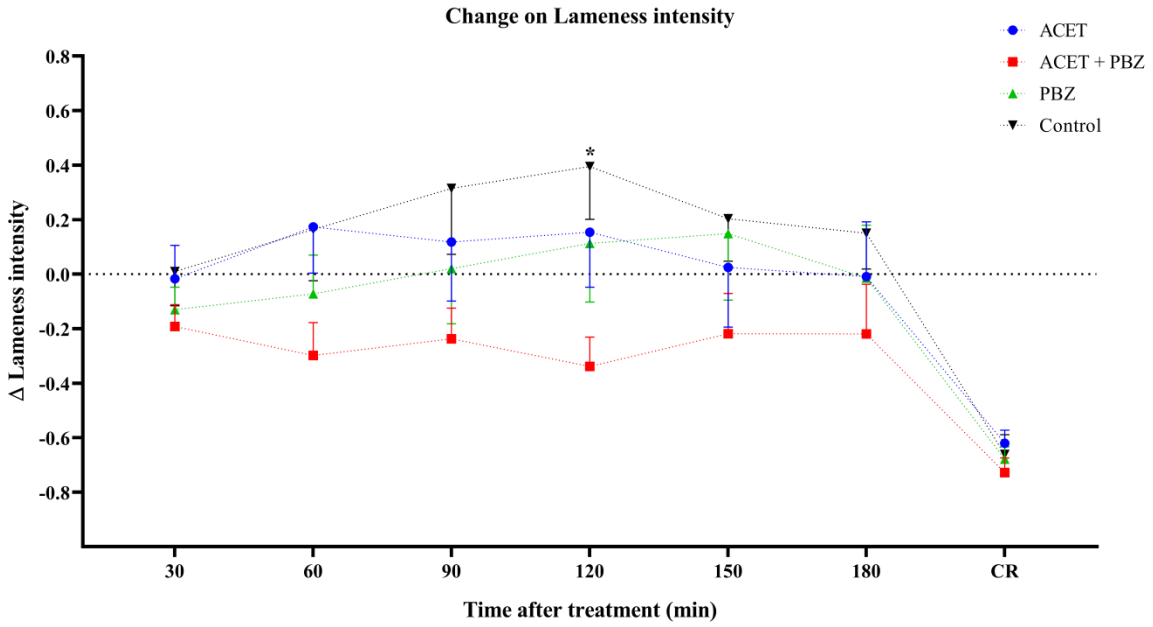

Figure 2 – Mean and standard error (*s.e*) of change on lameness intensity (ΔLI) form 12 horses with induced foot lameness and treated orally with acetaminophen (ACET, 20 mg Kg^{-1}), or acetaminophen associated with phenylbutazone (2.2 mg Kg^{-1}) administered intravenously (ACET+PBZ), with phenylbutazone (4.4 mg Kg^{-1}) intravenously only or without any treatment (Control) along 180 minutes of treatment. *****represent statistical difference between ΔLI from ACET + PBZ compared to Control group ($p<0.05$).

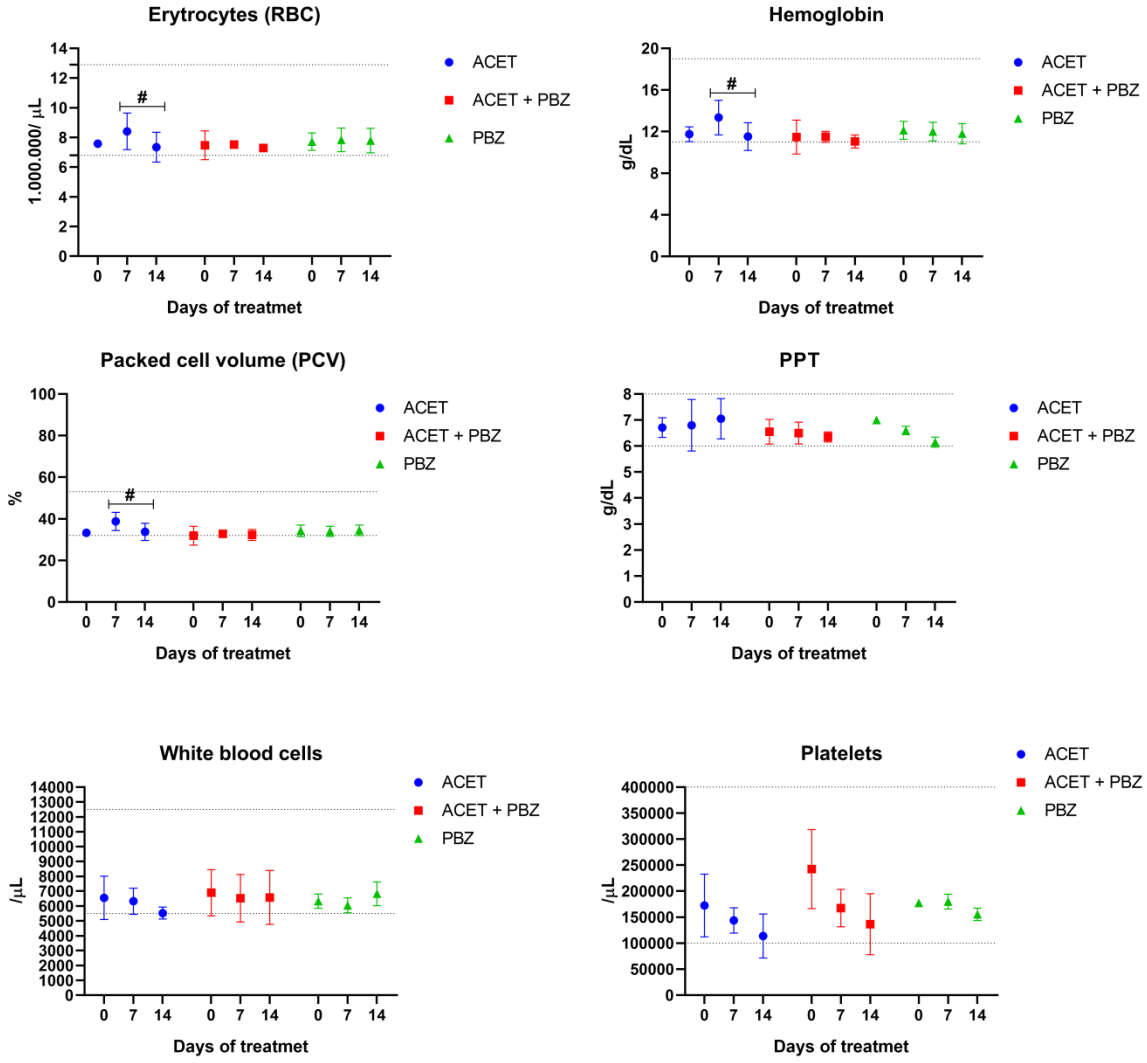

Figure 3 – Mean and *s.d* of complete blood count of 12 horses treated orally twice a day for 14 days with acetoaminophen (ACET, 20 mg Kg^{-1}), acetaminophen (20 mg Kg^{-1}) associated with phenylbutazone (ACET+PBZ, 2.2 mg Kg^{-1}) and with phenylbutazone (PBZ, 4.4 mg Kg^{-1}) only. Dot lines along x axis represent laboratory references. # Statistical difference ($p<0.05$) between mean of day 7 compared with day 14.

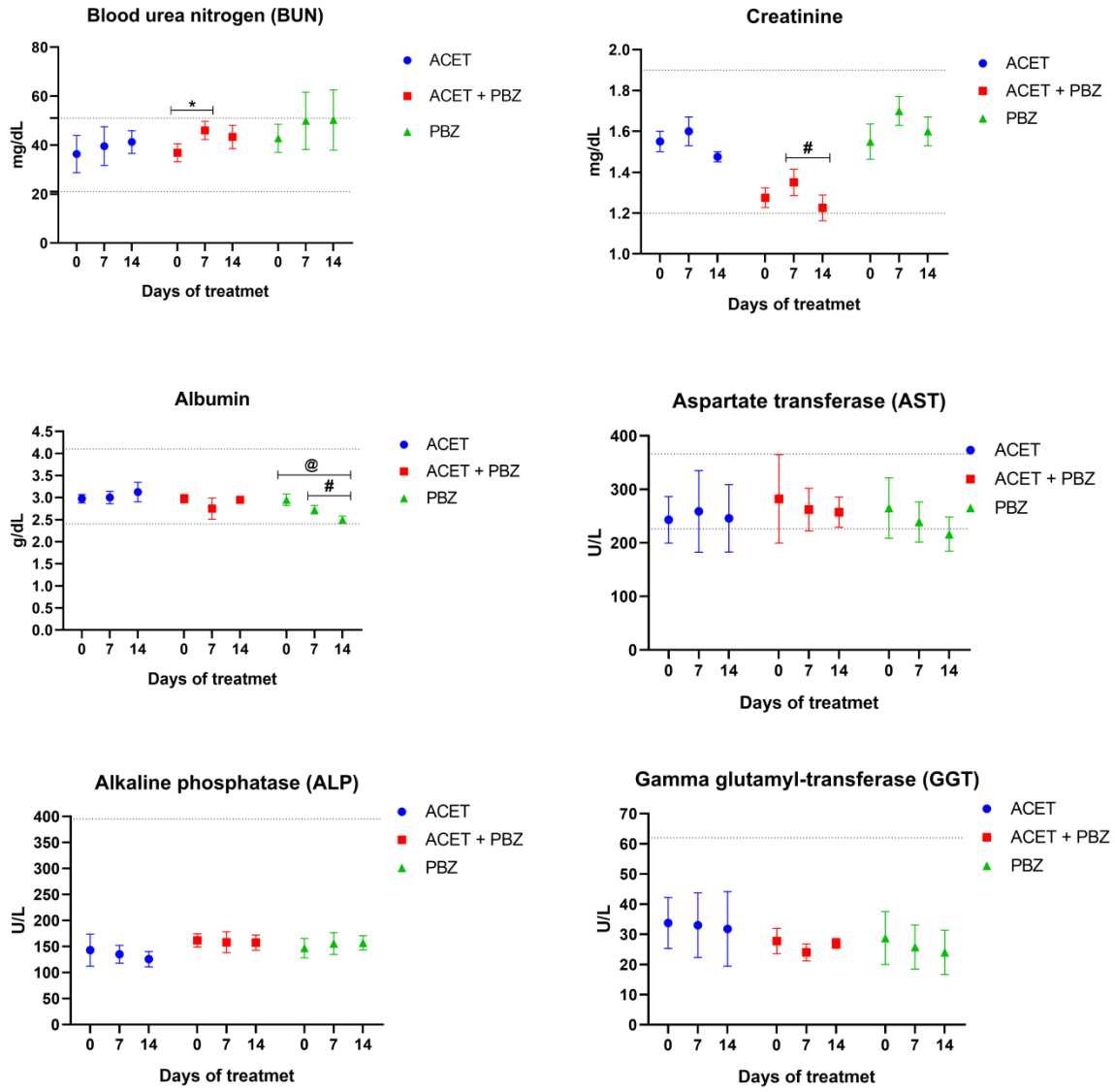

Figure 4 – Mean and *s.d* of serum biochemical paramenters of 12 horses treated orally twice a day for 14 days with acetoaminophen (20 mg Kg^{-1}), acetaminophen (20 mg Kg^{-1}) associated with phenylbutazone (2.2 mg Kg^{-1}) and with phenylbutazone (4.4 mg Kg^{-1}) only. Dot lines along x axis represent laboratory references.* Statistical difference ($p<0.05$) between mean of day 0 compared with day 7; @ statistical difference ($p<0.05$) between mean of day 0 compared with day 14; and # statistical difference ($p<0.05$) between mean of day 7 compared with day 14.

6. DISCUSSÃO

No primeiro estudo, o cloreto de amônio 2% demonstrou ser um fármaco de grande valor no manejo da dor crônica em equinos, uma vez que possibilitou uma analgesia parcial a completa por até 62 dias em diferentes casos de dor crônica que tem origem no casco em equinos. Acredita-se que uma melhor resposta deste fármaco poderia ser obtida associando ele a outras modalidades terapêuticas normalmente utilizadas no manejo da dor ligada aos cascos, como ferrageamento terapêutico e infiltrações com corticosteroides ou outros medicamentos de uso intrasinovial (DABAREINER et al., 2003; DABAREINER et al., 2005; GUTIERREZ-NIBEYRO et al., 2010). Outro fator que poderia proporcionar melhores respostas seria a utilização deste neurolítico em casos mais sutis de claudicações ligadas ao casco, uma vez que a maioria dos animais utilizados no presente estudo apresentavam alterações radiográficas de moderadas a severas. A observação dos melhores índices de analgesia entre os dias 12 e 19 sugere que seu melhor efeito clínico seja observado dias após a sua aplicação, ressaltando que este composto apresenta um mecanismo de ação diferente dos anestésicos locais (HARKINS et al., 1997; (CAMPOS et al., 2013).

Variáveis como volume utilizado e precisão na deposição do medicamento podem influenciar no início e grau de analgesia dos agentes neurolíticos como descrito na realização de bloqueios anestésicos (SCHUMACHER et al. 2013). Os resultados obtidos no presente estudo foram similares aos descritos após a injeção intraneural com álcool 98% (SCHNEIDER et al. 2014). Do contrário, outro estudo usando a aplicação perineural de álcool absoluto e álcool benzílico nos nervos palmares digitais demonstrou melhores e mais duradouros índices de analgesia do que os observados no presente estudo (NICOLETTI et al. 2007c). Comparados à neurectomia, procedimento cirúrgico sujeito a complicações, os agentes neurolítcos apresentam maior segurança, uma vez que a percepção da dor aguda se mantém presente após a aplicação perineural (HARKINS et al. 1997b; NICOLETTI et al. 2007c; CAMPOS et al. 2013a) e intraneural (SCHNEIDER et al. 2014) destes fármacos. Outras potenciais aplicações dos neurolíticos seriam no controle da dor durante a reabilitação de casos crônicos, como a laminite e síndrome do navicular, ou durante a recuperação após cirurgias ortopédicas, como a artrodese de articulação interfalangeana distal ou proximal, quando não há preocupação com

complicações como sepse ou falha dos implantes no período pós-operatório. O presente estudo utilizou uma pequena casuística que reflete a rotina de muitos clínicos dentro da medicina de equinos. Assim, um estudo prospectivo com mais casos, associado a técnicas mais sensíveis de diagnóstico por imagem, como ressonância magnética ou tomografia computadorizada, se faz necessário para melhor entendimento do efeito analgésico dos agentes neurolíticos no manejo da dor ligada ao casco em equinos.

No segundo estudo, a observação das alterações histológicas dos nervos digitais palmares após a injeção perineural do cloreto de amônio 2% permitiu um melhor entendimento do mecanismo de ação do fármaco. Verificou-se que a sua utilização induz uma resposta inflamatória local semelhante à aplicação de solução salina 0.9%, e que a degeneração Walleriana causada é semelhante à observada após injeção intraneural com álcool 98% (SCHNEIDER et al., 2014). Porém, o cloreto de amônio 2% apresentou menos reações locais após sua aplicação perineural do que as descritas para a utilização de compostos neurolíticos a base de álcool (NICOLETTI et al., 2007; SCHNEIDER et al., 2014; ESCODRO et al., 2018). Desta forma, o uso de agentes anestésicos antes, ou durante a aplicação de agentes neurolíticos seria de grande valor a fim de se evitar reações de desconforto durante a administração destes compostos (KOBAYASHI et al., 1997; VAN HOOGMOED e SNYDER, 2002). O efeito analgésico dos diferentes compostos neurolíticos pode ser relacionado com o grau de lesão induzida por eles. Assim, de acordo com o diagnóstico clínico, a utilização isoladamente dos neurolíticos pode não ser eficiente em aliviar a dor por completo.

No terceiro estudo, a associação do acetaminofeno com metade da dose usual da fenilbutazona possibilitou um efeito analgésico satisfatório no modelo de claudicação induzida utilizado, ressaltando os benefícios da utilização do paracetamol na medicina de equinos como descrito na medicina humana por décadas (ALTMAN, 2004; GRAHAM et al., 2013). A associação farmacológica avaliada apresentou resultados similares à utilização da fenilbutazona apenas, reafirmando o efeito sinérgico do paracetamol quando administrado juntamente com outros anti-inflamatórios não esteroides (WEST et al., 2011). O modelo experimental utilizado se mostrou eficaz, uma vez que permitiu a indução de estímulo mecânico consistente durante o período avaliado, de forma que não se observou alteração na intensidade da claudicação após indução no grupo controle. A indução de

claudicação gerou um aumento significativo na frequência cardíaca para todos os tratamentos quando comparada com os valores basais, e estes foram similares aos descritos após a indução da claudicação com pressão na ranilha em equinos (FOREMAN e RUEMMLER, 2011; FOREMAN e RUEMMLER, 2013). A possibilidade de utilizar um método de análise objetiva da claudicação, como o sistema de sensores inercias sem fio, possibilitou maior confiabilidade aos dados apresentados sobre o efeito analgésico e sinérgico do acetaminofeno associado à fenilbutazona, reforçando o potencial analgésico do paracetamol no manejo da dor em equinos (WEST et al., 2011; FOREMAN et al., 2016).

A administração, por 14 dias, de múltiplas doses do acetaminofeno com metade da dose terapêutica da fenilbutazona não provocou alterações fora dos valores de referência nos parâmetros hematológicos e bioquímicos avaliados nos animais do estudo. Também não se evidenciou alterações nestes parâmetros quando administrado múltiplas doses destes fármacos separadamente pelo mesmo período de tempo. Estes resultados reforçam a segurança da utilização de múltiplas doses do acetaminofeno (MERCER et al., 2020), e sugerem que a sua administração conjunta à fenilbutazona por até 14 dias induzem poucas alterações hematológicas e bioquímicas em equinos. Esta associação pode apresentar vantagem comparada com a utilização apenas de fenilbutazona no manejo da dor crônica em casos que necessitem longo período de tratamento (WEST et al., 2011). Isto porque, a utilização de menores doses da fenilbutazona está relacionada com menores efeitos adversos como úlceras gástricas, colite dorsal direita e nefrite tóxica quando comparada ao uso de doses regulares e superiores (MACALLISTER et al. 1993; DOUCET et al. 2008). Desta forma, baseado nos resultados deste estudo, estudos experimentais e clínicos avaliando diferentes protocolos de associação entre os anti-inflamatórios não-esteroides, principalmente os COX-2 seletivos, ou opióides, com o acetaminofeno são indicados.

7. CONCLUSÃO

O cloreto de amônio 2% nos nervos digitais palmares pode ser de grande utilidade clínica para proporcionar analgesia de parcial a completa por um período de até 62 dias em diferentes casos de claudicação crônica ligada ao casco dos equinos. A presença de alterações radiográficas de grau moderado a severo pode influenciar negativamente no efeito analgésico do cloreto de amônio 2%. A aplicação perineural dos nervos digitais palmares com cloreto de amônio 2% induziu degeneração Walleriana de moderada a severa e seu uso não apresentou interferência em neurectomias futuras.

A associação de acetaminofeno com metade da dose terapêutica da fenilbutazona pode ser administrado com segurança por até 14 dias. Esta associação produziu analgesia igual a superior à utilização dos fármacos de forma isolada. Mais estudos com diferentes protocolos de indução de claudicação deveriam ser realizados, para melhor avaliar o potencial analgésico do acetaminofeno associado ou não com outros anti-inflamatórios não-esteroidais, antes da recomendação de seu emprego na rotina clínica.

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ANEXOS

ANEXO A - Comprovante de publicação do artigo 1 no periódico Equine Veterinary Education. DOI: 0.1111/eve.12972

Equine Veterinary Education

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Original Article

Management of chronic foot lameness with 2% ammonium chloride on the palmar digital nerves

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Keywords: horse; distal interphalangeal joint; navicular disease; neurolytic; palmar digital nerve block; podotrochlear apparatus disease

Summary

This case series describes the analgesic effect of 2% ammonium chloride (2% AC) in horses with chronic foot pain. Ten horses with foot pain related to chronic laminitis (n = 1), bruised sole (n = 1), distal interphalangeal joint (DIPJ; n = 1), podotrochlear apparatus (PA; n = 4) and PA associated with DIPJ (PA + DIPJ; n = 3) received perineural injections with 3 mL of 2% AC on the palmar digital nerves. A Five horses with pain related to PA + DIPJ (n = 3), PA (n = 1) and DIPJ (n = 1) were treated with saline as control. The analgesic effect was evaluated as lameness improvement (LI) rate (%) using a body-mounted inertial sensor system, and was assessed at 5, 12, 19, 35, 47 and 62 days after treatment. Horses treated with 2% AC demonstrated a mean LI rate above 50% from Day 12 (63% ± 26) to Day 62 (65% ± 26). Control horses has a overall LI of 28% (± 23%) and a LI above 50% was evidenced in horses with PA + DIPJ (n = 2) and PA pain (n = 1) at different times. Horses with PA pain presented higher LI rates (72% ± 23) than that presented by horses with PA + DIPJ (51% ± 9) or DIPJ (51% ± 19). Horses with severe radiographic lesions of the navicular bone and DIPJ had the lowest LI rates after treatment. The 2% AC is a useful treatment to be included in the clinical management of chronic foot pain involving the podotrochlear apparatus with mild radiographic lesions.

Introduction

Chronic foot pain is an important cause of poor performance in a variety of equine sport modalities (Dabareiner *et al.* 2005; Murray *et al.* 2006). Chronic foot pain has traditionally been treated by corrective trimming and shoeing; administration of nonsteroidal anti-inflammatory drugs; or injection of corticosteroids and/or hyaluronic acid in the distal interphalangeal joint (DIPJ) and navicular bursa (NB) (Dabareiner *et al.* 2003; Gutierrez-Nibeyro *et al.* 2010).

In addition to medical treatment and shoeing practices, surgery can be done to treat chronic foot lameness. Although effective to alleviate foot pain, low pastern neurectomy is the last treatment option for horses with a poor response to conservative therapy (Maher *et al.* 2008). The structures involved and the surgical technique determine the success rate (Gutierrez-Nibeyro *et al.* 2010). Depending on the surgical technique, variable post-operative complications can occur (Maher *et al.* 2008; Gutierrez-Nibeyro *et al.* 2015).

The use of chemical substances to mimic neurectomy has been used in human medicine for the management of chronic pain of diverse aetiologies (Manchikanti *et al.* 2001; Rosen 2004; Koyyalagunta and Burton 2010). In veterinary medicine, equine practitioners usually apply neurolytic agents to provide long-term nerve block (Harkins *et al.* 1997). However, unlike the response to injection of local anaesthetics, complete limb desensitisation does not occur after perineural injection of neurolytic agents (Harkins *et al.* 1997; Campos *et al.* 2013). Even after intraneural injection of 98% ethyl alcohol or formaldehyde, skin sensitivity and thermal nociception persist (Schneider *et al.* 2014). In contrast, substantial to complete desensitisation of mechanic nociception of the foot has been demonstrated experimentally after injection of neurolytic compounds on the palmar nerves (Nicoletti *et al.* 2007; Schneider *et al.* 2014). Therefore, the present case series aimed to objectively describe the analgesic effect of 2% ammonium chloride[1] (2% AC) injected on the palmar digital nerves (PDN) of horses with chronic foot pain of different aetiologies.

Cases histories

After physical examination, 15 horses were equipped with a body-mounted inertial sensor system[2] to objectively assess lameness intensity, and to compare lameness intensity after diagnostic blocks. A diagnosis of chronic foot lameness was based on the presence of lameness for more than 2 months and a lameness improvement (LI) rate greater than 80% 10 min after the PDN block. Demographic information on the horses is presented in **Table 1**. Horses were involved in one of four exercise activities: equine assisted-therapy (for children and adults with health issues); police patrol horses; Showjumping horses and a retired horse. Horses involved in equine assisted-therapy were ridden at a walk or trot, for 3–4 h a day, 4 days a week. Police patrol horses participated in afternoon street patrols three times a week and had 2 days of mild exercise (walk, trot and gallop) in an arena. Showjumping horses were ridden 5 days per week, with 1 day a week for jumping exercises, and the other days of light riding only. The retired horse was kept in a paddock without an exercise routine. Horses were not treated with any other medication or corrective trimming or shoeing during the evaluation.

Lameness exam

Lameness evaluation was performed with a body-mounted inertial sensor system[2]. Horses were trotted by hand in a straight line on a hard surface (asphalt/concrete/compacted

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Short Communication

Histologic Evaluation of Palmar Digital Nerves after Perineural Injection of 2% Ammonium Chloride in Miniature Horses

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