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ABORDAGENS INTEGRATIVAS NA PREVENÇÃO DE RECAÍDA POR MORFINA EM RATOS: ORGANOTERÁPICO E ISOTERÁPICO

Santa Maria, RS
2024

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

Orientador: Prof[a] Dr[a] Marilise Escobar Burger

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Murilo Barboza Fontoura

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Santa Maria, RS
2024

‘’Inteligência é a capacidade de se adaptar às mudanças.’’

(Stephen Hawking)

RESUMO

ABORDAGENS INTEGRATIVAS NA PREVENÇÃO DE RECAÍDA POR MORFINA EM RATOS: ORGANOTERÁPICO E ISOTERÁPICO

AUTOR: Murilo Barboza Fontoura
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De acordo com a Organização Mundial de Saúde, o uso indiscriminado e recreacional de drogas psicoativas, especialmente opioides, têm causado um grave problema de saúde pública em nível mundial, e caracterizando a reconhecida crise dos opioides nos E.U.A. A drogadição caracteriza-se pela presença de fatores, dentre os quais, dependência (compulsão ao uso), tolerância, sintomas de abstinência na retirada e recaída que podem ser desencadeados após algumas exposições à droga. A recaída ao uso destas substâncias mostra-se como o principal obstáculo para o sucesso do tratamento de desintoxicação, uma vez que a maior parte dos indivíduos recorre ao uso da mesma após o período de detoxificação. De um modo geral, as abordagens farmacológicas utilizadas até o momento têm sido apenas sintomáticas, mostrando-se pouco eficazes quando a situação envolve drogadição. Nesse sentido, a busca por terapias integrativas, e entre estas, os tratamentos com medicamentos homeopáticos podem representar um avanço para o reestabelecimento da homeostase orgânica, uma vez que a desregulação desta homeostase se relaciona diretamente aos sintomas impulsivo-compulsivos, emocionais, sociais e físicos desenvolvidos pela drogadição. O presente estudo tem por objetivo avaliar o potencial terapêutico de duas preparações homeopáticas, ou seja, um isoterápico de morfina (ISO) e um organoterápico obtido a partir do cérebro total de carneiro saudável (ORG), sobre parâmetros comportamentais e moleculares consequentes à recaída por morfina em ratos. Para isso, os animais foram expostos à morfina no paradigma de preferência de lugar condicionado (PLC) para a indução inicial de comportamentos de preferência e busca pela droga, com subsequente tratamento com ISO ou ORG durante 14 dias. Após a observação da extinção da preferência pela morfina no paradigma de PLC, os animais foram desafiados a comportamento de recaída à preferência pela droga no mesmo aparato de PLC. Como esperado, os animais expostos à morfina apresentaram comportamento de busca pela droga, confirmando o forte poder hedônico da droga, o quais não mostraram prejuízos de memória, locomoção ou de ansiedade, confirmando que a busca pela droga foi por impulso hedônico. Ambos os medicamentos ISO e ORG foram capazes de prevenir a recaída pela morfina. Avaliações em nível molecular envolvendo o sistema dopaminérgico, glutamatérgico e opioide devem ser desenvolvidos na sequência desta dissertação, na busca de mecanismos relacionados aos benefícios comportamentais aqui observados. Em conclusão, até onde sabemos, este estudo mostra pela primeira vez que ambos medicamentos homeopáticos aqui estudados representam uma prática integrativa que pode ser útil no tratamento da drogadiçao por opioides, contribuindo para o reestabelecimento da homeostase orgânica comprometida pelo uso crônico da droga.

Palavras-chave: Opioides, drogas psicoativas, terapias naturais, paradigma de preferência de lugar condicionado (PLC).

ABSTRACT

INTEGRATIVE HEALTH PRACTICES ON MORPHINE-REINSTATEMENT IN RATS: ORGANOTHERAPIC AND ISOTHERAPIC

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The World Health Organization (WHO) determines drug addiction as a chronic and recurrent disease, constituting an alarming public health problem. Considering the recreational use of psychoactive drugs, such as morphine (MORPH), dependence (compulsion to use), tolerance, abstinence syndrome and relapse can be triggered after withdrawal. Reinstatement to opioid drug use is one of the main obstacles to the success of detoxification treatment, since most individuals fall back to drug use after the detoxification period. In general, the pharmacological treatments developed to date are only symptomatic, proving to be ineffective when the situation involves drug addiction. In this sense, natural therapies such as ultra-diluted medicines that aim to reestablish and integrate the organism into its homeostasis represent promising therapeutic potential in the treatment of this condition, since the dysregulation of the homeostatic balance is directly related to impulsive-compulsive, emotional, social and physical symptoms caused by the drug. This project aims to evaluate the therapeutic potential of both medicines on behavioral and molecular parameters related to MORPH reinstatement. Rats were exposed to MORPH in the conditioned place preference (CPP) paradigm to assess the drug's addictive and drug-seeking symptoms, and subsequently treated with both homeopathy medicines for 14 days. After MORPH-preference extinction, animals were challenged to MORPH reconditioning in the same CPP paradigm to evaluate MOPRH-reinstatement. Behaviors related to drug addiction, anxiety-like, memory and locomotion were assessed evaluated during drug withdrawal, followed by ex vivo assays. Our results showed MORPH-preference confirming the strong hedonic power of the drug. In the memory test and locomotor activity, no differences were observed between the groups, thus confirming the results of the CPP paradigm. Both ISO and ORG medicines prevented reinstatement-like behavior of MORPH. Molecular analysis are still being carried out. In conclusion, as far as we know, this study shows by the first time that both ultra-high dilution medicines may be an alternative which can contribute with opioid addiction reestablishing the body's physiological functions when exposed to toxic agents such as MORPH.

Keywords: Opioid addiction; psychoactive drugs; natural therapies; conditioned place preference (CPP).

LISTA DE ILUSTRAÇÕES

MANUSCRITO CIENTÍFICO

LISTA DE ABREVIATURAS E SIGLAS

ATV	Área tegmental ventral
DAT	Transportador de dopamina
DOR	Receptor opioide delta
DR	Receptor de dopamina
ISO	Isoterápico de morfina
KOR	Receptor opioide kappa
MOR	Receptor opioide mu
MORF	Morfina
NAc	Núcleo accumbens
OMS	Organização Mundial da Saúde
ORG	Organoterápico oriundo do cérebro total de carneiro saudável
PICS	Práticas Integrativas e Complementares em Saúde
PLC	Paradigma de preferência de lugar condicionado
PNPIC	Política Nacional de Práticas Integrativas e Complementares
SUS	Sistema Único de Saúde

SUMÁRIO

1. INTRODUÇÃO

O uso recreativo de drogas de abuso tem aumentado de forma alarmante em todo o mundo, com grande impacto devido à pandemia de COVID-19, provocando sérios problemas de saúde, uma vez que essas substâncias estão relacionadas com efeitos de hedonia, tolerância, dependência e abstinência, resultando em consequências médicas, psiquiátricas, cognitivas, judiciais e socioeconômicas (WESTOVER; PAUL; ROBERT, 2008; BERRIDGE; KRINGELBACH, 2015; BAIMEL; BORGLAND, 2015).

O circuito neural subjacente aos processos de recompensa, emoção e dependência inclui a via mesolímbica, que pode ser estimada por receptores de dopamina (D1R até D5R) e transportador (DAT), particularmente no *núcleo accumbens* (NAc) e área tegmental ventral (ATV) (GEIGER et al., 2009). O sistema dopaminérgico é um alvo bem conhecido de drogas com potencial aditivo, visto que a plasticidade neste circuito neural está intimamente envolvida no desenvolvimento da adição (HYMAN; MALENKA; NESTLER, 2006; KAUER; MALENKA, 2007). O aumento de monoaminas no NAc e na ATV está diretamente relacionado com os efeitos positivos das drogas psicoativas (NUTT et al., 2015; MILIANO et al., 2016).

Nesse contexto envolvendo propriedades reforçadoras, uma classe que merece grande destaque no âmbito drogadição são os opioides, frequentemente utilizados com o uso abusivo (WESTOVER; PAUL; ROBERT, 2008). Nos últimos anos, tanto as prescrições médicas quanto o uso não clínico dos opioides tem aumentado de forma progressiva, com altas taxas de mortalidade por overdose (SAMHSA, 2014; RELATÓRIO MUNDIAL SOBRE DROGAS DAS NAÇÕES UNIDAS, 2022). A abstinência de opioides inclui sintomas que muitas vezes não são suportados pelo indivíduo dependente (agitação, ansiedade, irritabilidade, dores musculares, insônia, sudorese, diarreia, calafrios, náuseas e vômitos), levando à compulsão (HUTCHINSON et al., 2011).

De modo geral, é importante ressaltar que os tratamentos farmacológicos até o presente momento desenvolvidos são obsoletos, mostrando-se pouco eficazes no cenário drogadição, visto que são úteis somente na redução de alguns sintomas de abstinência (MILANESI et al., 2023). Dessa forma a busca por novas estratégias terapêuticas torna-se essencial para o tratamento desta condição.

Práticas Integrativas e Complementares em Saúde (PICS) incluindo produtos naturais e terapias complementares ultra diluídas vem sendo utilizadas com frequência pela população para auxílio no tratamento de enfermidades (SHAHID et al. 2017). A homeopatia é fundamentada em 4 princípios: a lei do semelhante, a experimentação na pessoa sadia, as doses infinitesimais e o medicamento único (RAJENDRAN, 2018). De acordo com Nascimento et al. (2018), o uso deste sistema médico enunciado por Hipócrates no século IV a.C. vem sendo pesquisado e utilizado como uma prática integrativa e complementar no tratamento de inúmeras doenças, por meio de estímulos nas respostas biológicas dos indivíduos. Atualmente, ainda que os conhecimentos sobre homeopatia estejam expandindo, a literatura é carente de informações científicas que abordem os efeitos dos medicamentos homeopáticos.

1.1 HIPÓTESE

A drogadição por opioides é um distúrbio crônico recorrente caracterizado por dependência, tolerância, síndrome de abstinência e recaída. De fato, os opioides apresentam elevado poder hedônico, o qual se reflete em neuroadaptações, caracterizando a perda da homeostase. Por outro lado, a homeopatia é um sistema de medicina integrativa, cujos princípios de cura seguem leis como ultradiluição, dinamização e cura do semelhante pelo semelhante, o que engloba a isoterapia e a organoterapia. No pressuposto da isoterapia, uma substância que causa sintomas em um indivíduo saudável pode ser usada de uma forma altamente diluída e dinamizada para tratar sintomas semelhantes em um indivíduo doente (CORRÊA; SIQUEIRA-BATISTA; QUINTAS, 1997), ao passo que na organoterapia, a utilização de um órgão saudável de um animal (carneiro por exemplo) é capaz de reconstituir a homeostase deste mesmo homólogo doente (HORTA; CUNHA, 2021). Dessa forma, a hipótese desse estudo envolve a utilização das preparações homeopáticas aqui apresentadas na busca do estímulo orgânico para a obtenção da homeostase celular após a exposição à um agente tóxico como a morfina.

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Avaliar os possíveis efeitos benéficos do tratamento com medicamentos homeopáticos sobre parâmetros comportamentais e moleculares de recaída em animais previamente expostos à morfina (MORF).

1.2.2 Objetivos Específicos

a) Induzir a preferência pela MORF no Protocolo de Preferência condicionada de lugar (PCL) em ratos;

b) Tratar os animais expostos à MORF com isoterápico (ISO) ou organoterápico do cérebro total de ovinos saudáveis (ORG) após a retirada da droga, durante o período de abstinência;

c) Avaliar os comportamentos de recaída, após reexposição à MORF;

d) Avaliar comportamentos de ansiedade de memória e locomoção que possam falsear resultados observados no paradigma de PLC;

e) Avaliar marcadores moleculares dopaminérgicos, glutamatérgicos e opiodes associados à exposição à MORF.

1.3 JUSTIFICATIVA

O uso indevido crônico de opioides tem sido frequentemente associado ao desenvolvimento de drogadição devido ao seu alto poder aditivo e hedônico, impactando em graves problemas de saúde pública com alta morbidade elevados custos econômicos e sociais (RELATÓRIO MUNDIAL SOBRE DROGAS DAS NAÇÕES UNIDAS, 2022). A terapia empregada atualmente é baseada na

substituição de um opiaceo por um opioide que provoque sintomas de abstinência mais brandos, tais como a metadona e/ou a buprenorfina, cujo emprego têm apresentado baixa eficácia, também relacionados ao uso abusivo (MATTICK et al., 2014). Desse modo, é importante investigar novas estratégias terapêuticas que busquem minimizar os efeitos relacionados ao abuso de drogas, sendo os medicamentos homeopáticos uma promissora abordagem terapêutica. Além disso, tais benefícios já foram demonstrados experimentalmente, através da redução da compulsão e ansiedade frente à drogadição por anfetamina (SEGAT et al., 2019), além dos efeitos benéficos clínicos envolvendo transtornos do movimento como o Parkinson (HORTA; CUNHA, 2021), estimulando assim o presente estudo.

2. REVISÃO BIBLIOGRÁFICA

2.1 DROGADIÇÃO

As razões pelas quais o uso de substâncias aditivas se torna abusivo são complexas e multifatoriais, e são definidas pelo agente (droga), hospedeiro (usuário) e ambiente. O uso continuado destas pode promover quadros de dependência, tolerância, síndrome de abstinência e recaída (KOOB; VOLKOW, 2016). Inúmeras são as substâncias usadas de forma recreativa, incluindo desde drogas lícitas (nicotina, álcool) e ilícitas (anfetamina, cocaína, psicodélicos), assim como medicamentos (barbitúricos, benzodiazepínicos, opioides, anestésicos). Visando obter alterações prazerosas no humor e percepções, muitas substâncias são usadas em conjunto pelos usuários, sem considerar as diferentes interações e o potencial malefício a ser causado (MEASHAM; MOORE, 2009). Conforme descrito por Chao e Nestler (2004), todas as substâncias de uso abusivo são prejudiciais, com gravidade variável.

O elemento comum entre todas as drogas aditivas é a capacidade de promoção de recompensa (‘’reforço positivo’’) – efeitos de hedonia. Todas as substâncias que podem levar à adição ativam a via mesolímbica dopaminérgica que se estende (Fig. 1), via feixe medial do prosencéfalo, desde a área tegmental ventral (ATV) do mesencéfalo até o estriado ventral (NAc) e a região límbica (MALDONADO et al., 1997). O aumento na liberação de dopamina na via dopaminérgica ATV-accumbens é responsável pelas sensações gratificantes, que fazem com que os

indivíduos queiram repetir os efeitos recompensadores (DI CHIARA, 2002; (HYMAN; MALENKA; NESTLER, 2006; MARAMAI, 2016).

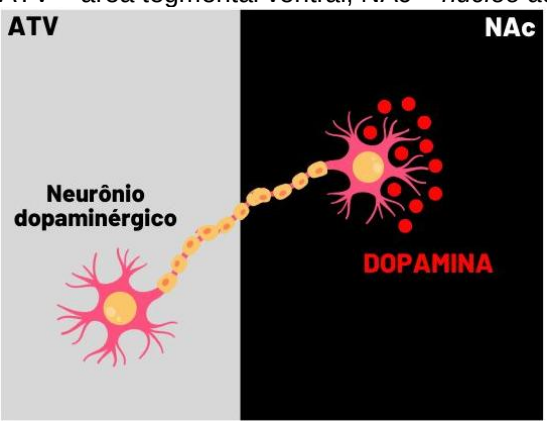
Nesse sentido, a toxicodependência tem sido conceituada como uma desordem psiquiátrica caracterizada por modificações neuroadaptativas e comportamentais crônicas, que desempenham um papel fundamental no aumento do risco de recaída por drogas, uma vez que modificam o sistema de recompensa (KOOB; VOLKOW, 2010; IKEMOTO; YANG; TAN, 2015). Nesse contexto, a drogadição descreve a condição humana na qual o desejo de sentir os efeitos das substâncias torna-se incontrolável e compulsivo, englobando fatores psicológicos e fisiológicos em desequilíbrio (JUPP; CAPRIOLI; DALLEY, 2013). A dependência impacta de forma negativa no estilo e qualidade de vida dos indivíduos (WEISS, 2005).

Um fator importante relacionado à dependência é a tolerância, desenvolvida ao longo do tempo. Pode ser definida como a ocorrência de uma diminuição do efeito farmacológico na administração repetida de uma substância em doses usuais (WILLIAMS et al., 2013). No caso dos opioides, por exemplo, a tolerância celular resulta em parte da dessensibilização dos receptores, uma vez que a ativação prolongada do receptor μ resulta em fosforilação por várias quinases intracelulares (BAGLEY et al., 2011; WILLIAMS et al., 2013), fazendo com que os indivíduos dependentes tenham que aumentar a dose consumida para voltar a experimentar os efeitos de recompensa.

Assim como a interrupção abrupta do uso de uma droga, a hiperatividade do sistema nervoso central decorrente da readaptação à ausência da substância que gerou dependência podem ser dois fatores preditores de abstinência (STAHL, 2014). A ocorrência da síndrome de abstinência é produzida pelo reajuste dos mecanismos homeostáticos em resposta ao uso repetido de uma droga, manifestando-se por sintomas que tendem a ser contrários aos efeitos originais produzidos pela droga (KOCHERLAKOTA, 2014). A síndrome de abstinência de opioides por exemplo, é muito desagradável, caracterizada por sinais e sintomas como compulsão, irritabilidade, acentuação da sensibilidade à dor, insônia, ansiedade, náuseas, diarreia, sudorese, hipertensão arterial, taquicardia e disforia (KORNOR; WAAL, 2005).

A recaída ao uso de drogas mostra-se como um dos principais obstáculos para o sucesso do tratamento de desintoxicação, uma vez que a maior parte dos indivíduos recorre ao uso destas após o período de detoxificação (BAILEY; CONNOR, 2005; WEISS, 2005; JUPP; CAPRIOLI; DALLEY, 2013). De um modo geral, ainda faltam tratamentos farmacológicos eficientes que consigam promover resultados satisfatórios para a condição drogadição. Dessa forma a busca por novas terapias é imprescindível para o tratamento de usuários adictos.

Figura 1. Circuito dopaminérgico mesolímbico como via final comum de recompensa. Abreviações: ATV = área tegmental ventral; NAc = *núcleo accumbens*; DA = dopamina.



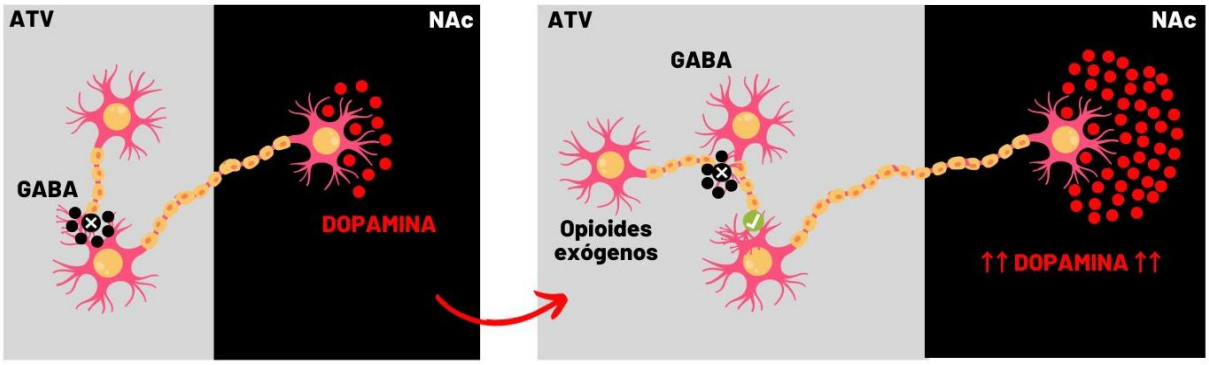
Fonte: Autor.

2.2 OPIOIDES

O tratamento da dor crônica é um problema biopsicossocial de difícil manejo, uma vez que esta é um componente presente em muitas doenças clínicas (WOOLF; SALTER, 2000). Os opioides constituem a base do tratamento da dor, já que exercem sua ação terapêutica ligando-se aos receptores opióides acoplados à proteína Gi, como mu (MOR), delta (DOR) e kappa (KOR). No entanto, seu uso indevido pode levar a sérios problemas de saúde pública e socioeconômica (BALLANTYNE; MAO, 2003). Os Estados Unidos, maior usuário de opioides prescritos no mundo, estão enfrentando uma epidemia com muitos danos e mortes relacionadas aos opioides, entre as quais aproximadamente 80 mil pessoas morreram por overdose em 2022 (RELATÓRIO MUNDIAL SOBRE DROGAS DAS NAÇÕES UNIDAS, 2022) com cerca de 70% dessas mortes envolvendo uma prescrição ou opioide ilícito.

A dependência de opioides é reconhecida como um transtorno psiquiátrico caracterizado por neuroadaptação crônica e alterações comportamentais de acordo com o Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-5 2013). A morfina, protótipo da classe, tem um poder altamente aditivo e atua por meio da estimulação da transmissão de dopamina em regiões mesolímbicas, como o estriado dorsal e ventral, exercendo um papel central no risco de recaída (KALIVAS; VOLKOW, 2005; KOOB; VOLKOW, 2010; MILANESI et al. 2023). Os opioides medeiam suas propriedades de reforço atuando por meio da diminuição da liberação do neurotransmissor inibitório GABA e consequente desinibição dos neurônios de recompensa dopaminérgicos (Fig. 2) (STAHL, 2014; ROSA et al., 2019; MILANESI et al. 2023). Ademais, Milanesi et al. (2019) demonstraram um envolvimento crucial da neurotransmissão glutamatérgica na adição de morfina. A morfina aumenta as sinapses excitatórias, promovendo a liberação de glutamato na fenda pré-sináptica (PARK, 2018).

Figura 2. Mecanismo de ação opioides. Fisiologicamente os neurônios GABAérgicos provocam inibição dos neurônios dopaminérgicos que têm origem na ATV e se projetam para o NAc. Os neurônios GABAérgicos podem ser inibidos por encefalinas endógenas, modulando a liberação de GABA. A administração de opióides exógenos diminui a liberação de GABA e desinibe os neurônios dopaminérgicos. O aumento da liberação de dopamina no NAc está relacionado com os efeitos de hedonia e capacidade de ocasionar dependência dos opioides. Abreviações: ATV = área tegmental ventral; NAc = *núcleo accumbens*.



Fonte: Autor.

2.3 HOMEOPATIA

A homeopatia é uma forma de medicina integrativa desenvolvida por Samuel Hahnemann no final do século XVIII (TEIXEIRA, 2006). Trata-se de um sistema de caráter holístico e vitalista, que visualiza o indivíduo como um todo, não em partes (OMS, 2018). Baseada em princípios como energia vital, similitude, e

experimentação em indivíduos saudáveis, a busca pela prática homeopática tem aumentado progressivamente mais pela população para amparo de patologias.

Nesta prática, entende-se que o dinamismo orgânico e psíquico saudável depende do equilíbrio da energia vital, e que o processo de adoecimento envolve o desequilíbrio desta energia ou força vital (ALVES, 2004; OMS, 2018). Os sintomas físicos, emocionais e mentais apontam para o adoecimento antes mesmo de aparecerem quaisquer alterações ou danos fisiológicos. O restabelecimento da saúde inicia-se a partir do equilíbrio da energia vital, o qual leva à melhoria dos sintomas e à presença da sensação de bem-estar (ERNST, 2002; ALVES, 2004; ALVES, 2008; OMS, 2018).

Diferentemente dos tratamentos alopáticos, que geralmente são prescritos com base no peso corporal de cada paciente, nas prescrições homeopáticas considera-se as características próprias de cada paciente (SHAHID et al. 2017). O tratamento homeopático torna-se assim pessoal, levando em conta a natureza individual de cada paciente e suas peculiaridades (personalidade, gostos, aversões, entre outros) (RAJENDRAN, 2018). Os medicamentos homeopáticos são originados de substâncias naturais, como plantas, minerais e produtos de origem animal, que são diluídos em concentrações muito baixas (ERNST, 2002; ALVES, 2004). Nas preparações medicamentosas homeopáticas, considera-se que a partir das diluições e dinamizações seriadas até a potência 12CH, a formulação ultrapassa o limite de Avogadro, ou seja, já não consta mais matéria da substância original dentro do medicamento (ERNST, 2002; KHUDA-BUKHSH et al., 2011; SAMADDER et al., 2013).

Conforme descrito na 3ª edição da Farmacopeia Homeopática Brasileira, um bioterápico é conceituado como uma preparação medicamentosa de uso homeopático obtida a partir de produtos biológicos, quimicamente indefinidos: secreções, excreções, tecidos e órgãos (patológicos/nosódios ou não patológicos/sarcódios/organoterápicos), produtos de origem microbiana e alérgenos. A isoterapia compreende o bioterápico cujo insumo ativo pode ser de origem endógena ou exógena (alérgenos, alimentos, cosméticos, medicamentos, toxinas), atuando especialmente pela lei dos semelhantes.

A isoterapia, uma classe de homeopatia, consiste na administração da mesma substância que causou os sintomas mórbidos inicialmente (quando administrada em sua forma integral), porém agora administrada como um tratamento, altamente diluído (VIGANÒ; NANNEI; BELLAVITE, 2015). As substâncias são tão excessivamente diluídas que nenhuma molécula original está presente, e acredita-se que seus efeitos biológicos estejam relacionados à bio-informação molecular transduzida via água (ALVES, 2004; WEBER et al., 2008; AGUIAR et al., 2014), podendo provocar efeitos nos seres vivos supressivos ou estimulantes em múltiplos níveis, incluindo a nível celular (GUEDES et al., 2011; QUTUBUDDIN et al., 2019).

No que diz respeito à organoterapia, os medicamentos organoterápicos têm como base a identidade biológica, reestabelecendo a função do órgão doente através do homólogo sadio (DE CASTILHOS, 2003; TEIXEIRA, 2013). Evidências descritas por Horta e Cunha (2021) utilizando organoterápicos oriundos do cérebro saudável de ovinos, demonstraram que pacientes acometidos pela doença de Parkinson apresentaram melhora nos sintomas de distúrbios motores e emocionais. Nessa lógica, extratos de cérebros de animais saudáveis servem para tratar doenças cerebrais.

Nesse contexto, é importante destacar que atualmente, a Política Nacional de Práticas Integrativas e Complementares (PNPIC) à saúde disponível no Sistema Único de Saúde (SUS), oferece para acesso da população nas redes de atenção à saúde, de forma integral e gratuita, 29 procedimentos de PICS, abrangendo serviços como homeopatia, fitoterapia, medicina tradicional chinesa/acupuntura, aromaterapia, meditação, musicoterapia, reiki, quiropraxia, entre outros (OMS, 2022). Apesar disso, estas abordagens terapêuticas ainda sofrem preconceitos, principalmente por parte dos profissionais que desconhecem sua profundidade e potencial terapêutico, intensamente demonstrados através de inúmeros casos clínicos na saúde humana e animal. Neste contexto, é importante ressaltar que atualmente a homeopatia é reconhecida por entidades profissionais dos mais diversos países e especialidades, destacando-se desde profissionais de saúde humana e animal, até profissionais de áreas rurais (MORENO, 2008; IN nº46, 2011; ZULIAN, 2017; RENOUX, 2021, p. 37). No geral, a medicina complementar e integrativa, especialmente a homeopatia, têm algumas soluções necessárias a

oferecer, e devem ser consideradas e implementadas pelas autoridades de saúde, uma vez que já vem sendo recomendadas pela Organização Mundial da Saúde (OMS) (Renoux, 2021, p. 37), para atuar na promoção da saúde (OMS, 2013).

Considerando a crise dos opioides como tema central deste estudo, qualquer preconceito em relação à homeopatia servirá apenas para agravar ainda mais esta grave situação, cujo tratamento farmacológico convencional ainda não se mostrou bem-sucedido. Embora o conhecimento sobre a homeopatia esteja em expansão, a literatura carece de informações científicas que abordem seus efeitos benéficos, especialmente envolvendo a grave situação de dependência à drogas psicoativas. Nessa lógica, o presente estudo teve como objetivo avaliar a influência de duas preparações homeopáticas: i) Isoterápico de morfina (ISO); e ii) Organoterápico preparado a partir de cérebro total de carneiro saudável (ORG), sobre as propriedades aditivas induzidas pela morfina, acessando também suas possíveis influências benéficas na recaída de opioides, considerada o principal fator relacionado à overdose e morte por estas substâncias.

3. DESENVOLVIMENTO

3. 1 MANUSCRITO CIENTÍFICO

Os resultados inseridos nessa dissertação apresentam-se sob a forma de manuscrito científico, o qual se encontra aqui estruturado. Os ítens Materiais e Métodos, Resultados, Discussão e Referências encontram-se no mesmo formato em que o manuscrito foi submetido para a publicação.

BENEFICIAL EFFECTS OF BOTH ISOTHERAPIC AND ORGANOTHERAPIC MEDICINES ON MORPHINE ADDICTION REINSTATEMENT IN RATS

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Abstract

Drug addiction is classified as a chronic and recurrent disease, constituting an alarming public health problem. Considering the current crisis of opioid substance abuse such as morphine (MORPH) in the world, reinstatement to this drugs use is one of the main obstacles to the success of detoxification treatment, since most individuals fall back to drug use after its withdrawal and detoxification period. In general, pharmacological approaches applied so far are they are just palliatives when the situation involves addiction. Thus, there is a need to seek integrative medicine, and among these, ultra-high dilution treatments, such as isotherapeutic (ISO) - the same substance that causes the damage is used as a medicine, and organotherapeutic (ORG) - tissue from healthy animals is used to treat the same sick counterpart. These medicines have promising potential in drug addiction treatment, since frequent relapses to addictive drugs involve holistic symptoms, especially mood disorders, and anxiety, as well as severe symptoms related to cravings that are difficult to control. Subsequently to the MORPH conditioned place preference (CPP) paradigm, rats were treated with ISO or ORG for 14 days during MORPH-CPP extinction and subsequently challenged to the drug to assess MORPH-CPP reinstatement. Besides confirming the robust hedonic power of the MORPH, our outcomes showed that both ISO and ORG medicines could prevent the MORPH-CPP reinstatement, indicating the potential for continuity of the studies, especially at the molecular and clinical level. In conclusion to our knowledge, this is the first study to demonstrate the beneficial influence of ultra-high dilution medicines as a possible treatment for opioid addiction, which we believe to present great relevance, since these medicines aren't related to toxicity or undesirable side effects. Clinical studies are needed to validate their use in rehabilitation centers for opioid addicts.

Keywords: Opioid; morphine; conditioned place preference (CPP); ultra-high dilution.

1. Introduction

Drug addiction is a global health, medical and socioeconomic concern that has been further aggravated by the COVID-19 pandemic.[46] According to The United Nations World Drug Report 2023,[60] the use of opioids is responsible for approximately 90.000 deaths attributed to drug abuse use disorders. Following the COVID-19 pandemic, the drug abuse death rate in the United States has grown exponentially, with the country in an alarming current scenario of public calamity promoted especially by the opioid epidemic.[27, 66] In this sense, opioid addiction is a chronic disorder that is characterized by multiple episodes of reinstatement, which are after conventional treatment and drug withdrawal. Such relapse episodes are motivated by neuroadaptive and behavioral alterations, which are related to tolerance, dependence and abstinence syndrome, due to drug withdrawal.[4, 29, 61]

Opioid reinstatement is the major challenge for the treatment of dependent users, especially because the currently available therapies such as Methadone, and Buprenorphine are in fact ineffective, since their administration has also been related to both recreational use and drug addiction.[36] In this sense, the treatment for opioid addiction shows up scarce and only soothing, and the development of medicines that may act in this condition is indispensable.

Morphine is a natural alkaloid, considered one of the main representatives of opioid drugs. Its pharmacological action involves the mu opioid receptor (MOR), whose activation can increase dopamine (DA) levels in the mesocorticolimbic brain areas, such as the ventral tegmental area (VTA) and Nucleus accumbens (NAc), which are closely involved in reinforcing properties of addiction and drug-seeking behaviors.[8, 13]

Given this scenario, over the years, our research group has shown experimental evidence of beneficial influences of different non-pharmacological treatments on behavioral, biochemical and molecular aspects related to both psychostimulant drugs-(amphetamine and cocaine) and opioid preference, especially observed in the prevention of the relapse and reinstatement for the drug, respectively.[2, 41, 49, 50, 56]

Considering integrative and complementary health practices (ICHP), ultra-high dilution medicines have been frequently used by the population in the treatment of different disturbances.[58] In this sense, the ultra-high diluted drug is based on 4 principles: the law of similarity, experimentation on the healthy person, infinitesimal doses (goes beyond Avogadro's number), and single medicine.[1, 17] Following previous studies,[23, 45, 56,] this medical system has been researched and used as an integrative and complementary tool in the treatment of different situations, since it can act through stimuli on the biological responses of the individual.

Isotherapy, a therapy that falls within the ICHP, consists of administering the same substance that caused the morbid symptoms initially (when administered in its entirety), but

now administered as a highly diluted treatment which can cause suppressive or stimulating effects on living beings at multiple levels,[69] including cellular levels. [22, 70]

Likewise, in organotherapy, organotherapeutic medicines are based on biological identity, reestablishing the function of the diseased organ through its healthy counterpart. [71, 72] Evidence using organotherapeutics from the healthy brain of sheep, demonstrated that patients affected by Parkinson's disease showed improvement in the symptoms of motor and emotional disorders. [23] According to this logic, extracts from the brains of healthy animals can be used to treat brain diseases.

In this context, it is important to emphasize that currently, ultra-high dilution medicines are recognized by professional entities from the most diverse countries and specialties, highlighting human and animal health professionals to professionals in rural areas.[25, 44, 67, 68] Complementary and alternative medicine have some of the needed solutions to offer, being recommended by the World Health Organization (WHO) to work in health promotion.[47, 64]

Considering the opioid crisis as the central theme of this study, any prejudice towards ultra-high dilution medicines will only serve to further aggravate this serious situation, whose conventional pharmacological treatment has not yet proven successful. Although knowledge about ultra-high treatment medicines is expanding, the literature lacks scientific information that addresses their beneficial effects, especially involving the severe situation of opioid addiction. In this logic, the current study aimed to evaluate the influence of two ultra-high dilution preparations: i) morphine isotherapic (ISO); and ii) an organotherapic prepared from whole healthy sheep brain (ORG), on the morphine-induced addictive properties, also accessing their possible beneficial influences on the opioid reinstatement, which is considered the main factor related to overdose and death by opioid.

2. Materials and methods

2.1. Animals

Thirty-two male Wistar rats (50 day-old) weighting 200±25g from the breeding facility of the Federal University of Santa Maria (UFSM), RS, Brazil, were kept in Plexiglas cages with food and water ad libitum, in a room with controlled temperature (22 ±2 ◦C) and on a 12-h light/dark cycle. Before starting the experimental protocols, the animals underwent 10 days of acclimatization. The Animal Ethical Committee of the Federal University of Santa Maria (7550111022-UFSM), which is affiliated with the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance approved all procedures. All efforts were made to minimize animal suffering and reduce the number of animals used in the experiment.

2.2. Drugs and solutions

Morphine sulfate pentahydrate (10mg/mL, Hipolabor®, Belo Horizonte, Minas Gerais, Brazil) was diluted in 0.9% sodium chloride solution to a standardized final concentration of 4 mg/kg/mL for conditioning and reconditioning phases.[42, 51] Saline was the standard solution of 0.9% NaCl. The preparation of medicines was made in the homeopathic Manipulation Pharmacy in the city of Santa Maria, Rio Grande do Sul, Brazil, involving 12 successive dilutions, following standards from Brazilian Homeopathic Pharmacopeia.[18] The solutions were prepared by dissolving 0.1mL of MORPH or total sheep brain in 9,9 mL of ethanol 70%. The vehicle solution was prepared the same way as both ultra-high dilution medicines, without feedstock. The following solutions were made by dissolving 1 (mass) part of the preceding solution in 99 (mass) parts of ethanol 70%. Each new solution obtained was prepared in a new, clean glass bottle and solutions were submitted to manually vigorous shaking in the vertical direction, 100 strokes in 15s between each diluting step.[16, 22, 56, 63] This procedure was repeated until a "concentration" of 10^{-24} mg/mL was reached.

2.3. Experimental protocol

After acclimatization, animals were randomly assigned to two experimental groups following intraperitoneal injections (i.p) (SAL 0.9% NaCl, i.p, n=8 per group; and MORPH 4 mg/kg/mL, i.p., n=24 per group) and submitted to the conditioned place preference (CPP) protocol with MORPH during 45 minutes for 5 days.[2, 35, 37, 56, 65] After the first CPP test (basal CPP) which was performed to verify MORPH preference,[40] the MORPH group was re-assigned according to the following treatment by gavage (p.o) for 14 days, 0,2 mL once a day, during the CPP extinction, resulting in four final groups: i) SALINE (i.p.)-VEHICLE (p.o); ii) MORPH (i.p)-VEHICLE (p.o); iii) MORPH (i.p)-ISO (p.o); iv) MORPH (i.p)-ORG (p.o). Following treatments, animals were reexposed to MORPH (4 mg/kg/mL, i.p.) for only 2 days in the CPP paradigm to observe MORPH-reinstatement.[42] 48 hours after the last MORPH injection, memory and locomotor impairments were observed in the Y-maze task, whereas anxiety-like symptoms related to MORPH abstinence were assessed on the elevated plus maze (EPM) paradigm.

2.4. Conditioned place preference (CPP)

The CPP procedure is a well-described behavioral model to assess the hedonic reinforcement effects of addictive drugs.[3, 21, 59] It uses three compartments in the boxes, which are separated by manual guillotine doors: two compartments of equal size (45 cm ×45 cm ×50 cm) with different visual clues: one with white floor and striped walls and the other with striped floor and smooth white walls, both compartments converging to a third neutral smaller compartment. The CPP procedure was performed following these steps: habituation,

pre-test, conditioning, test, extinction, reconditioning and reinstatement test. [9, 35, 37, 41, 59, 65] On day 1, rats were kept for 15 min. in each compartment for habituation. On the next day, we performed the pre-test, which consisted of letting the animal freely choose one of the compartments for 15 min. Animals that showed preference (more than 75%) for any compartment were excluded from the experimental protocol. For the next five consecutive days, animals were conditioned with SAL solution (0.9% NaCl, i.p.) placed for 45 min. in the compartment they spent more time during the pre-test, and then with the MORPH (4 mg/kg/mL, i.p.) in the opposite compartment of the CPP after 6 h the SAL conditioning with an interval of 6 h between each administration. The SAL group was injected with the saline solution on both sides of the apparatus. On the testing day, rats were individually placed in the center of the chosen chamber with free access to both compartments for 15 min, without any injection. Time spent in the drug-paired environment was interpreted as MORPH preference. For the extinction of morphine CPP, the animals were placed in the CPP apparatus every other day and were allowed to freely access all compartments for 15 min. The time spent in each compartment was recorded and the conditioning score was calculated in the same way as in the pre-test and test. This procedure was repeated until the calculated conditioning scores in two consecutive extinction tests became similar to those on the pre-conditioning day. After morphine-CPP extinction, animals were reconditioned with MORPH for only two days, just like the conditioning phase (MORPH 4 mg/kg/mL, i.p.) to induce drug reinstatement, which was observed 24 h after the last MORPH injection, in the CPP apparatus, similarly to previous MORPH-CPP for 15 min. Time spent in the drug-paired environment was interpreted as MORPH reinstatement.[40,59]

2.5. Y-maze task

The Y-maze apparatus consisted of three Y-shaped arms (32 cm (long) ×10 cm (wide) ×26 cm (walls)).[10] Briefly, each rat was placed in the center of the Y-maze and was allowed to freely explore the maze during 5 min. The total number and the sequence of arms entered were quantified. The total number of arm entries was used as a measure of locomotor activity for the animals. Alternation was defined as three consecutive entries in three different arms, and this measures the memory work. To minimize odor cues, the maze was wiped clean with ethanol 20°GL before each test. The percentage alternation score was calculated following the formula: (Total number of alternations) / (Total number of entries - 2) x 100%.[24]

2.6. Elevated plus maze (EPM) test

This behavioral test is performed to evaluate anxiety-related parameters, which are based on the innate fear that rodents have for open and elevated spaces.[43]The apparatus

was allocated in a room with low light intensity, presenting a cross shape, with two open arms (50cm×10cm) facing each other; and two arms of the same dimensions closed by side walls and opposite to each other. All arms have a central intersection (10cm×10cm). On the test day, animals were individually placed in the central intersection facing an open arm. The number of entries and the time spent in the open arms were quantified for 5 min. in EPM. The anxiety index was calculated as described in the following formula: Anxiety index (A.U.) = 1 - ((Open arms time / Total time) + (Open arms entries / Total entries)/2). Anxiety index values range from 0 to 1, where increased values indicate increased anxiety-like behavior.[12]

2.7. Statistical analysis

MORPH-CPP data before both ISO and ORG treatments were analyzed by Student's T-test. For all other analyses, one-way ANOVA followed by Duncan's test was used (Software package Statistic 8.0 for Windows). Values of $p < 0.05$ were considered statistically significant for all comparisons made. GraphPad Prism® (version 5.01) was used to create the figures.

3. Results

3.1. Behavioral assessments

3.1.1. Morphine-conditioned place preference (CPP) is shown in Figure 2.

Prelimaginarily, no animal showed place preference (greater than 60%) for any compartment of the CPP apparatus, therefore there was no exclusion of rats at the start of the experimental protocol, as observed in the pre-conditioning test (data not shown). As expected, behavioral observations performed following MORPH-CPP, Student's t-test showed that MORPH-conditioned animals spent a longer time in the drug-paired compartment of the SAL group, indicating MORPH preference (Fig. 2A). In the sequence, the same T-test showed that MORPH-CPP was extinguished in all groups on the day 21 (Fig. 2B).

Subsequently to MORPH-preference extinction and treatments, animals were challenged to drug-preference reinstatement in the same CPP paradigm. Student's t-test showed that the MORPH-exposed group remained longer in the drug-paired compartment than the SAL group, thus indicating MORPH-reinstatement (Fig. 2C). Interestingly, Duncan's test showed that among MORPH-exposed groups, both ISO and ORG treatments prevented MORPH-reinstatement in comparison to vehicle (Fig. 2C), indicating the beneficial influence of the ultra-high dilution medicines on the reinstatement-like symptoms, which are considered the greater concern in the addiction situation.

3.1.2. Anxiety-like symptoms evaluated in the elevated plus-maze (EPM) paradigm is shown in Figure 3.

Student's t-test (SAL versus MORPH-conditioned rats) and Duncan's test (comparisons among MORPH-VEHICLE, -ISO and -ORG groups) showed no differences in anxiety-like symptoms (anxiety index) (Fig. 3), reinforcing our findings observed in the CPP paradigm, which were not consequent to emotionality, but they were related to MORPH-induced hedonic effects.

3.1.3. Working memory and locomotor performance observed in the Y-maze task is shown in Figure 4.

Student's t-test (SAL versus MORPH-conditioned rats) and Duncan's test (comparisons among MORPH-VEHICLE, -ISO and -ORG groups) showed no differences in both % alternation and the total number of entries in the Y-maze arms (Figure 4A and 4B, respectively), indicating that the MORPH-preference observed in the CPP paradigm was not consequent of memory impairments (Fig. 4C) or locomotor damages.

4. Discussion

Drug reinstatement following periods of abstinence is the major obstacle to opioid addiction treatment success, constituting the biggest problem related to drug addiction,[7] since it has been related to high rates of mortality for opioids. Furthermore, understanding how different life experiences can lead to this common endpoint makes this condition even more complex.[15] Considering this, during abstinence, reintegration of psychoactive drugs is often precipitated by different factors, such as: i) re-exposure to environmental associated cues; ii) experience of stressful situations, and iii) drug-reexposure, which acts on the neuroadaptive dysregulation previously induced by its prolonged use.[57] In this logic, a better understanding of opioid-induced plasticity is needed to provide more effective therapies for opioid addiction disorder.[19]

In the last few years, our research group has invested efforts to understand different factors related to drug addiction leading to causal relationship between behavioral, biochemical and molecular parameters.[31, 32, 40, 49, 50, 54, 55, 56] In the current study, we investigated the influence of both isotherapic and organotherapic medicines on the prevention of morphine reinstatement in rats. As expected, our current findings confirmed the MORPH-induced conditioned place preference (MORPH-CPP), whereas this behavior was extinct 10 days following the last day of drug conditioning, in all experimental groups. Although the literature is rich in studies about innovative treatments involving opioid addiction, there are some previous studies involving MORPH addiction, as far as we know, our current outcomes are presenting the first time that both ultra-high dilution medicines were

able to prevent MORPH-reinstatement in animals which presented initially MORPH-CPP. These outcomes were not related to memory and/or locomotor impairments, as well to anxiety behavior as observed by the absence of locomotor change (through total number of arms entries of the Y-maze) and absence of memory impairment (by spontaneous alternation and anxiety index (A.I) in the Y-maze task and EPM test, respectively, reinforcing our current findings in the CPP paradigm, indicating that they are not referred to memory, anxiety and/or locomotor artifact.

In conventional pharmacotherapy, the use of medicines that exert a similar effect to the original disease or recreational drug, for example, has been frequent. In this context of similarities, psychostimulant drugs such as modafinil, methylphenidate and dextroamphetamine have been used to treat attention deficit and hyperactivity disorder (ADHD) treatment.[20] Also, nicotine is used as an isotherapic in the smoking treatment, [14, 28] confirming that a similar pharmacotherapy can minimize symptoms of the original disorder. Of particular importance, over many years opioid addiction treatment has been carried out through substitution therapy, that is, an addictive drug is replaced by another with similar action, which can develop hedonic effects and milder withdrawal symptoms and therefore easier to withdraw.[52] In this context, both methadone and buprenorphine have been used to treat opioid addiction, most time, replacing morphine, oxycodone, heroin and also fentanyl.[6, 26, 30] In the same line of thought, central nervous system (CNS) depressors such as benzodiazepine have been used in replacement of the alcohol absence that is also a depressant substance, confirming that a similar pharmacotherapy can minimize symptoms of the disorder.[33, 53] Thus, ultra-high dilution medicines, especially the MORPH-isotherapic (ISO), which were used in the current study to prevent MORPH-CPP reinstatement, follow this same principle of similarity. It is important to observe that these ultra-high dilution preparations are within the limit of Avogadro's number, evidencing that there is no presence of the MORPH molecule, whose characteristic reduces toxicity and adverse effects common in pharmacotherapy. Ultra-high dilution preparations, particularly isotherapic, were also used successfully in another approach,[56] which showed at the behavioral and molecular level, the beneficial effects of the amphetamine-isotropic on the toxicological aspects of amphetamine addiction in rats. Of particular importance to our findings, a recent study showed lasting clinical improvement in patients with Parkinson's disease following treatment with an organotherapic, i.e., an ultra-high diluted medicine obtained from the whole brain of healthy sheep, which promoted improvement in the neurodegenerative symptoms.[23]

Considering that the use of allopathic medicines is almost always accompanied by the development of adverse effects, classical pharmacology itself has been concerned with reducing harm and minimizing it, as can be evidenced through promising nanoformulations.[34, 39] In this context, this concern has encouraged the reduction of

medication dosages as well as their delivery in nanoformulations.[5,42] In the same line of reasoning, the ultra-high dilution and dynamization of medicinal substances can also favor the minimization of damage or adverse effects of medicines, whose system is managed in the preparation of ultra-high dilution medicines, according to the Brazilian Homeopathic Pharmacopoeia. In this scenario, integrative and complementary health practices (ICHP), including natural products, complementary therapies, and ultra-high diluted drugs, among others, are important, as they have been widely used by the population to aid in the treatment and collapse of illnesses.[58]

Resuming our experimental study, the CPP paradigm is a behavioral animal model frequently used to assess both preference and reinstatement of psychoactive drugs such as MORPH.[42, 59] This experimental paradigm shows advantages such as low training sessions, and sensitivity to low doses of drugs and it is not an invasive procedure.[3] Considering the drug reinstatement, three ways have been used to induce relapse in the CPP paradigm: i) re-exposing animals to environmental cues related to drug use; ii) recondition to the use of the addictive substance; iii) exposing the animal to an acute stressor stimulus.[38, 42, 59] Here, animals were reexposed to MORPH through an additional two days of the same MORPH dose in the CPP (4 mg/kg/mL) to check the MORPH-CPP reinstatement development. To reinforce our findings, MORPH-CPP was not related to emotionality or memory- and locomotion impairments, but it was related to the powerful hedonic effect of morphine, further extolling the strength of the ultra-dynamized and ultra-high dilution medicines evaluated here.

To the best of our knowledge, our current findings show for the first time that both ISO and ORG ultra-high dilution treatments can put forward homeostasis, especially when neurons from the mesolimbic brain area are exposed to MORPH toxicity. The continuation of advanced studies, especially at the molecular level, involving morphine drug addiction and treatment with both ISO and ORG should be developed soon.

Conclusion

The prevention and treatment of opioid addiction is urgent and, therefore, must be considered a public health priority. Our study shows that the treatments that are part of the Integrative and Complementary Health Practices (ICHP) are able to prevent morphine reinstatement, exerting neuroprotection. Based on this, it is needed to emphasize that additional clinical and toxicological studies must be carried out to investigate the influence of this holistic treatment known as ultra-high dilution medicines.

Author contributions (CRediT)

Murilo B. Fontoura, Domenika R. Rossato, Jéssica da Rosa, Leana E. M. Souza and Caroline S. Fischer, performed the experimental procedures and collected behavioral data.

Murilo B. Fontoura and Marilise E. Burger analyzed data, wrote the manuscript, designed the study and interpreted the data. Murilo B. Fontoura and Marilise E. Burger reviewed and edited the manuscript. Marilise E. Burger was responsible for the funding acquisition. All authors critically reviewed the content and approved the final version for publication.

Conflict of interests

The authors have no conflict of interests to declare.

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Ethical statement

The Animal Ethical Committee of the Federal University of Santa Maria (7550111022-UFSM), which is affiliated with the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance approved all procedures.

Data availability

Yes.

Figures

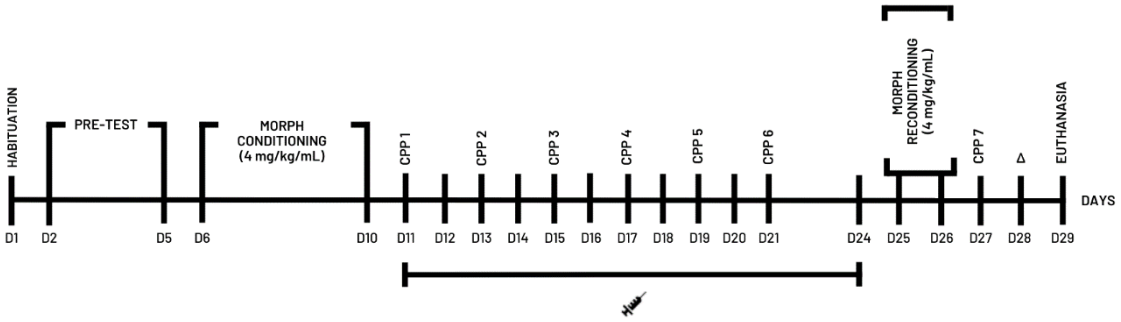

Fig. 1. Experimental design: After the pre-test, animals were conditioned with MORPH (4 mg/kg/mL) in the CPP paradigm for 5 days, when the 1st behavioral observation (CPP 1) was performed. Then, animals were treated with ISO, ORG or vehicle for 14 days when the MORPH- preference was monitored in the CPP apparatus every 2 days until its extinction (CPP 2- 6). At the sequence, rats were reexposed to MORPH (4 mg/kg/mL) / SALINE (1 mL/kg) for an additional two days and submitted to MORPH-CPP reinstatement (CPP 7) on day 27. After 24 h, memory and locomotor impairments evaluation (Y-maze task) and anxiety-like symptoms (EPM test) were observed. Symbols: 💉 = ISO, ORG or vehicle administration; Δ = Y-maze and EPM task. Abbreviations: MORPH: morphine; CPP: compartment place preference; ISO: MORPH-isotherapic; ORG: organotherapic medicine, EPM: elevated plus maze.

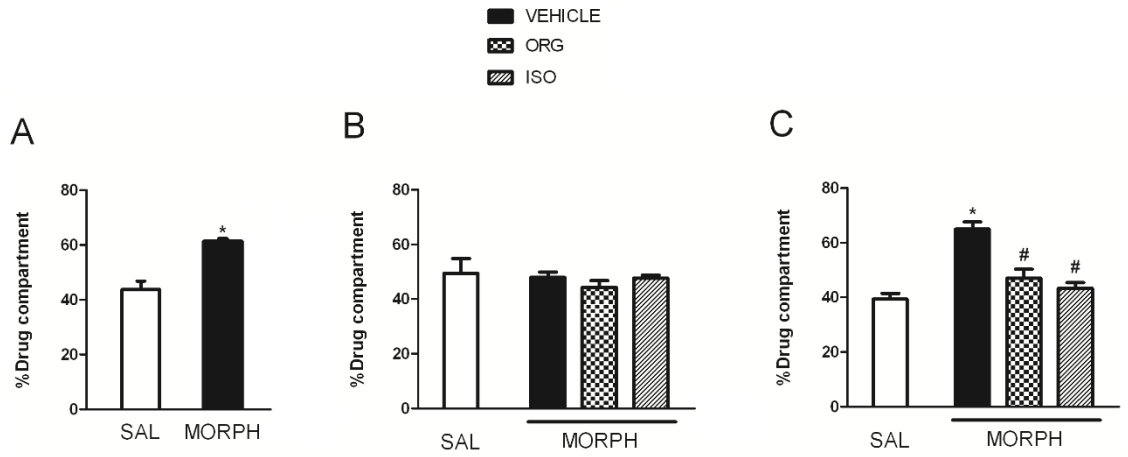

Fig. 2. Influence of ISO or ORG ultra-high dilution treatments on MORPH addictive parameters assessed in the conditioned place preference (CPP) test. Morphine-conditioned place preference (CPP) (**A**); Morphine preference extinction in CPP (**B**); Morphine reinstatement in CPP (**C**). Data are expressed as mean ± S.E.M. ($p < 0.05$). * indicates a significant difference from the saline group and # indicates a significant difference from the vehicle. Abbreviations: SAL = saline; MORPH = morphine; ORG = organotherapic; ISO = isotherapic.

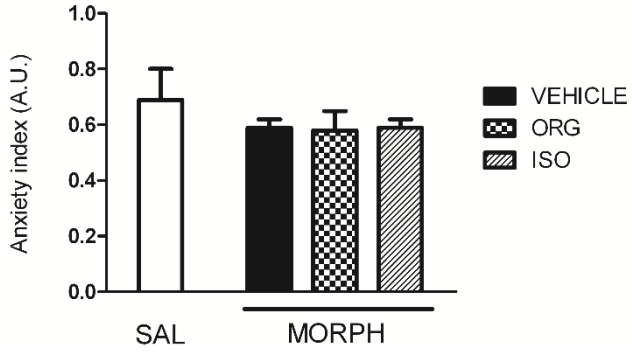

Fig. 3. Influence of both ISO or ORG ultra-high dilution treatments on anxiety behavior assessed in the elevated plus maze test (EPM). Data are expressed as mean ± S.E.M ($p < 0.05$). Abbreviations: SAL = saline; MORPH = morphine; ORG = organotherapic; ISO = isotherapic.

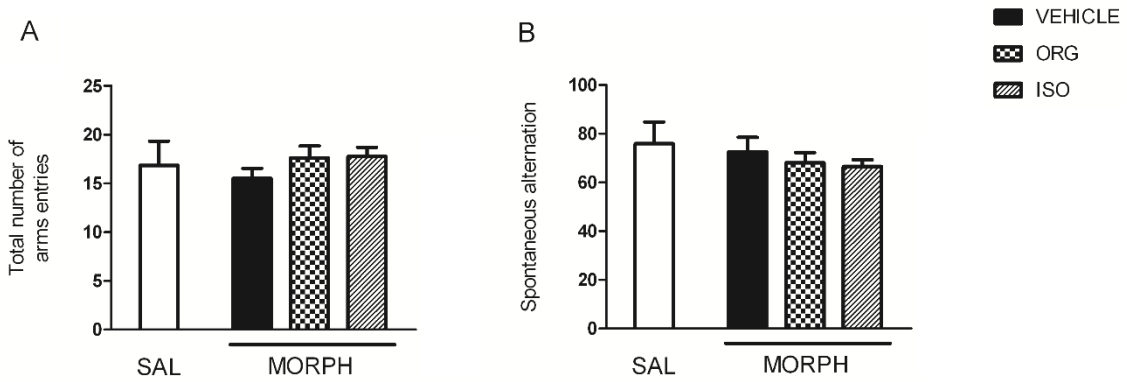

Fig. 4. Influence of both ISO or ORG ultra-high dilution treatments on locomotion and memory behavior were assessed in the Y-maze task. Total number of arms entries (A); Spontaneous alternation (B). Data are expressed as mean ± S.E.M ($p < 0.05$). Abbreviations: SAL = saline; MORPH = morphine; ORG = organotherapic; ISO = isotherapic.

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Integrative Medicine Research

BENEFICIAL EFFECTS OF BOTH ISOTHERAPIC AND ORGANOTHERAPIC MEDICINES ON MORPHINE ADDICTION REINSTATEMENT IN RATS

--Manuscript Draft--

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Abstract:	Addiction is classified as a recurrent disease, constituting an alarming public health problem. Considering the current crisis of opioid such as morphine (MORPH) in the world, reinstatement to opioid use is one of the main obstacles to the success of detoxification treatment, since most individuals fall back to drug use after its withdrawal. Thus, as drug addiction involves emotional, social and physical factors, there is a need to seek integrative medicine, and among these, ultra-high dilution treatments, such as isotherapeutic (ISO) and organotherapeutic (ORG). These medicines have promising potential in drug addiction treatment, since frequent relapses to addictive drugs involve holistic symptoms, especially mood disorders, and anxiety, as well as severe symptoms related to cravings that are difficult to control. Subsequently to the MORPH conditioned place preference (CPP) paradigm, rats were treated with ISO or ORG for 14 days during MORPH-CPP extinction and subsequently challenged to the drug to assess MORPH-CPP reinstatement. Besides confirming the hedonic power of the MORPH, our outcomes showed that both ISO and ORG could prevent the MORPH-CPP reinstatement, indicating potential for continuity of the studies at the molecular and clinical level. As far as we known, this is the first study to demonstrate the beneficial influence of ultra-high dilution medicines as a possible treatment for opioid addiction, which we believe to present relevance, since these medicines aren't related to toxicity or undesirable side effects. Clinical studies are needed to validate their use in rehabilitation centers for opioid addicts.
Suggested Reviewers:	Maria Josefina Carlucci majoc@qb.fcen.uba.ar
	Cleópatra Planeta majoc@qb.fcen.uba.ar Expert in dtrug addiction.
	Isabel Oliveira Horta isabeloh@gmail.com Expert in ultra-high dilution medicines and Integrative and Complementary Health Practices
	Bin Cong hbmubincong@126.com Expert in CPP (experimental model in drug addiction studies)
Opposed Reviewers:	

4. CONSIDERAÇÕES FINAIS

A partir dos resultados apresentados no manuscrito científico, o qual compõe a presente dissertação, demonstramos a influência benéfica de dois medicamentos homeopáticos na prevenção da recaída à morfina em ratos, reduzindo a busca dos animais pela droga aditiva. Através dos resultados experimentais obtidos, chegamos às seguintes conclusões:

- Os animais desenvolveram preferência (adição) e recaída por morfina;
- O tratamento com Isoterápico de morfina preveniu a recaída pela droga;
- O tratamento com Organoterápico (oriundo do cérebro total de carneiro saudável) preveniu a recaída por morfina;
- Os medicamentos homeopáticos exerceram efeitos benéficos em um modelo experimental de adição por opioide;
- Estudos adicionais envolvendo neuroadaptações em nível molecular dopaminérgico, glutamatérgico e opioide serão desenvolvidos para complementar a compreensão dos benefícios dos medicamentos homeopáticos, aqui apresentados.

ANEXO A - CERTIFICADO DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA

Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "ESTUDO DAS NEUROADAPTACOES MOLECULARES PROMOVIDAS POR CETAMINA E ORGANOTERÁPICO NA PREVENÇÃO DA RECAIDA POR DROGAS ADITIVAS", protocolada sob o CEUA nº 7550111022 (ID 004022), sob a responsabilidade de **Marilise Escobar Bürger** *e equipe; Caroline De Souza Fischer; Leana Eduarda Mezzomo De Souza; JAIME ARAMBURU; Domenika Rubert Rossato; Jessica Leandra Oliveira da Rosa; MURILO BARBOZA FONTOURA* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADA** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 24/01/2023.

We certify that the proposal "STUDY OF MOLECULAR NEUROADAPTATIONS PROMOTED BY KETAMINE AND ORGANOTHERAPY IN THE PREVENTION OF ADDITIVE DRUG RECURRENCE", utilizing 192 Heterogenics rats (192 males), protocol number CEUA 7550111022 (ID 004022), under the responsibility of **Marilise Escobar Bürger** *and team; Caroline De Souza Fischer; Leana Eduarda Mezzomo De Souza; JAIME ARAMBURU; Domenika Rubert Rossato; Jessica Leandra Oliveira da Rosa; MURILO BARBOZA FONTOURA* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **APPROVED** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 01/24/2023.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 10/2022 a 10/2027 Área: Departamento de Fisiologia E Farmacologia

Origem:	Biotério Central UFSM			
Espécie:	Ratos heterogênicos	sexo: Machos	idade: 30 a 50 dias	Quantidade: 192
Linhagem:	Wistar		Peso: 200 a 250 g	

Santa Maria, 03 de outubro de 2023

Dra. Patrícia Bräunig
Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Profa. Dra. Vania Lucia Loro
Vice-Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

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