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Dissertação de mestrado

Estudo pré-clínico de compostos organocalcogênios como estratégia terapêutica para a toxicidade da oxaliplatina em camundongos

Ketlyn Pereira da Motta

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Dedico esta dissertação a Deus e aos meus pais...

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RESUMO

MOTTA, Ketlyn Pereira da. **Estudo pré-clínico de compostos organocalcogênios como estratégia terapêutica para a toxicidade da oxaliplatina.** 2021. 142f. Dissertação (Mestrado em Bioquímica e Bioprospecção) - Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2021.

A oxaliplatina (OXA), fármaco quimioterápico amplamente utilizado no tratamento do carcinoma de colorretal, está associado ao desenvolvimento de quadros toxicológicos neurológicos e renais. Neste contexto, esforços foram direcionados para o estudo pré-clínico de compostos organocalcogênios como estratégia terapêutica para a toxicidade da OXA. Inicialmente, foi investigado o potencial terapêutico do 5 - ((4-metoxifenil) tio) benzo [c] [1,2,5] tiodiazol (MTDZ) frente a neuropatia periférica e suas comorbidades em camundongos *Swiss* machos e fêmeas expostos à OXA. Os animais receberam a OXA (10 mg/kg) por via intraperitoneal, nos dias 0 e 2 do protocolo experimental. A administração oral de MTDZ (1 mg/kg) foi realizada nos dias 2 a 14. A OXA causou prejuízo cognitivo, comportamento do tipo ansioso, hipersensibilidade mecânica e térmica em camundongos, sendo as fêmeas mais suscetíveis à sensibilidade térmica. O MTDZ reverteu a hipersensibilidade, o comprometimento cognitivo e o comportamento do tipo ansioso induzidos pela OXA. A OXA alterou a atividade das bombas iônicas: ATPase total, Na^+ K^+ - ATPase, Ca^{2+} - ATPase e Mg^{2+} - ATPase e aumentou os níveis de espécies reativas (ER) e a atividade da superóxido dismutase (SOD). O MTDZ foi capaz de regular a homeostase das ATPases totais, Mg^{2+} - ATPase e a atividade da SOD. Os resultados obtidos sugerem que o composto orgânico de enxofre (MTDZ) é promissor para o tratamento da neurotoxicidade induzida pelo quimioterápico OXA. No segundo

estudo, a toxicidade renal induzida pela OXA foi avaliada em camundongos machos *Swiss*. Nesta etapa, a relação entre o dano oxidativo renal induzido pela OXA e o potencial terapêutico do 7-cloro-4-(fenilselanil) quinolina (4-PSQ) foi estudada. Os camundongos receberam a OXA (10 mg / kg) ou veículo por via intraperitoneal (dias 0 e 2). A administração oral de 4-PSQ (1 mg / kg) ou veículo foi realizada nos dias 2 a 14. No dia 15, os animais foram submetidos à eutanásia, e os rins e sangue foram coletados. Os resultados revelaram um aumento nos níveis de ureia e um dano oxidativo renal significativo em camundongos expostos à OXA. A exposição ao quimioterápico aumentou a atividade das enzimas SOD, glutationa peroxidase (GPx) e glutationa-S-transferase (GST). A OXA causou uma redução nos níveis de tióis não-proteicos (NPSH) e a inibição da atividade das enzimas catalase (CAT) e glutationa redutase (GR). A atividade das enzimas Na^+ K^+ ATPase e δ-aminolevulinato desidratase (δ-ALA-D) foram inibidas pela OXA. O 4-PSQ diminuiu os níveis de ureia plasmática e o dano oxidativo renal induzidos pela OXA. As atividades de SOD, GPx, CAT, GR e Na^+ K^+ - ATPase foram restauradas pelo 4-PSQ. O conjunto de dados obtidos sugere que o 4-PSQ reduz o dano renal induzido pela OXA em camundongos, possivelmente devido ao seu potencial antioxidante. Baseado nos resultados obtidos, pode-se concluir que o 4-PSQ e o MTDZ são moléculas promissoras para o tratamento da toxicidade induzida pela OXA.

Palavras-chave: oxaliplatina; neuropatia periférica; toxicidade renal; estresse oxidativo.

ABSTRACT

MOTTA, Ketlyn Pereira da. **Preclinical study of organochalcogen compounds as a therapeutic strategy for oxaliplatin toxicity.** 2021. 142 f. Dissertation (Master in Biochemistry and Bioprospecting) - Biochemistry and Bioprospecting Postgraduate Program. Federal University of Pelotas, Pelotas, 2021.

Oxaliplatin (OXA), a chemotherapeutic drug widely used in the treatment of colorectal carcinoma, is associated with the development of neurological and renal toxicological conditions. In this context, efforts were directed towards the preclinical study of organochalcogen compounds as therapeutic strategies for the OXA toxicity. Initially, the therapeutic potential of 5 - ((4-methoxyphenyl) thio) benzo[c][1,2,5] thiodiazole (MTDZ) was investigated against peripheral neuropathy and its comorbidities in male and female Swiss mice exposed to OXA. The animals received OXA (10 mg/kg) intraperitoneally, on days 0 and 2 of the experimental protocol. The oral administration of MTDZ (1 mg/kg) was performed on days 2 to 14. OXA caused cognitive impairment, anxiety-like behavior, mechanical and thermal hypersensitivities in mice, with females being more susceptible to thermal sensitivity. MTDZ reversed OXA-induced hypersensitivity, cognitive impairment, and anxiety-like behavior. OXA altered the activity of ion pumps: total ATPase, Na^+ K^+ - ATPase, Ca^{2+} - ATPase and Mg^{2+} - ATPase and increased the levels of reactive species (ER) and superoxide dismutase (SOD) activity. MTDZ was able to regulate the homeostasis of total ATPases, Mg^{2+} - ATPase and SOD activity. The results obtained suggest that the organic sulfur compound (MTDZ) is promising for the treatment of neurotoxicity induced by OXA chemotherapy. In the second study, OXA-induced renal toxicity was evaluated in male Swiss mice. At this stage, the relationship between OXA-induced renal oxidative damage and the therapeutic potential of 7-chloro-4-(phenylselanyl)

quinoline (4-PSQ) was studied. The mice received either OXA (10 mg/kg) or vehicle intraperitoneally (days 0 and 2). Oral administration of 4-PSQ (1 mg/kg) or vehicle was performed on days 2 to 14. On day 15, animals were euthanized, and kidneys and blood were collected. The results revealed an increase in urea levels and significant renal oxidative damage in mice exposed to OXA. Exposure to chemotherapy increased the activity of SOD, glutathione peroxidase (GPx) and glutathione-S-transferase (GST). OXA caused a reduction in the levels of non-protein thiols (NPSH) and inhibition of the activity of the enzymes catalase (CAT) and glutathione reductase (GR). The activity of the enzymes Na^+ K^+ ATPase and δ-aminolevulinate dehydratase (δ-ALA-D) were inhibited by OXA. 4-PSQ decreased plasma urea levels and renal oxidative damage. SOD, GPx, CAT, GR and Na^+ K^+ - ATPase activities were restored by 4-PSQ. The dataset obtained suggests that 4-PSQ reduces OXA-induced kidney damage in mice, possibly due to its antioxidant potential. Based on the results obtained, it can be concluded that 4-PSQ and MTDZ are promising molecules in the treatment of toxicity induced by OXA.

Keywords: oxaliplatin; peripheral neuropathy; renal toxicity; oxidative stress.

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LISTA DE ABREVIATURAS E SIGLAS

4-PSQ	7-cloro-4- (fenilselanil) quinolina
ACh	Acetilcolina
AChE	Acetilcolinesterase
ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
CAT	Catalase
CFA	Completo Adjuvante de Freund
ERNs	Espécies reativas de Nitrogênio
EROs	Espécies Reativas de Oxigênio
ERs	Espécies Reativas
GPx	Glutationa peroxidase
GR	Glutationa redutase
GST	Glutationa S-transferase
MDA	Malondialdeído
MTDZ	5 - ((4-metoxifenil) tio) benzo [c] [1,2,5] tiodiazol
NCAM	Molécula neuronal de adesão celular
NPSH	Tióis não-proteicos
OXA	Oxaliplatina
PC	Proteína Carbonila
SOD	Superóxido dismutase
TBARS	Espécies Reativas ao Ácido Tiobarbitúrico
δ-ALA-D	Ácido delta-aminolevulinato desidratase

Sumário

1. Introdução

A dor é uma experiência sensitiva e emocional desagradável associada, ou semelhante àquela associada, a uma lesão tecidual real ou potencial (TREEDE, 2018). Por este motivo, atualmente é reconhecida como o quinto sinal vital, uma vez que está relacionada a estímulos que caracterizam diferentes doenças e auxiliam no tratamento adequado. Mundialmente, estima-se que 1 em cada 5 adultos sofra com algum tipo de dor, representando maior prevalência em idosos e afetando predominantemente o sexo feminino (TREEDE, 2018).

A dor neuropática, particularmente, é causada por uma lesão ou doença que afeta o sistema nervoso somatossensorial, considerada uma condição dolorosa crônica, comum, com grande impacto na qualidade de vida de seus portadores (NIEDZWIEDZ et al., 2019). O processo doloroso considerado neuropático pode se tratar de um dano a nível de sistema nervoso central ou periférico.

A neuropatia central se desenvolve imediatamente após o insulto ou pode ter um início tardio de 6 à 12 meses (WIDERSTRÖM-NOGA et al., 2017). Neste quadro patológico, a dor tende a se tornar crônica e por toda a vida, às vezes com graves consequências psicossociais e funcionais (SIDDALL et al., 2003; WIDERSTRÖM-NOGA et al., 2017). Já a neuropatia periférica é uma condição definida pela degeneração ou disfunção dos nervos periféricos, em seu trajeto da medula espinhal até a periferia, podendo ocasionar alterações motoras ou sensitivas e sua prevalência varia entre 2% e 10% da população (COLLOCA et al., 2017; FÉLIX; DE OLIVEIRA, 2010; MERKIES; FABER; LAURIA, 2015).

Sobretudo, em pacientes com câncer, a dor é um sintoma que afeta 70% das pessoas na fase avançada da doença, e isso ocorre porque a sobrevivência tem sido significativamente prolongada devido aos avanços na quimioterapia (STAROBOVA; VETTER, 2017). A neuropatia periférica é o

tipo de dor mais prevalente nos pacientes com câncer e que se submetem ao tratamento quimioterápico (SISIGNANO et al., 2014).

Embora a taxa de sobrevida tenha aumentado, a dor neuropática nestes pacientes é um efeito adverso comum, que afeta a qualidade de vida, leva à descontinuidade do tratamento, ocasiona a redução da dose e altera, com isso, o resultado e a efetividade do tratamento (BENNEDSGAARD et al., 2020; MERKIES; FABER; LAURIA, 2015). Corroborando a isso, estudos apontam o aumento de comorbidades nos pacientes expostos à quimioterapia, como: insônia, ansiedade, depressão e déficit cognitivo (DIBONAVENTURA et al., 2017; YOON; OH, 2018).

A oxaliplatina (OXA) é um fármaco quimioterápico de nova geração amplamente utilizado para tratar diferentes carcinomas, dentre eles o de colorretal (KANG et al., 2020). A OXA possui como principal sintoma adverso a dor neuropática (FITZMAURICE et al., 2017a).

Neste contexto, cabe ressaltar a necessidade de compreensão dos mecanismos fisiopatológicos envolvidos na neuropatia causada pela OXA. Além disso, os compostos de platina causam uma ampla gama de efeitos adversos, incluindo ototoxicidade, hepatotoxicidade, neurotoxicidade e nefrotoxicidade (ALMARZOOQI et al., 2015; BANO; IKRAM, 2017; KUMARAN et al., 2015; STAROBOVA; VETTER, 2017).

Uma vez que a etiologia do processo ainda é mal compreendida, e, subsequentemente, o manejo do paciente faz-se inadequado, torna-se de particular importância o aprofundamento do conhecimento da toxicidade causada pela OXA. Em vista disso, a modulação colinérgica, uma das mais antigas abordagens farmacológicas para o tratamento da dor, ainda é pouco explorada como possível mecanismo envolvido na neuropatia causada pela OXA.

Apesar disso, Pacini e colaboradores (2010) relataram que a estimulação de receptores nicotínicos evoca propriedades antineuropáticas. Além disso, resultados obtidos pelo nosso grupo de pesquisa revelaram alterações na

homeostase da enzima acetilcolinesterase (AChE) em camundongos machos que receberam a OXA (Reis et al., 2020b).

Perante isso, nosso grupo de pesquisa tem se dedicado na busca pela compreensão da toxicidade causada pela OXA a nível neurológico, hepático e renal; bem como, busca investigar novas estratégias terapêuticas capazes de modularem o sistema colinérgico e com isso tratarem sintomas adversos como a dor neuropática e a nefrotoxicidade causadas pelo quimioterápico OXA (LEMOS et al., 2021; MOTTA et al., 2021; REIS et al., 2020a, 2020b).

Neste contexto, compostos orgânicos contendo calcogênios vêm se destacando por apresentarem atividades relevantes em modelos de dor nociceptiva, inflamatória e neuropática e por modularem o sistema colinérgico (OLIVEIRA et al., 2020; THANKACHAN et al., 2015). Frente a isto, destacam-se como estratégias terapêuticas promissoras os compostos sintéticos 5-((4-metoxifenil)tio)benzo[c][1,2,5] tiodiazol (MTDZ) e 7-cloro-4-(fenilselanil) quinolina (4-PSQ).

O MTDZ exerce potencial atividade inibitória da AChE, podendo ser um protótipo interessante a ser investigado para o tratamento de patologias relacionadas às alterações na via colinérgica. Ademais, o 4-PSQ, um derivado de quinolina contendo selênio, possui diversas propriedades farmacológicas já descritas na literatura, incluindo a ação antioxidante e a habilidade de modular diferentes vias dentre elas a glutamatérgica, nitrérgica e serotoninérgica, sendo um composto promissor no tratamento de patologias relacionadas ao estresse oxidativo e estes sistemas (PINZ et al., 2016, 2018; REIS et al., 2017; SILVA et al., 2017; VOGT et al., 2018). Assim, considerando os resultados prévios obtidos por pesquisadores do Laboratório de Pesquisa em Farmacologia Bioquímica utilizando os compostos MTDZ e 4-PSQ, neste estudo buscou-se ampliar o conhecimento sobre o potencial farmacológico destes compostos organocalcogênios, com ênfase na neurotoxicidade e nefrotoxicidade causadas pela exposição à OXA em camundongos.

2. Objetivo

2.1 Objetivo Geral

Investigar o potencial terapêutico dos compostos organocalcogênios, MTDZ e 4-PSQ, frente a neuropatia periférica e suas comorbidades (ansiedade e déficits cognitivos) e a nefrotoxicidade induzidas pela OXA em camundongos.

2.2 Objetivos Específicos

- Investigar o potencial antinociceptivo, anti-inflamatório e antiedematogênico do MTDZ em modelos agudos de nocicepção;
- Avaliar o potencial farmacológico do MTDZ frente à neuropatia periférica e comorbidades (ansiedade e déficits cognitivos) associadas à exposição de OXA;
- Averiguar a contribuição da AChE no desenvolvimento da neuropatia periférica induzida pela OXA e a sua modulação pelo MTDZ;
- Elucidar a contribuição das ATPases totais, Na^+ K^+- ATPase, Ca^{2+}- ATPase e Mg^{2+}- ATPase no desenvolvimento da neuropatia periférica causada pela OXA e determinar a modulação destas pelo MTDZ no córtex, hipocampo e medula espinhal de camundongos;
- Observar o potencial antioxidante do MTDZ frente ao estresse oxidativo gerado pela exposição à OXA;
- Ampliar o conhecimento sobre o efeito farmacológico do 4-PSQ contra a nefrotoxicidade causada pela OXA;
- Determinar o efeito antioxidante do 4-PSQ contra o dano oxidativo renal induzido pela exposição ao quimioterápico OXA;
- Prospectar compostos organocalcogênios como potenciais estratégias terapêuticas para o tratamento da toxicidade associada ao uso da OXA.

3. Revisão Bibliográfica

3.1 Toxicidade de quimioterápicos

O câncer constitui um problema grave de saúde pública com impacto mundial e está entre as quatro principais causas de morte antes dos 70 anos de idade (BRAY et al., 2018). A incidência e a mortalidade por câncer estão em ascensão, em parte devido ao aumento da expectativa de vida e as mudanças de hábitos populacionais por conta do desenvolvimento socioeconômico (BRAY et al., 2018; FITZMAURICE et al., 2017b; HELM; RUDEL, 2020).

Atualmente, a quimioterapia constitui uma das modalidades de maior escolha para o tratamento do câncer. O tratamento quimioterápico pode ser descrito como o uso de substâncias químicas sistêmicas, isoladas ou em combinação, que tem como objetivo tratar e combater o câncer (HOFF, 2013). Apesar da eficácia do tratamento quimioterápico, sabe-se que boa parte deles estão ligados ao desenvolvimento de quadros toxicológicos de complexo tratamento (BENNEDSGAARD et al., 2020; DUBOIS et al., 2014; SALES et al., 2019).

Particularmente, o carcinoma de colorretal, responsável em 2018 por 10% dos novos casos de câncer e mortes em todo o mundo, possui como principal agente quimioterápico a OXA (BRAY et al., 2018). Envolvida em uma série de efeitos adversos derivados do seu tratamento, a OXA apresenta danos a nível neurológico, hepático, renal e gastrointestinal (BANO; IKRAM, 2017; CRUZADO et al., 2014; DI CESARE MANNELLI et al., 2013; TABASSUM et al., 2015).

Além da OXA, diversos outros quimioterápicos têm sido reportados por desencadearem efeitos adversos, dentre eles podemos mencionar: a cisplatina, o paclitaxel, a vincristina, e o bortezomibe (FLATTERS; DOUGHERTY; COLVIN, 2017; SISIGNANO et al., 2014; STOCKSTILL et al., 2020; TODD; LIPPARD, 2009). Embora muitos aspectos estejam esclarecidos sobre os sintomas desencadeados por quimioterápicos, há mecanismos intrínsecos ao desenvolvimento toxicológico que ainda possuem necessidade de elucidação.

3.1.1. Toxicidade renal causada pela OXA

A nefrotoxicidade causada pela OXA é pouco esclarecida. Por ser um fármaco de terceira geração dentre os derivados de platina, a OXA foi prospectada pela necessidade de amenizar os efeitos adversos causados por outros quimioterápicos contendo platina previamente desenvolvidos. Apesar disso, um número crescente de casos clínicos tem demonstrado insuficiência renal no decorrer do tratamento com a OXA, o que chama a atenção da comunidade científica (ALMARZOOQI et al., 2015; BANO; IKRAM, 2017; ITO et al., 2012).

Estudos sugerem que a OXA tem nefrotoxicidade aceitável em comparação com a cisplatina; entretanto, a exposição à OXA tem sido relacionada ao desenvolvimento de vacuolização tubular, necrose tubular, acidose tubular e lesão renal aguda (ALMARZOOQI et al., 2015; YAGHOBI JOYBARI et al., 2014). De fato, a exposição à platina causa dano ao DNA de células tubulares e glomerulares (KRÜGER et al., 2015).

O dano renal causado pela OXA possui fisiopatologia pouco conhecida, porém associa-se que o dano mitocondrial, a apoptose celular e o estresse oxidativo estejam inerentes ao processo toxicológico (FULCO et al., 2018; MANSOUR et al., 2002; YAGHOBI JOYBARI et al., 2014). Ainda, um estudo reportou a necessidade de investigação em modelos animais da nefrotoxicidade causada pela OXA, uma vez que a maior parte dos estudos aponta apenas casos clínicos ou ensaios celulares (KRÜGER et al., 2015).

3.2 Dor Neuropática

A dor crônica descrita em termos do seu tempo de duração (superior a 12 semanas), é uma condição clínica severa, associada a um quadro de lesão tecidual de difícil reparação. A classificação da dor crônica apresenta-se em duas categorias amplas: dor devido a doença ou dano tecidual (dor nociceptiva, como

osteoartrite) e dor causada por doença ou dano do sistema somatossensorial (dor neuropática) (BARON; TREEDE, 2007).

A dor neuropática, uma subclasse da dor crônica, é uma síndrome caracterizada por degeneração ou disfunção dos nervos. Uma primeira distinção que pode ser feita é o envolvimento de um nervo periférico, no caso de mononeuropatias, ou de múltiplos nervos periféricos, no caso de neuropatias multifocais e polineuropatias. Dentro desta distinção, a grande maioria dos casos se enquadra em quatro categorias: I) Lesão nervosa periférica focal e multifocal (traumática, isquêmica ou inflamatória); II) Polineuropatias periféricas generalizada (tóxicas, metabólicas, hereditárias ou inflamatória); III) Lesões no sistema nervoso central (acidente vascular cerebral, esclerose múltipla e lesão da coluna vertebral); IV) Complexos distúrbios neuropáticos (Complexo da síndrome da dor regional – CSDR) (BARON; TREEDE, 2007).

Dentre os sintomas mais frequentes mencionados pelos pacientes que sofrem com a dor neuropática estão a parestesia e a disestesia (STAROBOVA; VETTER, 2017). A parestesia é caracterizada pela sensação de formigamento ou dormência nas extremidades como mãos e pés. Já a disestesia é conhecida pela sensação de queimação e é uma condição clínica associada a perda da sensibilidade dos sentidos.

A sinalização destes sintomas decorrentes da dor neuropática ocorre da seguinte forma: a dor evocada pelos estímulos nos nociceptores é transduzida até a medula espinhal e, posteriormente, é modulada na medula espinhal e caracterizada como hiperalgesia e/ou alodínia (COLLOCA et al., 2017; CRUCCU et al., 2016). Podemos definir a hiperalgesia como sendo uma resposta exagerada a um estímulo doloroso e a alodínia uma dor produzida por um estímulo que normalmente não causa dor.

De forma mais aprofundada, os processos fisiopatológicos desencadeadores da hiperalgesia e alodínia estão interligados à excitabilidade aumentada dos neurônios espinhais. Estes neurônios produzem respostas aumentadas a muitas modalidades sensoriais e, logo, permitem que as fibras aferentes Aβ e Aδ

mecanossensíveis ativem os neurônios nociceptivos de segunda ordem (que transmitem informações sensoriais ao cérebro) e, por este mecanismo, expandam seus campos receptivos para que um determinado estímulo excite mais neurônios nociceptivos de segunda ordem, gerando a chamada sensibilização central (COLLOCA et al., 2017).

Em outras palavras, a dor neuropática modifica propriedades elétricas dos nervos sensoriais e este processo conduz a um desequilíbrio entre a sinalização central excitatória e inibitória, de modo que neurônios inibitórios e sistemas de controle descendente sejam prejudicados (COLLOCA et al., 2017; STAROBOVA; VETTER, 2017). Neste sentido, a transmissão de sinais sensoriais e os seus mecanismos de desinibição são alterados a nível dos neurônios do gânglio dorsal da medula espinhal.

Se tratando de neuropatias periféricas, centenas de etiologias diferentes já foram identificadas e com isso, a dificuldade de diagnóstico e intervenções terapêuticas é um agravante para o início de um tratamento eficaz. Durante as fases de diagnóstico, além do histórico do paciente, alguns outros pontos devem ser levados em consideração. O exame geral inclui a avaliação do aumento e deformidades nas articulações, presença de articulações hipermóveis e/ou pele hiperelástica, dentre outros (DYCK; OVIATT; LAMBERT, 1981). Por fim, o exame sensorial deve incluir a sensação de picada de agulha para documentar qualquer perda da função das fibras pequenas, sensação vibratória e sensação proprioceptiva para avaliar a funcionabilidade de fibras grandes (SIAO; KAKU, 2019).

Embora a combinação de histórico familiar, exames de imagem, testes auxiliares e avaliação sorológica sejam realizados para auxiliar no tratamento, cerca de 20% a 25% dos casos permanecem com a etiologia considerada incerta (WATSON; DYCK, 2015). Além disso, um estudo identificou que 64% das pessoas que tomam remédios prescritos descobriram que sua medicação para dor era inadequada, destes, 48% dos que sofrem de dor crônica (dentre elas a

dor neuropática) não tomam analgésicos e 14% pararam devido a efeitos colaterais (BREIVIK, 2017).

Sob esta perspectiva, destaca-se a importância do estudo da dor neuropática, uma vez que há necessidade de conhecimento da etiologia dos casos, dificuldade de diagnóstico, e o tratamento muitas vezes é inadequado ou produz efeitos colaterais. Ademais, atualmente não existe uma solução para qualquer tipo de dor neuropática, os tratamentos apenas visam minimizar os sintomas e não são específicos para este tipo de patologia.

Corroborando ainda a estes argumentos, uma metanálise de 2015 de ensaios que examinaram tratamentos farmacológicos para dor neuropática identificou que, embora haja forte recomendação para o uso de certos medicamentos (por exemplo, gabapentina, pregabalina e antidepressivos tricíclicos), os efeitos são relativamente modestos. O número necessário para produzir uma redução de 50% na dor para aqueles medicamentos com forte recomendação de uso varia entre 4 à 8 pacientes que devem ser tratados para que um paciente experimente pelo menos 50% de redução da dor quando a resposta ao placebo é subtraída (FINNERUP et al., 2015).

Sob essa perspectiva, evidencia-se a necessidade da busca por terapias alternativas, bem como, a compreensão mais precisa dos mecanismos fisiopatológicos envolvidos no desenvolvimento da dor neuropática, sobretudo na neuropatia periférica.

3.2.1 Neuropatia periférica e quimioterápicos

A neuropatia periférica é um sintoma adverso comumente relatado em pacientes expostos ao tratamento quimioterápico. A neuropatia aguda se desenvolve durante ou dentro de horas após o recebimento da quimioterapia e é caracterizada por parestesia em picada, alodínia e cãibras musculares, principalmente nas mãos e na região perioral (STAROBOVA; VETTER, 2017).

Neste período de neuropatia ainda considerada aguda, os estudos de condução nervosa são normais, indicando que não há perda axonal de grandes fibras mielinizadas, mas mostram descargas motoras repetitivas semelhantes à neuromiotonia (BENNEDSGAARD et al., 2020). O teste de excitabilidade do nervo mostra alterações proeminentes da excitabilidade do nervo correlacionadas aos sintomas sensoriais e que são bem modelados por uma desaceleração da inativação do canal de sódio (BENNEDSGAARD et al., 2020; HEIDE et al., 2018; PARK et al., 2011).

Rapidamente, o caso clínico dos pacientes submetidos ao tratamento quimioterápico tende a evoluir para uma neuropatia considerada crônica. A patogênese subjacente da polineuropatia crônica pode ser dividida em efeitos sobre o neurônio do gânglio dorsal, o axônio e a bainha de mielina ou células de Schwann (JORTNER, 2020; SOMMER; DOPPLER, 2015). Os mecanismos são diversos e incluem anormalidades endoteliais (BERGHOFF et al., 2003), interrupção da função das células de Schwann (GONÇALVES et al., 2017), disfunção capilar (OSTERGAARD et al., 2015), quebra da barreira hemato-nervosa (RICHNER et al., 2019), apoptose (GILL; WINDEBANK, 1998), estresse oxidativo elevado (JANG; LEE, 2011), efeitos tóxicos diretos (JORTNER, 2020), danos ao DNA mitocondrial (PODRATZ et al., 2011), perda de polímeros de neurofilamento (SCOTT; CLARK; ZOCHODNE, 1999) e transporte axonal prejudicado e perda de função dos microtúbulos (KOMIYA; TASHIRO, 1988).

Existem seis grupos de substâncias principais que causam danos aos neurônios sensoriais e motores periféricos, resultando no desenvolvimento da neuropatia periférica: os antineoplásicos à base de platina (OXA e cisplatina), os alcalóides da vinca (vincristina e vinblastina), as epotilonas (ixabepilona), os taxanos (paclitaxel, docetaxel), os inibidores de proteassoma (bortezomida) e drogas imunomoduladoras (talidomida) (STAROBOVA; VETTER, 2017).

Devido a taxa significativa de eficácia desses medicamentos para o tratamento de diversos tipos de carcinomas, a sobrevida dos pacientes passou a ser aumentada. Por conseguinte, o aumento de casos de pacientes que sofrem

com dor neuropática, proporcionalmente, também tem aumentado. Cerca de 68% dos pacientes expostos a quimioterapia tendem a desenvolver a neuropatia periférica no primeiro mês de tratamento (SERETNY et al., 2014).

Neste sentido, ressalta-se a necessidade de alternativas terapêuticas eficazes para tratar os pacientes submetidos a quimioterapia. Mais precisamente, fazem-se imprescindíveis tratamentos que enfoquem diretamente o mecanismo fisiopatológico desencadeador da neuropatia que é diferente dependendo do quimioterápico utilizado. A figura 1, de forma resumida, demonstra os principais mecanismos de ação dos agentes quimioterápicos desencadeadores da dor neuropática.

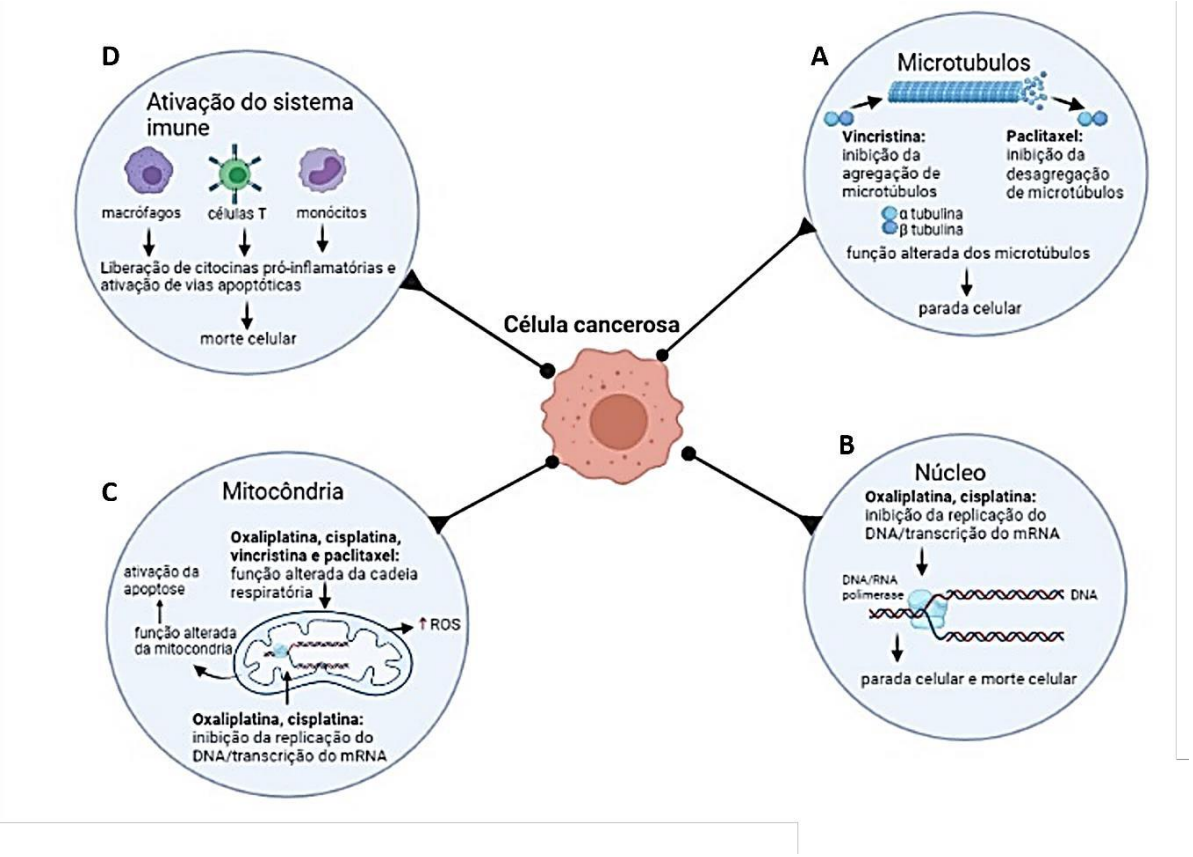

Figura 1 – Mecanismo de ação da vincristina, paclitaxel, oxaliplatina e cisplatina. (A) A vincristina previne a agregação dos microtúbulos, enquanto o paclitaxel previne a desagregação dos microtúbulos, um efeito que leva à

interrupção da divisão das células cancerosas e morte celular. (B) A oxaliplatina e a cisplatina se ligam ao ácido desoxirribonucléico (DNA) nuclear das células cancerosas, causando a interrupção da replicação do DNA e da transcrição do ácido ribonucléico (RNA) e subsequente interrupção da divisão celular cancerosa. Os adutos de DNA ativam as vias apoptóticas que induzem a morte celular e a degradação do tumor. (C) Todos os quatro agentes antitumorais alteram a função das mitocôndrias, seguida pela interrupção da função da cadeia respiratória e aumento da produção de espécies reativas de oxigênio (ROS). Além disso, a oxaliplatina e a cisplatina causam danos às mitocôndrias das células cancerosas ao se ligarem ao DNA mitocondrial, alterando a replicação e a transcrição do mDNA. (D) Todos os quatro agentes causam ativação de células do sistema imunológico, um efeito que provavelmente contribui para a degradação das células tumorais. Apenas alguns tipos representativos de células imunes são mostrados. (Adaptado de Starobova e Vetter, 2017)

3.2.2 Neuropatia periférica e Oxaliplatina

Os agentes quimioterápicos à base de platina, como a OXA e cisplatina, ambos listados na lista de medicamentos essenciais da Organização Mundial de Saúde (OMS), são usados para o tratamento de vários tumores sólidos. Particularmente, a OXA é um fármaco quimioterápico de terceira geração, amplamente utilizado em combinação com ácido folínico e 5-fluorouracil como parte do regime FOLFOX para o tratamento do carcinoma de colorretal (BANO; IKRAM, 2017; CRUZADO et al., 2014).

A OXA desencadeia um tipo de neuropatia periférica classificada como polineuropatia sensorial crônica. Quando administrada, a OXA, ainda na corrente sanguínea, sofre uma reação de deslocamento do grupo oxalato provocada por nucleófilos fracos (CVITKOVIC, 1998). Consequentemente, uma série de reações não-enzimáticas subsequentes originam intermediários instáveis, os quais têm o grupo 1,2-DACH-platina deslocado por hidrólise. Por fim, os

metabólitos ativos formados são o monocloro-DACH platina, dicloro-DACH platina e monoaqua-1,2-DACH platina, moléculas que reagem com o DNA.

No meio intracelular, a OXA cria ligações cruzadas intra-fita de DNA ligando-se a duas bases de guanina ou um par guanina-adenina (FAIVRE et al., 2003). Não obstante, a OXA ainda pode inibir a transcrição de DNA / produção de RNAm ao interagir com fatores de transcrição, inibição de RNA polimerases e pela criação de adutos de DNA nucleossômico que conduzem à morte celular (TODD; LIPPARD, 2009). Em suma, a OXA é capaz de alterar a síntese, replicação e transcrição do DNA.

O oxalato além de dar início a essa cascata de reações danosas ao DNA também pode contribuir para o desenvolvimento da neuropatia periférica. Segundo Starobova e Vetter (2017) a geração do metabólito oxalato é uma das poucas características que distinguem a OXA da cisplatina, e esse metabólito tem sido relacionado à elevada sensibilidade ao frio demonstrada em pacientes expostos a OXA.

Dentre outros mecanismos que contribuem para o desenvolvimento da dor neuropática induzida por OXA pode-se mencionar a alteração na excitabilidade axonal devido à disfunção do canal iônico, a desregulação da homeostase do cálcio e a função alterada dos canais potenciais do receptor transiente (SAŁAT, 2020). Além disso, o estresse oxidativo tem sido reportado como um fator responsável pela disfunção das células neuronais e gliais e apoptose (SAŁAT, 2020).

Por este motivo, ainda, vale destacar, que a neuropatia aguda transitória causada pela OXA ocorre em cerca de 90% dos pacientes poucas horas após a infusão, e a neuropatia periférica crônica foi estimada em cerca de 70% dos pacientes submetidos ao tratamento (STAROBOVA; VETTER, 2017). Sendo assim, a neuropatia causada pela OXA apresenta elevada incidência e é um efeito adverso preocupante com necessidade de tratamentos eficazes.

3.3 Estresse oxidativo e oxaliplatina

Tem sido amplamente demonstrado que a produção de espécies reativas de oxigênio e nitrogênio (EROs e ERNs) por quimioterápicos antineoplásicos, contribui para indução de apoptose em células cancerosas (RADI; CASSINA; HODARA, 2002). Apesar deste ser um mecanismo de morte celular importante para o tratamento do câncer afim de inibir o crescimento tumoral, sabe-se que, paralelamente, ele está inerente ao desenvolvimento da dor neuropática.

A OXA, especialmente, possui como um dos seus principais mecanismos fisiopatológicos o desenvolvimento de estresse oxidativo (STAROBOVA; VETTER, 2017). Neste sentido, diferentes estudos têm proposto que a OXA pode aumentar a concentração de espécies reativas no organismo e inibir a atividade de defesas antioxidantes importantes (NASSINI et al., 2011; REIS et al., 2020a; WASEEM; PARVEZ; TABASSUM, 2017; ZANARDELLI et al., 2014).

Uma das vias de apoptose induzida pela OXA inicia pela liberação do citocromo c dos complexos mitocondriais. Quando isto ocorre os elétrons em transferência na cadeia fosforilativa são desviados para o O_2 e são formados os radicais superóxido. Esses radicais livres gerados possuem diversos alvos celulares, sendo os lipídeos celulares seus alvos primários. Após reações de lipoperoxidação são gerados os produtos primários, radicais peroxil e alcoxil, e os produtos secundários são os aldeídos como o malondialdeido (MDA).

Joseph e colaboradores (2008) observaram que a OXA é capaz de danificar nociceptores, sendo que o mecanismo principal sugerido para o dano neural se desencadeia diretamente pelo estresse oxidativo. Não obstante, um estudo do nosso grupo de pesquisa demonstrou pela primeira vez que o tratamento com OXA levou ao depósito de platina na medula espinhal de camundongos e este acúmulo provavelmente está interligado ao aumento de espécies reativas nos animais (REIS et al., 2020a).

3.4 Comorbidades e transtornos psiquiátricos associados ao tratamento quimioterápico

A disfunção cognitiva e os transtornos psiquiátricos induzidos por quimioterapia são sintomas adversos comuns em pacientes com câncer. No início, os sintomas tendem a se manifestar com declínio na atenção e nas habilidades visuoespaciais, porém, posteriormente, tendem a progredir ao desenvolvimento de ansiedade, depressão e patologias cognitivas (TANNOCK et al., 2004).

Em relação ao déficit cognitivo, particularmente, a prevalência medida após a quimioterapia varia de 15 a 75% dos pacientes com tumores sólidos ((VARDY; ROURKE; TANNOCK, 2007; WEFEL; SCHAGEN, 2012). As funções cognitivas mais afetadas pelo tratamento quimioterápico reportadas são a atenção/concentração, função executiva, verbal ou visual, aprendizagem e velocidade de processamento (ARGYRIOU et al., 2011; FALLETI et al., 2005; JANELSINS et al., 2011; STEWART et al., 2006).

Estudos têm demonstrado que os distúrbios emocionais e transtornos psiquiátricos tendem a persistir mesmo após a interrupção do tratamento quimioterápico ocasionando prejuízos na qualidade de vida e na capacidade de trabalho destes indivíduos sobreviventes do câncer (ZHANG et al., 2017). Além disso, DiBonaventura (2017) em uma pesquisa multimodal observou a prevalência de comorbidades em pacientes com dor neuropática; dentre as comorbidades destacaram-se: ansiedade (38%), depressão (34,5%), insônia (34,2%) e condições relacionadas à dor, como fibromialgia (10,1%) e artrite reumatóide (14,5%).

Neste sentido, embora os mecanismos envolvidos na evolução destes quadros patológicos sejam pouco conhecidos, algumas lacunas têm sido esclarecidas, pelo menos em parte, quanto a determinados quimioterápicos. No regime FOLFOX, utilizado no tratamento do carcinoma de colorretal, foi demonstrado que o 5-fluorouracil pode induzir inflamação aguda e lesão vascular, seguida por dano tardio aos tratos mielinizados no cérebro (HAN et al., 2008). Além disso, tanto o 5-fluorouracil quanto a OXA podem diminuir a neurogênese e/ou a proliferação das células do hipocampo (SHARPE et al.,

2012). Por outro lado, foi observado em modelos animais que a administração combinada de ambas as drogas causa mais toxicidade em comparação com qualquer um dos agentes separadamente e que as tarefas dependentes do hipocampo, a memória contextual do medo e a memória de referência espacial são as principais áreas afetadas (FARDELL et al., 2012).

Corroborando a estes estudos, testes de imagem do lobo frontal e hipocampo de pacientes submetidos a quimioterapia apresentaram atrofia da substância branca sugerindo que o comprometimento cognitivo frontal-hipocampal predomina afetando principalmente a memória (DE RUITER et al., 2012). Além disso, a relação entre o declínio cognitivo relacionado à quimioterapia e os efeitos colaterais da doença/tratamento e fatores psicológicos tem sido investigada.

No caso de fatores psicológicos relacionados ao declínio cognitivo em pacientes com câncer, sugere-se que a depressão ou a ansiedade seja causada pelo diagnóstico do câncer e o processo da doença, e isso afeta o declínio cognitivo. Sob essa perspectiva, a depressão é sugerida como uma variável imprescindível para o desenvolvimento do declínio cognitivo (HUTCHINSON et al., 2012). A relação entre as mudanças na função cognitiva e o estado emocional ainda não está clara e faltam estudos que verifiquem isso (KRYNETSKIY et al., 2013).

Confirmando essa relação entre a cognição e os transtornos emocionais, um estudo recente do nosso grupo de pesquisa observou a correlação entre o comportamento tipo-ansioso de camundongos com o déficit cognitivo evidenciado pelo prejuízo da memória a longo prazo causado pelo tratamento quimioterápico (REIS et al., 2020b). Além disso, este mesmo estudo demonstrou o aumento dos níveis plasmáticos de corticosterona e evidenciou a diminuição da atividade da enzima $Na^+ K^+$ - ATPase após a exposição de camundongos à OXA; logo, estes fatores podem estar contribuindo para o desenvolvimento patológico (REIS et al., 2020b).

3.5 Papel da via colinérgica na dor neuropática

A acetilcolina (ACh), um neurotransmissor e neuromodulador da via colinérgica, é considerada uma das moléculas mais proeminentes no sistema nervoso central e periférico. A ACh destaca-se por controlar os ramos simpáticos e parassimpáticos do sistema nervoso autônomo no gânglio e, assim, mediar a funcionalidade de "descanso e alerta" (TIWARI et al., 2013). Cientificamente, a via colinérgica é especialmente interessante devido à sua rica plasticidade, exemplificada pela alteração da expressão de receptores nicotínicos e muscarínicos.

De acordo com alguns autores, a ativação de receptores nicotínicos pela acetilcolina pode aliviar a dor e agonistas nicotínicos têm eficácia analgésica semelhante aos opioides (UMANA et al., 2017). Além disso, compostos colinérgicos produzem efeitos antinociceptivos em macaco rhesus, gato e rato (KATAYAMA; HIROKAWA; TANAKA, 1984; PERT; KUHAR; SNYDER, 1975) e a administração de inibidores de colinesterases que permeiam a barreira hematoencefálica produz analgesia e aumento da analgesia de fármacos opioides (LEWIS; ENGELMAN, 1983).

Sugere-se que o aumento glicêmico sanguíneo em diabéticos é capaz de causar um acréscimo da concentração de glicose que, por sua vez, afeta o equilíbrio osmótico nos neurônios, reduz a produção de ACh e leva a decomposição da bainha de mielina (KUZUMOTO et al., 2006). Esta sequência de ações ocasiona danos somatossensoriais, além de proporcionar o acúmulo de espécies reativas, fator intrínseco ao desenvolvimento de neuropatia (KUZUMOTO et al., 2006). Adicionalmente, em modelo de dor neuropática induzida pela OXA em camundongos foi relatado um aumento na atividade da enzima AChE, demonstrando uma alteração na via colinérgica por este fármaco (REIS et al., 2020b).

Ademais, estudos recentes demonstraram o envolvimento, principalmente, dos receptores nicotínicos de ACh no desenvolvimento de dor neuropática (HONE; MCINTOSH, 2018; KIGUCHI et al., 2018). No genoma

humano, já foram descritas mais de 16 subunidades que codificam o receptor nicotínico. As subunidades α e β existentes nos receptores nicotínicos se reúnem em várias combinações para formar distintos subtipos de receptores, os quais são predominantemente expressos nos neurônios e apresentam diferentes sensibilidades frente a situações dolorosas (HONE; MCINTOSH, 2018).

Particularmente, o conjunto α6β4, tem sido mencionado por estar envolvido no processamento sensorial de informações derivadas da dor e, sugere-se que a estimulação deste receptor poderia inibir, paralelamente, os receptores purinérgicos, como o P_2X, através de reações ainda não identificadas (HONE; MCINTOSH, 2018). De fato, estudos relatam que fármacos que inibem receptores do tipo P_2X já vem sendo comercializadas para o tratamento da dor crônica (HONE; MCINTOSH, 2018; NORTH; JARVIS, 2013). Apesar da importância de estudar fármacos analgésicos que apresentem ações por meio da via colinérgica, pesquisadores têm observado a necessidade de especificidade destas drogas nos receptores-alvo, uma vez que, fármacos com ação colinérgica já têm sido mencionados por produzirem efeitos colaterais cardiovasculares e gastrointestinais (HONE; MCINTOSH, 2018).

3.6 Potencial terapêutico de compostos orgânicos contendo calcogênios

Substâncias antioxidantes são moléculas com capacidade de proteger o organismo contra a ação deletéria de espécies reativas geradas pela ação do próprio organismo ou durante processos patológicos. Os antioxidantes podem ser definidos como moléculas que, mesmo presentes em baixas concentrações em relação ao substrato oxidável, podem retardar significativamente ou inibir o processo oxidativo (HALLIWELL et al., 1995).

O estresse oxidativo decorre de um desequilíbrio entre a geração de compostos oxidantes e a atuação dos sistemas de defesa antioxidante. Neste sentido, o estresse oxidativo é um estado danoso diretamente associado ao desenvolvimento de quadros clínicos, como é o caso da neuropatia periférica

causada por quimioterápicos. A ação oxidativa atua promovendo prejuízos moleculares em componentes celulares como proteínas, lipídios e DNA (LOSADA-BARREIRO; BRAVO-DÍAZ, 2017).

Sob essa perspectiva, a síntese de compostos orgânicos, área responsável por cerca de 85% dos medicamentos disponíveis, representa um importante papel na descoberta e no desenvolvimento de novos fármacos (BARREIRO; FRAGA, 2014). Destes, 62% são compostos heterocíclicos, ganhando destaque aqueles pertencentes ao grupo dos calcogênios, tais como o selênio (Se), o telúrio (Te) e o enxofre (S) por serem descritos como potenciais agentes antioxidantes (MOTTA; FORNO; ÁVILA, 2020).

Biologicamente, o S é um átomo com potencial antioxidante, além disso constitui uma série de moléculas essenciais pois pode atuar como cofator em diversos processos enzimáticos fundamentais combatendo o estresse oxidativo. Moléculas derivadas do tiodiazol, composto de dois átomos de carbono, dois de nitrogênio e um de enxofre, têm se destacado por apresentarem atividade antimicrobiana, antitumoral, anti-inflamatória e antioxidante (FEIJÓ et al., 2012; HANIF et al., 2012). Ademais, compostos contendo S apresentam ação antioxidante com potencial para modular a enzima AChE, tem se mostrado interessantes no tratamento da dor (THANKACHAN et al., 2015).

Em vista disso, nosso grupo de pesquisa tem dedicado esforços para investigar as propriedades farmacológicas do composto 5-((4-metoxifenil)tio)benzo[c][1,2,5] tiodiazol (MTDZ), uma nova molécula derivada do tiodiazol, que já apresentou ação inibitória da enzima AChE ($I_{máx}$ ~ 90%) em um ensaio *in vitro* (DOS SANTOS et al., 2020). SANTOS e colaboradores. (2020) sugeriram que a promissora atividade anticolinesterásica do MTDZ se deve, além do grupamento tiodiazol, à presença do grupo metóxi, doador de elétrons na posição para. Posto isto, foi hipotetizada a possibilidade de uma promissora atividade do MTDZ frente a neuropatia periférica induzida por OXA em um modelo animal utilizando camundongos machos e fêmeas.

Ainda, o Se, um calcogênio essencial, se encontra associado com o correto funcionamento de processos metabólicos celulares importantes e tem sido um elemento importante em diversas patologias. Compostos orgânicos contendo Se apresentam ação antioxidante, anti-inflamatória, antinociceptiva, ansiolítica e participam da proteção contra a ação nociva de metais pesados (como a platina) e xenobióticos (BRÜNING et al., 2009; OLIVEIRA et al., 2020; REIS et al., 2017; SAVEGNAGO et al., 2013; SILVA et al., 2017; ZARZECKI et al., 2017). Desta forma, conjecturamos que o 4-PSQ, um derivado de quinolina contendo Se, seria uma promissora molécula para o tratamento do dano renal causado pela OXA devido as suas atividades já mencionadas na literatura.

O 4-PSQ é uma molécula multialvo e apresenta diversos efeitos promissores. Podemos destacar que o 4-PSQ exerce ação anti-inflamatórias agudos e efeitos antinociceptivos que estão correlacionados com sua propriedade antioxidante (PINZ et al., 2016) e a modulação dos sistemas serotonérgico, nitrérgico e glutamatérgico (SILVA et al., 2017). Recentemente, foi demonstrado que este composto exerce ações potenciadoras de memória em ratos idosos por meio da modulação do sistema colinérgico e da plasticidade sináptica, aumentando as moléculas de adesão de células neurais (NCAM) e os níveis de polissialil transferase no córtex cerebral e hipocampo (BARTH et al., 2019). Igualmente importante, o 4-PSQ reduziu a captação de glutamato no córtex cerebral e protegeu contra o comportamento relacionado à ansiedade induzido pelo cainato em camundongos (REIS et al., 2017). Além disso, o 4-PSQ revelou potencial vantagem terapêutica no tratamento das lesões de pele em um modelo de dermatite atópica em camundongos (VOSS et al., 2017).

Em relação a neuropatia periférica causada pela OXA, o 4-PSQ reduziu a hipersensibilidade mecânica e térmica e normalizou a alteração na atividade enzimática de defesas antioxidantes (REIS et al., 2020a). Foi demonstrado ainda que a OXA levou a um prejuízo cognitivo e ao comportamento tipo-ansioso em camundongos machos e que estas alterações apresentam correlação positiva significativa entre si; sendo o 4-PSQ uma estratégia terapêutica para o prejuízo

neurológico (REIS et al., 2020b). Adicionalmente, em um modelo animal, foi observada a hepatotoxicidade frente a exposição à OXA, evidenciada pelo aumento da atividade plasmática de aspartato e alanina aminotransferase e do exame histopatológico (LEMOS et al., 2021). O 4-PSQ foi capaz de reverter o dano oxidativo hepático e de minimizar a lesão hepática induzida pela OXA.

De fato, nossos resultados ajudaram a expandir o conhecimento sobre os mecanismos envolvidos na fisiopatologia da neurotoxicidade e hepatotoxicidade induzida por OXA e demonstram o potencial terapêutico do 4-PSQ. Logo, todos estes fatores relacionados ao potencial multiterapêutico do 4-PSQ contribuíram à especulação do 4-PSQ, agora, como um potencial tratamento à nefrotoxicidade causada pela OXA.

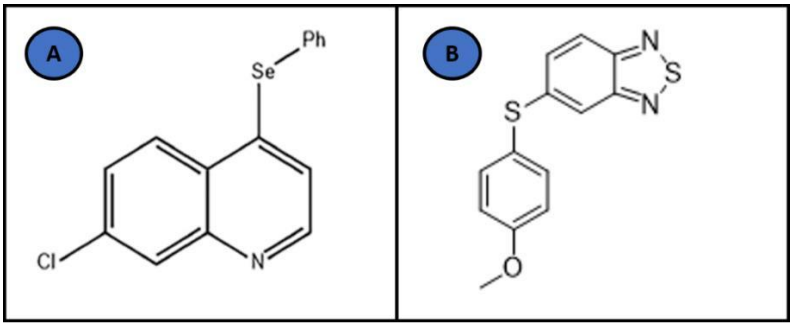

Figura 2 – Estrutura química do (A) 7-cloro-4- (fenilselanil) quinolina (4-PSQ) e do (B) 5 - ((4-metoxifenil) tio) benzo [c] [1,2,5] tiodiazol (MTDZ).

4. Artigos Científicos

Os principais resultados que fazem parte desta dissertação estão apresentados sob a forma de artigo científico e manuscrito, os quais se encontram assim organizados. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se nos próprios artigos.

O artigo científico encontra-se publicado na revista *Canadian Journal of Physiology and Pharmacology* e o manuscrito está submetido para publicação na revista *Chemico-Biological Interactions*.

4.1 Capítulo 1 - Manuscrito

Target Enzymes in Oxaliplatin-induced Peripheral Neuropathy in Swiss mice: A New Acetylcholinesterase Inhibitor as Therapeutic Strategy

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Conflicts of Interest: The authors declare no competing financial interests.

Abstract

In the present study it was hypothesized that 5-((4-methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ), a new acetylcholinesterase inhibitor, exerts antinociceptive action and reduces the oxaliplatin (OXA)-induced peripheral neuropathy and its comorbidities (anxiety and cognitive deficits). Indeed, the acute antinociceptive activity of MTDZ (1 and 10 mg/kg; per oral route) was observed for the first time in male Swiss mice in formalin and hot plate tests and on mechanical withdrawal threshold induced by Complete Freund's Adjuvant (CFA). To evaluate the MTDZ effect on OXA-induced peripheral neuropathy and its comorbidities, male and female Swiss mice received OXA (10 mg/kg) or vehicle intraperitoneally, on days 0 and 2 of the experimental protocol. Oral administration of MTDZ (1 mg/kg) or vehicle was performed on days 2 to 14. OXA caused cognitive impairment, anxious-like behaviour, mechanical and thermal hypersensitivity in animals, with females more susceptible to thermal sensitivity. MTDZ reversed the hypersensitivity, cognitive impairment and anxious-like behaviour induced by OXA. Here, the negative correlation between the paw withdrawal threshold caused by OXA and acetylcholinesterase (AChE) activity was demonstrated in the cortex, hippocampus, and spinal cord. OXA inhibited the activity of total ATPase, Na^+ K^+ - ATPase, Ca^{2+} - ATPase and altered Mg^{2+} - ATPase in the cortex, hippocampus, and spinal cord. OXA exposure increased reactive species (RS) levels and superoxide dismutase (SOD) activity in the cortex, hippocampus, and spinal cord. MTDZ modulated ion pumps and reduced the oxidative stress induced by OXA. In conclusion, MTDZ is an antinociceptive molecule promising to treat OXA-induced neurotoxicity since it reduced

nociceptive and anxious-like behaviours, and cognitive deficit in male and female mice.

Keywords: Neuropathy, Neurotoxicity, Oxaliplatin, Acetylcholinesterase, Sulfur, ATPases.

1. Introduction

Peripheral neuropathy is one of the main adverse effects caused by current chemotherapy treatment [1]. Particularly, oxaliplatin (OXA), the main chemotherapy drug used to treat colorectal carcinoma, has a high incidence of development of neurotoxic conditions, among which peripheral neuropathy stands out [2]. Due to its neurotoxicity, OXA has also been associated with the development of other comorbid neurological conditions in patients, such as cognitive deficit and anxious behaviour [3,4].

Although not all mechanisms involved in the development of peripheral neuropathy caused by OXA are identified, it is known that oxalate metabolites can alter the functioning properties of voltage-dependent Na^+ channels, resulting in a prolonged open state of the ion channels and hyperexcitability of dorsal root ganglion sensory neurons [5]. Regardless of knowledge of damage to ion channels, little has been reported about ATPases, ion pumps essential for the conduction of nerve impulses, such as Na^+ K^+ - ATPase, Ca^{2+} - ATPase, Mg^{2+} - ATPase and total ATPase, being a possible pathophysiological mechanism to be explored.

Furthermore, mitochondrial dysfunction and oxidative damage in patients exposed to OXA have been observed [5,6,7]. In this context, the acetylcholinesterase (AChE) has been reported as an important cholinergic enzyme sensitive to oxidative stress [8,9]. From this perspective, the association between the cholinergic system and the development of painful processes has received the attention of researchers [10–13].

Recently, our research group revealed that the AChE activity was increased by OXA exposure in the cortex of male mice, suggesting that this enzyme might be implicated in the physiopathology of the OXA-induced peripheral neuropathy and that inhibitors of AChE activity can be a promising therapeutic strategy [14]. Reinforcing this theory, Kawashiri and collaborators [15] demonstrated that repeated administration of donepezil, a cholinesterase inhibitor for Alzheimer's disease, attenuated OXA-induced peripheral neuropathy.

Given that organic compounds containing sulfur come to be highlighting for presenting relevant activities in nociceptive, inflammatory and neuropathic pain models and for modulating the cholinergic system [16,17], our research group has dedicate special attention to the study of pharmacological properties of the 5-((4-methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ), a new sulfur-containing compound. MTDZ exerts potential inhibitory activity of AChE and it can be an interesting prototype to be investigated for the treatment of neuropathic pain caused by OXA exposure [16].

Based in these considerations, the hypothesis of the present study is that MTDZ exerts antinociceptive action and reduces the OXA-induced peripheral neuropathy and its comorbidities (anxiety and cognitive deficits) by inhibiting AChE activity. In addition, MTDZ could modulate ion pumps and reduce the oxidative stress induced by OXA exposure in male and female mice.

2. Materials and methods for pre-clinical study

2.1 Animals

The tests were carried out using male and female adult *Swiss* mice (25-30 g). Animals were maintained at 22 ± 2 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00 a.m.). The animals were experimentally divided into 9 to 10 animals per group.

2.2 Ethics Statement

All experiments were performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506-2017).

2.3 Drugs

MTDZ (Fig. 1) was synthesized and characterized as described by Santos et al. [16]. The purification of the compound MTDZ was performed by column chromatography (only with hexane). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker (300 MHz and 75 MHz, respectively) spectrometer. OXA was obtained from Eurofarma® pharmaceutical company. All other chemicals used in this study were of analytical grade and obtained from Sigma Aldrich, (St. Louis, MO, USA). The MTDZ compound was dissolved in canola oil vehicle, meloxicam and formaldehyde were diluted with 0.9% saline and the OXA was prepared with 5% glucose. In view of the degree of toxicity of the management with antineoplastic chemotherapy, the researchers used personal protective equipment with low permeability and a laminar flow hood.

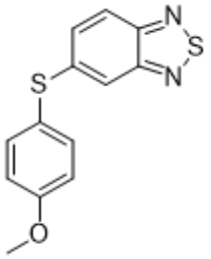

Fig. 1 - Chemical structure of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ).

2.4 Evaluation of the acute antinociceptive effects of MTDZ

First, the effect of MTDZ was evaluated in acute pain models in *Swiss* male mice (25-30g) to minimize the total number of animals. The dose-effect of the compound was observed through the formalin, Complete Freund's Adjuvant (CFA), and hot plate tests. The time–response curve of the MTDZ was evaluated through the CFA test. To screen the effect of MTDZ on acute nociceptive behaviours, the compound was tested at the doses of 1 and 10 mg/kg by oral route (p.o. - intragastric gavage). The doses and intervals of administration of the compound were chosen in view of previous studies (to minimize the use of animals) which used organic compounds containing chalcogens or sulfur and which showed promising effects in nociception tests [14,18–22]. The oral route was chosen in view of the invasive exposure that cancer patients are already submitted to and the ease and practicality that this route offers.

2.4.1 Formalin-induced nociception

The formalin test was carried out as described by Hunskaar and Hole [23]. Mice were pretreated with MTDZ (1 and 10 mg/kg, per oral route (p.o.)), canola oil (vehicle, 10

mL/kg; p.o.) or the reference drug meloxicam (10 mg/kg; p.o.)). After 30 minutes, the animals received the intraplantar (i.pl.) administration of formalin (2.5 %, v/v; 20 μL/paw) in the right hind paw and saline solution (0.9 %, w/v; 20 μL/paw) in the left paw. The animals were individually placed in an acrylic box and duration of paw licking was recorded at 0-5 min (first phase) and 15-30 min (second phase). The first phase, called the neurogenic phase, represents the irritating effect of formalin on sensory C-fibers. The second phase, called the inflammatory phase, is characterized by the release of inflammatory mediators. After the period of observation, mice were killed, and the paw edema was measured. Paws were removed and weighed. The paw edema was measured by comparing the difference between the weight of the formalin injected paw and the weight of the contralateral paw (saline-treated paw). Data were expressed as pain behaviour (seconds) for the neurogenic and inflammatory phases and for the edematogenic evaluation data were expressed as paw edema (mg).

2.4.2 Hot Plate Test

The hot-plate test was carried out as described by Woolfre and MacDonald [24]. Animals were placed in a heated metal plate maintained at 55 ± 1 °C. The latency of nociceptive responses, such as licking or shaking one of the paws or jumping, was recorded. Cut-off was limited to 45 s. The change in thermal withdrawal latencies was recorded before treatment and at 30 min after treatment with MTDZ (1 and 10 mg/kg, p.o.), morphine (10 mg/kg, p.o.) or the canola oil vehicle (10 mL/kg, p.o.). The delta of latency (Δt) was calculated for each animal: Δt (s) = post-treatment latency – pre-treatment latency.

2.5 Time–response curve of MTDZ on mechanical withdrawal threshold induced by Complete Freund's Adjuvant (CFA)

The mice received an i.pl. injection of CFA (1 mg/mL *Mycobacterium tuberculosis*, 20 μL) in the right hind paw and saline solution (0.9%, 20 μL/paw) in the left paw. After 24h, animals received vehicle (canola oil, p.o.), MTDZ (1 and 10 mg/kg, p.o.) or meloxicam (10 mg/kg, p.o.). The nociceptive response was verified using the von Frey test at 30 minutes, 2, 4, 6 and 24 hours after treatment. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (digital aesthesiometer, Insight, São Paulo, Brazil) adapted with a polypropylene tip. This test described by Alamri et al. [25] determines mechanical hyperalgesia through the paw withdrawal threshold. Before induction with CFA, the animals were submitted to baseline evaluation of mechanical sensitivity to rule out possible false results during the behavioural test. Data were expressed as withdrawal threshold (g).

2.6 Evaluation of the MTDZ effects on OXA-induced peripheral neuropathy and its comorbidities

2.6.1 Experimental Design

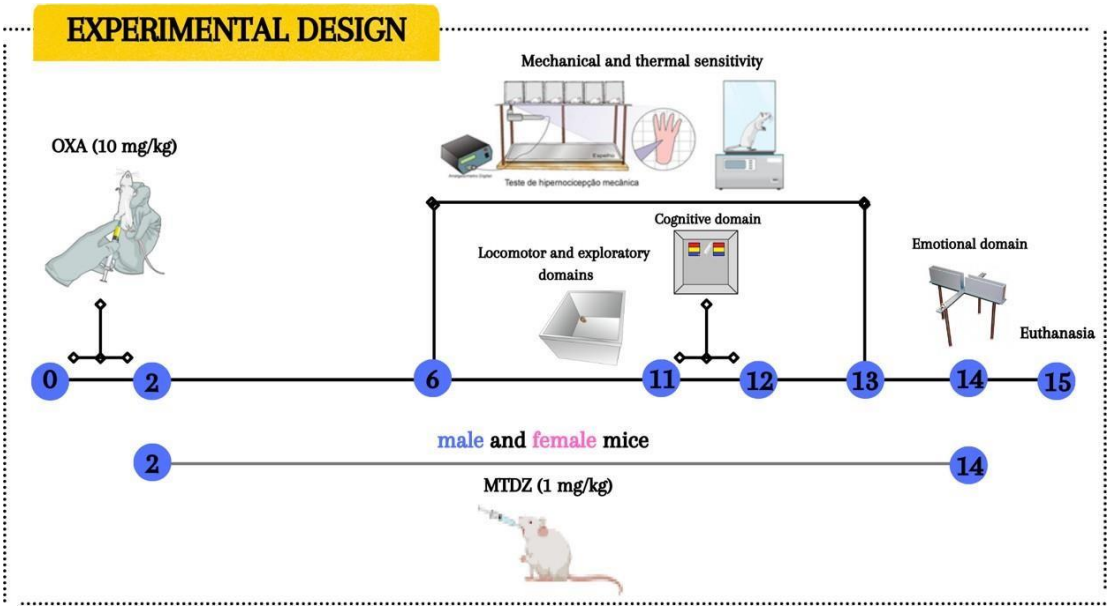

Fig. 2 - The experimental design. Male and female mice received oxaliplatin (OXA) (10 mg/kg) or 5% glucose solution by intraperitoneal (i.p.) route (days 0 and 2), and they were per oral route treated for 12 days with MTDZ (1 mg/kg) or canola oil (1 mL/kg). On day 0, the animals were submitted to basal von frey and hot plate tests. On the 6th and 13th day, the von frey and hot plate tests were performed (mechanical and thermal sensitivity, respectively). Open field test (locomotor and exploratory domains) was performed on the 11th day. Object recognition test (cognitive domain) was performed on the 11th and 12th days. On the 14th day, the elevated plus-maze test (emotional domain) was performed. On the 15th day the animals were euthanized for *ex vivo* assays.

The male and female mice were randomly divided into four group (n = 9 to 10 animals): I) Control; II) OXA; III) MTDZ; and IV) OXA + MTDZ. As shown in Fig.2, on the zero and second days, the animals of the Control and MTDZ groups received 5%

glucose solution (10 mL/kg, i.p.), while the animals of the OXA and OXA + MTDZ groups received OXA (10 mg/kg, i.p.). Animals received 12 administrations in total. Daily, from the second day, the Control and OXA groups received canola oil (10 mL/kg p.o.), while mice of the MTDZ and OXA + MTDZ groups received MTDZ (1 mg/kg, p.o.), up to the fourteenth day. The dose of 1 mg/kg of MTDZ was chosen after previous nociceptive tests, since it was effective in reducing nociceptive, inflammatory and edematogenic responses; furthermore, this was the lowest dose tested with promising effects. Twenty-four hours after the last treatment, the animals were anesthetized and euthanized by inhalation of isoflurane anesthetic. Cerebral cortex, hippocampus and spinal cord were collected and used for *ex vivo* assays. It is noteworthy that the present study sought to encompass, in addition to treatments, the sexual statistical bias to enrich the discussion of pain sensitivity and intrinsic pathophysiological processes. Above all, the sexual variable was used due to the lack of studies investigating the sex factor in models of neuropathic pain caused by OXA. The experimental design was organized according to previous studies [14,26].

2.6.2 Behavioural procedures

2.6.2.1 Measurement of Mechanical Sensitivity

Mechanical sensitivity was carried out in mice according to the method described in item 2.5. For this test, mice were placed individually inside acrylic cages with wire grid floors 30 min before the start of testing performed in a quiet room. The observation of mechanical sensitivity was performed at baseline (on day zero) and during the experimental protocol (on days sixth and thirteenth). The paw withdrawal threshold was

measured by applying the polypropylene tip perpendicular to the middle of the plantar surface of the hind paw at a constant progressive pressure until paw withdrawal, and the pressure value was automatically recorded. Data were expressed as withdrawal threshold (g).

2.6.2.2 Measurement of Thermal Sensitivity

Thermal sensitivity was tested in mice as reported by Woolfe and MacDonald [24], and as provided in item 2.4.2 with some modifications. The observation of thermal sensitivity was performed before the treatments and on the sixth and thirteenth days of the experimental protocol. For this, animals were placed in a glass box on a heated metal plate maintained at 52 ± 1 °C. The latency of nociceptive responses such as licking or shaking one of the paws or jumping was recorded as the reaction. To avoid damage to the paws of animals, time standing on the plate was limited to 45 s. Results were expressed as latency (s).

2.6.2.3 Assessment of locomotor and exploratory domains

The open field test evaluates the general locomotor and exploratory behaviours of mice. The open field was made of plywood and surrounded by 30 cm-high walls. The floor of the open field, 45 cm long and 45 cm wide, was divided by masking tape markers into 9 squares (3 rows of 3). Mice were evaluated on the eleventh day, before the object recognition task. In this test, each animal was placed at the center of the open field and observed for 4 min to record the locomotor (number of segments crossed with the four

paws) and exploratory (number of rearing on the hind limbs) activities [27]. Mice were tested only once.

2.6.2.4 Assessment of cognitive domain

The object recognition task was carried out according to the method previously described by Stangherlin et al. [28]. This task has been widely used to evaluate short-term (STM) and long-term (LTM) memories. The task was performed in an open field apparatus on the eleventh and twelfth days of the experimental protocol. On the day of the task (the twelfth day of the experimental protocol) each animal underwent a 5 min habituation session in the absence of objects, where we performed the test to assess the domain of locomotion and exploration of the animals. Posteriorly, four objects named A1, A2, B and C were used. The A1 and A2 objects were two identical balls, the B object was a cube, and the C object was a square. The objects used were made of plastic material, measuring 10 x 10 cm (length x height) and had the following color pattern: blue, red, and yellow. During the training, the animals were placed for 5 min in the arena containing two identical objects (objects A1 and A2) to explore. Exploration was accounted when the animal directed its nose around 2 cm of the object while sniffing, touching, or looking at it. In the presence of a familiar object (A1) and a new object (B), 1.5 h after training, the STM of mice was evaluated. The time to explore was defined in
5 min, enough to measure learning and recognition memory. In turn, LTM was assessed 24 h after training. For this, the mice were placed to explore a familiar object (A1) and a new object (C) for 5 min. Time spent exploring each object was reported. Data were

expressed as a percentage of the exploratory preference and calculated as follows: Training = (A2/(A1+A2))×100; STM = (B/(A1+B))×100; LTM = (C/(A1+C))×100.

2.6.2.5 Assessment of emotional domain

The elevated plus-maze apparatus consists of two opposed open arms (16 cm x 5 cm) and two opposed closed arms (16 cm x 5 cm x 10 cm) mounted at an angle of 90º, all facing a central platform (5 cm x 5 cm) elevated 50 cm from the floor. This test is widely validated to measure anxiety in rodents [29]. On the fourteenth day, all animals were evaluated in the elevated plus-maze test. Each animal was placed individually at the center of the apparatus facing one of the open arms. The frequency of entries into either open or closed arms and the time spent in each type of arm were measured for 5 min. The anxiolytic effects of a drug are illustrated by a significant statistical increase of parameters in open arms. The data were expressed as percentage of entries (with the four paws) into, and time spent in the open arms in relation to the total number of entries and time, respectively, in both open and enclosed arms. The total number of entries into the enclosed arms was also recorded.

2.6.3. Biochemical assays

2.6.3.1 Sample preparation

The cerebral cortex, hippocampus and spinal cord were separated and washed with cold saline solution (0.9%). To determine the activity of AChE, the cerebral and spinal cord structural samples were prepared in 0.25 mol/L sucrose buffer (1:10 w/v) and centrifuged at 900 × g for 10 min to produce a supernatant (S1). For the other biochemical

analyses, the other samples were homogenized in 50 mmo/L Tris HCl pH 7.4 and centrifuged at 900 × g for 10 min to produce a supernatant (S2).

2.6.3.2 AChE activity

The AChE activity was assayed following a modified method of Ellman [30], using acetylthiocholine as the substrate. The reaction mixture (2 mL final volume) contained S1 (100 µL), 100 mM K^+-phosphate buffer, pH 7.5 and 1 mM 5,5′-dithiobis-nitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5′-dithio-bis-nitrobenzoate, measured spectrophotometrically at 412 nm during 2 min. The enzyme was pre-incubated for 2 min at 25 °C. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide. The enzymatic activity was expressed as µmol/h/mg protein.

2.6.3.3 ATPases activity

Total ATPase activity was assayed in an incubation medium comprising all the salt substrates needed for the ionic pumps to take place: 30 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 3 mM ATP, and 50 µL of S2 samples. Controls to correct for non-enzymatic substrate hydrolysis were prepared by adding sample preparations after the reactions were stopped with TCA [31]. The color reaction was assayed spectrophotometrically at 650 nm. The enzyme activities were expressed in nmol of Pi/min/mg of protein.

2.6.3.4 Na^+ K^+ - ATPase and Mg^{2+} - ATPase activity

The reaction mixture containing S1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl and 50 mM Tris/HCl, pH 7.4, in a final volume of 500 μL was used. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samples were performed under the same conditions with the addition of 0.1 mM ouabain. Considering that ouabain is an inhibitor of the Na^+ K^+ pump, it was possible to observe in this technique the enzyme activity related to the Mg^{2+} pump. To determine the Mg^{2+}-ATPase activity, ouabain (1 mM) was added to the reaction medium. The reactions was initiated by adding ATP and was stopped after 30 min of incubation by the addition of 10% TCA. The samples were incubated at 37 °C for 30 min and the incubation was stopped by adding 10% TCA with 10 mM $HgCl_2$. Enzyme activity was calculated from the difference between amounts of inorganic phosphate (Pi) found after incubation in the absence and presence of ouabain. Released inorganic phosphate (Pi) was measured according Fiske and Subbarow [32]. The color reaction was assayed spectrophotometrically at 650 nm. Results were expressed as nmol Pi/mg protein/min.

2.6.3.5 Ca^{2+} - ATPase activity

Ca^{2+}-ATPase activity was measured as previously described [33] with minor modifications [31]. The Ca^{2+}-ATPase activity was assayed in an incubation medium comprising 30 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 3 mM ATP, and 50 μL of S2. The activity was determined by subtracting the activity measured in the presence of Ca^{2+} from that was determined in the absence of Ca^{2+} (no added Ca^{2+} plus 0.1 mM EDTA). The enzyme activities were expressed in nmol of Pi/min/mg of protein.

2.5.3.7 Reactive Species (RS) levels

The RS levels were determined using a spectrofluorimetric method, using 2′,7′-dichlorofluorescein diacetate (DCHF-DA) assay according to Loetchutinat et al. [34]. The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 nm (with 488 nm excitation) 60 min after the addition of DCHF-DA to the medium. RS levels were expressed as arbitrary units of fluorescence.

2.6.3.8 Superoxide Dismutase (SOD) activity

SOD activity was assayed spectrophotometrically [35]. This method is based on the capacity of SOD to inhibit autoxidation of 4 mM epinephrine (pH 2.0 to pH 10.0). Briefly, S1 was diluted 1:10 (v/v) for determination of SOD activity. S2 aliquot was added to a 0.05 M Na_2CO_3 buffer, and enzymatic reaction was started by adding of epinephrine. The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 37°C. Results were expressed as Unit (U)/mg protein**.**

2.6.3.9 **Protein quantification**

The protein concentration was measured by the method of Bradford [36], using bovine serum albumin as the standard.

2.7 Statistical analysis

The normality of the data was evaluated using the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism 7.0 software (San Diego, CA, USA). Statistical analysis was performed using one-way ANOVA (for the evaluation of the acute antinociceptive effects of MTDZ) or two-way ANOVA (for data from the evaluation of the MTDZ effects on OXA-induced peripheral neuropathy and its comorbidities and biochemical assays) followed by Tukey's multiple comparisons test. Data were expressed as mean ± standard error of the mean (S.E.M.). Main effects are presented only when the higher second order interaction was non-significant. Values of $P < 0.05$ were considered statistically significant. Pearson's correlation coefficient was used for correlation analysis.

3. Results

3.1 Evaluation of the acute antinociceptive effects of MTDZ

3.1.1 Formalin-induced nociception

MTDZ showed antinociceptive activity in the neurogenic and inflammatory phases of the formalin test, when compared with the control group (Fig. 3a and 3b, respectively). In the neurogenic phase, the animals pretreated with MTDZ, at the doses of 1 and 10 mg/kg, reduced the licking time (around 56 %) (Fig. 3a). Meloxicam (10 mg/kg), the reference drug, was not able to reduce the formalin-induced licking time in the first phase, when compared to the control group.

In the inflammatory phase, the MTDZ, at the doses of 1 and 10 mg/kg, reduced the licking time (around 57%) when compared with the control group (Fig. 3b). At the

same time, meloxicam (10 mg/kg), in the second phase of the test, did not reduce formalin-induced paw licking and bite time when compared to the control group.

MTDZ markedly reduced the formalin-induced paw edema formation by 35 % (1 mg/kg) and 33 % (10 mg/kg), when compared with the control group (Fig. 3c). Meloxicam (10 mg/kg) reduced the formalin-induced paw edema formation by 32%.

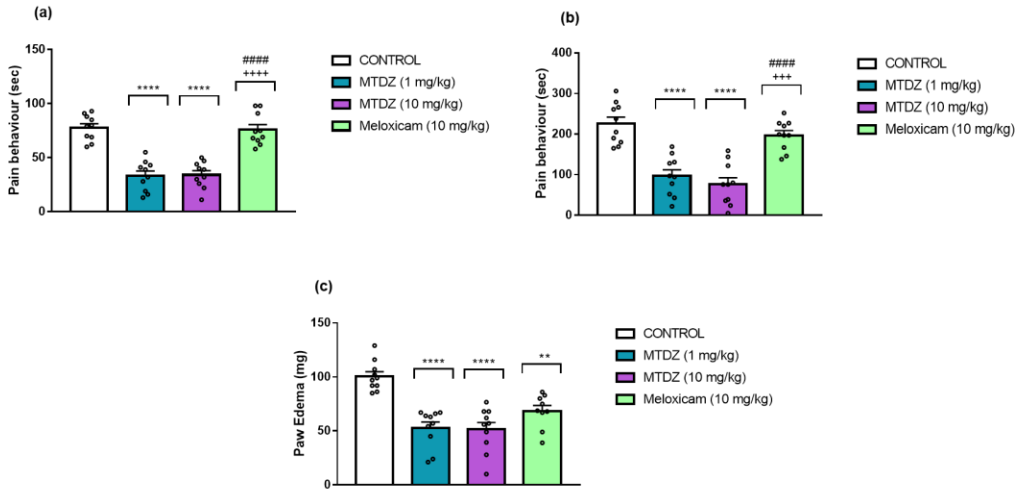

Fig. 3 - Effects of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) on formalin-induced nociception. Inhibition of the formalin-induced pain behaviour (sec) in the neurogenic (a) and inflammatory (b) phases, and (c) paw edema (mg). Data are reported as mean ± S.E.M of 10 animals per group. Statistical analysis was performed by one -way ANOVA followed by Tukey's test. (**) denotes $P < 0.01$, (****) denotes $P < 0.0001$ when compared with the control group. ($^{\#\#\#\#}$) denotes $P < 0.0001$ when compared with the MTDZ group (1 mg/kg). ($^{+++}$) denotes $P < 0.001$ and ($^{++++}$) denotes $P < 0.0001$ when compared with the MTDZ group (10 mg/kg).

3.1.2 Hot Plate Test

Effects of acute treatment of the MTDZ on response latency to thermal stimulus in the hot-plate test in mice are demonstrated in Figure 4. The acute treatment with MTDZ (1 and 10 mg/kg) (Fig. 4) decreased the response latency to thermal stimulus in comparison with the control group in 97% and 78%, respectively. Morphine, a drug with a central effect used as a reference for the hot plate test, at the dose of 10 mg/kg, did not present an antinociceptive response to thermal stimulation when compared to the control group. MTDZ (1 and 10 mg/kg) was more effective in reducing the nociceptive response to thermal stimulus than morphine (10 mg/kg).

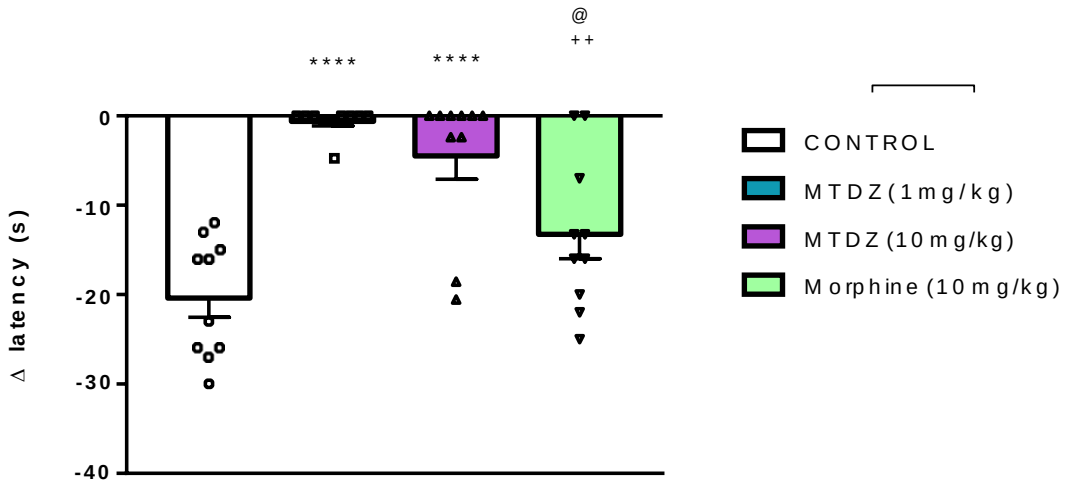

Fig. 4 - Effects of acute treatment of the 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) on response latency to thermal stimulus in the hot-plate test in mice. Data are reported as mean ± S.E.M of 10 animals per group. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. (****) denotes $P < 0.0001$ when compared with the control group. ($^{++}$) denotes $P < 0.01$ when compared with the MTDZ group (1 mg/kg). ($^{@}$) denotes $P < 0.05$ when compared with the MTDZ group (10 mg/kg).

3.1.3 Time–response curve of MTDZ on mechanical withdrawal threshold induced by Complete Freund's Adjuvant (CFA)

The results illustrated in Figure 5 indicate the antinociceptive effect exerted by the MTDZ against the inflammatory process triggered by CFA in the mechanical sensitivity test. The animals treated with MTDZ demonstrated a reduction in the mechanical hypersensitivity induced by CFA, with maximum inhibition of 91% and 102% after half an hour of treatment, at the doses of 1 and 10 mg/kg, respectively. In parallel, the meloxicam (10 mg/kg) reduced mechanical sensibility in 65%. In the period ranging from 0.5 hour to 6 hours after treatment, the compound MTDZ and meloxicam remain showing an antinociceptive effect in relation to the group that received CFA. Twenty-four hours after treatment with MTDZ (1 and 10 mg/kg) or meloxicam (10 mg/kg), no antinociceptive activity in the process triggered by the CFA was observed.

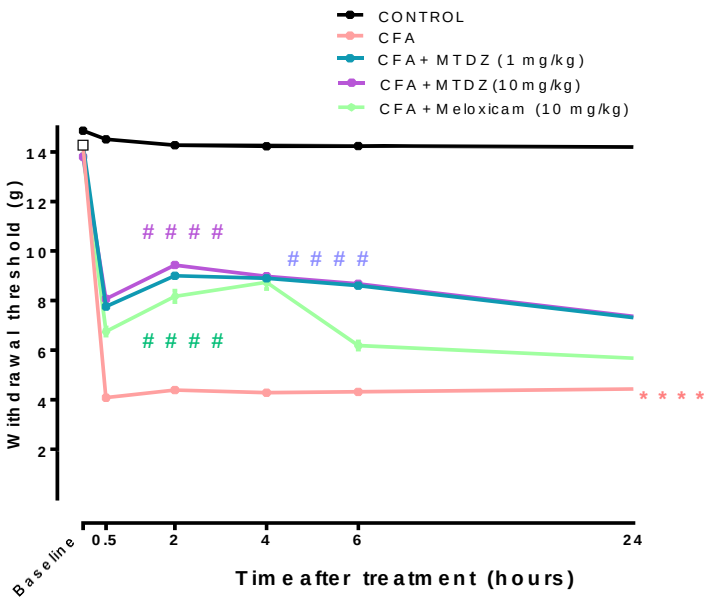

Fig. 5 – Effect of the 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) and Complete Freund's Adjuvant (CFA) on the paw withdrawal threshold to mechanical stimulus in the von Frey test. Data are reported as mean ± S.E.M of 10 animals per group. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. (****) denotes $P < 0.0001$ when compared with the control group. ($^{\#\#\#\#}$) denotes $P < 0.0001$ when compared with the CFA group (1 mg/mL).

3.2 Evaluation of the MTDZ effects on OXA-induced peripheral neuropathy and its comorbidities

3.2.1 Measurement of Mechanical Sensitivity

No difference in OXA-induced mechanical hypersensitivity was observed between male and female mice. On day 06, OXA exposure reduced the paw withdrawal threshold by 54% and 52% in males and females mice, respectively. Likewise, on day 13 of the experimental protocol, OXA reduced the paw withdrawal threshold by 49.5% and 53.5% in males and females, respectively. On days 06 and 13, treatment with MTDZ reversed the mechanical hypersensitivity induced by OXA, in both sexes.

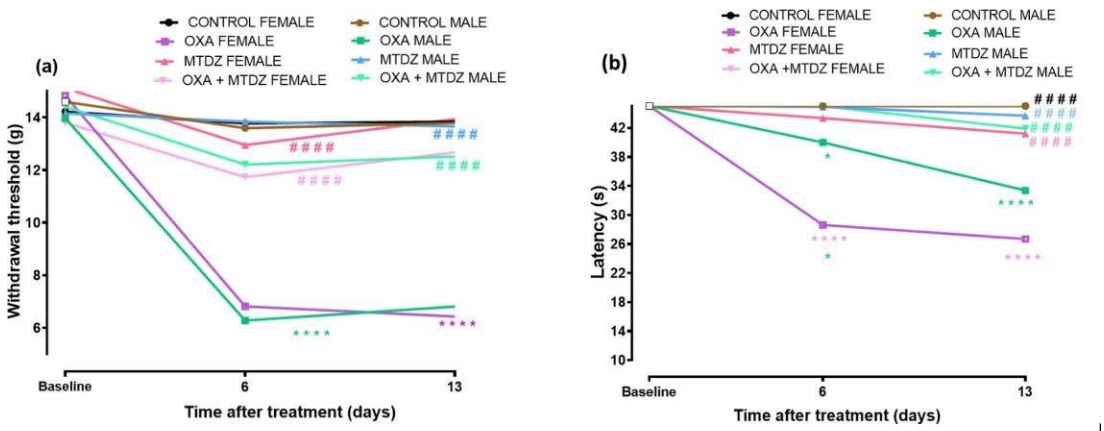

Fig. 6 - Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on the (a) paw withdrawal threshold to mechanical stimulus in the von Frey test and (b) on the latency to thermal stimulus in the hot plate test. Data are reported as mean ± S.E.M of 10 animals per group. Statistical analysis was performed by two-way ANOVA followed by Tukey's test. (*) denotes P < 0.05 and (****) denotes $P < 0.0001$ when compared with the control group. ($^{\#}$) denotes $P < 0.05$, ($^{\#\#\#}$) denotes $P < 0.001$ and ($^{\#\#\#\#}$) denotes $P < 0.0001$ when compared with the OXA group (10 mg/kg).

3.2.2 Measurement of Thermal Sensitivity

The hot plate test revealed that female mice are more sensitive to OXA-induced thermal nociception than male mice (about 28.5%) (Figure 6b). MTDZ protected against OXA-induced thermal hypersensitivity, regardless of sex.

3.2.3 Assessment of locomotor and exploratory domains and body weight

The number of crossings and rearing in the open field is presented in supplementary

material. The data analysis revealed that the treatment of mice with MTDZ and/or OXA did not cause any significant change in the number of crossings or rearing. Treatment with OXA caused the loss of body weight in male and female mice. The treatment of MTDZ (*per se*) did not alter the body weight gain (see in the supplementary material).

3.2.4 Assessment of cognitive domain

In the object recognition task, during the habituation phase, no significant difference among groups in the percentage of exploratory preference was observed (Table 1). OXA administration reduced (around 50% for male and female) the exploratory preference for the new object when compared with the control group (Table 1). At the same time, MTDZ treatment (1 mg/kg, p.o.) reversed the reduction in this memory parameter induced by OXA.

In LTM, OXA administration reduced the exploratory preference of male (71%) and female (41.5%) mice when compared with the control group (Table 1). MTDZ treatment restored the percentage of preference for a novel object when compared with the OXA group, regardless of gender.

Table 1. Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on habituation phase, short-term and long-term memories in the object recognition task.

Groups	Habituation phase (exploratory preference %)		Short-term memory (exploratory preference %)		Long-term memory (exploratory preference %)	
	Male	Female	Male	Female	Male	Female
Control	56.6 ± 1.2	49.2 ± 2.2	79.3 ± 3.3	74.9 ± 3.9	61.6 ± 3.9	61.1 ± 4.0
OXA	54.8 ± 4.6	49.3 ± 1.9	39.9 ± 4.1 ****	38.5 ± 5.7 ****	17.9 ± 3.5 ***	35.6 ± 2.0 **
MTDZ	49.4 ± 1.6	52.9 ± 1.2	76.8 ± 4.0####	71.2 ± 3.3####	59.7 ± 5.2	60.1 ± 3.1
OXA + MTDZ	51.7 ± 2.0	55.8 ± 2.0	74.0 ± 3.8####	75.3 ± 3.9####	64.4 ± 5.0####	57.9 ± 3.4##

Data are reported as mean ± S.E.M of 9 to 10 animals per group. Statistical analysis was performed by two -way ANOVA followed by Tukey's test. (**) $P < 0.01$, (***) $P < 0.001$ and (****) $P < 0.0001$ denotes significance levels when compared with the control group. ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with OXA group.

3.2.4 Assessment of emotional domain

No difference on emotional domain was observed between male and female mice. OXA reduced the percentage of time spent in the open arms (Table 3), percentage of open arm entries (Table 2) and number of dives (Table 2) by 80%, 71% and 77%, respectively for male, and 87 %, 80% and 75%, respectively for female, when compared OXA group with the control group. MTDZ treatment (1 mg/kg, p.o.) reversed the decrease of the percentage of time spent in the open arms, of open arm entries and number of dives altered by the treatment with OXA in the elevated plus-maze test, regardless of gender.

Table 2. Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on behavioural parameters in the elevated plus-maze test in mice.

Groups	% Time spent in the open arms		% Open arm entries		Number of dives	
	Male	Female	Male	Female	Male	Female
Control	30.2 ± 2.9	28.9 ± 3.2	35.0 ± 3.0	36.0 ± 3.5	8.2 ± 0.8	7.5 ± 1.4
OXA	5.9 ± 1.4****	3.7 ± 1.0****	10.2 ± 1.3****	7.1 ± 1.6****	1.8 ± 0.4***	1.9 ± 0.6***
MTDZ	23.4 ± 2.9 $^{\#\#\#}$	25.3 ± 2.7 $^{\#\#\#}$	30.2 ± 3.0$^{\#\#\#\#}$	30.6 ± 3.1 $^{\#\#\#\#}$	6.2 ± 1.2$^{\#}$	6.4 ± 1.1$^{\#}$
OXA + MTDZ	22.8 ± 2.0 $^{\#\#\#}$	21.1 ± 2.6 $^{\#\#\#}$	27.2 ± 3.8 $^{\#\#\#}$	28.7 ± 4.1 $^{\#\#\#}$	8.4 ± 1.3 $^{\#\#\#}$	6.7 ± 0.4 $^{\#}$

Data are reported as mean ± S.E.M of 9 to 10 animals per group. Statistical analysis was performed by two -way ANOVA followed by Tukey's test. (***) $P < 0.001$ and (****) $P < 0.0001$ denote significance levels when compared with the control group; ($^{\#}$) $P < 0.05$, ($^{\#\#\#}$) $P < 0.001$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with OXA group.

3.3 Biochemical Assays

3.3.1 AChE activity

As shown in Figure 7a, an increase around of 49% (male) and 36% (female) in the activity of the AChE was observed in the cerebral cortex of mice exposed to OXA, when compared with the control group. In contrast, treatment with MTDZ (1 mg/kg, p.o.) was able to normalize the AChE activity of male and female mice.

Similarly, in the hippocampus an increase in AChE activity around 125% (males) and 95% (females) was observed in animals that received treatment with OXA when compared with the control group. MTDZ treatment normalized the hippocampal AChE activity in both sexes (Figure 7b).

In the spinal cord of animals exposed to OXA there was an increase in the activity of the AChE enzyme in 68% (males) and 120% (females) when the data were compared with the control group. AChE activity was normalized by MTDZ treatment (Figure 7c).

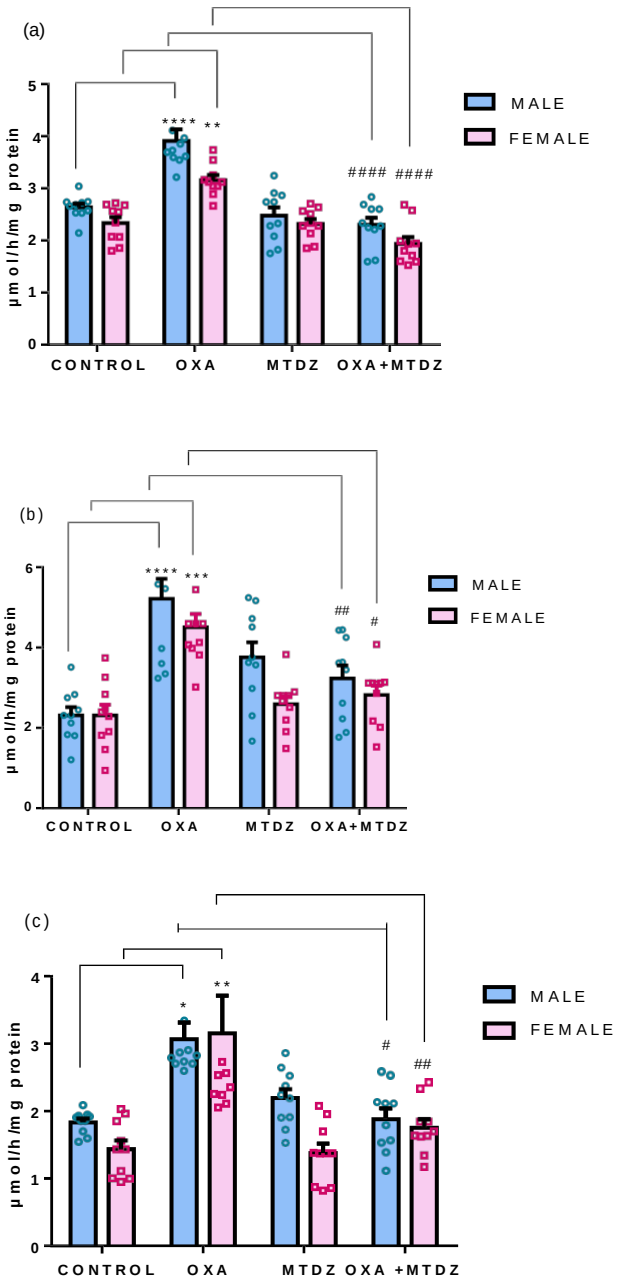

Fig. 7 - Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on the AChE activity in the (a) cerebral cortex, (b) hippocampus and (c) spinal cord. Data are reported as mean ± S.E.M of 10 animals per group. Statistical analysis was performed by two -way ANOVA

followed by Tukey's test. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ and (****) $P < 0.0001$ denote significance levels when compared with the control group; ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with OXA group.

3.3.1.1 Correlation between AChE activity and paw withdrawal threshold after OXA treatment

Pearson's correlation test was performed to test the correlation between AChE activity and the paw withdrawal threshold observed in the OXA-induced mechanical hyperalgesia test. The results illustrated in Fig. 8a showed the correlation coefficients between the AChE activity (µmol/h/mg protein) in the cerebral cortex of male and female mice and the mechanical hyperalgesia (r= -0.223; $P < 0.0001$). Fig. 8b showed the correlation coefficients between the AChE activity (µmol/h/mg protein) in the hippocampus of male and female mice and the paw withdrawal threshold (g) (r = -0.6705; $P < 0.0001$). Fig. 8c showed the correlation coefficients between the AChE activity (µmol/h/mg protein) and the paw withdrawal threshold (g) in the spinal cord of male and female mice (r = -0.7152; $P < 0.0001$). Indeed, all evaluated parameters were negatively correlated. In this case, the negative correlation of the data demonstrates an inversely proportional relationship between the paw withdrawal threshold (g) and AChE activity. In other words, the greater the AChE activity, the lower the paw withdrawal threshold and, consequently, greater mechanical sensitivity.

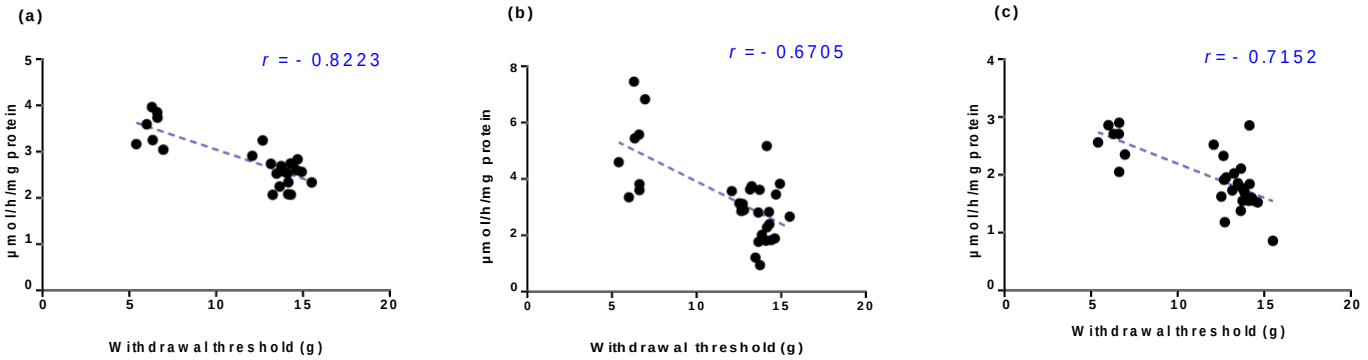

Fig. 8 - Correlation between AChE activity and paw withdrawal threshold after OXA treatment. Correlation coefficients between the withdrawal threshold (g) and the activity of the AChE expressed in µmol/h/mg protein in the cerebral cortex (a) hippocampus (b) and spinal cord (c). Pearson correlation (r) and *P*-value were used to verify the correlation between data.

3.3.2 ATPases activity

OXA inhibited the activity of the total ATPases in cerebral cortex of male (48%) and female (74%) mice (Fig. 9a); and in the spinal cord of male (48%) and female (36%) mice (Fig. 9b), when compared with the control group. MTDZ (1 mg/kg, p.o.) treatment was able to restore the activity of ATPases in the cerebral cortex and spinal cord of both sexes of mice.

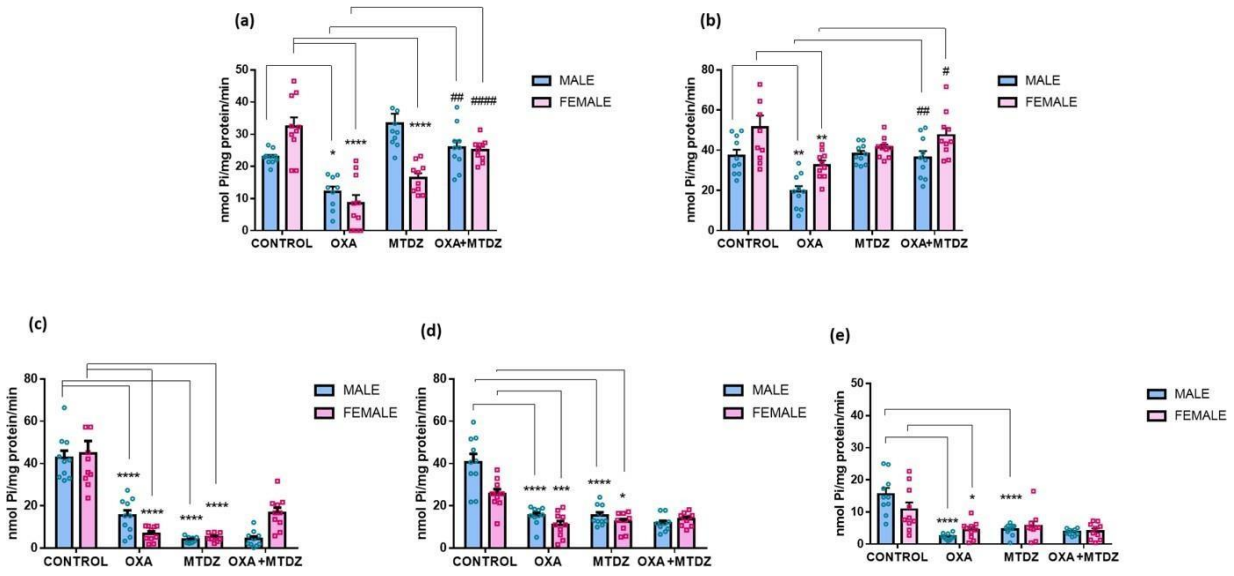

Fig. 9 - Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on the ATPases activity in the (a) cerebral cortex and (b) spinal cord and the Na^+ K^+ - ATPase activity in the (c) cerebral cortex, (d) hippocampus, (e) spinal cord. Data are reported as mean ± S.E.M of 9 to 10 animals per group. Statistical analysis was performed by two-way ANOVA followed by Tukey's test. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ and (****) $P < 0.0001$ denote significance levels when compared with the control group. ($^{\#}$) $P < 0.05$, ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with the OXA group.

3.3.3 Na^+, K^+ - ATPase activity

OXA exposure inhibited the activity of Na^+, K^+ - ATPase in the cerebral cortex (64% for male and 85% for female mice) (Fig. 9c); in the hippocampus (62% for male

and 57% for female mice) (Fig. 9d) and in the spinal cord (85% for male and 59% for female) (Fig. 9e) of mice, when compared with the control group. MTDZ (1 mg/kg, p.o.) treatment was unable to normalize the Na^+, K^+ - ATPase activity in the analyzed tissues.

3.3.4 Ca^{2+} - ATPase activity

As shown in Fig. 10a, OXA exposure inhibited the activity of the Ca^{2+}- ATPase in the cerebral cortex of mice (65% for male and 73% for female) when compared with the control group. In parallel, in the spinal cord (Fig. 10b) of the animals treated with OXA, an inhibition of the Ca^{2+}- ATPase activity was observed in both male (69%) and female (59%) mice. MTDZ (1 mg/kg, p.o.) treatment was not able to normalize this enzyme activity in any of the analyzed structures.

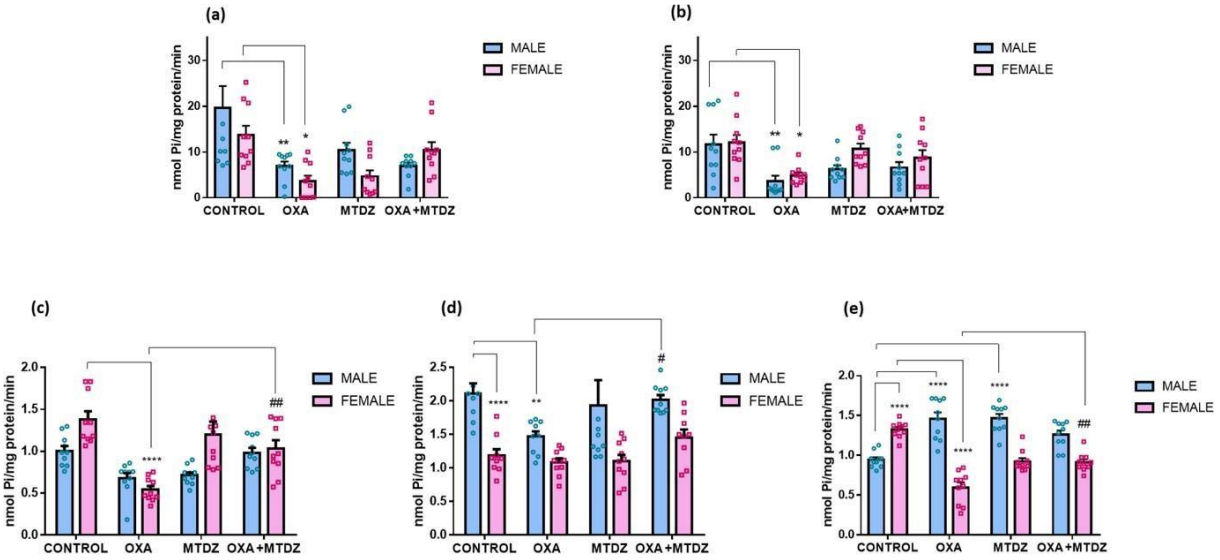

Fig. 10 - Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on the Ca^{2+} - ATPase activity in the

(a) cerebral cortex, (b) spinal cord and Mg^{2+} -ATPase activity in the (c) cerebral cortex, (d) hippocampus and (e) spinal cord. Data are reported as mean ± S.E.M of 9 to 10 animals per group. Statistical analysis was performed by two-way ANOVA followed by Tukey's test. (*) $P < 0.05$, (**) $P < 0.01$ and (****) $P < 0.0001$ denote significance levels when compared with the control group. ($^{\#}$) $P < 0.05$ and ($^{\#\#}$) $P < 0.01$ denote significance levels when compared with the OXA group.

3.3.5 Mg^{2+} *- ATPase activity*

The treatment with OXA inhibited the activity of Mg^{2+} - ATPase in the cerebral cortex, hippocampus and spinal cord of mice. In the cerebral cortex (Fig. 10c), a decrease in Mg^{2+} - ATPase enzyme activity was observed in females (about 61%) treated with OXA specifically. On the other hand, in the hippocampus (Fig. 10d) there was a reduction in enzyme activity in male mice treated with OXA (30.5%). MTDZ (1 mg/kg p.o) normalized the Mg^{2+} - ATPase activity in cerebral cortex of female, hippocampus of male and spinal cord of female (Fig. 10c and d).

In the spinal cord (Fig. 10e), OXA treatment increased the Mg^{2+} - ATPase activity of male mice (54.4%) and inhibited this enzyme activity of female mice (55%). The treatment with MTDZ only normalized the spinal cord Mg^{2+} - ATPase of female mice.

3.3.6 RS levels

Data analysis revealed an increase in the levels of RS in the cerebral cortex (143% for male and 45% for female) (Fig. 11a), hippocampus (258% for male and 171% for female) (Fig. 11b) and spinal cord (65% for male and 46% for female) (Fig. 11c) of mice

exposed to OXA, when compared with mice of the control group. The results showed that treatment with MTDZ (1 mg/kg, p.o.) reduced the RS levels in the cerebral cortex of male (44%), in the hippocampus of male (65%) and female (46%), and in the spinal cord of male (37%) mice.

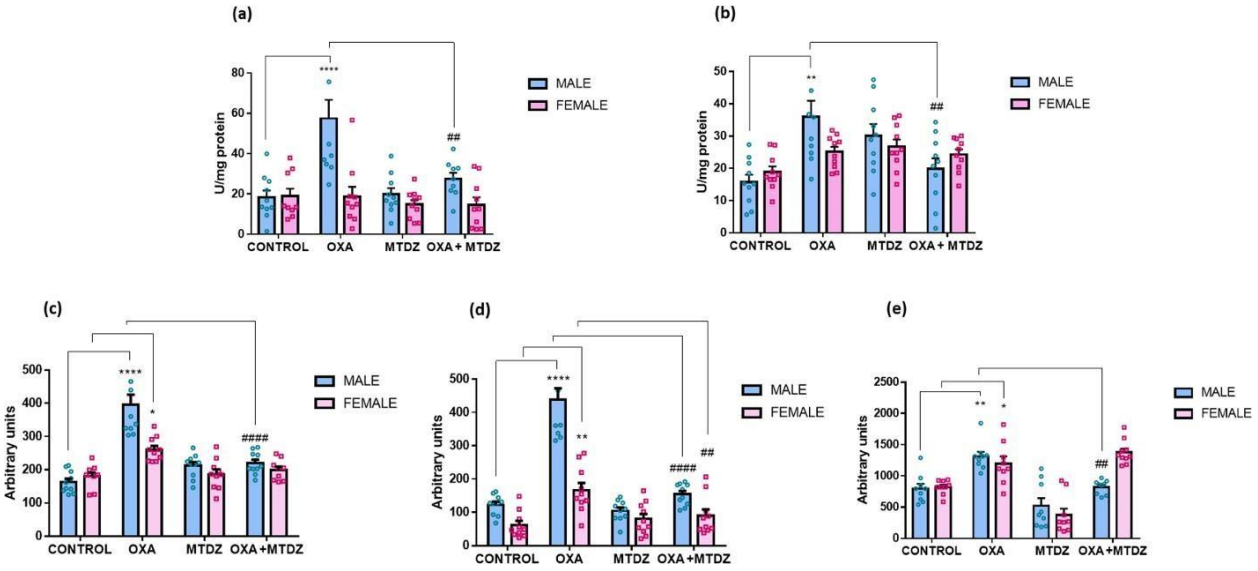

Fig. 11 – Effect of 5-((4 methoxyphenyl)thio)benzo[c][1,2,5] thiodiazole (MTDZ) (1 mg/kg, p.o.) and oxaliplatin (OXA) (10 mg/kg, i.p.) on the reactive species (RS) levels in the (a) cerebral cortex, (b) hippocampus and (c) spinal cord and the superoxide dismutase (SOD) activity in the (d) cerebral cortex and (e) hippocampus. Data are reported as mean ± S.E.M of 9 to 10 animals per group. Statistical analysis was performed by two-way ANOVA followed by Tukey's test. (*) $P < 0.05$, (**) $P < 0.01$ and (****) $P < 0.0001$ denote significance levels when compared with the control group. ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#\#}$) $P < 0.0001$ denote significance levels when compared with the OXA group.

3.3.7 SOD activity

As shown in Fig. 11d and 11e, OXA administration increased the SOD activity in the cerebral cortex (214%) and hippocampus (128%) of male mice. Female SOD activity was not altered by OXA exposure. MTDZ (1 mg/kg, p.o.) restored the cerebral cortex and hippocampus SOD activity of male mice exposed to OXA at the control levels.

4. Discussion

Results of the present study confirmed, for the first time, the hypothesis that MTDZ exerts antinociceptive action and reduces the OXA-induced peripheral neuropathy, anxiety, and cognitive deficits by inhibiting AChE activity. Indeed, a previous study revealed the compound MTDZ as a cholinergic modulator, by its ability to inhibit the AChE activity [16]. Furthermore, our findings indicated the pharmacological modulation of ion pumps as potential targets for OXA-induced neurotoxicity. MTDZ normalizes the total ATPase and Mg^{2+} - ATPase activities and it reduces the oxidative stress induced by OXA exposure in mice.

Our results in the formalin test, demonstrated that MTDZ reduced the nociceptive behaviour in the neurogenic and inflammatory phases and showed an anti-edematogenic effect by reducing the formation of paw edema. These results suggest that MTDZ could be inhibiting the direct activation of the nociceptive fibers, which release substance P, glutamate, nitric oxide, aspartate, glutamate, prostacyclins and prostaglandins, which stimulate the spinal cord and cause pain and edema [37,39]. Here, considering the obtained results, MTDZ showed more promising effects in the neurogenic and inflammatory phases in the formalin test than meloxicam, a reference drug for the treatment of pain and inflammation. In paw edema, the anti-edematogenic responses of

treatments (MTDZ and meloxicam) were similar. These findings are in agreement with studies that reported the therapeutic effect of sulfur-containing compounds against states of nociception and inflammation [18,40].

To assess the central-level effect of the MTDZ, the hot plate test was performed. MTDZ reduced the nociceptive behaviour from the thermal stimulus in the hot-plate test, suggesting central action of this compound. The thermal stimulus in the hot-plate is a reliable model to investigate the central effect of analgesic drugs with selectivity for opioid-like drugs [41,42]. Comparing to morphine (10 mg/kg), the compound MTDZ has a greater antinociceptive effect in this test. Results like this reinforce the promising antinociceptive effect that MTDZ triggers. Together, results of formalin and hot-plate tests indicate that the MTDZ exerts antinociceptive effect at the peripheral and central levels.

Of particular importance in this study, MTDZ also reduced the inflammatory process triggered by CFA in the mechanical sensitivity test. Given that CFA induces a stable local inflammatory response with severe pain around the injected area [43], it was possible to infer that MTDZ could be a promising molecule for inflammatory pain treatment. In addition, the antinociceptive effect of MTDZ is fast and of long lasting (0.5 to 6-24 hours).

Considering these results, a model of peripheral neuropathy was performed to broaden the understanding of the antinociceptive activity of MTDZ in male and female mice. All harmful effects induced by OXA can damage sensory neurons, which leads to neuropathic pain characterized by an increase in mechanical and thermal sensitivity [44]. Of particular importance in this study, female showed a greater sensibility to

thermal stimulus after OXA exposure than male mice. A recent study revealed a physiological change in pain in female rodents by botezomib, a chemotherapeutic agent [45]. However, no study to date has reported the relationship of thermal sensitivity between male and female mice exposed to OXA. In view of this, it is the first time that OXA-induced exacerbation of thermal sensitivity in female mice has been reported. Indeed, studies in rodents have shown greater pain sensitivity in females due to greater vulnerability to oxidative stress [46,47]. Importantly, our results revealed MTDZ as a promisor treatment to mechanical and thermal sensitivities caused by OXA, regardless of gender.

Given that OXA-induced peripheral neuropathy is a complex disorder, it is seems to be associated with a decline in cognitive function and emotional disorders [3,26]. Our results are in line with others showed that OXA reduced preference for the novel object in the object recognition task [26,49]. MTDZ reversed the cognitive impairment generated by OXA in male and female mice. We hypothesize that the modulation of AChE by MTDZ contributed to this pharmacological effect. Impaired cholinergic neurotransmission is known to contribute to cognitive dysfunction [50,51]. Thus, therapies that can minimize OXA-induced cognitive damage can be focused on modulating AChE.

Here, results of elevated plus-maze test showed that OXA treatment caused an anxious-like behaviour in both male and female mice. These results are in agreement with a previous study that demonstrated anxiety caused by OXA in male mice [26]. Also, recent studies have addressed that the risk of developing anxiety and other psychological disorders is higher in colorectal cancer survivors [52,53]. Changes in

AChE were related to cognitive decline and anxious-like behaviours in different experimental models [21,58,59]. In fact, in a previous study it was shown that OXA caused alterations in the activity of the AChE enzyme [14]. However, very little about the correlation between changes in the cholinergic pathway and neuropathic pain has been reported, and there are even few scientific approaches that can discuss changes in ion pumps necessary for the cholinergic synapse and neurotoxicity triggered by OXA.

In the present study, we observed that OXA increased AChE activity in the cerebral cortex, hippocampus, and spinal cord of male and female mice. Our results are in agreement with Ferrier et al. [60] that demonstrated OXA reduced ACh levels and increased choline levels in the cerebral cortex of rats. Likewise, increased AChE activity in the cerebral cortex of mice exposed to OXA has been reported, suggesting that it can be implicated in the pathophysiology of OXA-induced peripheral neuropathy and comorbidities [14]. It is important to emphasize that a negative correlation between AChE and paw withdrawal threshold after treatment with OXA was observed. While a decrease in the paw withdrawal threshold is observed, showing mechanical hyperalgesia, an increase in the activity of the AChE enzyme is verified. It is one of the most relevant findings of our study since peripheral neuropathy remains the most common adverse effect in patients exposed to OXA and, therefore, it suggests a pathophysiological mechanism involved in the neurotoxicity caused by OXA and justify the MTDZ pharmacological potential in this experimental model.

In the present study, we also focused our efforts on observing the activity of ATPases. We found that OXA treatment caused a decrease in total ATPase activity in the cerebral cortex and spinal cord of male and female mice. Studies evaluating the

effect of OXA in brain ATPase activities are scarce; however, Waseem et al. [61] observed the mitochondrial damage evidenced by the decrease in the activity of total ATPases in the liver of rats. The treatment with OXA, by damaging the activity of total ATPases, can lead to impairment of energy-dependent metabolic functions to occur and further reduce the electrical potential of cells [61]. Furthermore, a recent study by our research group demonstrated the accumulation of platinum in the spinal cord of mice exposed to OXA [14] and the accumulation of metals may be associated with inhibition of the activity of total ATPases [62]. Even so, the MTDZ compound showed important activity by reversing the inhibition of total ATPases activity in the cortex and spinal cord.

Also equivalent to this result, we evaluated the activity of Na^+ K^+ - ATPases responsible for the regulation of neuronal cell volume in neuronal and synaptic plasticity. Our results demonstrated that OXA inhibited the enzymatic activity of Na^+ K^+ - ATPase in cerebral cortex, hippocampus, and spinal cord of male and female mice. Indeed, the inhibition of Na^+ K^+- ATPase activity has been considered an important factor for the progression of peripheral neuropathy caused by OXA [63,64]. Given these results, we hypothesized that the damage to ATPases also interferes with cholinergic activity.

Our results are in agreement with other experimental studies that observed a inhibition of Na^+ K^+ - ATPase activity after exposure to OXA [26,65]. Although the modulation of Na^+ K^+ - ATPase is an important pharmacological target for the treatment of neurotoxicity caused by OXA, the compound MTDZ was not able to reverse the damage. Although it does not specifically modulate the enzyme Na^+ K^+ - ATPase,

MTDZ seems to compensate for the energetic and synaptic loss when it demonstrates an effect through the total ATPases.

Ca^{2+}- ATPase is crucial for calcium control and intracellular signaling [66]. In the present study, the inhibition of Ca^{2+}- ATPase activity in the cerebral cortex and spinal cord of male and female mice exposed to OXA was demonstrated. Our results suggest that inhibition of Ca^{2+} - ATPase activity by OXA administration can lead to cellular Ca^{2+} dysregulation affecting neuronal homeostasis and intracellular signaling pathways. In fact, previous studies have shown that oxalate (metabolite of OXA) is a calcium chelator. The action of oxalate on calcium levels has been associated as a determining factor for the development of neuropathic pain [67–69]. Despite this, it is the first time that the inhibition of OXA-induced Ca^{2+} - ATPase enzyme activity has been reported. According to Vizi [70], the absence of Ca^{2+} leads to an overproduction of ACh, which may be an explanation for the increased activity of the AChE enzyme.

In addition, MTDZ selectively modulated Mg^{2+} - ATPase in female cortex, male hippocampus, and female spinal cord, when the activity was altered. The concentration of Mg^{2+} also contributes to neurotoxicity and disorders associated with peripheral neuropathy [71]. Thus, we hypothesize that these may be specific mechanisms of action of MTDZ which can be explored for treatments that require specificity.

Considering that oxidative stress is one of the main mechanisms involved in peripheral neuropathy caused by OXA, we tried to observe some oxidative parameters [60,73]. Our results demonstrated that OXA induction leads to an exacerbation of RS in the cortex, hippocampus, and spinal cord of male and female mice. In fact, the increase in RS levels has been associated with the loss of enzymatic antioxidant defenses, which

may be responsible for the neurodegeneration and pain seen in peripheral neuropathy [74]. Importantly, although the mechanisms involved between cholinergic damage and the maintenance of neuropathic pain are little understood, it is assumed that somatosensory damage focusing on the cholinergic pathway may start in situations of oxidative stress [75]. Treatment with MTDZ was able to reverse the increase in RS levels in male cortex, male and female hippocampus, and male spinal cord.

In line with results obtained in a previous study of our research group [14], an increase in the activity of SOD, an important antioxidant defense involved in the conversion of superoxide anion to hydrogen peroxide, was observed in male, but not in female mice, exposed to OXA. The increase of SOD activity seems like a compensatory mechanism to high RS levels. It is important to emphasize that this result can partially explain the thermal hypersensitivity shown by females in relation to males. We hypothesize that the lag of SOD activity in females may be harming the conversion of the superoxide anion, increasing oxidative damage. In fact, studies have related thermal hypersensitivity to damage to antioxidant defenses [45,76]. MTDZ normalized the SOD activity in the cortex and hippocampus of male mice, corroborating the results of RS levels and hot-plate test.

5. Conclusion

Our study correlates, for the first time, paw withdrawal threshold and AChE activity and suggest MTDZ, an AChE inhibitor, as therapeutic strategy. Here, we revealed that the ion pumps inhibition seems to be involved in the toxicity induced by OXA exposure. The antinociceptive potential of the MTDZ was demonstrated using experimental models

of acute nociception and inflammation, as well as OXA-induced peripheral neuropathy in mice. MTDZ is a promising molecule for OXA-induced neurotoxicity since it reduced nociceptive behaviours and comorbidities associated with cognitive deficit and anxiety in male and female mice. Although this is an initial study aimed at investigating the effect of the MTDZ compound, we emphasize that combination therapies are necessary for the most effective management of chemotherapy-induced peripheral neuropathy treatment and its comorbidities. The pharmacological actions of MTDZ seem to be related to its antioxidant and anti-inflammatory activities, as well as its ability to modulate AChE and total ATPases. To summarize current knowledge, the present research has helped to expand the understanding of the mechanisms involved in the pathophysiology of OXA toxicity in male and female mice, as well as suggest a potential treatment to peripheral neuropathy and comorbidities associated to OXA exposure.

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7. Contribution Statement

K.P.M and E.A.W. conceived and designed research. B.F.S. and N.L.C.D. synthesized the compound. K.P.M. conducted experiments. K.P.M. and E.A.W. analyzed data. K.P.M. and E.A.W. wrote the manuscript. C.L. and E.A.W. supervised the experiments. All authors critically reviewed the content and approved the final version for publication.

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4.2 Capítulo 2 - Artigo científico

Canadian Science Publishing

ARTICLE

7-Chloro-4-(phenylselanyl) quinoline reduces renal oxidative stress induced by oxaliplatin in mice

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Abstract: The object of this study was to evaluate the relationship between oxidative damage induced by oxaliplatin (OXA) and the therapeutic potential of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) in kidney of mice. Mice received OXA (10 mg/kg) or vehicle intraperitoneally (days 0 and 2). Oral administration of 4-PSQ (1 mg/kg) or vehicle was performed on days 2 to 14. On day 15 the animals were euthanized and the kidneys and blood were collected. The effect of OXA and (or) 4-PSQ on urea, thiobarbituric acid reactive species, nonprotein thiol (NPSH), and protein carbonyl (PC) levels were investigated. Moreover, renal superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), δ-aminolevulinic acid dehydratase (δ-ALA-D), and Na^+,K^+ ATPase activities were evaluated. Our findings revealed an increase on urea levels and significant renal oxidative damage in OXA-induced mice. OXA exposure increased SOD, GPx, and GST activities and caused a reduction on NPSH levels and CAT and GR activities. Na^+,K^+ ATPase and δ-ALA-D activities were reduced by OXA. 4-PSQ decreased plasmatic urea levels and renal oxidative damage. SOD, GPx, CAT, GR, and Na^+,K^+ ATPase activities were restored by 4-PSQ. 4-PSQ may be a good prototype for the treatment of OXA-induced renal injury.

Key words: oxaliplatin, selenium, nephroprotection, quinoline, oxidative stress.

Résumé : Cette étude avait pour but d'évaluer le lien entre les dommages oxydatifs entraînés par l'oxaliplatine (OXA) et le potentiel thérapeutique de la 7-chloro-4-(phénylsélanyl) quinoline (4-PSQ) dans le rein chez la souris. Les souris ont reçu de l'OXA (à 10 mg/kg) ou le véhicule par voie intrapéritonéale aux jours 0 et 2. Nous avons procédé à l'administration orale de 4-PSQ (1 mg/kg) ou du véhicule aux jours 2 à 14. Au jour 15, nous avons euthanasié les animaux et prélevé les reins et du sang. Nous avons étudié l'effet de l'OXA et/ou de la 4-PSQ sur les taux de produits réactifs de l'acide thiobarbiturique, des thiols non protéiques (NPSH) et du carbonyl protéique (PC). En outre, nous avons évalué l'activité de la superoxyde dismutase (SOD), de la catalase (CAT), de la glutathion peroxydase (GPx), de la glutathion réductase (GR), de la glutathion S-transférase (GST), de la déshydratase de l'acide δ-aminolévulinique (δ-ALA-D) et de la Na^+/K^+ ATPase. Nos résultats ont révélé une augmentation des taux d'urée, et des dommages oxydatifs importants dans les reins des souris OXA. L'exposition à l'OXA entraînait une hausse de l'activité de la SOD, de la GPx et de la GST, ainsi qu'un abaissement des taux de NPSH de même que de l'activité de la CAT et de la GR. L'OXA entraînait un abaissement de l'activité de la Na^+/K^+ ATPase et de l'δ-ALA-D. Mais la 4-PSQ entraînait une diminution des taux d'urée plasmatique et des dommages oxydatifs rénaux. De fait, l'activité de la SOD, de la GPx, de la CAT, de la GR et de la Na^+/K^+ ATPase se rétablissait avec la 4-PSQ. En conclusion, la 4-PSQ pourrait constituer un bon prototype pour le traitement des lésions rénales entraînées par l'OXA. [Traduit par la Rédaction]

Mots-clés : oxaliplatine, sélénium, néphroprotection, quinoline, stress oxydatif.

1. Introduction

According to American Cancer Society, Surveillance and Health Services Research (Miller et al. 2016), chemotherapy treatment is used by 56% of patients with cancer; however, toxicity is a common adverse effect caused by several chemotherapeutic drugs and often results in cessation of therapy (Alexander et al. 2017; Bano and Ikram 2017; Watne 1970). Currently, according to the Global Burden of Disease Cancer Collaboration (Fitzmaurice et al. 2017), cancer is the second leading cause of death worldwide, and colorectal carcinoma represents the main cause of death for women with cancer. Also, this carcinoma is the second and third leading cause of cancer among women and men, respectively (Bray et al. 2018). Oxaliplatin (OXA), a platinum-based chemotherapeutic agent, is a key component of first line chemotherapy in colorectal carcinoma in both the adjuvant and metastatic contexts (Nichetti et al. 2019).

In this sense, platinum-based chemotherapeutic compounds are highly potent anticancer drugs and are widely used in the therapy. Nevertheless, platinum compounds cause a wide range

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of side effects including ototoxicity, hepatotoxicity, neurotoxicity, and nephrotoxicity (Almarzooqi et al. 2015; Bano and Ikram 2017; Kumaran et al. 2015; Starobova and Vetter 2017; Tabassum et al. 2015). Nephrotoxicity induced by treatment with platinum compounds, such as OXA, is a dose-limiting toxicity that negatively influences patients' quality of life.

Studies suggest that the OXA has acceptable nephrotoxicity as compared with cisplatin, however recent case reports suggest various forms of renal toxicity related to OXA, such as renal tubular vacuolization, tubular necrosis, renal tubular acidosis, and acute renal injury (Almarzooqi et al. 2015; Yaghobi Joybari et al. 2014). Indeed, cases of acute kidney injury after OXA administration were reported in patients (Vyskocil et al. 2019). According to recently published data, more studies are needed to investigate the mechanisms involved in the nephrotoxicity induced by OXA, with the aim to improve the tolerability of this cancer therapy (Hubbard 2016; Vyskocil et al. 2019).

The physiopathology processes induced by treatment with OXA are multi-factorial, including production of reactive species, altered mitochondrial function, and activation of apoptosis pathways (Colloca et al. 2017; Kanat et al. 2017; Starobova and Vetter 2017). Indeed, it has been reported that the OXA causes oxidative damage, and in this context, the oxidative stress also has been described as one of the main mechanisms involved in the development of toxicity induced by OXA in the kidneys (Kawashiri et al. 2019; Lu et al. 2019; Scatena et al. 2012). Thus, antioxidant molecules may be promising in the treatment or prevention of OXA-induced renal damage.

Thus, we highlight the 7-chloro-4-(phenylselanyl) quinoline (4-PSQ), a quinoline derivative containing selenium, which has several pharmacological properties already described in the literature including but not limited to antioxidant action (Barth et al. 2019; Pinz et al. 2016, 2018; Reis et al. 2017; Silva et al. 2017; Vogt et al. 2018; Voss et al. 2017). 4-PSQ elicits acute anti-inflammatory and antinociceptive effects that are correlated with its antioxidant property (Pinz et al. 2016) and to the modulation of serotonergic, nitrergic, and glutamatergic systems (Silva et al. 2017). Recently, we showed that this compound exerts memory enhancer actions in aging rats through modulation of cholinergic system and synaptic plasticity by enhancing the neural cell adhesion molecule and polysialyl transferase levels in the cerebral cortex and hippocampus (Barth et al. 2019). Equally important, 4-PSQ reduced glutamate uptake in cerebral cortices and protected against kainate-induced anxiety-related behaviour in mice (Reis et al. 2017). In addition, 4-PSQ revealed potential therapeutic advantage in the treatment and management of atopic dermatitis-like skin lesions in mice (Voss et al. 2017).

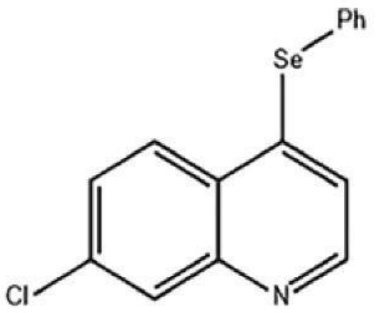
In view of our continued interest in the pharmacology of this compound and regarding (*i*) the high number of patients diagnosed with colorectal cancer; (*ii*) the need for more studies to investigate molecular mechanisms involved in the nephrotoxicity induced by treatment with OXA; and (*iii*) the demand for therapeutic alternatives that attempt to improve and (or) prevent the renal toxicity caused by cancer therapy with OXA; the aim of this study was to evaluate the effect of 4-PSQ against the renal toxicity induced by OXA. In this sense, parameters of the damage caused by renal and oxidative stress were investigated. In addition, we measured the involvement of sulfhydryl enzymes, which are sensitive to oxidative stress.

2. Materials and methods

2.1. Animals

The tests were carried out using male adult Swiss mice (25–30 g). Animals were maintained at 22 ± 2 °C with free access to water and food, under a 12-h light:dark cycle (with lights on at 0600).

Fig. 1. Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ).



2.2. Ethics statement

All experiments were performed in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEEA 4506-2017). Animals were treated in accordance with *Guide for the Care and Use of Laboratory Animals* (8th edition, National Academies Press).

2.3. Drugs

4-PSQ (Fig. 1) was prepared and characterized in our laboratory (Duarte et al. 2017). Nuclear magnetic resonance analysis (^{1}H and ^{13}C) showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9%) was determined by gas chromatography coupled with mass spectrometry (Duarte et al. 2017).

4-PSQ and OXA were dissolved in canola oil and in 5% glucose solution, respectively. OXA was purchased from commercial sources. All other chemicals used in this study were of analytical grade and obtained from standard commercial suppliers. Mice received 4-PSQ treatment by oral route (p.o., intragastric gavage) and OXA by intraperitoneal (i.p.) route, at a constant volume of 10 mL/kg of body weight.

2.4. Experimental protocol

The mice were randomly divided into four groups: (*i*) control; (*ii*) OXA; (*iii*) 4-PSQ; and (*iv*) OXA + 4-PSQ. On day 0 and day 2, the animals of the control and 4-PSQ groups received 5% glucose solution (10 mL/kg, i.p.), while the animals of the OXA and 4-PSQ + OXA groups received OXA (10 mg/kg, i.p.). On the second day, after 30 min of the treatment with OXA, mice from the control and OXA groups received canola oil (10 mL/kg p.o.), while mice of the 4-PSQ and 4-PSQ + OXA groups received 4-PSQ (1 mg/kg, p.o.), up to the fourteenth day. Twenty-four hours after the last treatment, the animals were anesthetized and euthanized by inhalation of isoflurane anesthetic. Blood and kidneys were collected and used for ex vivo assays. The dose of 4-PSQ and the treatment protocol were based on previous studies (Pinz et al. 2018; Reis et al. 2020). As other studies evaluating the pharmacological actions of the 4-PSQ have been carried out (Pinz et al. 2018; Reis et al. 2020) and to minimize the number of animals used, a dose–response curve was not performed in the present study.

2.5. Ex vivo assays

The kidney samples were collected to determine oxidative stress markers, such as thiobarbituric acid reactive species (TBARS), nonprotein thiol (NPSH), protein carbonyl (PC) levels, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD),

and catalase (CAT) activities. Furthermore, renal δ-aminolevulinic acid dehydratase (δ-ALA-D) and Na^+,K^+ ATPase activities were evaluated. For these analyses, samples were homogenized in 50 mmol/L Tris HCl pH 7.4, and centrifuged at 900*g* for 10 min to yield a supernatant (S1). Specifically, for PC assay for the renal samples were not centrifuged. Plasma was obtained by centrifugation at 900*g* for 10 min (hemolyzed plasma was discarded) and used for urea levels determination.

2.5.1. Urea levels

To evaluate the renal damage caused by OXA and the pharmacological potential of 4-PSQ, the level of plasmatic urea was determined (MacKay and MacKay 1927). This biochemical assay was performed using commercial test kit (Bioclin®). Urea levels were expressed in U/dL.

2.5.2. TBARS levels

An aliquot of S1 (200 μL) was added to the reaction mixture containing 500 μL TBA (0.8%), 200 μL sodium dodecyl sulfate (SDS, 8.1%), and 500 μL acetic acid (pH 3.4), and was incubated for 2 h at 95 °C. TBARS levels were determined in accordance with a method previously described in the literature (Ohkawa et al. 1979). Malondialdehyde reacts with TBA to generate a colored product that can be measured spectrophotometrically at 532 nm. Results were expressed as nmol malondialdehyde/g tissue.

2.5.3. PC content

An increase of carbonyl content is found in oxidatively-modified proteins (Yan et al. 1995). An aliquot of a diluted homogenate (Tris–HCl buffer, pH 7.4, in a proportion of 1:8 (*v*/*v*)) was mixed with 10 mM dinitrophenylhydrazine or 2 M HCl (Levine et al. 1990). After 1 h of incubation at room temperature in the dark, denaturing buffer 150 mM sodium phosphate buffer, pH 6.8, containing SDS (3%), heptane (99.5%), and ethanol (99.8%) were added. The tubes were shaken with a vortex mixer for 40 s and centrifuged at 900*g* for 15 min. Following this procedure, the pellet (protein isolated) was washed twice with ethyl acetate/ethanol 1:1 (*v*/*v*) and suspended in denaturing buffer. Absorbance was measured at 370 nm in a spectrophotometer. Total carbonylation was calculated using a molar extinction coefficient of 22 000 M^{-1}/cm^{-1}. Results were expressed as nmol carbonyl content/mg protein.

2.5.4. NPSH content

The NPSH content, an indirect measure of reduced glutathione (GSH) (Pês et al. 2016; Saccol et al. 2017), was evaluated using the method of Ellman (1959) and reported in μmol/g tissue. A sample of S1 was mixed (1:1, *v*/*v*) with 10% trichloroacetic acid (TCA). The samples were centrifuged at 900*g* for 10 min. After the centrifugation, the protein pellet was discarded, and free-thiol groups were determined in the clear supernatant. An aliquot of supernatant (200 μL) was added in Tris buffer (0.4 M, pH 8.9) and 10 mM 5,5′-dithiol-bis(2-nitrobenzoic acid). The color reaction was measured at 412 nm. Results were expressed as μmol NPSH/g tissue.

2.5.5. Antioxidant enzymes

2.5.5.1. SOD activity

SOD activity was assayed spectrophotometrically (Misra and Fridovich 1972). This method is based on the capacity of SOD to inhibit autoxidation of 4 mM epinephrine (pH 2.0 to pH 10.0). Briefly, S1 was diluted 1:10 (*v*/*v*) for determination of SOD activity. S1 aliquot was added to a 0.05 M Na_2CO_3 buffer, and enzymatic reaction was started by adding of epinephrine. The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 37 °C. Results were expressed as Unit (U)/mg protein.

2.5.5.2. CAT activity

CAT activity was assayed spectrophotometrically in accordance with a method previously described in the literature (Aebi 1984), which involves monitoring the consumption of hydrogen peroxide (H_2O_2) in the homogenate at 240 nm. The enzymatic reaction was initiated by adding an aliquot of S1 and the substrate (H_2O_2) to a concentration of 0.3 mmol/L in a medium containing 50 mM potassium phosphate buffer, pH 7.0. Results were expressed as U/mg protein (1 U decomposes 1 μmol H_2O_2/min at pH 7.0 at 25 °C).

2.5.5.3. GPx activity

GPx activity was assayed spectrophotometrically by the method of Wendel (1981), which involves monitoring the dismutation of H_2O_2 in the presence of S1 at 340 nm. S1 was added in a system composed of GSH, NADPH, and GR, and the enzymatic reaction was initiated by the addition of H_2O_2. In this assay, the enzymatic activity is indirectly measured by NADPH decay. H_2O_2 is reduced and generates oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by the GR present in the medium at the expense of NADPH. The enzymatic activity was expressed as nmol NADPH/min per mg protein.

2.5.5.4. GR activity

GR plays a role in maintaining adequate amounts of GSH. GR activity in S1 was determined spectrophotometrically (Carlberg and Mannervik 1985). In this assay, GR activity was determined through consuming NADPH to reduce GSSG; the oxidation of NADPH was followed for 2 min at 340 nm. Enzyme aliquots were preincubated in the presence of NADPH for 5 min to trace the nonenzymatic oxidation, after which GSSG was added to start the reaction. To correct for nonspecific oxidation of NADPH, the blank cuvette contained all the assay components except GSSG. Thus, GR activity is proportional to the decay of NADPH. Results were expressed as nmol NADPH/min per mg protein.

2.5.5.5. GST activity

GST plays a physiological role in initiating the detoxification of potential toxic agents and has antioxidant action. The reaction mixture contained an aliquot of the homogenized tissue (S1), buffer sodium phosphate (0.1 M, pH 7), GSH (100 mM), and 1-chloro-2, 4-dinitrobenzene (CDNB) (100 mM), which was used as a substrate. GST activity was assayed through the conjugation of GSH with CDNB at 340 nm (Habig et al. 1974). Results were expressed in nmol CDNB conjugated/min per mg protein.

2.5.6. Sulfhydryl enzymes sensitive to oxidative stress

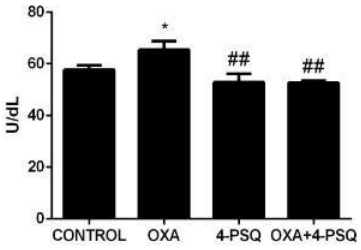
2.5.6.1. δ-ALA-D activity

δ-ALA-D activity was measured through the rate of product (porphobilinogen (PBG)) formation (Sassa 1982). An aliquot of S1 was preincubated for 10 min at 37 °C with 1 M potassium phosphate buffer, pH 6.8. The enzymatic reaction was initiated by adding the substrate aminolevulinic acid (ALA) and incubated for 1 h at 37 °C. The reaction was stopped by adding 10% TCA solution with 10 mM $HgCl_2$. The reaction product was measured at 555 nm using Ehrlich's modified reagent and values were expressed as nmol PBG/mg protein per hour.

2.5.6.2. Na^+,K^+ ATPase activity

The reaction mixture containing S1, 3 mM $MgCl_2$, 125 mM NaCl, 20 mM KCl, and 50 mM Tris/HCl, pH 7.4, in a final volume of 500 μL, was used. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Control samples were performed under the same conditions with the addition of 0.1 mM

Fig. 2. Effects of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on plasma urea levels of mice exposed to oxaliplatin (OXA). Each column represents mean ± standard error of the mean ($n = 7$). Statistical analysis was performed by one-way ANOVA followed by Newman–Keuls' test. (*) denotes $p < 0.05$ as compared with the control group and (##) denotes $p < 0.01$ as compared with the OXA group.



ouabain. The samples were incubated at 37 °C for 30 min and the incubation was stopped by adding 10% TCA with 10 mM $HgCl_2$. Enzyme activity was calculated from the difference between amounts of inorganic phosphate found after incubation in the absence and presence of ouabain. The color reaction was assayed spectrophotometrically at 650 nm. Results were expressed as nmol inorganic phosphate/mg protein per minute.

2.5.7. Protein quantification

The protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

2.6. Statistical analysis

The normality of the data was evaluated using the D'Agostino and Pearson omnibus normality test. Statistical analysis was performed using GraphPad Prism version 6.0 software (San Diego, CA, USA). Data were analyzed by one-way analysis of variance (ANOVA) followed by the Newman–Keuls test when appropriate. Data were expressed as mean ± standard error of the mean. Differences between group means were considered statistically significant at $p < 0.05$.

3. Results

3.1. Urea levels

Figure 2 shows the effects of OXA and (or) 4-PSQ treatments on plasmatic urea levels of mice. The results revealed that OXA administration, at the dose of 10 mg/kg, increased (13.4%) the plasmatic urea levels when compared with control group (one-way ANOVA: $F_{[3,24]} = 5.629$, $p = 0.0046$). Statistical analysis showed that 4-PSQ (1 mg/kg) treatment reversed the increase in urea levels caused by OXA exposure. Urea levels were not altered in plasma of mice treated only with 4-PSQ.

3.2. TBARS levels

Figure 3*a* demonstrates the TBARS levels in kidney of mice submitted to OXA and (or) 4-PSQ treatments. The OXA administration (10 mg/kg) increased the TBARS levels in the kidney by 24% when compared with control group. The analysis of the results revealed that 4-PSQ (1 mg/kg) reversed the TBARS levels increased in the kidney of OXA-induced mice (one-way ANOVA: $F_{[3,24]} = 6.816$, $p = 0.0018$). 4-PSQ alone did not change the TBARS levels in the kidney when compared with control group.

3.3. PC content

The effects of OXA and (or) 4-PSQ treatments on PC content are shown in Fig. 3*b*. OXA exposure (10 mg/kg) increased renal PC content by 89% when compared with control group. The analysis of the results revealed that 4-PSQ (1 mg/kg) reversed the PC content increase in the kidney of OXA-induced mice (one-way ANOVA: $F_{[3,24]} = 4.979$, $p = 0.0079$). No alteration on renal PC content in animals exposed only to 4-PSQ was observed.

3.4. NPSH content

Figure 3*c* illustrates the effects of OXA and (or) 4-PSQ treatments on the NPSH content in kidney of mice. Renal NPSH content was decreased by OXA (10 mg/kg) exposure by 17.7% when compared with the control group. 4-PSQ (1 mg/kg) treatment significantly reversed the decrease of NPSH content in the kidney of mice exposed to OXA (one-way ANOVA: $F_{[3,24]} = 4.388$ $p = 0.0135$). The 4-PSQ alone did not change the NPSH levels in the kidney.

3.5. Antioxidant enzymes

3.5.1. SOD activity

The effects of OXA and (or) 4-PSQ treatments on SOD activity in kidney of the mice are shown in Fig. 4*a*. The results demonstrated the induction with OXA (10 mg/kg) increased SOD activity by 93.6% when compared with control group (one-way ANOVA: $F_{[3,24]} = 5.178$; $p = 0.0067$). The 4-PSQ (1 mg/kg) treatment restored the SOD activity increased by OXA administration in kidney. No change on the renal SOD activity was verified after the treatment with 4-PSQ alone.

3.5.2. CAT activity

Figure 4*b* demonstrates the effects of OXA and (or) 4-PSQ treatments on the CAT activity in kidney of mice. OXA administration, at the dose of 10 mg/kg, decreased the CAT activity by 13% in kidney of mice (one-way ANOVA: $F_{[3,24]} = 19.55$; $p < 0.0001$). Analysis of the results revealed that 4-PSQ (1 mg/kg) significantly restored the CAT activity reduced by OXA administration in kidney. 4-PSQ increased (13.09%) the renal CAT activity when compared with control group.

3.5.3. GPx activity

The effects of OXA and (or) 4-PSQ treatments on renal GPx activity in mice are shown in Fig. 4*c*. OXA (10 mg/kg) exposure increased GPx activity by 35.6% compared with that mice from the control group. 4-PSQ reverted the increase of GPx activity in kidney of OXA-induced mice (one-way ANOVA: $F_{[3,24]} = 4.684$, $p = 0.0103$). GPx activity remained unaltered in the kidneys of mice that received only 4-PSQ (1 mg/kg).

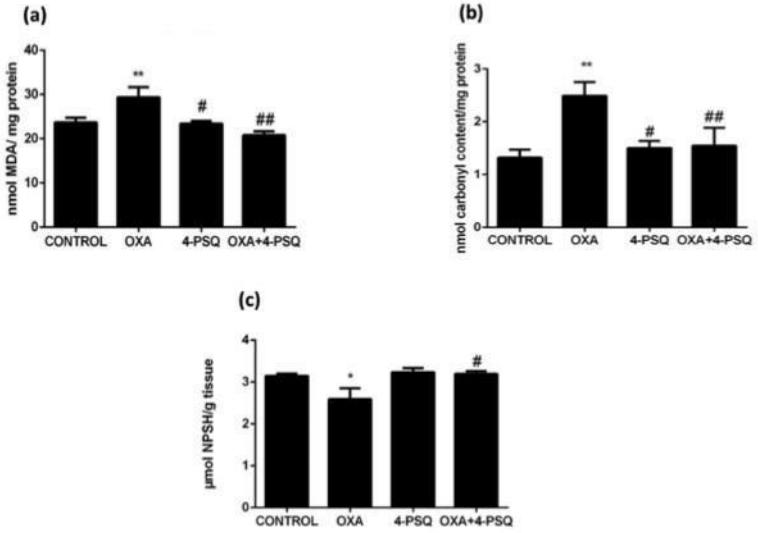
3.5.4. GR activity

Figure 4*d* shows the effects of OXA and (or) 4-PSQ treatments on the GR activity in kidney of mice. Results demonstrated that OXA (10 mg/kg) injections decreased GR activity in kidney (17.7%) when compared with the control group. 4-PSQ (1 mg/kg) treatment significantly restored the GR activity (one-way ANOVA: $F_{[3,24]} = 6.823$, $p = 0.0017$). No change on the renal GR activity was verified after the treatment only with 4-PSQ.

3.5.5. GST activity

Figure 4*e* demonstrates the effects of OXA and (or) 4-PSQ treatments on the GST activity in kidney of mice. OXA (10 mg/kg) administration increased (26%) the activity of GST in kidney of mice in comparison with the control group (one-way ANOVA: $F_{[3,24]} = 8.811$, $p = 0.0004$). Results showed that treatment with 4-PSQ (1 mg/kg) did not restore the renal enzyme activity to normal levels. Treatment with 4-PSQ per se increased the renal GST activity when compared with the control group.

Fig. 3. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on (*a*) renal thiobarbituric acid reactive species, (*b*) protein carbonyl, and (*c*) nonprotein thiol (NPSH) levels of mice exposed to oxaliplatin (OXA). Each column represents mean ± standard error of the mean from seven animals for each group. Statistical analysis was performed by one-way ANOVA followed by Newman–Keuls' test. (*) denotes $p < 0.05$ and (**) denotes $p < 0.01$ when compared with the control group. (#) denotes $p < 0.05$ and (##) denotes $p < 0.01$ when compared with the OXA group. MDA, malondialdehyde.



3.6. Sulfhydryl enzymes sensitive to oxidative stress

3.6.1. *δ-ALA-D activity*

Figure 5*a* illustrates effects of the administration of OXA and 4-PSQ, at the dose of 10 mg/kg, on δ-ALA-D activity in kidney of mice. OXA (10 mg/kg) administration decreased the renal δ-ALA-D activity by 38% when compared with control group (one-way ANOVA: $F_{[3,24]} = 3.483$, $p = 0.0314$). Statistical analysis revealed that 4-PSQ (1 mg/kg) treatment did not restore δ-ALA-D activity reduced by OXA exposure. The 4-PSQ per se did not change the δ-ALA-D activity in the kidney of mice.

3.6.2. *Na^+,K^+ ATPase activity*

The effects of OXA and (or) 4-PSQ treatments on Na^+,K^+ ATPase activity in mice are shown in Fig. 5*b*. Animals exposed to OXA (10 mg/kg) exhibited a reduction of 40.6% on the activity of renal Na^+,K^+ ATPase when compared with control group (one-way ANOVA: $F_{[3,24]} = 5.104$, $p = 0.0071$). OXA-induced alterations on the activity of the renal Na^+,K^+ ATPase were normalized to control levels by 4-PSQ (1 mg/kg) treatment. No alteration on renal Na^+,K^+ ATPase activity was observed after treatment only with 4-PSQ.

4. Discussion

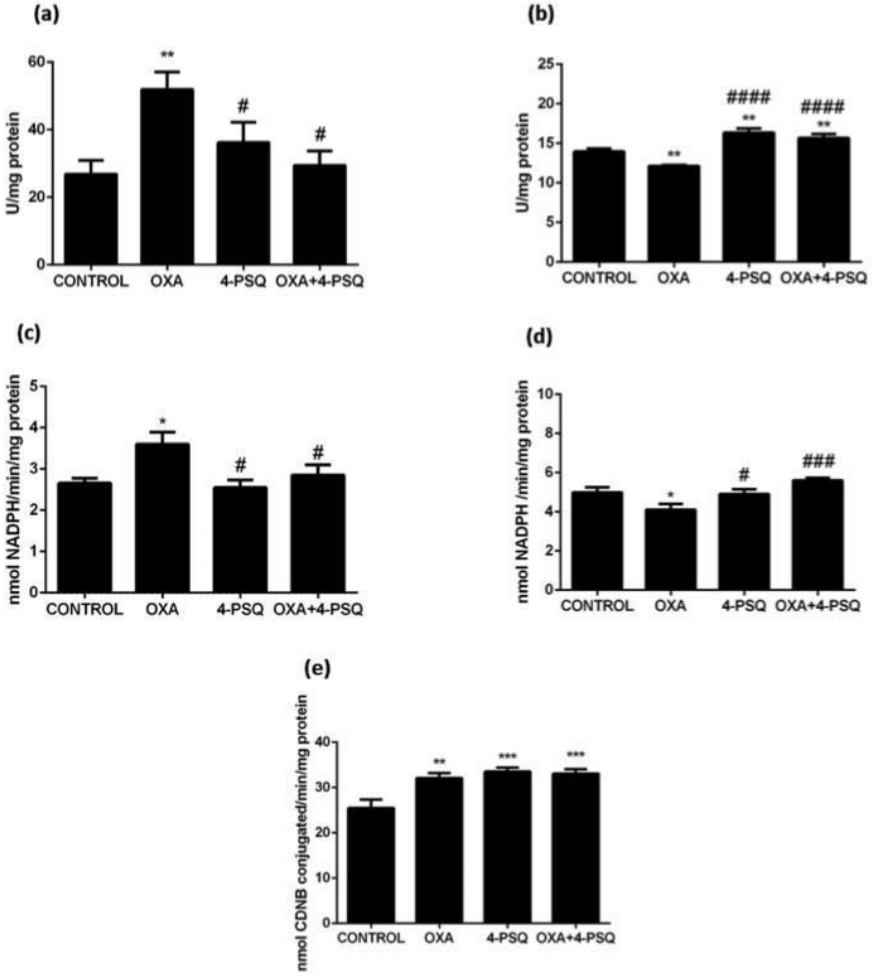
Studies suggest that the OXA has acceptable nephrotoxicity as compared with other platinum-based chemotherapeutic agents (de Gramont et al. 2000; Yaghobi Joybari et al. 2014); however, increasing researches have shown a high nephrotoxic potential of the OXA in cancer patients (Ito et al. 2012; Jain et al. 2015; Vyskocil et al. 2019). Investigating the mechanism of OXA nephrotoxicity induction, we hypothesized that treatment with 4-PSQ may reduce the nephrotoxic effects of this chemotherapeutic agent. Indeed, here we demonstrated that the administration of OXA caused renal injury, as evidenced by increase of urea levels in the plasma and oxidative damage in the kidney of mice. Taken together, the results of our study shown that 4-PSQ could be considered a protective compound in OXA-induced kidney injury, because this multi-target molecule decreased the plasmatic urea levels and renal oxidative damage as evidenced by reduction of lipid and protein oxidation. 4-PSQ restored the activities of SOD, GPx, CAT, GR, and Na^+,K^+ ATPase, as well as NPSH levels altered by OXA exposure.

In this study, the OXA administration showed severe side effects on mice kidney tissue. Essentially, the onset of renal insufficiency begins days after the nephrotoxic agent exposure and it is manifested by increased serum creatinine and urea levels (Miller et al. 2010; Rysz et al. 2017). Accordingly, results of the present study revealed an increase in plasma urea levels in mice exposed to OXA. These findings reflect impairments on the clearance of the nonprotein nitrogen substances indicating renal dysfunction. Indeed, the urea level, an important marker of acute kidney injury, is used for monitoring the renal function in patients receiving treatment with platinum-based chemotherapy drugs (Ozkok and Edelstein 2014).

To increase knowledge about OXA-induced renal toxicity, we investigate the mechanisms that could be involved in this damage. The harmful pathophysiological processes OXA induced are multi-factorial including upregulation of N-methyl D-aspartate receptors, production of reactive species resulting in altered mitochondrial function, and activation of apoptosis pathways. It has been reported that oxidative stress is implicated in the pathophysiology of numerous diseases (Colloca et al. 2017; Kanat et al. 2017; Starobova and Vetter 2017; Steller et al. 2018). Indeed, our findings demonstrated that OXA treatment caused oxidative damage in macromolecules such as lipids and proteins.

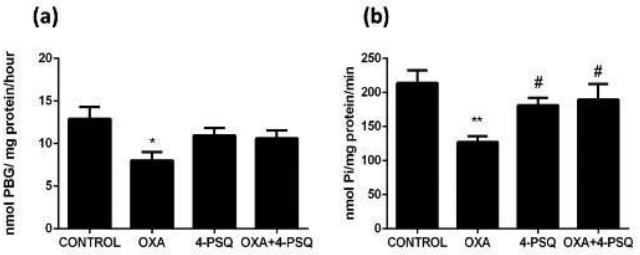
Here, the lipid peroxidation and PC levels were increased after the administration of OXA in mice. Proteins and lipids are the main target of the reactive species in the cells due to their abundance and high rate of the reaction. In this sense, the PC are

Fig. 4. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on (*a*) superoxide dismutase, (*b*) catalase, (*c*) glutathione peroxidase, (*d*) glutathione reductase, and (*e*) glutathione S-transferase activities in kidney of mice exposed to oxaliplatin (OXA). Each column represents mean ± standard error of the mean ($n = 7$). Statistical analysis was performed by one-way ANOVA followed by Newman–Keuls' test. (*) denotes $p < 0.05$, (**) denotes $p < 0.01$, and (***) denotes $p < 0.001$ when compared with the control. (#) denotes $p < 0.05$, (###) denotes $p < 0.001$, and (####) denotes $p < 0.0001$ when compared with the OXA group. CDNB, 1-chloro-2,4-dinitrobenzene.



biomarkers of oxidative damage due to their relative early formation and stability (Dalle-Donne et al. 2003). Based on the evidence from the present study, it is believed that the oxidative damage contributes to nephrotoxicity observed in animals exposed to OXA. Thus, we evaluated the possible changes induced by treatment with OXA on the enzymatic antioxidant defenses, as changes in homeostasis of antioxidant enzymes have been related to oxidative stress (Muller et al. 2006). Enzymatic or nonenzymatic antioxidants are molecules that inhibit oxidative attack in the proteins, lipids, carbohydrates, and DNA through neutralizing free radicals. Several antioxidant enzymes are involved in cellular protection including SOD, the most common antioxidant enzyme, which is responsible for catalyzing the reaction that converts superoxide anion radical to H_2O_2 in the mitochondria and cytosol cellular level. The SOD enzyme is considered an important marker to renal injury induced by oxidative stress (Marklund 1984; Schieber and Chandel 2014); however, the H_2O_2 is still considered a reactive molecule that causes damage and needs to be neutralized. The CAT is responsible for increasing the conversion speed of H_2O_2 to water (Steller et al. 2018). Our results evidenced that the oxidative damage caused by OXA led to an increase in the renal SOD activity and inhibition of CAT activity. The treatment with OXA caused changes in the homeostasis of the enzymatic antioxidant defenses evaluated. Our results demonstrated that the increase of SOD and reduced activity

Fig. 5. Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on (*a*) δ-aminolevulinic acid dehydratase and (*b*) Na^+,K^+ ATPase activities in kidney of mice exposed to oxaliplatin (OXA). Each column represents mean ± standard error of the mean ($n = 7$). Statistical analysis was performed by one-way ANOVA followed by Newman–Keuls' test. (*) denotes $p < 0.05$ and (**) denotes $p < 0.01$ when compared with control group. (#) denotes $p < 0.05$ when compared with the OXA group. PBG, porphobilinogen; Pi, inorganic phosphate.



of CAT in OXA-treated animals could be justified as a compensatory action against the oxidative damage. Otherwise, it was possible to observe that 4-PSQ was able to restore the activity of the decreased CAT enzyme. In this sense, it is hypothesized that 4-PSQ is, directly or indirectly, modulating CAT activity. In line with our results, it is important to highlight that many studies have shown that excessive oxidative stress causes changes in the homeostasis of the enzymatic antioxidant defenses (Foyer and Noctor 2005; He et al. 2017; Li et al. 2018; Vergani et al. 2004). It is also possible to relate the oxidative damage caused by OXA to mitochondrial impairment (Di Cesare Mannelli et al. 2013). The increase in reactive nitrogen or oxygen species in the body leads to mitochondrial impairment. The oxidizing action promotes the collapse of the potential mitochondrial membrane ($\Delta\psi m$) and the suppression of the mitochondrial complex, releasing a series of oxygen radicals such as the superoxide anion radical (Di Cesare Mannelli et al. 2013; Waseem et al. 2017). In addition, another contributing factor to the increase in SOD activity in the OXA group may be associated with the increase in the superoxide anion radical and hydroxyl radicals (Barnes et al. 2019; Helm and Rudel 2020). Studies have linked the increase in these radical species to irreparable DNA damage. Among the damages, cell death and genomic instability can be mentioned (Barnes et al. 2019; Helm and Rudel 2020).

Besides that, we indirectly demonstrated that OXA, at a dose of 10 mg/kg, causes impairment in GSH redox cycle. GSH, which is the main NPSH quantified in the NPSH test, is also considered a marker of oxidative damage (Varnes et al. 1984). Of particular importance, our data revealed that OXA reduced GSH levels in the kidney tissue. It was evidenced that the depletion of GSH causes irreversible damage to cells as it acts directly or indirectly in many biological processes including protein synthesis, metabolism, and cell protection (Forman et al. 2009). The GSH depletion caused by OXA could be explained by the increasing renal GPx activity observed in the present study. GPx protects against damage effects of H_2O_2 and is an important enzyme for the maintenance of intracellular concentration of GSH. At the same time, the reduction in GSH content in the kidneys of mice treated with OXA could partially result from a direct interaction of OXA with GSH. In fact, owing to the high affinity of platinum compounds, the SH groups are oxidized, leading to cytotoxicity (Bortolatto et al. 2014; Kuhlmann et al. 1997). In addition, here we evidenced a reduction on GR activity, which plays a role in maintaining adequate amounts of GSH, in kidney of mice exposed to OXA. These findings are reinforced by previously published studies that showed oxidative damage caused by OXA through of the depletion of antioxidant defenses (Areti et al. 2014; Flatters et al. 2017).

Besides, the GST also constitutes the GSH redox cycle and is present in both the adrenal gland and kidneys (Ralf 2000). It has been suggested that the biotransformation products are more cytotoxic than OXA for the cell. According to this data, our results evidenced an increase on the GST activity in kidneys of OXA-induced mice. These results reaffirm our theory that one of the main mechanisms involved in OXA-induced toxicity occurs through growing oxidative damage that leads to depletion of GSH of the kidney cells.

Understanding that OXA causes oxidative damage directly to kidneys cells, we investigated the direct relationship between the activity of the sulfhydryl enzymes, Na^+, K^+ ATPase and δ-ALA-D, and the treatment with OXA. Researchers have demonstrated that sulfhydryl enzymes are sensitive to oxidative agents, which act in the SH groups by inhibiting the activity of these enzymes (Allan et al. 2016; Farina et al. 2003; Monti et al. 2017). The δ-ALA-D catalyzes the condensation of two molecules of ALA to form PBG, which is a precursor of heme, cytochromes, and other hemoproteins. δ-ALA-D catalyzes the second step in the porphyrin and heme biosynthetic pathway and the activity is inhibited by reactive species. Consequently, this leads to the accumulation of the ALA substrate, which could enhance reactive species production and exacerbate the development of oxidative stress (Rocha et al. 2012). In the present study, the activity of δ-ALA-D was inhibited by OXA and this is another evidence of the OXA-induced oxidative damage in the kidneys.

Another sulfhydryl enzyme used as a marker of renal oxidative damage is the Na^+,K^+ ATPase (Dobrota et al. 1999). Na^+,K^+ ATPase is found in large amounts in the basolateral membrane of the proximal tubules renal and plays a key role in the active translocation of sodium and potassium ions across the membrane and in the secondary active transport of several other solutes. In kidneys, the reactive species act as important second messengers, and the excessive increase of these molecules causes inhibition of the Na^+,K^+ ATPase. Indeed, the activity of this enzyme is sensitive to balance redox (Liu et al. 2017). In light of this, increasing experimental evidence suggests that platinum-based chemotherapeutic agents can inhibit Na^+,K^+ ATPase activity (Huliciak et al. 2014; Kubala et al. 2014). The association between reduced expression of β1 subunit Na^+, K^+ ATPase protein and OXA resistance in cancer cells has been previously demonstrated (Tummala et al. 2009). Here, our results demonstrated that the Na^+,K^+ ATPase activity in kidney of mice was inhibited by OXA exposure. Besides, the alteration of Na^+,K^+ ATPase activity harms the function of renal excretion and increases the oxidative stress, which could exacerbate renal damage caused by treatment with OXA. According to our results, we can suppose that the OXA-induced renal

oxidative damage could be related to the inhibition of the δ-ALA-D and Na^+,K^+ ATPase activities.

In this sense, it is necessary to search for pharmacological alternatives to minimize nephrotoxicity for the patients undergoing oncologic treatment with the chemotherapeutic agent OXA. In this sense, we investigated the therapeutic potential of 4-PSQ on the OXA-induced renal impairments. As expected, 4-PSQ protected against the renal damage induced by OXA exposure. Of particular importance, in this study 4-PSQ reestablished the GSH levels, reduced lipid peroxidation and PC levels in the renal tissue, as well as reduced urea levels in the plasma of OXA-induced mice. Therefore, our results showed that the treatment with 4-PSQ was effective against the OXA-induced renal oxidative damage. Indeed, 4-PSQ has received the attention of researchers because of their important pharmacological actions that are correlated with its antioxidant property (Pinz et al. 2016, 2018; Savegnago et al. 2013).

4-PSQ, when administered after OXA, balanced the activity of antioxidant enzymes SOD, GPx, GR, and CAT in kidneys of mice. The selenium is recognized as an essential dietary element for mammals, with physiological roles as a structural component of several antioxidant enzymes, participation in metabolic pathways, including the thyroid hormones metabolism, and immune function (Nogueira and Rocha 2011). In accordance with this, organoselenium compounds have received the attention of researchers because of their broad pharmacological potential (Nogueira et al. 2004; Nogueira and Rocha 2011). In line with this research, the 4-PSQ besides restoring the renal oxidative damage, also reestablished the enzymatic antioxidant defenses altered by OXA.

The treatment with 4-PSQ also reestablished Na^+,K^+ ATPase sulfhydryl enzyme activity after OXA exposure. This is another hypothesis to explain the effect of 4-PSQ in the OXA-induced nephrotoxicity. Studies showed that decreased Na^+,K^+ ATPase activity in the proximal tubule cause cellular apoptosis and reduction of tubular transport of glucose, amino acids, electrolytes, and water. In addition, it leads to renal injury with the decrease of the kidney excretion function (Doucet 1988; Ratliff et al. 2016). Our results demonstrated that the treatment with 4-PSQ reestablished the Na^+,K^+ ATPase activity and, with this, the renal cell functionality.

Importantly, our research group also showed that 4-PSQ elicits antinociceptive, neuroprotective, and antioxidant effects in animal models (Pinz et al. 2018; Reis et al. 2017; Vogt et al. 2018). The present experimental data demonstrated that 4-PSQ is a promising molecule as an alternative therapy for OXA-induced nephrotoxicity. The pharmacological actions of 4-PSQ seem to be related to its antioxidant and anti-inflammatory activities, as well as its ability to modulate the serotonergic, nitrergic, glutamatergic, and cholinergic systems (Barth et al. 2019; Pinz et al. 2016, 2018; Reis et al. 2017; Silva et al. 2017).

5. Conclusion

The bioactivity of selenium-based quinolines emerges as a new important avenue for drug development targeting the management of OXA-induced nephrotoxicity. To summarize the current knowledge, 4-PSQ reduced the renal damage induced by OXA, and its antioxidant action seems to be involved in this pharmacological effect; however, further studies are required to elucidate the other mechanisms involved in this pharmacological action of 4-PSQ.

Conflict of interest

The authors declare no competing financial interests.

Contribution statement

KPM, BBL, ASR, JJP, and EAW conceived and designed research. GB and DA synthesized the compound. KPM, BBL, JJP, and ASR conducted experiments. KPM, JJP, and ASR analyzed data. KPM, ASR, JJP, and EAW wrote the manuscript. CL and EAW supervised the experiments. All authors critically reviewed the content and approved the final version for publication.

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5. Discussão

Os resultados fornecidos na presente dissertação ampliam o conhecimento a respeito da toxicidade gerada pelo quimioterápico OXA. Mais especificamente, nossos achados fornecem subsídios científicos consistentes sobre mecanismos envolvidos na fisiopatologia da nefrotoxicidade e da neuropatia periférica desencadeada pela exposição a OXA em camundongos. Ademais, dois novos compostos organocalcogênios apresentam-se como promissores tratamentos para os respectivos processos toxicológicos causados pela OXA.

A partir das evidências fornecidas no manuscrito (capítulo 1) algumas propriedades farmacológicas do composto MTDZ frente a modelos de nocicepção aguda foram elucidadas. Ainda, cabe destacar que o MTDZ se mostrou promissor em um modelo de neuropatia periférica causada por OXA em camundongos machos e fêmeas. Notavelmente, fármacos que tratam a dor neuropática ainda são considerados um desafio para a clínica medicinal, uma vez que a maior parte dos pacientes não experimentam o alívio suficiente da dor e/ou quando experimentam, desenvolvem tolerância ao medicamento – o que é o caso dos fármacos opioides utilizados para o tratamento da dor oncológica (BARON; BINDER; WASNER, 2010). Além disso, gabapentina, pregabalina e antidepressivos tricíclicos, comumente utilizados no tratamento da dor neuropática, não são capazes de diminuir potencialmente a dor nos pacientes visto que não são fármacos específicos para intervir na neuropatia periférica (FINNERUP et al., 2015).

Neste sentido, o presente estudo pré-clínico revelou que o MTDZ é um protótipo promissor para o tratamento da neuropatia periférica causada pela OXA, uma vez que este composto reduziu a sensibilidade mecânica e térmica em camundongos machos e fêmeas. Apesar da especificidade do MTDZ ainda não ter sido plenamente investigada, o presente estudo revelou pela primeira vez que o aumento da atividade da AChE é um mecanismo interligado ao

desenvolvimento da dor neuropática perante a indução com OXA, e que o MTDZ por ser um agente anticolinesterásico é capaz de normalizar a atividade enzimática. A correlação negativa observada entre o limiar de retirada da pata (hiperalgesia mecânica) e a atividade da AChE é um dos achados extremamente relevantes deste estudo, e que contribui para a compreensão dos processos decorrentes da exposição a OXA.

Tendo em vista a deficiência de estudos que investiguem o fator do sexo em modelos de dor neuropática causada pela OXA, nesta dissertação buscou-se englobar, além dos tratamentos, o viés estatístico sexual afim de enriquecer a discussão da sensibilidade a dor e processos fisiopatológicos intrínsecos a essa variável. Neste sentido, consolidou-se a hipótese da disparidade entre as sensibilidades à dor dependentes do sexo: em camundongos fêmeas, a OXA exacerbou a sensibilidade térmica em comparação aos machos. O possível mecanismo envolvido nesta elevada sensibilidade térmica em fêmeas foi discutido e, em parte, atribuído ao não aumento da atividade da enzima SOD em fêmeas perante a necessidade de defesa ao dano oxidativo demonstrado pelo aumento dos níveis de ERs.

A complexidade de processos hormonais, fisiopatológicos e metabólicos que podem estar envolvidos na disparidade à sensibilidade a dor entre camundongos machos e fêmeas perante a indução com OXA é imensa. E em contrapartida, evidencia-se uma lacuna de pesquisas que investiguem o fator sexo frente a exposição a OXA (REIS et al., 2020a). O estudo mais próximo da discussão da variável sexual em relação a OXA foi reportado por Rimola et al. (2020), porém nesta pesquisa os autores apenas avaliaram a hiperalgesia mecânica a qual – semelhantemente ao manuscrito 1 – não apresentou diferenças estatísticas entre machos e fêmeas.

Além disso, por meio dos testes do reconhecimento do objeto e do labirinto em cruz elevada foi observado que a exposição a OXA gerou nos camundongos machos e fêmeas déficit cognitivo e comportamento tipo-ansioso. Este comportamento comórbido está em consonância a um estudo anterior do

nosso grupo de pesquisa que relatou a correlação entre o desenvolvimento do déficit cognitivo e o comportamento tipo-ansioso em camundongos machos expostos a OXA (REIS et al., 2020b). A neurotoxicidade causada pela OXA é transcendente, e por conta disso a diminuição da qualidade de vida em pacientes expostos à quimioterapia tem sido tão abordada. Logo, os danos neurológicos decorrentes da exposição a este quimioterápico podem se perfazer a outros quadros patológicos preocupantes, como a ansiedade e o dano à memória, levando a um quadro de piora da situação clínica.

Para uma melhor compreensão do quadro neurotóxico causado pela OXA é necessário considerar o aumento da incidência de comorbidades em pacientes submetidos ao tratamento quimioterápico (DIBONAVENTURA et al., 2017; HAN et al., 2008; SHARPE et al., 2012). Particularmente, a OXA ainda possui necessidade de esclarecimento científico quanto ao desenvolvimento de comorbidades durante e após a exposição à quimioterapia. Visto que o carcinoma de colorretal é o segundo de maior incidência mundial e a necessidade de utilização de OXA tende a aumentar, devido ao aumento da expectativa de vida, faz-se necessária a avaliação do dano neurológico causado pela exposição a quimioterapia (CRUZADO et al., 2014; NICHETTI et al., 2019b).

Por conta disso, enzimas dependentes de ATP foram avaliadas no presente estudo com o intuito de analisar o quadro neurotóxico causado pela OXA; bem como, investigar o potencial terapêutico do MTDZ. A OXA alterou de forma significativa a atividade das ATPases totais, Na^+ K^+ - ATPase, Ca^{2+} - ATPase e Mg^{2+} - ATPase. As ATPases são enzimas com estruturas rotativas que utilizam hidrólise de ATP para bombear prótons através de uma membrana contra um gradiente eletroquímico, a fim de atingir uma determinada função celular.

De maneira geral, as ATPases formam um grande grupo de proteínas que estão envolvidas em vários processos celulares especializados, como o enovelamento de proteínas (RANSON; WHITE; SAIBIL, 2001), transporte intracelular (HIROKAWA; NODA; OKADA, 1998), degradação de proteínas

(LANGER, 2000), início da replicação do DNA (LEE; P BELL, 2000), reparo do DNA (YANG, 2000), remodelação do DNA (CARUTHERS; MCKAY, 2002) ou transporte de íons (NISHI; FORGAC, 2002). Além disso, moléculas que inibem a atividade de ATPases têm sido consideradas potenciais alvo na terapia contra o câncer uma vez que podem combater o crescimento tumoral, como é o caso da OXA (BENOIT; BRIENZA; DUBOIS, 2006; HOLLIDAY, 2017; NISHI; FORGAC, 2002).

Por outro lado, o comprometimento da atividade das ATPases pode ocasionar neurotoxicidade, principalmente devido ao transporte de informação prejudicado no trajeto axonal dependente da bomba de íons, sendo uma das causas de comorbidades neurológicas e da neuropatia. Além do já discutido em torno desta temática no manuscrito 1, é importante salientar que a conjugação de um fármaco à quimioterapia que possua a proposta de minimizar essa gama de efeitos adversos é extremamente vantajosa ao paciente. De fato, fármacos que modulam a atividade de ATPases têm sido desenvolvidos como novas terapias na clínica, exemplos de fármacos com este tipo de atividade são: digoxina, benzimidazóis, bafilomicina A1, ciprofloxacino (CHÈNE, 2002).

Dada a relevância de fármacos com ação às ATPases, o composto orgânico contendo enxofre (MTDZ) apresentou efeito modulador das ATPases totais e, seletivamente, modulou a Mg^{2+} - ATPase no córtex de fêmeas, hipocampo de machos e medula de fêmeas, quando a atividade se apresentava alterada. Desta forma, conjecturamos que o MTDZ pode ser capaz de modular especificamente a Mg^{2+} - ATPase perante efeitos deletérios causados pela OXA, o que demonstra a ação especializada do composto. Por outro lado, o MTDZ, num contexto generalizado, normalizou a atividade das ATPases totais sendo capaz de reparar o dano à bomba iônica e com isso modular processos dependentes de ATPases.

Não obstante, visto que a OXA possui como um dos principais mecanismos de toxicidade o desenvolvimento de estresse oxidativo, avaliamos os níveis ERs e a atividade da enzima SOD, relevante na dismutação do radical

ânion superóxido (STAROBOVA; VETTER, 2017). Como era esperado, a OXA levou ao aumento de ERs em ambos os sexos e elevou a atividade da SOD em machos. O MTDZ, em contrapartida, foi capaz de normalizar os níveis de ERs no córtex de machos, hipocampo de machos e fêmeas e medula de machos, além de atenuar a atividade da SOD no córtex e hipocampo de machos.

Quanto a nefrotoxicidade causada pela OXA, o estudo desenvolvido nesta dissertação revelou que a OXA gerou danos oxidativos em lipídios, proteínas e tióis não-proteicos (capítulo 2). A OXA alterou a atividade de enzimas de defesa e sensíveis ao estresse oxidativo, promovendo ainda alteração na homeostase do ciclo da glutationa e inibindo a δ-ALA-d e a Na^+ K^+ - ATPase nos rins. O rimé um órgão envolvido na manutenção da homeostase plasmática, os glomérulos alveolares filtram todo o volume plasmático - a cada 30 minutos todo o sangue do corpo é filtrado - e reabsorvem aproximadamente 99% do filtrado de volta ao plasma. Os produtos residuais são excretados na urina enquanto outras substâncias são mantidas em circulação.

Nesta perspectiva, os níveis de ureia são um dos principais marcadores de dano renal, visto que por meio deste ensaio é possível avaliar a função de filtração glomerular dos rins. Sendo assim, alterações nos níveis de ureia plasmática podem demonstrar insuficiência renal (BANO; IKRAM, 2017; MANSOUR et al., 2002). No presente estudo foi observado que a OXA causou lesão renal, uma vez que aumentou os níveis de ureia plasmáticos, e que o 4-PSQ demonstrou exercer atividade renoprotetora.

Ainda, devido ao trabalho excessivo de limpeza e reabsorção que os rins exercem, sua função metabólica requer um grande consumo de oxigênio molecular (O_2) devido ao transporte ativo. De forma dimensional, em relação a demanda metabólica de O_2, os rins perdem apenas para o coração em termos do consumo e oferta de oxigênio (KATZ, 1982). Embora o consumo de oxigênio seja extremamente necessário para o bom funcionamento renal, sabe-se, por conseguinte, que este consumo está atrelado a produção de espécies reativas

que levam a danos renais em condições tóxicas ou patológicas (KATZ, 1982; RATLIFF et al., 2016).

Neste sentido, o estresse oxidativo renal tem sido reportado frequentemente como a razão para a progressão da doença renal, para doença renal crônica e para o estágio final da doença renal que requer terapia substitutiva (RYSZ et al., 2017). Sobretudo a nefrotoxicidade é uma das complicações mais comuns na quimioterapia, levando a lesão nos néfrons, variando de um quadro clínico assintomático até insuficiência renal terminal (ITO et al., 2012; YAGHOBI JOYBARI et al., 2014).

A OXA tem sido pouco estudada em termos do comprometimento renal uma vez que sua aplicação clínica veio como uma opção metodológica para um tratamento menos danoso aos rins em comparação a cisplatina (BANO; IKRAM, 2017). Entretanto, o número crescente de complicações renais em pacientes expostos a OXA tem chamado atenção (BANO; IKRAM, 2017; DE GRAMONT et al., 2000; ITO et al., 2012; VYSKOCIL et al., 2019; YAGHOBI JOYBARI et al., 2014). Em contrapartida, um fármaco com potencial antioxidante - como o 4-PSQ - se tornaria promissor no combate ao dano renal causado pela quimioterapia.

Nosso estudo revelou que a exposição a OXA em camundongos machos levou a danos oxidativos severos nos rins dos animais. Os ensaios bioquímicos de TBARS, PC e NPSH demonstraram que a OXA causou danos às macromoléculas como proteínas, lipídios e tióis não proteicos. Particularmente, os lipídios são alterados pela ação oxidativa das ERs, liberando cetonas e malondialdeído (MDA), que é o produto final da peroxidação lipídica. O MDA reage com a deoxiadenosina e desoxiguanosina no ácido desoxirribonucleico (DNA) levando a danos no DNA exacerbando o prejuízo aos rins (COPPOLINO et al., 2019; KRÜGER et al., 2015). Além disso, as proteínas oxidadas são transformadas em produtos de proteína de oxidação avançada, produtos estes de alto peso molecular, os quais se tornam insolúveis ao citosol podendo levar a doença renal (COPPOLINO et al., 2019).

Importantemente, no presente estudo o 4-PSQ foi capaz de diminuir os danos aos lipídios, proteínas e NPSH (sensível ao estresse oxidativo) causados pela OXA. De fato, o 4-PSQ é uma molécula multialvo com alto potencial antioxidante (PINZ et al., 2016, 2018; REIS et al., 2017; VOGT et al., 2018). A atividade antioxidante do 4-PSQ a nível renal é demonstrada com promissores efeitos pela primeira vez no nosso trabalho. De fato, o 4-PSQ possui capacidade de modular enzimas antioxidantes como já demonstrado em estudos anteriores do nosso grupo de pesquisa (REIS et al., 2020a, 2020b; VOGT et al., 2018).

Além disso, o 4-PSQ modulou a atividade de enzimas de defesa antioxidante SOD e CAT; bem como, a atividade das enzimas GPx, GR e GST. Esses ensaios demonstraram que o 4-PSQ age por diferentes vias de detoxificação e de combate ao dano oxidativo, gerando efeitos capazes de proteger contra o prejuízo da função renal. Por estes motivos o 4-PSQ é uma interessante alternativa terapêutica para o dano renal causado pela OXA.

Por fim, outro dado relevante do presente estudo refere-se ao efeito do 4-PSQ em normalizar a atividade das enzimas sulfidrílicas δ-ALA-d e Na^+ K^+ - ATPase. A Na^+ K^+ - ATPase, de particular importância neste estudo, é uma enzima extremamente relevante no contexto renal uma vez que, localizada no aspecto basolateral das células tubulares, desempenha um papel fundamental na translocação ativa de Na^+ e K^+ através desta membrana, bem como no transporte "ativo secundário" de vários outros solutos.

Aqui, a OXA inibiu a atividade da Na^+ K^+ - ATPase nos rins dos animais. A atividade da Na^+ K^+ - ATPase renal inibida acarreta mudanças no transporte de Na^+ ou K^+, indicando a participação dessa enzima no desenvolvimento de doença renal crônica, interferindo na reabsorção de Na^+ ou carga secretora de K^+ (KATZ, 1982). É interessante relatar que atualmente além da análise de ureia, creatinina e outros marcadores plasmáticos de dano renal, a hiperpotassemia tem sido um fator determinante para a necessidade de início a hemodiálise (CHEN; KNICELY; GRAMS, 2019). Logo, a OXA parece diminuir a funcionabilidade renal por meio da inibição das enzimas δ-ALA-d e Na^+ K^+ -

ATPase. Particularmente, na $Na^+ K^+$ - ATPase a OXA favorece o dano renal por meio de mudanças na bomba de Na^+ e K^+. Por outro lado, 4-PSQ atuou nas enzimas sulfidrílicas, δ-ALA-d e $Na^+ K^+$ - ATPase, sendo capaz de reverter o efeito deletério da OXA por meio deste mecanismo.

Para resumir o conhecimento atual desta dissertação, foi demonstrado neste estudo que a OXA levou a prejuízos severos a nível renal e neurológico. Observou-se que a OXA elevou a sensibilidade mecânica e térmica em camundongos machos e fêmeas, causou prejuízos cognitivos e comportamento tipo-ansioso, além de alterar a atividade de ATPases e enzimas antioxidantes. Neste quesito, o MTDZ foi um protótipo interessante para o tratamento da neurotoxicidade causada pela OXA. Em contrapartida, a OXA gerou danos renais por meio da inibição de enzimas antioxidantes e sulfidrílicas, dano oxidativo às macromoléculas o que também foi evidenciado pelo aumento plasmático dos níveis de ureia. O 4-PSQ se mostrou um tratamento eficaz frente a nefrotoxicidade causada pela OXA.

6. Conclusões

Baseado nos resultados obtidos pode-se sugerir que:

- Nosso estudo correlaciona, pela primeira vez, a sensibilidade mecânica induzida pela OXA e a ativação da enzima AChE e sugere o MTDZ, um inibidor da AChE, como estratégia terapêutica;
- A inibição das bombas iônicas parece estar envolvida na toxicidade induzida pela exposição à OXA;
- O MTDZ é uma molécula promissora para a neurotoxicidade induzida por OXA, uma vez que reduziu comportamentos nociceptivos e comorbidades associadas ao déficit cognitivo e comportamento ansioso em camundongos machos e fêmeas;
- As ações farmacológicas do MTDZ parecem estar relacionadas às suas atividades antioxidante e anti-inflamatória, bem como à sua capacidade de modular as enzimas AChE e ATPases totais;
- Os resultados obtidos expandiram a compreensão dos mecanismos envolvidos na fisiopatologia da toxicidade causada pela OXA em camundongos machos e fêmeas, bem como destacaram o potencial terapêutico do MTDZ para a neuropatia periférica e comorbidades associadas à exposição OXA;
- O 4-PSQ reduziu o dano renal induzido pela OXA, e sua ação antioxidante parece estar envolvida neste efeito farmacológico;

- Mais estudos são necessários para elucidar os outros mecanismos envolvidos nesta ação farmacológica do 4-PSQ.

7. Perspectivas

Mais estudos serão necessários para investigar os mecanismos de ação envolvidos no efeito terapêutico do MTDZ. Ademais, nosso grupo de pesquisa tem se dedicado ao estudo da toxicidade desenvolvida por quimioterápicos; bem como, busca elucidar processos fisiopatológicos intrínsecos ao desenvolvimento da dor neuropática causada por estes. Neste sentido, pretende-se estudar o efeito farmacológico de 4-PSQ e MTDZ na redução da dor oncológica e comorbidades associadas, causadas pela vincristina em camundongos, considerando o envelhecimento e o estresse como fatores de risco.

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Título	Estudo pré-clínico de compostos organocalcogênios como estratégia terapia para toxicidade da oxaliplatina em camundongos	Nome da Instituição	Universidade Federal de Pelotas
		Data de apresentação esperada	2021-12-01
Nome do instrutor	Ethel Antunes Wilhelm		

DETALHES ADICIONAIS

Número de referência do pedido	N / D	A pessoa / organização solicitante para aparecer na licença	Ketlyn Pereira da Motta

Título, descrição ou referência numérica da	A cloro-4- (fenilselanil) quinolina reduz o estresse	Título do artigo / capítulo de origem da porção	N / D
(s) parte (s)	oxidativo renal induzido pela oxaliplatina em camundongos.	Autor da (s) porção (ões)	N / D
Editor de porção (ões)	N / D	Problema, se republicar um artigo de uma série	N / D
Volume de série ou monogra a	N / D	Data de publicação da porção	2021-05-20
Página ou intervalo de páginas de parte	1102-1111		

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