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Campus de São José do Rio Preto

Diego Dias dos Santos

EFEITO DA INIBIÇÃO DA PROTEÍNA GALECTINA-3 NA TOXICIDADE HEPÁTICA INDUZIDA PELA CISPLATINA EM RATOS

São José do Rio Preto, SP
2023

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biociências, junto ao Programa de Pós-Graduação em Biociências, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

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RESUMO

A galectina-3 (Gal-3) é uma lectina de ligação a β-galactosídeo, que atua como um mediador pleiotrópico com inúmeras funções exercidas intra e extracelularmente, entre outras funções, como aquelas relacionadas à resposta inflamatória, tumorigênese e imunidade inata e adquirida. Diferentes modelos experimentais de hepatotoxicidade mostram um papel essencial da Gal-3 no estresse oxidativo, lesão e fibrose hepática, e nesse sentido, compostos capazes de inibir a expressão de Gal-3 têm sido utilizados como uma possível ferramenta no tratamento de doenças inflamatórias. Assim, avaliamos o efeito da inibição da Gal-3 pela pectina cítrica modificada (MCP) em modelo experimental de toxicidade hepática induzida pela cisplatina. Ratos machos Wistar foram distribuídos em quatro grupos experimentais com n = 6 animais/grupo. Os animais foram tratados via oral (gavagem) com salina (grupos SHAM e CIS) ou MCP (100 mg/kg/dia) (grupos MCP e MCP+CIS) por 7 dias. Após este período, os animais receberam por injeção intraperitoneal cisplatina (10 mg/kg/dia) ou salina por 3 dias. Após 6 horas da última dose de cisplatina, os animais foram eutanasiados para coleta de sangue e fígado. Os animais dos grupos CIS e MCP+CIS apresentaram perda de peso significativa, efeito corroborado pela redução acentuada no armazenamento de glicogênio nos hepatócitos, em comparação com os grupos SHAM e MCP. A cisplatina também produziu um aumento acentuado no influxo de leucócitos no fígado, degeneração hepática com aumento de corpos apoptóticos, níveis de ROS e ativação de STAT3 nos hepatócitos. Sistemicamente, os grupos CIS e MCP+CIS apresentaram aumento nos níveis plasmáticos de IL-6, IL-10, e biomarcadores de toxicidade hepática (ARG1, GSTα, SDH). Os níveis reduzidos de Gal-3 no fígado do grupo MCP+CIS foram associados ao aumento dos níveis de MDA e da expressão do complexo respiratório mitocondrial I. Além disso, o grupo MCP+CIS apresentou níveis plasmáticos aumentados de IL-1β, TNF-α e GOT1. A terapia com MCP antagonizou eficientemente a Gal-9 no fígado, mas não a Gal-1, que apresentou níveis aumentados. Em conclusão, a redução dos níveis endógenos de Gal-3 nos hepatócitos favorece o processo de morte celular e aumento do estresse oxidativo no modelo agudo de toxicidade induzida pela cisplatina.

Palavras-chave: Citocinas. Hepatotoxicidade. Inflamação. Peroxidação lipídica. Mitocôndrias. Pectina cítrica modificada. Espécies reativas de oxigênio.

ABSTRACT

Galectin-3 (Gal-3) is a β-galactoside-binding lectin, which acts as a pleiotropic mediator with numerous functions exerted intra and extracellularly, among other functions such as those related to the inflammatory response, tumorigenesis, and innate and acquired immunity. Different experimental models of hepatotoxicity show an essential role for Gal-3 in oxidative stress, injury, and liver fibrosis, and in this sense, compounds capable of inhibiting Gal-3 expression have been used as a possible tool in the treatment of inflammatory diseases. Thus, we evaluated the effect of Gal-3 inhibition by modified citrus pectin (MCP) in an experimental model of cisplatin-induced liver toxicity. Male Wistar rats were distributed into four experimental groups with n = 6 animals/group. The animals were treated orally (gavage) with saline (SHAM and CIS groups) or MCP (100 mg/kg/day) (MCP and MCP+CIS groups) for 7 days. After this period, the animals received cisplatin (10 mg/kg/day) or saline by intraperitoneal injection for 3 days. Six hours after the last cisplatin dose, the animals were euthanized for blood and liver collection. Animals in the CIS and MCP+CIS groups showed significant weight loss, an effect corroborated by the marked reduction in glycogen storage in hepatocytes, compared to the SHAM and MCP groups. Cisplatin also produced a marked increase in the influx of leukocytes into the liver, hepatic degeneration with an increase in apoptotic bodies, ROS levels and STAT3 activation in hepatocytes. Systemically, the CIS and MCP+CIS groups showed increased plasma levels of IL-6, IL-10, and liver toxicity biomarkers (ARG1, GSTα, SDH). Reduced levels of Gal-3 in the liver of the MCP+CIS group were associated with increased levels of MDA and expression of the mitochondrial respiratory complex I. In addition, the MCP+CIS group showed increased plasma levels of IL-1β, TNF- α and GOT1. MCP therapy efficiently antagonized Gal-9 in the liver, but not Gal-1, which showed increased levels. In conclusion, the reduction of endogenous Gal-3 levels in hepatocytes favors the process of cell death and increased oxidative stress in the acute model of cisplatin-induced toxicity.

Keywords: Cytokines. Hepatotoxicity. Inflammation. Lipid peroxidation. Mitochondria. Modified citrus pectin. Reactive oxygen species.

LISTA DE ILUSTRAÇÕES

Figura 1 – Estrutura morfológica e histológica do fígado 15

Figura 2 – Estrutura da família das galectinas 20

Figura 3 – Expressão e formação da proteína galectina-3 21

Figura 4 – Esquema representativo da pectina cítrica 24

Figura 5 – Mecanismo de inibição da proteína galectina-3 pela pectina cítrica modificada 25

MANUSCRITO: *Galectin-3 is a key hepatoprotective molecule against the deleterious effect of cisplatin*

Figure 1 – *Experimental model of cisplatin-induced toxicity ……* 33

Figure 2 – *Systemic inflammatory response in the cisplatin-induced acute toxicity model …….....* 39

Figure 3 – *Deleterious effects of cisplatin on the liver ……....* 40

Figure 4 – *pSTAT3 expression in hepatocytes ……* 41

Figure 5 – *Analysis of Gal-3 expression in the liver ……....* 43

Figure 6 – *Analysis of oxidative stress in the liver ……..* 44

Figure 7 – *Analysis of Gal-1 and Gal-9 expressions in the liver ……...* 46

LISTA DE ABREVIATURAS E SIGLAS

a.u. Unidades arbitrárias (do inglês, *arbitrary unit*)

ANOVA Análise de variância

ARG1 Arginase-1

ATP Adenosina tri-fosfato

BSA Albumina de soro bovino (do inglês, *bovine serum albumine*)

CIS Cisplatina

CRD Domínio de reconhecimento de carboidrato (do inglês, *carbohydrate recognition domain*)

Gal-1 Galectina-1

Gal-3 Galectina-3

Gal-9 Galectina-9

GH Hormônio do crescimento (do inglês, *growth hormone*)

GOT1 Aspartato aminotransaminase-1

GTSα Glutationa-S-tranferase alfa

H&E Hematoxilina e eosina

H_2O_2 Peróxido de hidrogênio

i.p. Intraperitoneal

IL Interleucina

KCl Cloreto de potássio

LGALS3 Gene codificante da proteína Galectina-3

MCP Pectina cítrica modificada (do inglês, *modified citrus pectin*)

MDA Malondialdeído

MFI Intensidade média de fluorescência (do inglês, *mean fluorescent intensity*)

MIP Proteína inflamatória de macrófago (do inglês, *macrophage inflammatory protein*)

NF-κB Fator nuclear kappa B (do inglês, *factor nuclear kappa B*)

p.o. Via oral

PAS Ácido periódico de Schiff (do inglês, *periodic acid-Schiff*)

PBS Tampão fosfato (do inglês, p*hosphate-buffered saline*)

pSTAT3 Transdutor de sinal e ativador de transcrição 3 fosforilado (do inglês, *phospho-STAT3*)

ROS Espécies reativas de oxigênio (do inglês, *reactive oxygen species*)

SEM Média e desvio padrão (do inglês, *standard error of the mean*)

SDH Sorbitol desidrogenase

STAT3 Transdutor de sinal e ativador de transcrição 3 (do inglês, *signal transducer and activator of transcription 3*)

TNF-α Fator de necrose tumoral alfa (do inglês, *tumor necrosis factor-alpha*)

LISTA DE SÍMBOLOS

%	Porcentagem
g	Grama
h	Hora
kDa	Quilodalton
kg	Quilograma
mg	Miligrama
mL	Mililitro
mm²	Milímetro quadrado
ng	Nanograma
nm	Nanômetro
ºC	Grau celsius
pg	Picograma
rpm	Rotação por minuto
µL	Microlitro
µm	Micrômetro
µM	Micromolar

SUMÁRIO

PREFÁCIO 12

1. INTRODUÇÃO 13

1.1. Fígado 13

1.2. Cisplatina 17

1.3. As Galectinas 19

1.3.1. Galectina-3 21

1.4. Pectina Cítrica Modificada 24

2. OBJETIVOS 28

2.1. Geral 28

2.2. Específicos 28

3. CAPÍTULO 1 (manuscrito publicado em revista indexada *Life Sciences*) 29

1. Introduction 31

2. Material and Methods 32

3. Results 38

4. Discussion 46

5. Conclusion 49

References 50

4. CONCLUSÃO 56

REFERÊNCIAS 58

ANEXO A – Aprovação do Comitê de Ética para o Uso de Animais 68

ANEXO B – Artigos publicados durante o período de Mestrado 69

ANEXO C – Ata da defesa pública da Dissertação de Mestrado 72

PREFÁCIO

Esta dissertação está sendo apresentada na forma de artigo de revista internacional indexada, intitulado “Galectin-3 is a key hepatoprotective molecule against the deleterious effect of cisplatin”. O referido artigo foi publicado na revista internacional indexada “*Life Sciences*”, com fator de impacto 6.78, da editora Elsevier.

A introdução apresenta uma revisão da literatura sobre os aspectos morfológicos, histológicos e fisiológicos do fígado. Além disso, são apresentados tópicos sobre os efeitos da cisplatina, que causa processos inflamatórios por hepatotoxicidade em altas dosagens, causando extresse oxidativo relacionados a elevação de biomarcadores de lesão hepática e outras transaminases. Em seguida, apresentamos a pectina cítrtica modificada, carboidrato rico em β-galactosídeos e que atua como inibidor da proteína galectina-3. Na sequência, são apresentados o objetivo geral e específicos do trabalho. O artigo está incluído em inglês, seguindo as normas de formatação recomendada pela referida revista e, por último, as conclusões em português.

1. INTRODUÇÃO

1.1. Fígado

O fígado (Figura 1A) é o maior órgão e glândula interna do corpo, com peso aproximado de 1,5 kg, representando quase 2,5% do peso corporal de um humano adulto (ROSS; PAWLINA, 2006). Localizado na cavidade abdominal, é protegido pela caixa torácica, e é envolvido pela capsula de Glisson, constituída principalmente de tecido conjuntivo fibroso, que também é revestida pelo peritônio visceral, que se adere ao diafragma e a outros órgãos (ROSS; PAWLINA, 2006).

Anatomicamente, o fígado é dividido em quatro lobos (direito, esquerdo, quadrado e caudado) (ROSS; PAWLINA, 2006). É um órgão responsável pela captação, armazenamento e distribuição de nutrientes, vitaminas, e proteínas plasmáticas, como albuminas, lipoproteínas, glicoproteínas, protrombinas, fibrinogênio, α-globulinas e β-globulinas, provenientes da corrente sanguínea (MANCO; ITZKOVITZ, 2021). Além disso, atua também como glândula exócrina e endócrina, produzindo a secreção biliar e também modificando ação de hormônios, como a tiroxina, GH, insulina e glucagon (TREFTS; GANNON; WASSERMAN, 2017). Também possui função de regularizar a homeostase corporal, no fornecimento e armazenamento de substâncias, metabolizando proteínas, carboidratos e lipídeos, e excretando o ferro e cobre oriundos da degradação de hemácias. A atuação na metabolização de fármacos e toxinas é imprescindível, principalmente por ser o primeiro a receber metabólitos e nutrientes (TREFTS; GANNON; WASSERMAN, 2017).

Histologicamente, é possível observar no fígado uma arquitetura específica em seu tecido parenquimatoso, havendo agrupamento de vasos de médio calibre localizados na periferia do lóbulo hepático (Figura 1B), como ramos da artéria e veia hepática, e de ductos biliares, que constituem a tríade portal (Figura 1C). Já ao centro do lóbulo, é possível observar a veia centrolobular (MANCO; ITZKOVITZ, 2021). Os lóbulos hepáticos são constituídos de cordões de hepatócitos (unidade funcional do fígado), intercalados de capilares sinusoides (Figura 1D). Esses capilares são compostos de células endoteliais (Figura 1E) que exibem grandes aberturas em seu citoplasma, vários poros ou fenestras desprovidas de diafragma em sua membrana, e são separadas umas das outras por lacunas intercelulares amplas que proporcionam a troca de substâncias entre o sangue e as células

hepáticas, e levam o sangue rico em gás carbônico e metabólitos a veia centrolubular, onde posteriormente desembocam na veia cava inferior (ROSS; PAWLINA, 2006). Essa intimidade física facilita duas funções centrais do fígado em seu papel de filtro hematológico vital: a produção de proteínas do plasma sanguíneo e a concomitante captação endocítica de lipídios, fatores de crescimento e outros agentes tróficos pelos hepatócitos (SCHULZE; SCHOTT; CASEY; TUMA *et al.*, 2019). Embora essencial, essa excepcional capacidade de purificação coloca o fígado em risco, tornando-o altamente suscetível a danos decorrentes da exposição excessiva a gordura, álcool, drogas e outras toxinas, bem como a uma série de patógenos, como o vírus da hepatite (SCHULZE; SCHOTT; CASEY; TUMA *et al.*, 2019).

Os hepatócitos (Figura 1C e E) são células poligonais que constituem aproximadamente 80% da população celular do fígado (MANCO; ITZKOVITZ, 2021; WANG; LIANG; GRAVOT; THORLING *et al.*, 2017). Como toda célula epitelial, os hepatócitos possuem domínios de membrana plasmática apical (canalicular) e basolateral (sinusoidal) compostos por proteínas de superfície, canais e distintos receptores. Dentro do domínio sinusoidal possui diferentes receptores tirosina quinases, como o receptor EGF (fator de crescimento epidérmico), os principais receptores de eliminação de lipídios e ferro, como o receptor de lipoproteína de baixa densidade (LDLR) e o receptor de transferrina (TfR), bem como numerosos ácidos biliares transportadores de captação. No domínio canalicular, predominam os transportadores de cassete de ligação ao ATP (*ATP-binding cassette*) e de efluxo de ácidos biliares (SCHULZE; SCHOTT; CASEY; TUMA *et al.*, 2019). Os hepatócitos também armazenam glicogênio, gotículas lipídicas, e por apresentarem alta atividade metabólica, são abundantes em organelas citoplasmáticas, tais como mitocôndrias, retículo endoplasmático liso e rugoso, complexo de Golgi e peroxissomos (SCHULZE; SCHOTT; CASEY; TUMA *et al.*, 2019). Considerando que estas células são chaves na execução das funções do fígado, elas podem sofrer danos por diversas vias e as organelas intracelulares podem fazer parte da célula disfuncional, com alterações que incluem dano mitocondrial por sobrecarga de ácidos graxos livres e β-oxidação insuficiente, bem como ativação do metabolismo peroxissomal (DI CIAULA; CALAMITA; SHANMUGAM; KHALIL *et al.*, 2021).

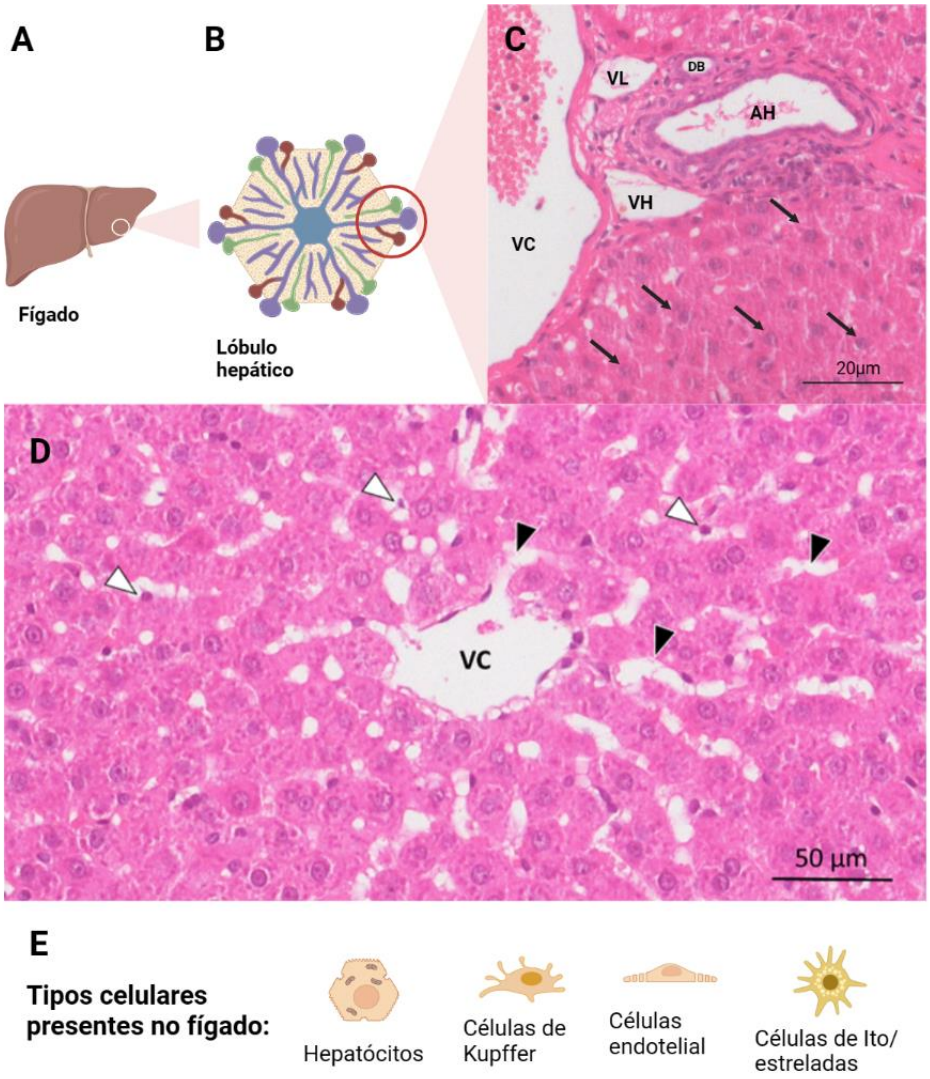


Figura 1. Estrutura morfológica e histológica do fígado. Esquema geral do fígado (A) e de um lóbulo hepático (B), destacando os elementos da tríade portal a nível histológico (C). Sinusóides hepáticos (cabeças de seta pretas) percorrem e separam os cordões de hepatócitos, bem como as células de Kupffer ou macrófagos sinusoidais estrelados (cabeças de seta brancas) são observadas em associação às células endoteliais dos sinusoides (D). Além dos hepatócitos (setas pretas), o fígado também é povoado por células de Kupffer, células endoteliais que revestem seus capilares sinusoides e células de Ito (C e E). **Abreviações: AH:** ramo da artéria hepática; **VL:** vaso linfático**; VH:** ramo da veia hepática; **VC:** veia centrolobular. **Coloração:** hematoxilina e eosina. Figura autoral (2023).

Além dos hepatócitos, outros tipos celulares estão presentes no fígado, como as células de Kupffer (Figura 1C e D), que representam os macrófagos residentes do fígado e componentes do sistema fagocítico mononuclear (ELCHANINOV; VISHNYAKOVA; MENYAILO; SUKHIKH *et al.*, 2022); e também células estreladas hepáticas (células de Ito) (Figura 1E), uma população de células mesenquimais que constitui 5-10% do número total de células no fígado (KHOMICH; IVANOV; BARTOSCH, 2019).

As células de Kupffer apresentam importante função de barreira, impedindo que patógenos e seus subprodutos tóxicos (por exemplo, endotoxinas) entrem na circulação sistêmica (ELCHANINOV; VISHNYAKOVA; MENYAILO; SUKHIKH *et al.*, 2022). Estima-se que estas células constituam 30-35% da população de células hepáticas e estão tipicamente localizadas dentro do lúmen dos capilares sinusoidais aderentes ao endotélio, o que lhes fornece acesso imediato a agentes estranhos imunogênicos que chegam com a circulação portal (PROTZER; MAINI; KNOLLE, 2012). As células de Kupffer também participam do metabolismo de proteínas e lipídios, bem como da eliminação de células apoptóticas da circulação (ELCHANINOV; VISHNYAKOVA; MENYAILO; SUKHIKH *et al.*, 2022).

As células estreladas hepáticas estão localizadas no espaço perissinusoidal (espaço de Disse) e são circundadas por hepatócitos e células endoteliais sinusoidais (KHOMICH; IVANOV; BARTOSCH, 2019). Suas principais funções são secreção de componentes da lâmina basal (laminina, proteoglicanos e colágeno tipo IV) e armazenamento de retinoides (vitamina A) no citoplasma em gotículas lipídicas (PUCHE; SAIMAN; FRIEDMAN, 2013). Uma vez ativadas, as células estreladas perdem a capacidade de armazenar retinoides e mudam seu fenótipo para proliferar, contrair e regular positivamente a síntese de componentes da matriz extracelular, metaloproteinases, citocinas pró-fibrogênicas, fatores de crescimento e proteínas morfogenéticas, que por sua vez regulam e afetam a arquitetura do tecido (KHOMICH; IVANOV; BARTOSCH, 2019).

Algumas doenças são capazes de afetar o fígado, causando processos inflamatórios de forma aguda ou crônica, onde o dano causado ao fígado pode ser progressivo, levando a deterioração da função hepática, podendo resultar em doença ou a insuficiência hepática (KANEL; KORULA, 2010). A lesão aguda pode ser evidenciada através do aumento de transaminases séricas, mostrando o grau

inflamatório do tecido, pela deposição de gordura nos hepatócitos, bem como necrose e inflamação, detalhes característicos em fígados que sofrem por toxicidade induzida por drogas (KANEL; KORULA, 2010).

Algumas proteínas presentes na corrente sanguínea podem atuar como biomarcadores da lesão hepática, tais como o sorbitol desidrogenase (SDH), enzima citosólica envolvida no metabolismo de carboidratos que converte sorbitol em frutose (UMBAUGH; JAESCHKE, 2021); a glutationa-S-transferase alfa (GTSα) que atua no metabolismo e desintoxicação de metabólitos reativos, como N-acetil-p-benzo-quinona imina (NAPQI) (CHURCH; KULLAK-UBLICK; AUBRECHT; BONKOVSKY *et al.*, 2019; UMBAUGH; JAESCHKE, 2021); a arginase-1 (ARG1), enzima hidrolase que catalisa a hidrólise da arginina em ureia e ortinina (BAILEY; HOLDER; PATEL; DEVLIN *et al.*, 2012; CHURCH; KULLAK-UBLICK; AUBRECHT; BONKOVSKY *et al.*, 2019); e o aspartato aminotransferase (GOT1), proteína envolvida no processo de transferência de redutores na forma de NADH do citosol para a mitocôndria, facilitando o processo de fosforilação oxidativa (KREMER; NELSON; LIN; YAROSZ *et al.*, 2021). Esses biomarcadores são comumente avaliados para diagnóstico e alterações em seus níveis plasmáticos são sugestivos de lesão hepática (CHURCH; KULLAK-UBLICK; AUBRECHT; BONKOVSKY *et al.*, 2019).

1.2. Cisplatina

A cisplatina (cis-diaminodicloroplatina(II)), também conhecida como cloreto de Peyrone desde o final do século XIX (em homenagem a Michele Peyrone, responsável pela sua primeira síntese), é um agente quimioterápico utilizado para o tratamento de uma ampla variedade de tumores, como o testicular, ovariano, de bexiga, cabeça e pescoço, esôfago, pulmão, mama, colo do útero, estômago e próstata, bem como linfomas de Hodgkin e não Hodgkin, neuroblastoma, sarcomas, mieloma múltiplo, melanoma, e mesotelioma (ABUZINADAH; AHMAD, 2020; CALLEJO; SEDÓ-CABEZÓN; JUAN; LLORENS, 2015; CORADINI; CIGANA; SELISTRE; ROSITO *et al.*, 2007; DILRUBA; KALAYDA, 2016; FLOREA; BÜSSELBERG, 2011; GHOSH, 2019). Intracelularmente, esta droga atua fazendo ligações covalentes entre os átomos de platina nas bases purinas do DNA, particularmente nos resíduos de guanosina e adenosina, realizando cruzamento inter/intra-cadeias, inibindo a divisão celular nas fases G1, S ou G2-M e causando

danos diretos ao DNA, e consequentemente, induzindo morte celular das células tumorais por vias apoptóticas e não apoptóticas (ABUZINADAH; AHMAD, 2020; CORADINI; CIGANA; SELISTRE; ROSITO *et al.*, 2007; DILRUBA; KALAYDA, 2016; FLOREA; BÜSSELBERG, 2011). Em relação às vias apoptóticas, elas podem ser extrínsicas por meio do aumento da produção do ligante indutor de apoptose relacionado ao fator de necrose tumoral (TRAIL) pelas células imunes, ou intrínsecas envolvendo a proteína supressora de tumor p53 ou mediado pela proteína p73 associada à p53 (FLOREA; BÜSSELBERG, 2011).

A resposta terapêutica inicial com cisplatina muitas vezes leva a uma remissão completa da doença, resposta parcial ou estabilização da doença. No entanto, a terapia com cisplatina está associada também com efeitos adversos graves incluindo nefro e hepatotoxicidade dose-limitante, neuropatia sensorial periférica cumulativa, ototoxicidade devido a dano irreversível das células pilosas no órgão de Corti, bem como náuseas e vômitos (DILRUBA; KALAYDA, 2016; PINGILI; PAWAR; CHALLA; KODALI *et al.*, 2019).

Os efeitos hepatotóxicos da cisplatina são causados principalmente pela ativação da cascata do estresse oxidativo, com elevação de transaminases e da bilirrubina na circulação (PINGILI; PAWAR; CHALLA; KODALI *et al.*, 2019; POWIS, 1982; UN; UGAN; KOSE; BAYIR *et al.*, 2020). Os níveis de glutationa e glutationa redutase diminuem significativamente, enquanto a glutationa peroxidase, a catalase e a gama-glutamil transpeptidase apresentam aumento significativo após terapia com cisplatina (FLOREA; BÜSSELBERG, 2011). Também é relatado que o tratamento com cisplatina pode aumentar o nível do citocromo P450 e a enzima citocromo P450-2E1 também é responsável pela lesão hepática (CARO; CEDERBAUM, 2004; FLOREA; BÜSSELBERG, 2011).

A cisplatina induz estresse oxidativo pela formação de espécies reativas de oxigênio (ROS), que diminui a capacidade redox da célula, aumenta a quantidade de radicais hidroxila e superóxido que dependem da concentração da cisplatina e do tempo de exposição (BROZOVIC; AMBRIOVIĆ-RISTOV; OSMAK, 2010). A produção de ROS está associada com a indução da peroxidação lipídica, depleção de grupos sulfidrila, alteração de diferentes vias de transdução de sinal, homeostase do cálcio, entre outras ações, que podem causar danos ao DNA e, consequentemente, apoptose de células (FLOREA; BÜSSELBERG, 2011). Havendo

a formação e o acúmulo de dano oxidativo causado por ROS, a função respiratória mitocondrial pode ser afetada, causando disfunção celular (BROZOVIC; AMBRIOVIĆ-RISTOV; OSMAK, 2010).

A alteração da função mitocondrial compromete também o equilíbrio pró-oxidante/antioxidante, com aumento de ácidos graxos não metabolizados no citosol como consequência do bloqueio da β-oxidação dos ácidos graxos e consequente estimulação da produção de ROS (BEGRICHE; MASSART; ROBIN; BONNET *et al.*, 2013; MASARONE; ROSATO; DALLIO; GRAVINA *et al.*, 2018). As disfunções mitocondriais são frequentemente acompanhadas por alterações ultraestruturais consideráveis, como megamitocôndrias, perda de cristas e formação de corpos de inclusão paracristalinos na matriz mitocondrial (SEKI; KITADA; YAMADA; SAKAGUCHI *et al.*, 2002).

1.3. As Galectinas

As galectinas, representantes das lectinas tipo S, são caracterizadas pela sua afinidade aos β-galactosídeos pela conservação evolutiva de uma sequência conservada de 130 aminoácidos no domínio reconhecedor de carboidrato – CRD (LEFFLER; CARLSSON; HEDLUND; QIAN *et al.*, 2002; SANJURJO; BROEKHUIZEN; KOENEN; THIJSSEN, 2022). Embora as galectinas possam se ligar a uma ampla gama de glicanos (polissacarídeos), cada galectina tem uma preferência diferente que contribui para suas atividades biológicas específicas (HIRABAYASHI; HASHIDATE; ARATA; NISHI *et al.*, 2002). Ao mesmo tempo, como seus glicanos ligantes são encontrados em muitos tipos de células e diferentes galectinas podem se ligar aos mesmos ligantes, elas podem apresentar atividade pleiotrópica e redundante, semelhante às citocinas (SANJURJO; BROEKHUIZEN; KOENEN; THIJSSEN, 2022).

Em mamíferos, 15 membros dessa família foram descritos e clonados (LIU; RABINOVICH, 2010). Com base na organização estrutural, as galectinas são classificadas em três subfamílias: prototípicas, quiméricas e do tipo repetições em sequência (*tandem-repeats*). As galectinas prototípicas (galectinas-1, -2, -5, -7, -10, -11, -13, -14, e -15) são compostas por um único tipo de CRD (~15 kDa) e podem se dimerizar, formando homodímeros com dois CRDs idênticos (~30 kDa) (Figura 2A). As *tandem-repeats* (galectinas-4, -6, -8, -9 e -12) apresentam dois CRDs distintos

conectados por uma região de ligação com 50-70 aminoácidos (Figura 2B). A terceira subfamília quimérica tem como único representante a galectina-3 (~30 kDa) e possui um CRD e um domínio aminoterminal rico em resíduos de prolina, glicina e tirosina (Figura 2C), além de seu potencial de oligomerização a partir da porção N-terminal (Figura 3).

As galectinas não possuem uma sequência-sinal clássica e podem ser secretadas por mecanismos pouco conhecidos (DELACOUR; KOCH; JACOB, 2009; HUGHES, 1997). São encontradas no citoplasma e no núcleo, mas também no meio extracelular e na superfície celular. Atuando no meio extracelular por meio da ligação a glicoproteínas ou glicolipídios, as galectinas desencadeiam vias de sinalização intracelulares e regulam interações célula-célula e célula-matriz extracelular (PERILLO; MARCUS; BAUM, 1998; VASTA; AHMED; NITA-LAZAR; BANERJEE *et al.*, 2012). Várias funções são atribuídas a essas proteínas, que incluem: adesão celular, regulação do crescimento celular, embriogênese, metástase, splicing do pré-RNAm, imunomodulação, inflamação e apoptose (COOPER; ILARREGUI; PESOA; CROCI *et al.*, 2010; RABINOVICH; TOSCANO, 2009).

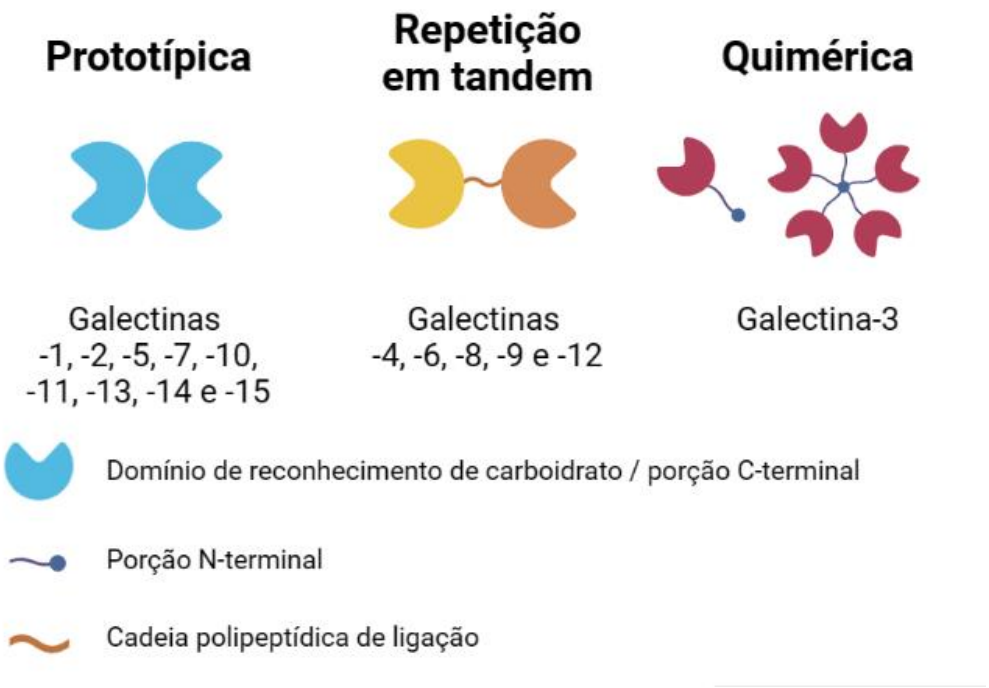


Figura 2. Estrutura da família das galectinas. Representação esquemática das diferentes subfamílias das galectinas: prototípicas (A), do tipo repetição em tandem (B) e quimérica (C). Figura autoral (2022).

1.3.1. Galectina-3

A proteína Gal-3 (Figura 2C e 4), codificada pelo gene *LGALS3* no cromossomo 14 humano, também denominada MAC-2, CBP-35, mL-34, L-29, hL-31, epsilon BP e proteína ligante de IgE, atua como um mediador pleiotrópico com inúmeras funções exercidas intra e extracelularmente (RADOSAVLJEVIC; VOLAREVIC; JOVANOVIC; MILOVANOVIC *et al.*, 2012). A expressão endógena dessa proteína foi observada em vários tipos celulares, especialmente nas relacionadas com a resposta inflamatória, tais como neutrófilos, mastócitos, macrófagos, linfócitos e eosinófilos (ANDRADE; COVRE; RAMOS; HAZARBASSANOV *et al.*, 2018; GE; BAHAIE; KANG; HOSSEINKHANI *et al.*, 2010; GIL; COOPER; ROSIGNOLI; PERRETTI *et al.*, 2006; MARBACK; DE FREITAS; PARANHOS JUNIOR; BELFORT JUNIOR, 2007). Dentre os vários papéis descritos para a Gal-3, destacam-se os relacionados com a resposta inflamatória, tumorigênese, imunidade inata e adquirida (GRUSON; KO, 2012; RADOSAVLJEVIC; VOLAREVIC; JOVANOVIC; MILOVANOVIC *et al.*, 2012).



Figura 3. Expressão e formação da proteína Galectina-3. Esquema representando o gene da Gal-3 (*LGALS3*), sua estrutura primária com um CRD e região N-terminal por onde pode sofrer oligomerização, formando um pentâmero. Figura autoral (2022).

Nos processos inflamatórios a Gal-3 atua na maioria das vezes como uma citocina pró-inflamatória, ativando os mastócitos e os basófilos (ZUBERI; FRIGERI; LIU, 1994), participando no processo de migração dos eosinófilos (GE; HA; LIU; RAO *et al.*, 2013; RAO; WANG; ZUBERI; SIKORA *et al.*, 2007), induzindo a liberação de citocinas pelos monócitos (JENG; FRIGERI; LIU, 1994), a quimiotaxia (SANO; HSU; YU; APGAR *et al.*, 2000) e o processo de fagocitose dos macrófagos *in vitro* (SANO; HSU; APGAR; YU *et al.*, 2003).

Estudos *in vivo*, utilizando modelo experimental de peritonite em camundongos nocautes para Gal-3, mostraram que após injeção intraperitonial de tioglicolato a migração de leucócitos reduziu significantemente para a cavidade peritoneal em relação aos animais selvagens, demonstrando o importante papel dessa proteína no recrutamento dos leucócitos (COLNOT; RIPOCHE; MILON; MONTAGUTELLI *et al.*, 1998; HSU; YANG; PAN; YU *et al.*, 2000). Portanto, a proteína Gal-3 pode interferir positivamente no recrutamento dos leucócitos da circulação sanguínea para os locais de inflamação.

É importante destacar que a Gal-3 pode apresentar papeis pró ou anti-apoptótico dependendo de sua localização (intra ou extracelular), estado de ativação e do tipo celular estudado (FERNÁNDEZ; ILARREGUI; RUBEL; TOSCANO *et al.*, 2005; HSU; YANG; PAN; YU *et al.*, 2000; WANG; GRAY; HAUDEK; PATTERSON, 2004). A atividade anti-apoptótica dessa proteína ocorre no compartimento intracelular (WANG; GRAY; HAUDEK; PATTERSON, 2004), ao contrário do efeito pró-apoptótico realizado pela proteína exógena (FERNÁNDEZ; ILARREGUI; RUBEL; TOSCANO *et al.*, 2005). Essa atividade intracelular está relacionada com a significante similaridade na sequência de aminoácidos da Gal-3 com a proteína Bcl-2, molécula envolvida na inibição da apoptose (AKAHANI; NANGIA-MAKKER; INOHARA; KIM *et al.*, 1997) e na capacidade de regular a integridade da mitocôndria (MATARRESE; FUSCO; TINARI; NATOLI *et al.*, 2000; YU; FINLEY; RAZ; KIM, 2002). Já a atividade extracelular, demonstrada em neutrófilos humanos, está

relacionada com a ativação da cascata de sinalização MAPK pela proteína p38 (FERNÁNDEZ; ILARREGUI; RUBEL; TOSCANO *et al.*, 2005).

Diferentes modelos experimentais de hepatotoxicidade evidenciam um papel importante da Gal-3 nos processos de estresse oxidativo, injúria e fibrose hepática (ABU-ELSAAD; ELKASHEF, 2016; DRAGOMIR; SUN; MISHIN; HALL *et al.*, 2012; IACOBINI; MENINI; RICCI; BLASETTI FANTAUZZI *et al.*, 2011). Um desses estudos mostrou, após administração de acetaminofeno em camundongos, aumento dos níveis de óxido nítrico sintase indusível (iNOS), IL-1β, proteína inflamatória de macrófagos (MIP)-2, MIP-3α, metaloproteinase da matriz-9, bem como do receptor para Gal-3 (CD98) no fígado (DRAGOMIR; SUN; MISHIN; HALL *et al.*, 2012). Já os animais nocautes para Gal-3 mostraram redução significativa desses marcadores pró-inflamatórios, exceto IL-1β, demonstrando que a Gal-3 possui um papel na inflamação e lesão no fígado após intoxicação por acetaminofeno. Por outro lado, estudos mostraram que a Gal-3 tem um papel protetor na hepatite induzida por citomegalovírus murino (MCMV) (STOJANOVIC; MILOVANOVIC; ARSENIJEVIC; STRAZIC GELJIC *et al.*, 2019). Os camundongos nocautes para Gal-3 apresentaram hepatite mais exacerbada em relação aos animais selvagens caracterizada por maior número de focos inflamatórios e necróticos, aumento dos níveis séricos de alanina aminotransaminase, apoptose e necroptose de hepatócitos, e replicação viral. O tratamento com a Gal-3 recombinante reduziu a inflamação, especialmente a necrose dos hepatócitos em camundongos nocautes para Gal-3 infectados com MCMV.

Além da Gal-3, membros de outras subfamílias das galectinas, tais como a Gal-1 e Gal-9, têm sido descritas com papéis importantes na biologia hepática. Estudos mostram que a Gal-1 é essencial para uma regeneração hepática eficiente após hepatectomia parcial por meio da regulação da inflamação hepática, proliferação de células hepáticas e controle do armazenamento de lipídios no fígado em regeneração (POTIKHA; ELLA; CERLIANI; MIZRAHI *et al.*, 2016). Por outro lado, a ausência de Gal-9 exacerba o dano hepatocelular induzido por modelo de isquemia-reperfusão, efeito suprimido pela administração de Gal-9 recombinante (HIRAO; UCHIDA; KADONO; TANAKA *et al.*, 2015).

1.5. Pectina cítrica modificada

As pectinas são polissacarídeos naturais complexos formados por monômeros de ácido galacturônico unidos entre si por ligações glicosídicas, derivadas especialmente das cascas de frutas cítricas (ELIAZ; RAZ, 2019; LECLERE; CUTSEM; MICHIELS, 2013). As pectinas são moléculas grandes (60-300 kDa) e possuem três regiões centrais de polissacarídeos pécticos: homogalacturonano (HG; 65% da composição da molécula), ramnogalacturonano-I (RG-I; 20-35% da composição) e galacturonanos substituídos (GS) (Figura 4) (LATTIMER; HAUB, 2010; LECLERE; CUTSEM; MICHIELS, 2013). Entre os GS, encontra-se o ramnogalacturonano-II (RG-II), distinto do RG-I. O RG-II tem quatro tipos de cadeias de oligossacarídeos estruturalmente diferentes e compostas por 12 tipos de resíduos glicosídicos. Em humanos, devido à sua composição, a pectina atua como uma fibra dietética, não sendo digerida enzimaticamente no intestino delgado, mas pode ser degradada pela microbiota do cólon (LATTIMER; HAUB, 2010). Ela mantém sua ação gelificante no trato digestivo, de modo que retarda a digestão (LATTIMER; HAUB, 2010).

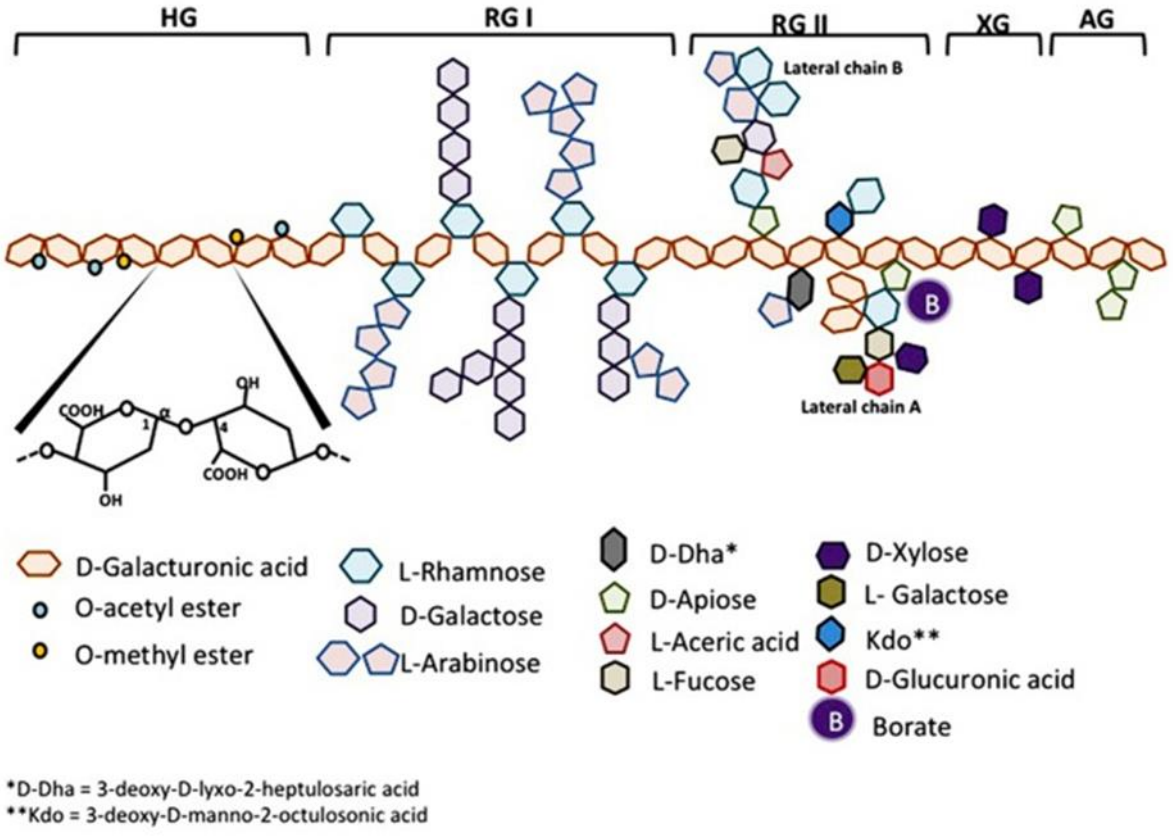


Figura 4. Esquema representativo da pectina. Polímero formado por monômeros de ácido galacturônico unidos entre si por ligações glicosídicas. **AG**, arabinogalactano; **HG**, homogalacturonano; **RG**, ramnogalacturonano; **XG**, xilogalacturonano. Figura retirada de (LECLERE; CUTSEM; MICHIELS, 2013).

Entretanto, a pectina cítrica pode sobrer modificações, tornando-se a pectina cítrica modificada (MCP). Este processo ocorre a partir de alterações de pH e reação enzimática, causando despolimerização da estrutura polissacarídica e desesterificação das regiões HG, clivagem de açúcares neutros, regiões ramificadas e remoção de resíduos de arabinose, formando assim uma molécula com baixo peso molecular (15 kDa) (LECLERE; CUTSEM; MICHIELS, 2013). Com isso, a MCP pode ser absorvida pelo epitélio intestinal e prosseguir para a corrente sanguínea (ELIAZ; RAZ, 2019; LECLERE; CUTSEM; MICHIELS, 2013).

A MCP vem sendo utilizada como tratamento preventivo de doenças cardiovasculares, renais, hepáticas, tumores e até mesmo no tratamento funcional contra a obesidade (ELIAZ; RAZ, 2019; LECLERE; CUTSEM; MICHIELS, 2013). O Pectasol-C, uma forma de MCP, tem peso molecular entre 5 e 10 kDa e é comercializado como um suplemento de saúde natural e vários estudos relatam também sua capacidade de inibir a progressão do câncer, inflamação e fibrose (ELIAZ; HOTCHKISS; FISHMAN; RODE, 2006).

Muitas destas ações protetoras da MCP estão relacionadas com sua capacidade de antagonizar a ação da proteína Gal-3, por ser uma molécula rica em resíduos β-galactosídeos que tem alta afinidade pelo CRD desta lectina (Figura 5). Assim, a MCP pode regular a bioatividade da Gal-3 no meio intra e extracelular, e consequentemente suas ações pró-inflamatória, fibrótica e metastática, atuando como um fator de proteção para doenças cardiovasculares, renais, hepáticas e diferentes tipos de tumores (DONG; ZHANG; HU; ZHENG *et al.*, 2018; ELIAZ; RAZ, 2019).

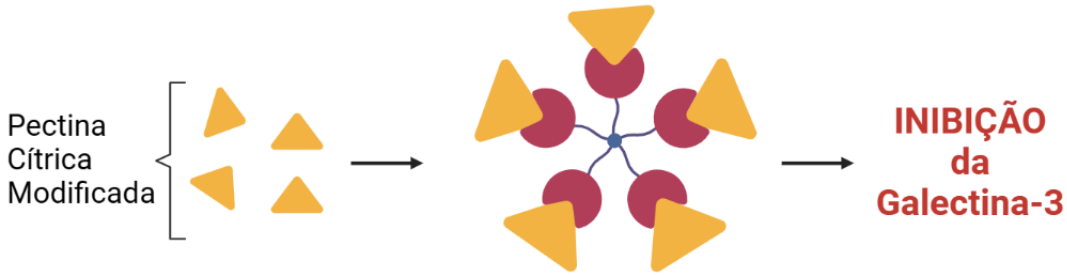


Figura 5. Mecanismo de inibição da proteína galectina-3 pela pectina cítrica modificada. Figura autoral (2022).

A administração de MCP em modelos experimentais de tumorigênese *in vivo* e *in vitro*, envolvendo tumores de bexiga urinária (FANG; LIU; NING; DAN LIU *et al.*, 2018), cólon (LIU; HUANG; YANG; LU *et al.*, 2008), ovário (HOSSEIN; HALVAEI; HEIDARIAN; DEHGHANI-GHOBADI *et al.*, 2019; HOSSEIN; KESHAVARZ; AHMADI; NADERI, 2013; WANG; LI; YU; ZHANG *et al.*, 2020), pele (INOHARA; RAZ, 1994), próstata (CONTI; VEXLER; HAGOEL; KALICH-PHILOSOPH *et al.*, 2018; PIENTA; NAIK; AKHTAR; YAMAZAKI *et al.*, 1995) e tireoide (MENACHEM; BODNER; PASTOR; RAZ *et al.*, 2015), evidenciaram a ação inibitória deste composto via Gal-3 nos processos de metástase e proliferação celular. Além disso, a segurança e eficácia da terapia com MCP (Pectasol-C) foi demonstrada em estudo prospectivo de fase II em pacientes com câncer de próstata não metastático com base nos níveis do antígeno prostático específico (PSA) (KEIZMAN; FRENKEL; PEER; KUSHNIR *et al.*, 2021). Os pacientes (n = 59) receberam 4,8 gramas de MCP em cápsulas 3 x ao dia, por seis meses. Após seis meses, 78% responderam à terapia, com PSA diminuído/estável em 58% (n = 34), ou melhora de duplicação do PSA em 75% (n = 44). A progressão da doença durante os primeiros 6 meses foi observada em apenas 22% (n = 13), com progressão do PSA em 17% (n = 10) e PSA e progressão radiológica em 5% (n = 3). Nenhum paciente desenvolveu toxicidade de grau 3 ou 4.

Outro ensaio clínico examinou a eficácia do bloqueio da Gal-3 com Pectasol-C em pacientes que sofrem de osteoartrite (OA) de joelho (ANDREWS; FERNANDES; BROWNMILLER; HANNA *et al.*, 2020). Os pacientes (n = 50) foram randomizados para receber MCP ou placebo (n = 25/cada) na dose de 4 g em cápsulas duas vezes ao dia por 12 semanas. Os pacientes de ambos os grupos apresentaram características basais semelhantes quanto à gravidade da OA, níveis séricos de Gal-3 e controle da dor, indicando que a terapia de 12 semanas com MCP não melhorou significativamente a doença em comparação com o placebo.

Já estudos relacionados com a fibrose miocárdica mostram que o tratamento com a MCP melhora a disfunção cardíaca, inibindo efeitos inflamatórios que ocorrem por meio da regulação negativa da expressão de Gal-3, e pela supressão da

ativação da via de sinalização TLR4/MyD88/NF-κB (XU; ZHANG; YANG; SUN *et al.*, 2020). Também, estudos pré-clinicos relacionados com a progressão da fibrose hepática induzida por tetracloreto de carbono em ratos, mostraram que a terapia com MCP atenua esse processo (ABU-ELSAAD; ELKASHEF, 2016). O tratamento com MCP nas doses de 400 ou 1200 mg/dia atenuaram a fibrose hepática por meio de efeito antioxidante (redução dos níveis de malondialdeído), da inibição de ativação de células estreladas hepáticas mediada por Gal-3 e indução de apoptose (ABU-ELSAAD; ELKASHEF, 2016).

Nesse sentido, estratégias terapêuticas que visem a inibição das ações deletérias da Gal-3, e possível modulação de Gal-1 e Gal-9, tais como o uso da MCP, são promissoras e têm sido mostradas na literatura em diferentes modelos experimentais de obesidade (MARTÍNEZ-MARTÍNEZ; CALVIER; ROSSIGNOL; ROUSSEAU *et al.*, 2016; MARTÍNEZ-MARTÍNEZ; LÓPEZ-ÁNDRES; JURADO-LÓPEZ; ROUSSEAU *et al.*, 2015), hipertensão (IBARROLA; MARTÍNEZ-MARTÍNEZ; SÁDABA; ARRIETA *et al.*, 2017; MARTÍNEZ-MARTÍNEZ; CALVIER; FERNÁNDEZ-CELIS; ROUSSEAU *et al.*, 2015; MARTÍNEZ-MARTÍNEZ; IBARROLA; FERNÁNDEZ-CELIS; CALVIER *et al.*, 2018), fibrose cardiorrenal e hepática (ABU-ELSAAD; ELKASHEF, 2016; CALVIER; MARTINEZ-MARTINEZ; MIANA; CACHOFEIRO *et al.*, 2015; KOLATSI-JOANNOU; PRICE; WINYARD; LONG, 2011), em especial na toxicidade induzida pela cisplatina (LI; YANG; LI; FENG, 2018). Neste último estudo, as análises de creatinina sérica e histológicas mostraram que o pré-tratamento dos camundongos com MCP a 1% diluída em água aumentou a função renal e atenuou a fibrose renal após lesão induzida por cisplatina. Além disso, o papel protetor da inibição de Gal-3 na lesão renal foi mediado pela proteína quinase C-α (PKC-α), que promoveu apoptose celular e síntese de colágeno I pelas células epiteliais humnas renais da linhagem HEK293.

Apesar de vários estudos contribuírem para a compreensão do papel da Gal-3 na inflamação e lesão hepática, os mecanismos moleculares pelos quais essa proteína modula as respostas celulares, particularmente na toxicidade hepática induzida pela cisplatina, ainda não são conhecidos.

2. OBJETIVOS

2.1. Geral

Avaliar o efeito da inibição da Gal-3 por meio do tratamento farmacológico com a MCP, na toxicidade hepática induzida pela droga quimioterápica cisplatina em ratos.

2.2. Específicos

Utilizando o modelo experimental de toxicidade hepática induzida pela cisplatina em ratos Wistar, associado ou não ao tratamento farmacológico com a MCP, os seguintes objetivos específicos foram avaliados:

- Resposta inflamatória sistêmica por meio da quantificação dos leucócitos do sangue periférico e dosagem das citocinas IL-1β, IL-6, IL-10, e TNF-α;
- Alterações morfológicas do fígado por meio de análises histológica e histoquímica;
- Dosagem dos biomarcadores de dano hepático ARG1, GOT1, GSTα e SDH no plasma;
- Expressão da STAT-3 fosforilada e da Gal-3 no fígado por imuno-histoquímica e western blotting;
- Possíveis alterações da expressão de Gal-3 nos hepatócitos por imunocitoquímica ultraestrutural e microscopia eletrônica de transmissão;
- Produção de espécies reativas de oxigênio (ROS) no fígado por fluorescência;
- Expressão dos complexos mitocondriais da cadeia de transporte de elétrons nos homogenatos de fígado por ensaio multiplex;
- Dosagem de atividade da enzima antioxidante catalase nos homogenatos de fígado;
- Dosagem dos níveis de malondialdeído nos homogenatos de fígado pelo método do ácido tiobarbitúrico (TBA);
- Possível efeito da MCP na expressão das Gal-1 e Gal-9 no fígado por imuno-histoquímica e western blotting.

3. CAPÍTULO 1 (manuscrito publicado na revista indexada *Life Sciences*)

Galectin-3 is a key hepatoprotective molecule against the deleterious effect of cisplatin

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Abstract

Aims: Evaluate the role of galectin-3 in the liver using an acute model of cisplatin-induced toxicity.

Material and Methods: Modified citrus pectin (MCP) treatment was used to inhibit galectin-3. Rats were distributed into four groups: SHAM, CIS, MCP and MCP+CIS. On days 1-7, animals were treated by oral gavage with 100 mg/kg/day of MCP (MCP and MCP+CIS groups). On days 8, 9 and 10, animals received intraperitoneal injection of 10 mg/kg/day of cisplatin (CIS and MCP+CIS groups) or saline (SHAM and MCP groups).

Key findings: Cisplatin administration caused a marked increase in hepatic leukocyte influx and liver degeneration, and promoted reactive oxygen species production and STAT3 activation in hepatocytes. Plasma levels of cytokines (IL-6, IL-10), and hepatic toxicity biomarkers (hepatic arginase 1, α-glutathione S-transferase, sorbitol dehydrogenase) were also elevated. Decreased galectin-3 levels in the livers of animals in the MCP+CIS group were also associated with increased hepatic levels of malondialdehyde and mitochondrial respiratory complex I. Animals in the MCP+CIS group also exhibited increased plasma levels of IL-1β, TNF-α, and aspartate transaminase 1. Furthermore, MCP therapy efficiently antagonized hepatic galectin-9 in liver, but not galectin-1, the latter of which was increased.

Significance: Reduction of the endogenous levels of galectin-3 in hepatocytes favors the process of cell death and increases oxidative stress in the acute model of cisplatin-induced toxicity.

Abbreviations

ARG1: hepatic arginase 1; CIS: cisplatin; GOT1: aspartate transaminase 1; GSTα: α-glutathione S-transferase; IL: interleukin; MCP: modified citrus pectin; MDA: malondialdehyde; ROS: reactive oxygen species; SDH: sorbitol dehydrogenase; STAT3: signal transducer and activator of transcription 3; TNF-α: tumor necrosis factor-α.

Keywords: hepatotoxicity, inflammation, lipid peroxidation, mitochondria, modified citrus pectin, reactive oxygen species.

1. Introduction

Galectin-3 (Gal-3) is a chimera-type β-galactoside-binding lectin characterized by a carbohydrate recognition domain (CRD) linked to another N-terminal domain, the latter of which can form oligomers (DONG; LI; WANG; CHEN, 2020; SEYREK; RICHTER; LAVRIK, 2019). This lectin acts as a pleiotropic mediator with numerous exerted intra- and extracellular functions (DONG; LI; WANG; CHEN, 2020; RADOSAVLJEVIC; VOLAREVIC; JOVANOVIC; MILOVANOVIC *et al.*, 2012). Among the various roles described for Gal-3, those related to the inflammatory response, tumorigenesis, and innate and acquired immunity are the most well-understood (GRUSON; KO, 2012; RADOSAVLJEVIC; VOLAREVIC; JOVANOVIC; MILOVANOVIC *et al.*, 2012). In inflammatory processes, Gal-3 predominantly acts as a pro-inflammatory cytokine. For example, it activates mast cells and basophils (ZUBERI; FRIGERI; LIU, 1994), facilitates eosinophil migration (GE; HA; LIU; RAO *et al.*, 2013; RAO; WANG; ZUBERI; SIKORA *et al.*, 2007), induces cytokine release by monocytes (JENG; FRIGERI; LIU, 1994), promotes chemotaxis (SANO; HSU; YU; APGAR *et al.*, 2000) and enables phagocytosis by macrophages in vitro (SANO; HSU; APGAR; YU *et al.*, 2003). Gal-3 also plays an important role in apoptosis, which is a fundamental component of the tissue-repair phase of inflammatory response (SEYREK; RICHTER; LAVRIK, 2019). Notably, Gal-3 can have either pro- or anti-apoptotic roles depending on its location and activation state, as well as the cell type in question (FERNÁNDEZ; ILARREGUI; RUBEL; TOSCANO *et al.*, 2005; HSU; YANG; PAN; YU *et al.*, 2000; WANG; GRAY; HAUDEK; PATTERSON, 2004).

Different experimental hepatotoxicity models have shown an essential role for Gal-3 in oxidative stress, injury, and hepatic fibrosis (ABU-ELSAAD; ELKASHEF, 2016; DRAGOMIR; SUN; MISHIN; HALL *et al.*, 2012; IACOBINI; MENINI; RICCI; BLASETTI FANTAUZZI *et al.*, 2011). For example, in mouse models, acetaminophen administration increased levels of inducible nitric oxide synthase, IL-1β, macrophage inflammatory protein-2 (MIP-2), MIP-3α, matrix metalloproteinase-9, and the Gal-3 receptor CD98 in the liver (DRAGOMIR; SUN; MISHIN; HALL *et al.*, 2012). However, Gal-3 knockout mice exhibited a significant reduction in the expression of these pro-inflammatory markers, except for IL-1β, demonstrating that Gal-3 plays a role in

inflammation and liver damage after acetaminophen toxicity. As a result, compounds capable of inhibiting Gal-3 expression have been investigated as possible tools for treating inflammatory diseases (ABU-ELSAAD; ELKASHEF, 2016; ELIAZ; RAZ, 2019; HARRISON; MARRI; CHALASANI; KOHLI *et al.*, 2016; SUMIDA; YONEDA, 2018). In this context, we highlight modified citrus pectin (MCP), a complex polysaccharide rich in water-soluble beta-galactose, which can act as a Gal-3 inhibitor and thus help treat inflammatory diseases and liver damage (ELIAZ; RAZ, 2019).

Despite several studies that have improved our understanding of the role of Gal-3 in inflammation and liver injury, the molecular mechanisms by which this protein modulates cellular responses, particularly in cisplatin-induced liver toxicity, are not yet known. Cisplatin, a platinum-based anticancer agent (BROWN; KUMAR; TCHOUNWOU, 2019), can promote hepatotoxicity at high doses (ABD RASHID; ABD HALIM; TEOH; BUDIN *et al.*, 2021; PINGILI; PAWAR; CHALLA; KODALI *et al.*, 2019). These effects are primarily caused by oxidative stress associated with elevated levels of circulating transaminases, glutathione peroxidase, catalase, gamma-glutamyl transpeptidase, and bilirubin, as well as reductions in the levels of glutathione and glutathione reductase (ABD RASHID; ABD HALIM; TEOH; BUDIN *et al.*, 2021; PINGILI; PAWAR; CHALLA; KODALI *et al.*, 2019). Thus, in this work, we evaluated the role of Gal-3 in the liver using an acute model of cisplatin-induced toxicity (LUCCHI; SASSO; SENA; SANTOS *et al.*, 2022; SENA; SASSO; SANCHES; FRANCO *et al.*, 2022), with or without inhibition of this lectin through MCP treatment.

2. Material and methods

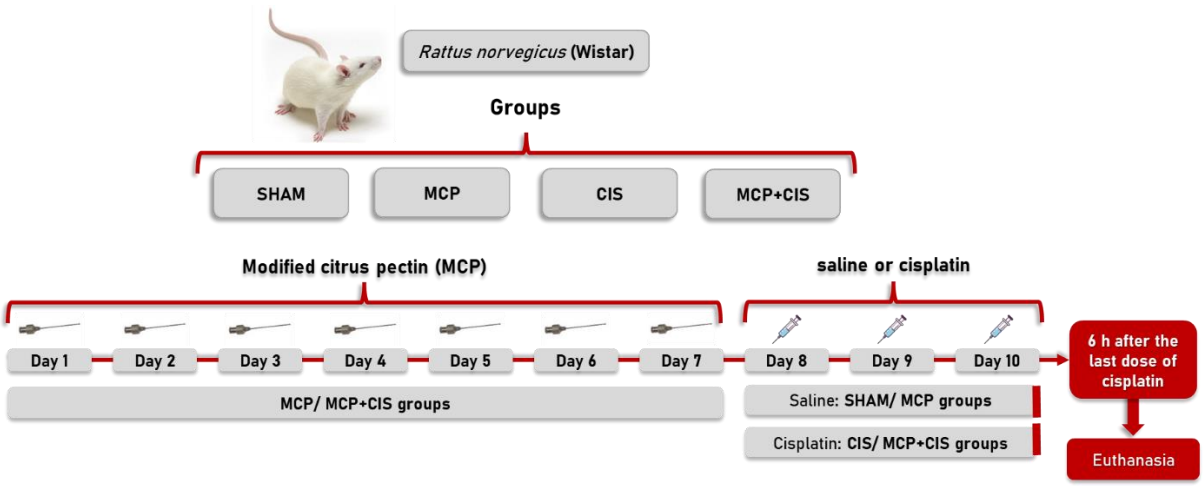
2.1. Animals

Male Wistar rats (250–350 g), aged 60–70 days, were housed under a 12h/12h light/dark cycle with a controlled temperatures of 23–25 °C and *ad libitum* access to water and food. Animals were carefully manipulated to ensure acclimatization and stress reduction for seven days before the experiments. All procedures were approved by the Ethics Committee in Animal Experimentation of the Federal University of São Paulo (UNIFESP; CEUA number 5533211220) and performed in

accordance with the protocols established by the National Council for the Control of Animal Experimentation (CONCEA).

2.2. Experimental model of cisplatin-induced toxicity

The animals were randomly distributed into four experimental groups (n = 5-6 animals/group): the sham (control) group received only sterile saline solution administration; the CIS group received intraperitoneal injections of cisplatin (10 mg/kg) on each of three days, totaling 30 mg/kg (CUNHA; REIS; MACEDO; MACHADO *et al.*, 2020; LUCCHI; SASSO; SENA; SANTOS *et al.*, 2022; SENA; SASSO; SANCHES; FRANCO *et al.*, 2022); the MCP group received 100 mg/kg/day of MCP (PectaSol-C, EcoNugenics, USA) by oral gavage for seven days (LI; YANG; LI; FENG, 2018), followed by i.p. administration of saline; and the MCP+CIS group received 100 mg/kg/day of MCP for seven days, followed by i.p. administration of cisplatin. Six hours after the last cisplatin administration, the animals were euthanized by decapitation immediately following the onset of anesthesia with isoflurane, and the



blood and liver were harvested (Fig. 1).

Fig. 1. Experimental model of cisplatin-induced toxicity. Animals were distributed in four groups: SHAM, CIS, MCP and MCP+CIS. On days 1-7, male rats from MCP and MCP+CIS groups received 100 mg/kg/day of MCP by oral gavage. On days 8-10, animals received intraperitoneal injection of saline (SHAM and MCP groups) or cisplatin (10 mg/kg/day) (MCP+CIS and CIS groups). Six hours after the last cisplatin administration, the animals were euthanized for blood and liver analysis.

2.3. Quantification of peripheral blood leukocytes

Whole blood was obtained by cardiac puncture using a syringe with 10% EDTA. Ten µL was then diluted 1:10 in Turk solution to quantify total leukocytes in a Neubauer chamber. An additional 10 µL was spotted onto a glass slide and distended for Fast Panoptic staining (Laborclin®, São Paulo, Brazil), which enables differential counting of neutrophils, lymphocytes, and monocytes. The remaining whole blood was centrifuged at 15,000 rpm for 10 minutes to collect the plasma for multiplex analysis.

2.4. Histological and histochemical analysis

Liver samples were fixed in 4% buffered paraformaldehyde for 48 hours. Subsequently, the samples were dehydrated in an increasing ethanol gradient, diaphanized in xylol, and embedded in paraffin blocks. The blocks were cut to a thickness of 4 µm with a rotating microtome and sections were adhered to glass slides. The sections were stained with hematoxylin and eosin (H&E) for standard histological analysis and by the periodic acid Schiff (PAS) stain to study the preservation of hepatic glycogen. All photomicrographs were obtained using an AXIOSKOP 2 microscope (Carl Zeiss, Jena, Germany). Quantitative analysis of apoptotic bodies in the liver sections was performed in the liver sections in a blinded fashion, with counting performed using a high-power objective (40×). Values are reported as the mean ± standard error (SEM) of the number of apoptotic bodies/mm^2 of tissue.

2.5. Immunohistochemical analysis

Expression of phospho-STAT3 (pSTAT3) and galectins was examined in 4-µm histological sections of liver tissue adhered to 4% silanized slides. Endogenous peroxidases were inactivated using a 3% hydrogen peroxide solution for 20 minutes at room temperature in the dark. After washing in distilled water, sections were incubated with citrate buffer, pH 6.0, at 96 °C for 60 minutes, followed by 4% BSA in PBS for 20 minutes at room temperature. After washing in PBS, sections were incubated overnight with the following rabbit polyclonal antibodies diluted 1:200 in 1% bovine serum albumin (BSA)/PBS: anti-pSTAT3-S727 (ABclonal, Woburn, MA, USA), anti-Gal-1 and anti-Gal-3 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), and anti-Gal-9 (Abcam, Waltham, MA, USA). A negative control of the reaction was

performed by omitting the primary antibody. After washing, the sections were incubated with peroxidase-conjugated goat anti-rabbit secondary antibody (Thermo Fisher Scientific) and detected through a colorimetric reaction with the substrate 3,3'-diaminobenzidine. Finally, the sections were counterstained with Carazzi's hematoxylin. Quantitative analysis of Gal-3 positive cells in the liver sections was performed in the liver sections in a blinded fashion using a high-power objective (40×). Values are reported as the mean ± SEM of the number of cells/mm^2 of tissue. For pSTAT3 analysis, slides were scanned using the Aperio® CS2 slide scanner equipment (Leica Microsystems, Wetzlar, Germany), and positive cells were quantified using QuPath software version 0.3.2 (University of Edinburgh, UK). Values are reported as the mean ± SEM of the number of positive nuclei/mm^2 of tissue.

2.6. Ultrastructural immunocytochemical analysis

Liver samples were fixed in 2% paraformaldehyde and 2% glutaraldehyde diluted in 0.1% sodium cacodylate buffer (pH 7.4) for 24 hours at 4°C. Samples were washed in sodium cacodylate, dehydrated through a graded methanol series, and embedded in LR Gold (Sigma Aldrich Corp., St. Louis, MO, USA). To detect Gal-3, ultrathin liver sections (~90 nm) were submitted for immunocytochemistry, as previously described (GIL; LA; PERRETTI; OLIANI, 2006). Briefly, anti-Gal-3 rabbit polyclonal antibody (1:100; Invitrogen, Thermo Fisher Scientific), followed by goat anti-rabbit IgG antibody (1:50) conjugated to 15-nm colloidal gold particles (British Biocell, Cardiff, UK), were used. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a ZEISS EM900 electron microscope (Carl Zeiss, Jena, Germany). Randomly selected areas of hepatocytes were imaged and analyzed using Axiovision software (Carl Zeiss). The density of immunogold particles (number of gold particles/μm^2) was calculated and reported as the mean ± SEM of 20–40 cells per experimental condition.

2.7. Multiplex assay

Plasma samples were submitted for multiplex assays using the RLI1MAG-92K (rat liver injury magnetic bead panel) and RECYTMAG-65K (rat cytokine/chemokine magnetic bead panel) for MILLIPLEX MAP® panels (EMD Millipore Corporation, Billerica, MA, USA). Liver damage was evaluated via four targets: hepatic arginase 1

(ARG1), aspartate transaminase 1 (GOT1), α-glutathione S-transferase (GSTα), and sorbitol dehydrogenase (SDH). For plasma cytokines, four targets were evaluated: interleukin-1β (IL-1β), IL-6, IL-10, and tumor necrosis factor-α (TNF-α).

To determine the impact of experimental conditions on the mitochondrial electron transport chain complexes I (NADH–ubiquinone oxidoreductase), III (ubiquinone cytochrome c oxidoreductase), and V (ATP synthase) were affected somehow by different experimental conditions, we performed a multiplex assay with liver homogenates using the Rat/Mouse Oxidative Phosphorylation (OXPHOS) Magnetic Bead MILLIPLEX MAP Panel (RM0XSMAG-17K; EMD Millipore Corporation). Liver homogenates were prepared from 0.1 g of sample macerated in 1 mL of mitochondrial/cell lysis buffer available in the kit, using a portable homogenizer (Uniscience, Sao Paulo, Brazil). Subsequently, the samples were incubated on ice and centrifuged for 10 minutes at 14,000 rpm, and the supernatants were stored at -70ºC.

Protein detection (25 µL of plasma or liver homogenates) was performed using Luminex® technology, with Milliplex Analyser Xponent 3 equipment (EMD Millipore Corporation), according to the manufacturer's instructions. Data are presented as the mean ± SEM of each target in ng/mL (biomarkers of hepatic toxicity), pg/mL (plasma cytokines), or median of fluorescence intensity (MFI) (complexes I, III, and V).

2.8. Detection of ROS

Liver histologic sections (4 µm) were diaphanized in xylol, re-hydrated in decreasing ethanol graduations and water, and incubated with 5 µM of CellROX Reagent (CellROX Green Reagent, ThermoFisher Scientific) for 30 minutes at 37°C. Slides were washed with PBS, mounted with Fluoroshield containing DAPI (Sigma-Aldrich), and analyzed using a Nikon Eclipse Ci-S fluorescence microscope (Nikon, Tokyo, Japan). ROS levels were imaged in ten fields/experimental condition using a 40× objective. ROS fluorescence intensity was quantified using ImageJ software (NIH, USA) and the values were reported in arbitrary units (a.u.; range: 0–255). All MFI values are displayed as mean ± SEM.

2.9. Catalase activity

Liver samples were homogenized in 10 mM potassium phosphate buffer (4°C, 1.36 g l^{-1} KH_2PO_4 and 1.78 g l^{-1} Na_2HPO_4, titrated to pH 7.4, supplemented with 30 mM KCl and 1 mM EDTA), using a Polytron Teflon homogenizer (Uniscience), and stored at -70°C until assays were performed. Protein content was determined using an aliquot of tissue homogenate with BSA as a standard (BRADFORD, 1976). The remaining homogenates were centrifuged at 12,000 rpm for 15 minutes at 4°C and catalase activity was analyzed in the resulting supernatants using standard spectrophotometric assays as described previously (GÓTH, 1991). Briefly, the catalytic activity of catalase was assayed by tracking the decomposition rate of H_2O_2 at 240 nm for 3 min. Catalase activity data are presented as mean ± SEM (U/mg of protein).

2.10. Levels of hepatic malondialdehyde (MDA)

To measure hepatic MDA levels, liver homogenates under different experimental conditions. Homogenates were prepared from 0.1 g of sample macerated in 1 mL of homogenization solution (1% KCl). Subsequently, the samples were centrifuged for 10 minutes at 4,000 rpm and the resulting supernatants were stored at -70ºC. To measure the levels of MDA in the samples, the following reagents were added to each microtube: 50 µL of homogenate, 750 µL of 20% acetic acid, 100 µL of 8% sodium dodecyl sulfate, 750 µL of 0.8% thiobarbituric acid, and 350 µL of distilled water. To generate the standard curve, 3 solutions containing known concentrations of MDA were prepared: tube 1 (300 nM/mL), tube 2 (30 nM/mL), and tube 3 (3 nM/mL); 50 µL of each standard was used and mixed with the remaining solutions as described above. A blank tube was obtained by mixing the incubation solutions but omitting the homogenate and standard solutions. The microtubes were incubated for 60 minutes in a dry bath at 95ºC and the reaction was stopped by transferring the microtubes to ice. Samples were centrifuged for 10 minutes at 4,000 rpm, and 200 µL was added to a 96-well plate. The plate was read at 532 nm using a Biotek 800 TS absorbance reader (Agilent Technologies, Santa Clara, CA, USA). MDA concentration data are shown as mean ± SEM in nM/mL.

2.11. Western blotting

Liver samples were frozen at -70 °C and processed for western blot analysis as previously described (LUCCHI; SASSO; SENA; SANTOS *et al.*, 2022). Nitrocellulose membranes were incubated overnight at 4°C with the following rabbit polyclonal rabbit antibodies diluted 1:1000 in a 1% BSA/Tris-buffered saline solution: anti-STAT3 and anti-pSTAT3-S727 (ABclonal), anti-Gal-1 and anti-Gal-3 (Invitrogen, Thermo Fisher Scientific), and anti-Gal-9 (Abcam). Mouse monoclonal anti-β-actin antibody (Sigma-Aldrich) diluted 1:2000 was also used. To detect target proteins, peroxidase-conjugated secondary antibodies (Thermo Fisher Scientific) were used for 60 minutes, and the signal was detected with chemiluminescence reagents (ClarityWestern ECL Substrate, Bio-Rad Laboratories, Hercules, CA, USA) using UVITEC equipment (UVITEC, Cambridge, UK). Protein expression analysis was performed using ImageJ software and data were displayed as the mean ± SEM of the relative expression of target proteins normalized to β-actin in arbitrary units (a.u.).

2.12. Statistical analysis

Data were analyzed using GraphPad Prism software version 8.2.1. The results first underwent descriptive analysis and normality determination (Kolmogorov–Smirnov test). For samples with normal distributions, an one-way analysis of variance (ANOVA) test with Bonferroni's post-test for multiple comparations was applied. For samples with non-normal distributions, the Kruskal–Wallis's test was performed, followed by Dunn's post-test. In all cases, a p-values < 0.05 were considered significant.

3. Results

3.1. MCP does not reduce the systemic inflammatory response induced by cisplatin administration

The acute model of cisplatin-induced toxicity was characterized by changes in blood leukocyte profiles. Specifically, six hours after the last dose of cisplatin, a significant increase and decrease in the proportion of neutrophils and lymphocytes, respectively, was observed in the CIS and MCP+CIS groups compared to the SHAM and MCP groups (Fig. 2A, B). No alterations in the proportion of monocytes (Fig. 2C) or the total number of blood leukocytes (Fig. 2D) were detected in any group.

Analysis of plasma cytokines showed significant increases in the levels of IL-6 and IL-10 in animals in the CIS, and MCP+CIS groups compared to their respective controls (Fig. 2F, G), indicating that cisplatin-induced inflammatory processes were not abolished by MCP. Additionally, there were significant increases in the plasma levels of liver damage biomarkers such as ARG1, GSTα, and SDH in the CIS and MCP+CIS groups (Fig. 2I, J, L). Furthermore, the MCP+CIS group was the only group in which significant increases in IL-1β (Fig. 2E), TNF-α (Fig. 2H), and the biomarker GOT1 (Fig. 2K) were detected compared to the SHAM and MCP groups.

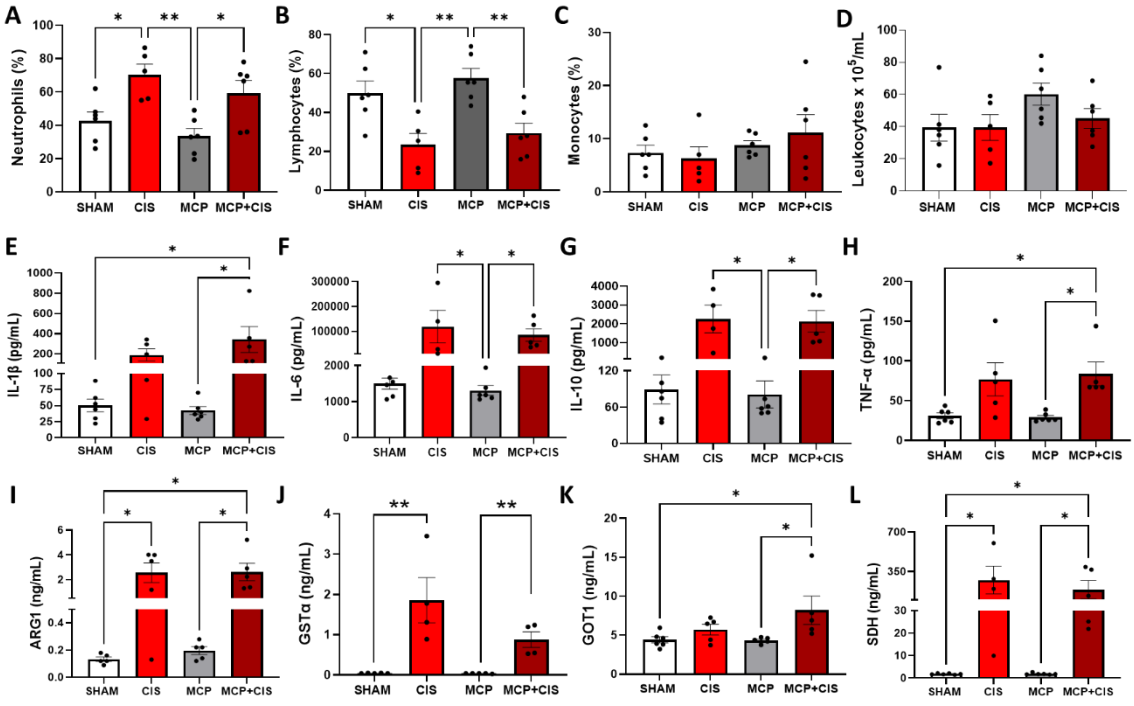


Fig. 2. Systemic inflammatory response in the cisplatin-induced acute toxicity model. The proportion of blood neutrophils (**A**), lymphocytes (**B**), and monocytes (**C**). Quantification of peripheral blood leukocytes (**D**). IL-1β (**E**), IL-6 (**F**), IL-10 (**G**), TNF-α (**H**), ARG1 (**I**), GSTα (**J**), GOT1 (**K**), and SDH (**L**) levels in plasma. The data represent the mean ± SEM of the proportion of leukocytes (%) or the protein concentration in pg/mL (**D-H**) or ng/mL (**I-L**). Dots on the bar graphs represent individual rats (n = 5-6/group). * $p < 0.05$, ** $p < 0.01$ (ANOVA with Bonferroni's post-test in A, B, E, I, and K; Kruskal–Wallis with Dunn's post-test in F, G, H, J, and L; unpaired t-test in D).

3.2. Characterization of hepatic damage caused by the acute cisplatin exposure

To characterize the morphological changes induced by acute cisplatin toxicity, histological, histochemical, and immunohistochemical analyses of the liver were performed. The administration of cisplatin in the CIS and MCP+CIS groups disrupted the hepatic lobules compared to the SHAM and MCP controls; sinusoids were congested with red blood cells, suggesting hepatic edema, and there was evidence of hepatic cord degeneration due to the presence of several apoptotic bodies (Fig. 3A-D). These findings were corroborated by the significant increase in the influx of leukocytes into the liver and the number of apoptotic bodies in the CIS and MCP+CIS groups compared to their respective controls (Fig. 3I, J). Furthermore, PAS staining showed a marked loss of glycogen presence in hepatocytes after cisplatin administration (CIS and MCP+CIS) compared to controls (SHAM and MCP) (Fig. 3E-H, K). These findings were confirmed by the densitometric analysis of positive PAS staining (purple color) in hepatocytes (Fig. 3K).

The animals were weighed on days 1, 7, and 10 to monitor them for possible weight loss. As expected, the control groups gained weight throughout the experiment. In contrast, the animals in the CIS and MCP+CIS groups exhibited significant weight loss on day 10 after three days of cisplatin administration (Fig. 3L).

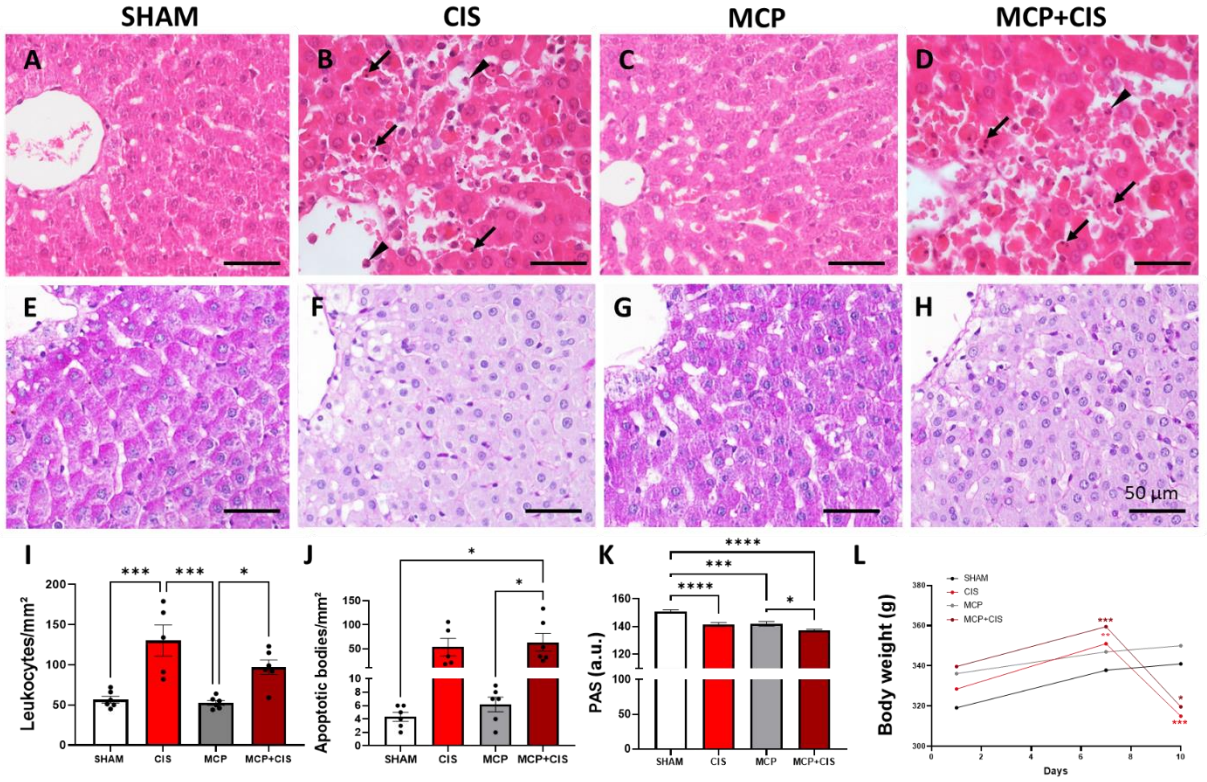


Fig. 3. Deleterious effects of cisplatin on the liver. **A, C:** SHAM and MCP groups. Healthy livers show a typical arrangement of hepatocyte cords between hepatic sinusoids. **B, D:** CIS and MCP+CIS groups. Hepatic cords were disorganized with dilated sinusoids and the presence of apoptotic bodies (arrows) and leukocyte influx (arrowheads). **E, G:** SHAM and MCP groups show PAS-positive hepatocytes with glycogen accumulation evidenced by purple cytoplasmic staining. **F, H:** CIS and MCP+CIS groups with PAS-negative hepatocytes. Stains: H&E (A–D); PAS (E–H). Scale bars: 50 µm. **I-L:** Data represent mean ± SEM of leukocytes and apoptotic bodies/mm², PAS positivity (a.u.), and body weight (g). Dots on the bar graphs represent individual animals (n = 5-6/group). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (ANOVA with Bonferroni's post-test in I, J and L; Kruskal–Wallis with Dunn's post-test in K).

The deleterious effects of cisplatin were associated with the activation of STAT3 in hepatocytes, indicated by phosphorylation of STAT3 at serine 727 and demonstrated by the immunohistochemical staining (Fig. 4). Hepatocytes from animals in the CIS and MCP+CIS groups showed significant increases in pSTAT3-positive nuclei compared to those from the SHAM group (Fig. 4A-D, F). STAT3 immunoreactivity

was not detected in the negative control (no primary antibody) (Fig. 4E). These histological observations were corroborated by molecular analyses showing high pSTAT3 protein levels in hepatic homogenates after cisplatin administration with and without MCP treatment (Fig. 4G, H).

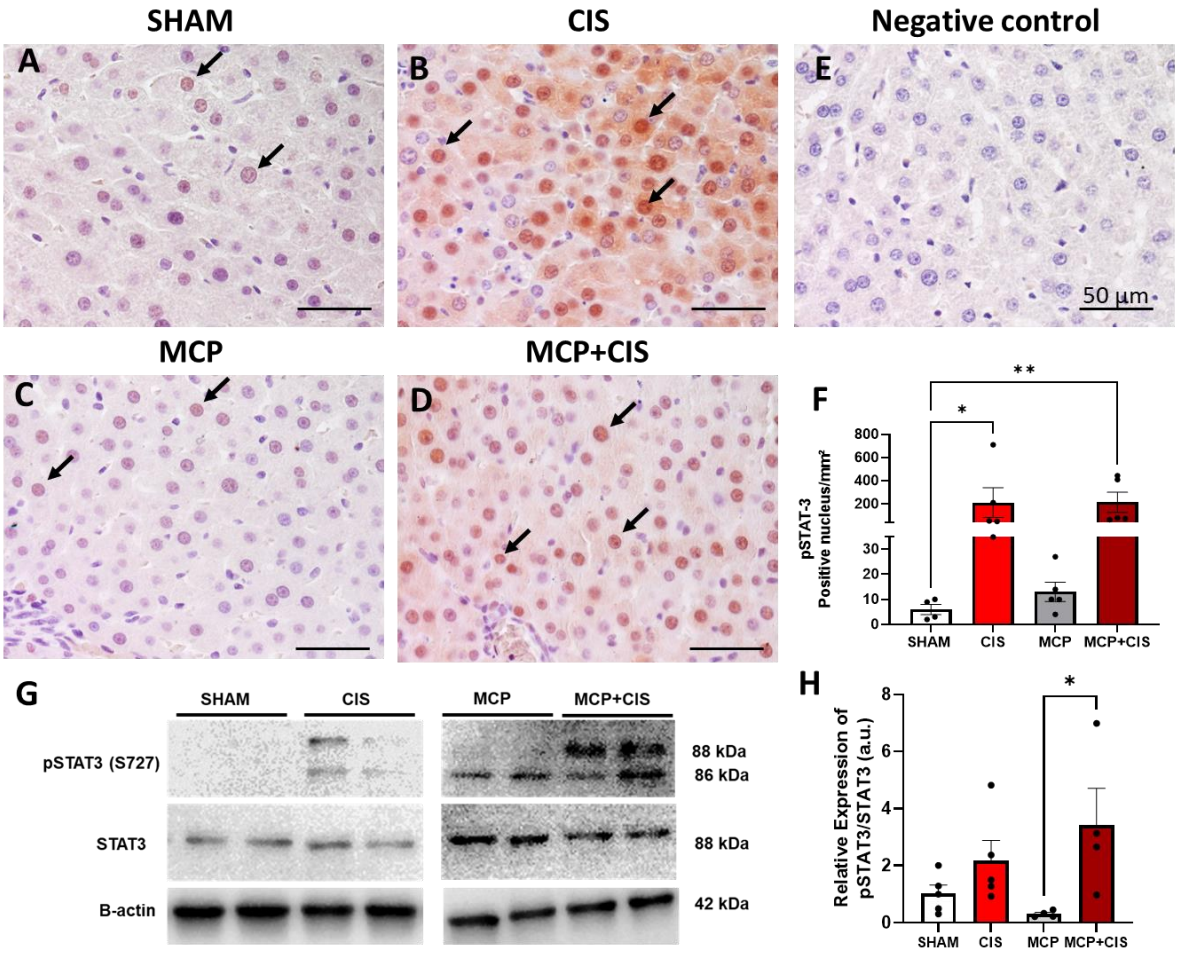


Fig. 4. pSTAT3 expression in hepatocytes. **A-D:** The CIS and MCP+CIS groups exhibited more hepatocytes with pSTAT3-positive nuclei (arrows) than the SHAM and MCP groups. **E:** Negative immunostaining control. Counterstain: Carazzi's hematoxylin. Scale bars: 50 µm. The data represent the mean ± SEM of pSTA3-positive nuclei/mm^2. Dots on the bar graphs represent individual animals (n = 5-6/group). * $p < 0.05$, ** $p < 0.01$ (Kruskal–Wallis with Dunn's post-test). **G**: Elevated levels of p-STAT3 (S727) protein detected in livers from the CIS and MCP+CIS groups confirm histological findings. **H:** The data represent the mean ± SEM of relative expression of pSTAT3 normalized to total STAT3 in arbitrary units (a.u.) (n=

4-5/group). β-actin was used as a protein loading control. * $p < 0.05$ (ANOVA with Bonferroni's post-test).

3.3. Gal-3 plays an essential role in liver maintenance

To better understand the deleterious effects of cisplatin on the liver and the possible role of Gal-3 in this context, we investigated the expression of this protein by western blotting and immunohistochemistry. As expected, immunoblots of liver homogenates revealed marked decreases in Gal-3 levels in animals from the MCP-treated groups compared to the SHAM and CIS groups (Fig. 5A, B). Immunohistochemistry also demonstrated higher Gal-3 expression levels in the portal triad structures of SHAM animals compared to those of MCP animals, such as cholangiocytes (bile duct epithelial cells) and endothelial cells (in the hepatic artery and portal vein), as well as in the surrounding stroma (Fig. 5C). On the other hand, the CIS and MCP+CIS groups showed an intense influx of Gal-3-positive leukocytes and Kupffer-like cells in hepatic sinusoids (Fig. 5C). Quantification of Gal-3-positive leukocytes in the liver showed a significant increase in the CIS group compared to the SHAM and MCP groups (Fig. 5D). On the other hand, no alterations were detected in the number of Gal-3-positive Kupffer-like cells between groups (Fig. 5E).

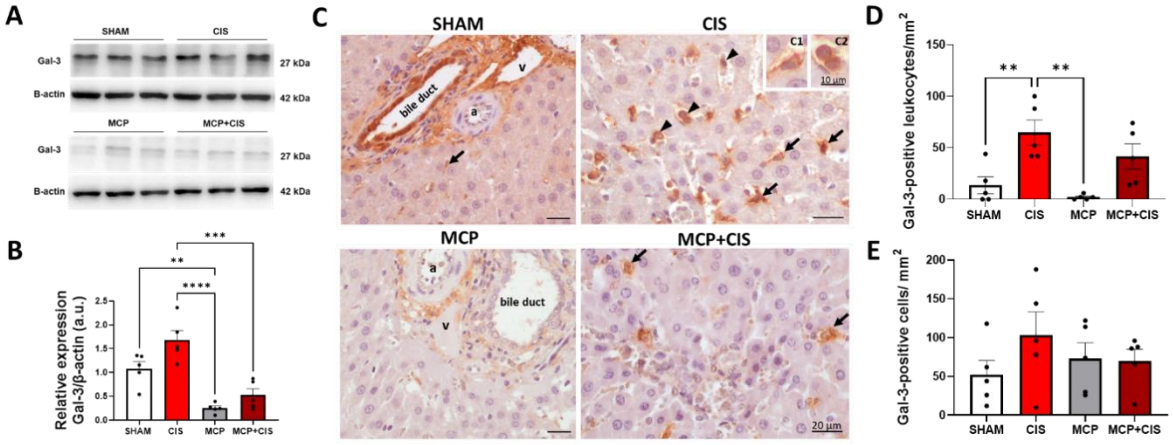


Fig. 5. Analysis of Gal-3 expression in the liver. **A:** Immunoblot analysis of liver homogenates shows decreased levels of Gal-3 in the MCP- and MCP+CIS-treated groups compared to the SHAM and CIS groups. **B:** The data represent the mean ± SEM of relative Gal-3 expression normalized to that of β-actin in arbitrary units (a.u.) (n = 5/group). ** $p < 0.01$, *** $p < 0.001$ **** $p < 0.0001$ (ANOVA with Bonferroni's

post-test). **C:** Higher expression of Gal-3 was detected in the portal triads (branch of bile duct, hepatic artery [a], and portal vein [v]) of animals in the SHAM vs. MCP group. The hepatic sinusoids of animals in the CIS and MCP+CIS groups exhibited Gal-3-positive leukocytes (arrowheads) and Kupffer-like cells (arrows). **C1, C2:** Higher-magnification images of Gal-3-positive Kupffer-like cells and leukocytes. Counterstain: Carazzi's hematoxylin. Scale bars: 20 μm; 10 μm (C1, C2). **D, E:** Quantification of Gal-3-positive leukocytes and Kupffer-like cells. Data indicate mean ± SEM. ** $p < 0.01$ (ANOVA with Bonferroni's post-test).

Given that cisplatin induces oxidative stress to generate cell damage and that mitochondria are activated during ROS release and lipid peroxidation (ADEMOWO; DIAS; BURTON; GRIFFITHS, 2017), ROS levels were evaluated in the liver. Similar to previous results, animals in the CIS and MCP+CIS groups exhibited higher levels of ROS fluorescence than those in the SHAM group (Fig. 6A-E). Furthermore, animals in the MCP+CIS group showed higher levels of MDA than those in the SHAM group, suggesting activation of lipid peroxidation processes (Fig. 6F). However, no changes in the activity of catalase, an antioxidant enzyme, were detected between the groups (Fig. 6G).

Despite the weak immunoreactivity for Gal-3 in hepatocytes, ultrastructural analysis of these cells revealed the presence of this protein in the cytoplasm, predominantly in mitochondria (Fig. 6H-K). Quantification of gold particles-labeled Gal-3 revealed a marked reduction of this protein in the hepatocytes of animals in the CIS and MCP+CIS groups than those in the the SHAM group (Fig. 6H-K, L).

We also investigated the expression of mitochondrial electron transport chain enzyme complexes using a multiplex assay. Analysis of liver homogenates revealed a significant increase in the expression of complex I (NADH–ubiquinone oxidoreductase) in the MCP+CIS group compared to the SHAM group (Fig. 6M), demonstrating that the reduction in Gal-3 is associated with the imbalance of this enzyme complex. No changes were detected in the expression of complexes III or V across groups (Fig. 6N, O).

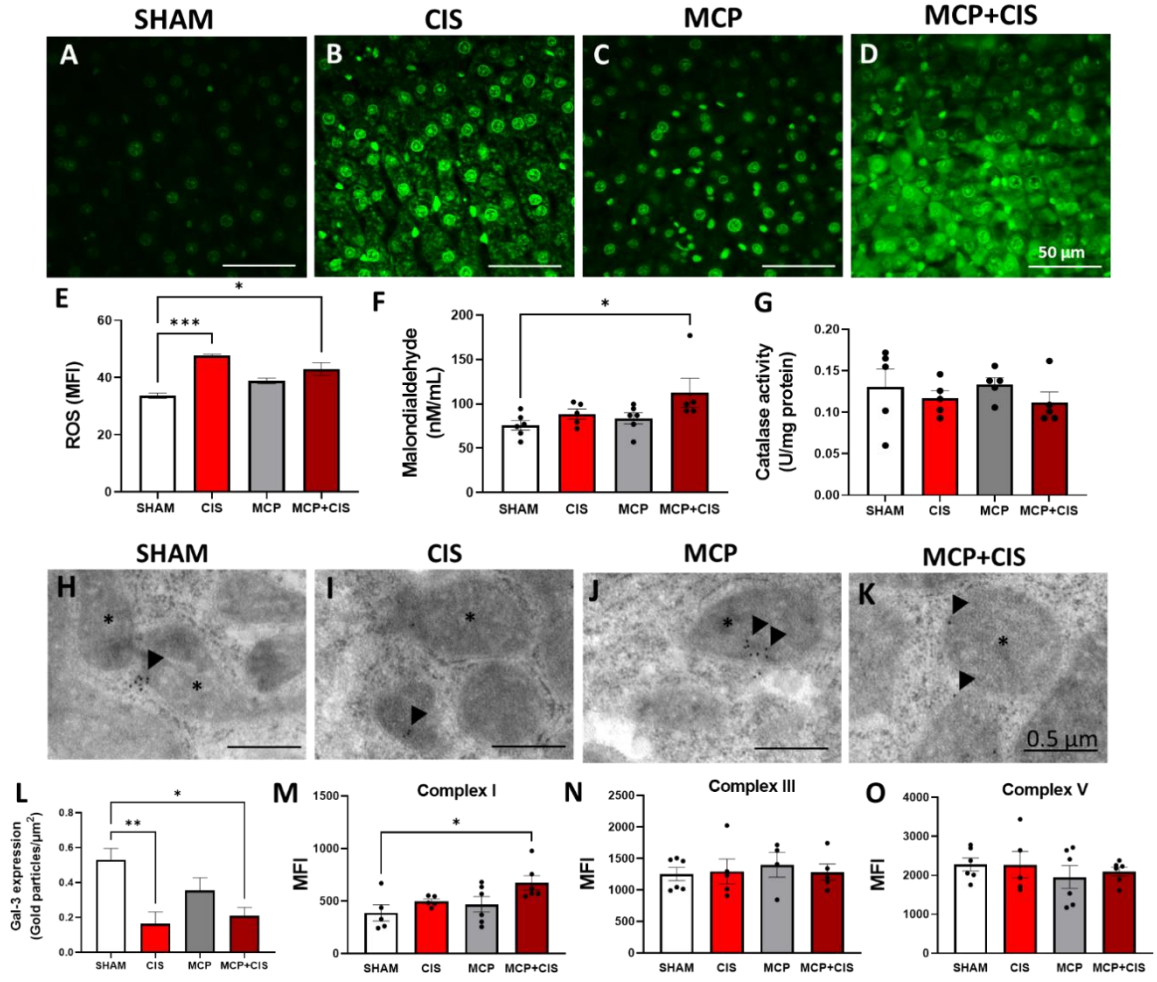


Fig. 6. Analysis of oxidative stress in the liver. **A-D:** ROS fluorescence intensity in the livers of animals in the CIS (B) and MCP+CIS (D) groups compared to the SHAM (A) and MCP (C) groups. Scale bars: 50 µm. **E:** Levels of ROS (MFI). **F:** MDA levels (nM/mL). **G:** Catalase activity (U/mg of protein). Data are mean ± SEM and the dots on the bar graphs represent individual animals (n = 5-6/group). * $p < 0.05$, *** $p < 0.001$ (Kruskal–Wallis with Dunn's post-test). **H-K:** Immunocytochemistry for Gal-3 in hepatocytes showing cytoplasm-associated gold particles (arrows) localized particularly in mitochondria (*). Scale bars: 0.5 µm. **L:** Quantification of Gal-3 immunogold particles in hepatocytes (gold particles/µm^2). **M-O:** Expression of complexes I, III, and V of the hepatic mitochondrial electron transport chain (MFI). Data indicate mean ± SEM and the dots on the bar graphs represent individual values (n = 5-6/group). * $p < 0.05$, ** $p < 0.01$ (ANOVA, Bonferroni's post-test in E, F and M; Kruskal–Wallis, Dunn's post-test in L).

3.4. Gal-1 and Gal-9 expressions are differentially regulated by MCP in hepatocytes

Although most studies of MCP have specifically focused on its Gal-3 antagonism, MCP can bind to and modulate the bioactivity of any galectin since it contains abundant β-galactose (ELIAZ; RAZ, 2019). To characterize the effect of MCP on other galectins, we investigated Gal-1 and Gal-9 levels in the liver. Immunoblot analysis of liver homogenates showed that MCP differentially regulated both galectins. Specifically, the MCP-treated groups demonstrated higher and lower levels of Gal-1 and Gal-9, respectively, than the SHAM and CIS groups (Fig. 7A-C). These results were confirmed by immunohistochemical analysis. As shown in Fig. 7D, hepatocytes from animals in the MCP and MCP+CIS groups exhibited higher levels of Gal-1 than those in the SHAM and CIS groups. On the other hand, hepatocytes from animals in the SHAM and CIS groups demonstrated higher levels of Gal-9 than those in the MCP-treated groups (Fig. 7E).

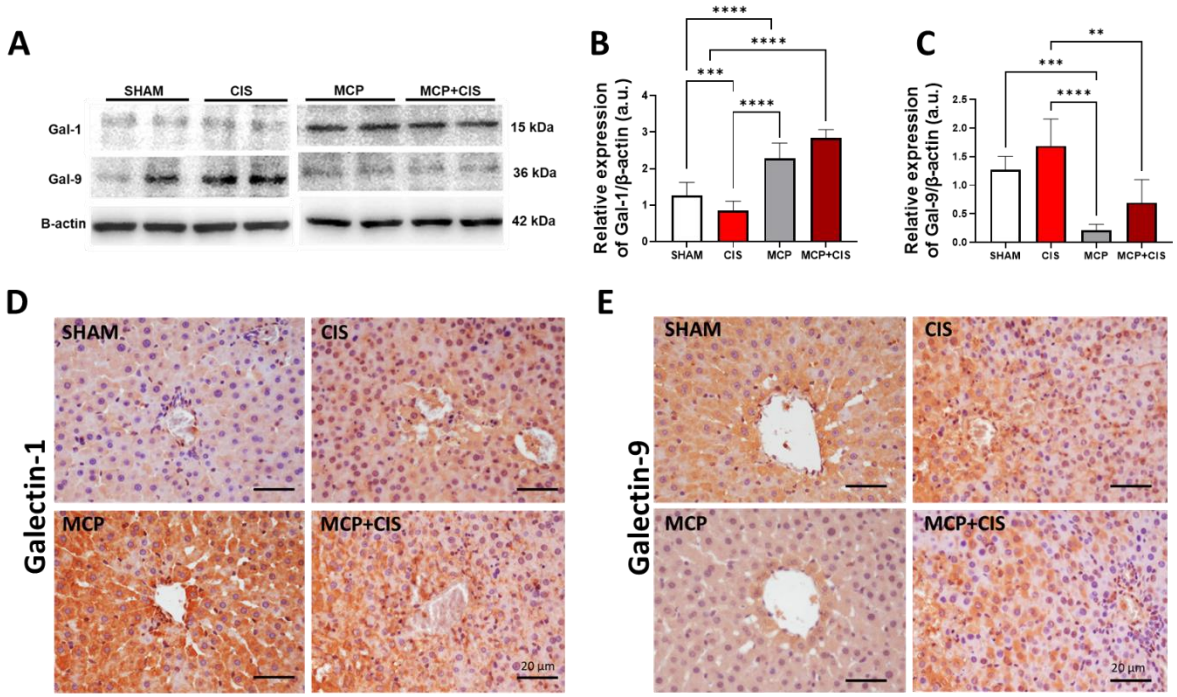


Fig. 7. Analysis of Gal-1 and Gal-9 expressions in the liver. **A:** Immunoblot analysis of Gal-1 and Gal-9 in liver homogenates. **B, C:** The data represent the mean ± SEM of relative expression of Gal-1 or Gal-9 normalized to β-actin in arbitrary units (a.u.) (n = 3/group). ** $p < 0.01$, *** $p < 0.001$ **** $p < 0.0001$ (ANOVA, Bonferroni's post-test). **D:** Higher expression of Gal-1 detected in hepatocytes from MCP-treated

groups compared to SHAM and CIS groups. **E:** Lower expression of Gal-9 detected in hepatocytes from MCP-treated groups compared to SHAM and CIS groups. Counterstain: Carazzi's hematoxylin. Scale bars: 20 μm.

4. Discussion

Given that the role of Gal-3 in cisplatin-induced hepatotoxicity is not entirely defined, this study sought to understand how pharmacologic Gal-3 inhibition with MCP affected the liver in a Wistar rat model of cisplatin-induced toxicity.

Although we did not observe any alterations in the total number of peripheral blood leukocytes between groups, the cisplatin-treated groups exhibited marked increases in the proportion of neutrophils. This finding was consistent with the elevated number of neutrophils that had infiltrated the livers of cisplatin-treated animals, a result also observed in previous studies (LUCCHI; SASSO; SENA; SANTOS *et al.*, 2022). In addition, cisplatin significantly increased the levels of plasma IL-6 and IL-10, as well as those of the hepatic damage biomarkers ARG1, GSTα, and SDH, compared to the control group, indicating an acute inflammatory process induced by the toxicity of this drug. Our findings corroborate other studies that also showed increases in cytokines (IL-1, IL-6, IL-10, IL-18, and TNF-α) and liver damage biomarkers (ARG1, GSTα, and SDH) after cisplatin administration (GAO; YU; LI; CHEN *et al.*, 2019; KIM; SO; LEE; PARK *et al.*, 2008; LUCCHI; SASSO; SENA; SANTOS *et al.*, 2022).

Drug toxicity is the leading cause of acute liver failure and generally presents as elevated levels of specific hepatic proteins in serum, such as ARG1, GSTα, and SDH (CHURCH; KULLAK-UBLICK; AUBRECHT; BONKOVSKY *et al.*, 2019). ARG1, GSTα, and SDH are enzymes that catalyze the hydrolysis of arginine to urea and orthenine, the conjugation of glutathione to various electrophiles, and the conversion of sorbitol into fructose, respectively (CHURCH; KULLAK-UBLICK; AUBRECHT; BONKOVSKY *et al.*, 2019; UMBAUGH; JAESCHKE, 2021). Increased levels of these enzymes in serum are associated with hepatic cell injury and death (CHURCH; KULLAK-UBLICK; AUBRECHT; BONKOVSKY *et al.*, 2019). Furthermore, animals in the MCP+CIS group showed more pronounced inflammatory responses and liver damage, with increased plasma levels of IL-1β, TNF-α, and GOT1. GOT1 is an

enzyme that transfers reducing equivalents in the form of NADH from the cytoplasm to the mitochondria (BERK; WADA; HORIO; POTTER *et al.*, 1990; KREMER; NELSON; LIN; YAROSZ *et al.*, 2021) and its alteration is suggestive of cytotoxic effects on both the liver and mitochondrial function (BAILEY; HOLDER; PATEL; DEVLIN *et al.*, 2012).

Given these observations, our next step was to evaluate animal's liver tissue. Histological analysis showed structural liver damage in the groups treated with cisplatin, predominantly in the hepatocyte cords, as well as an increase in the number of apoptotic bodies as described in previous studies (BAKIR; YAZGAN; İBILOĞLU; ELBEY *et al.*, 2015; LUCCHI; SASSO; SENA; SANTOS *et al.*, 2022; OZKOK; EDELSTEIN, 2014). In addition, the animals that received cisplatin showed a marked loss in hepatocellular glycogen and body weight compared to their respective control groups, effects associated with cisplatin adverse effect-associated food restriction (i.e., malaise) (AMMER; NATOCHIN; DAVID; RUMRICH *et al.*, 1993; SINGH; PATIL; NORONHA; JOSHI *et al.*, 2021).

Immunohistochemical studies revealed an increase in pSTA3-positive nuclei in the hepatocytes of animals in the CIS and MCP+CIS groups, indicative of STAT3 activation. STAT3 is a cytoplasmic transcription factor that belongs to the Janus kinase/signal transducer and activator of transcription pathway and plays a crucial role in liver injury. STAT3 can be activated by several peptides, particularly the IL-6 family of cytokines (KASEMBELI; BHARADWAJ; ROBINSON; TWEARDY, 2018; ZHAO; QI; YU, 2021). Thus, the high circulating levels of IL-6 in the acute toxicity model promote the increased expression of pSTAT3 in hepatocytes, representing a protective mechanism that enable cell proliferation to counteract cisplatin-induced liver damage (HORIGUCHI; ISHAC; GAO, 2007; ZHAO; QI; YU, 2021). Once phosphorylated, the activated STAT protein translocates to the nucleus as a dimer where it binds to the target genes and modulates their transcription (GAO; WANG; LAFDIL; FENG, 2012).

As expected from the previous studies (LI; YANG; LI; FENG, 2018), MCP treatment reduced the hepatic levels of Gal-3 compared to non-MCP-treated groups (SHAM

and CIS), as shown by immunoblots. These MCP-mediated reductions in Gal-3 levels were particularly evident in portal triad structures (bile ducts, blood vessels, and surrounding stroma), as well as in infiltrating leukocytes. Ultrastructural analysis of hepatocytes revealed a significant reduction in cytoplasmic Gal-3 in animals from the CIS and MCP+CIS groups, particularly in mitochondria. Hepatocytes from the CIS and MCP+CIS animals also showed significant increases in ROS production compared to those from SHAM and MCP animals. Oxidative stress is a prevalent mechanism of cisplatin-induced cytotoxicity (BENTLI; PARLAKPINAR; POLAT; SAMDANCI *et al.*, 2013; D'ORIA; SCHIPANI; LEONARDINI; NATALICCHIO *et al.*, 2020; OZKOK; EDELSTEIN, 2014; QU; LIN; WANG; LIU *et al.*, 2014), and induces the formation of reactive oxygen species (ROS), such as hydroxyl and superoxide radicals that depend on cisplatin concentration and exposure time (BROZOVIC; AMBRIOVIĆ-RISTOV; OSMAK, 2010). In turn, ROS promote lipid peroxidation, depletion of sulfhydryl groups, and alteration of different signal transduction pathways, among other actions, which can cause DNA damage and, consequently, cell apoptosis (FLOREA; BÜSSELBERG, 2011).

Animals in the MCP+CIS group showed a marked increase in hepatic MDA levels and mitochondrial respiratory chain complex I expression, indicating that loss of Gal-3 exacerbates mitochondrial dysfunction and oxidative stress. Previous studies have shown that Gal-3 localizes to the endoplasmic reticulum-mitochondria interface where it preserves the integrity of the mitochondrial network and modulates the cellular bioenergetics and the unfolded protein response (COPPIN; JANNIN; AIT YAHYA; THUILLIER *et al.*, 2020). Additionally, Gal-3 interacts with the F1 and F0 subunits of ATP synthase, which is located in the inner mitochondrial membrane, as well as iron-sulfur cluster assembly enzyme, a scaffold protein involved in the assembly of iron-sulfur clusters in complexes I, II, and III. Taken together, these data suggest that Gal-3 affects the mitochondrial OXPHOS complexes (COPPIN; JANNIN; AIT YAHYA; THUILLIER *et al.*, 2020; KIM; KIM; YOO; HONG *et al.*, 2008).

Gal-3-induced apoptosis inhibition also protects mitochondrial integrity, inhibits mitochondrial pore opening during permeability transitions, and facilitates cytochrome c release and subsequent activation of caspase-3 (FUKUMORI; OKA; TAKENAKA;

NANGIA-MAKKER *et al.*, 2006; MATARRESE; FUSCO; TINARI; NATOLI *et al.*, 2000; YU; FINLEY; RAZ; KIM, 2002). Thus, deletion of *LGALS3* (the gene encoding Gal-3) sensitizes various human epithelial cells to apoptosis (CHENG; HUANG; CHEN; TSAI *et al.*, 2011; SHI; HE; KUCHENBECKER; YOU *et al.*, 2007; WONGKHAM; JUNKING; WONGKHAM; SRIPA *et al.*, 2009; XU; GU; GONG; GUO *et al.*, 2013), whereas *LGALS3* overexpression protects them from apoptosis (TAKENAKA; FUKUMORI; YOSHII; OKA *et al.*, 2004). Gal-3 also plays a protective role in murine cytomegalovirus (MCMV)-induced hepatitis, as Gal-3 deficient mice exhibit more pronounced liver damage, higher serum levels of alanine aminotransaminase, and higher viral titers than control mice (STOJANOVIC; MILOVANOVIC; ARSENIJEVIC; STRAZIC GELJIC *et al.*, 2019). Moreover, MCMV induces higher NF-κB-mediated production of TNF-α in Gal-3 knockout hepatocytes, in turn augmenting apoptosis (STOJANOVIC; MILOVANOVIC; ARSENIJEVIC; STRAZIC GELJIC *et al.*, 2019).

Finally, our study showed that MCP treatment elicits up- and downregulation of Gal-1 and Gal-9 levels, respectively, in hepatocytes. Notably, galectins play key roles in inflammation and fibrosis-associated liver pathologies (BACIGALUPO; MANZI; RABINOVICH; TRONCOSO, 2013). Gal-1 is essential for efficient liver regeneration following partial hepatectomy through the regulation of liver inflammation, hepatic cell proliferation, and the control of lipid storage in the regenerating liver (POTIKHA; ELLA; CERLIANI; MIZRAHI *et al.*, 2016). The absence of Gal-9 exacerbated ischemia-reperfusion-induced hepatocellular damage, which was suppressed by the administration of recombinant Gal-9 (HIRAO; UCHIDA; KADONO; TANAKA *et al.*, 2015). Thus, increased Gal-1 expression levels in the livers of MCP-treated animals, particularly those that received cisplatin, may be interpreted as a compensatory mechanism that maintains the hepatoprotective function of these galectins in this setting.

5. Conclusion

Overall, our findings revealed that Gal-3 is a key hepatoprotective agent against the deleterious effects of cisplatin. Furthermore, MCP therapy efficiently antagonizes

both Gal-3 and Gal-9 in the liver, a novel finding that will have utility in future studies seeking to target the effects of Gal-9.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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4. CONCLUSÃO

Os resultados deste trabalho mostram que o modelo experimental de toxicidade hepática induzida pela cisplatina:

1. Altera o perfil leucocitário evidenciado pelo aumento e diminuição significativos da proporção de neutrófilos e linfócitos, respectivamente, nos grupos CIS e MCP+CIS;
2. Induz resposta inflamatória sistêmica e hepatotoxicidade evidenciadas pelo aumento significativo dos níveis plasmáticos de IL-6, IL-10, ARG1, GTSα e SDH nos grupos CIS e MCP+CIS;
3. Induz alteração morfológica hepática caracterizada por desorganização e degeneração dos cordões de hepatócitos, com aumento dos corpos apoptóticos, influxo de leucócitos, bem como redução do armazenamento de glicogênio nos hepatócitos após administração da cisplatina;
4. Aumenta a ativação da STAT3 produção de ROS nos hepatócitos dos grupos CIS e MCP+CIS;
5. Reduz a expressão de Gal-3 nos hepatócitos, especialmente nas mitocôndrias;

Além dos efeitos acima, o tratamento com a MCP no modelo experimental de toxicidade hepática induzida pela cisplatina:

6. Exacerba a resposta inflamatória sistêmica e hepatotoxicidade pelo aumento dos níveis plasmáticos de IL-1β, TNF-α e GOT1 no grupo MCP+CIS;
7. Reduz os níveis hepáticos totais de Gal-3 no fígado nos grupos MCP e MCP+CIS;
8. Causa um desbalanço na respiração mitocondrial pelo aumento da expressão do complexo enzimático I no grupo MCP+CIS;
9. Aumenta os níveis de MDA no fígado, evidenciando ativação do processo de peroxidação lipídica;
10. Aumenta os níveis hepáticos totais de Gal-1 e reduz os de Gal-9 nos grupos MCP e MCP+CIS.

Por fim, nossos achados revelaram que Gal-3 é um agente hepatoprotetor chave contra os efeitos deletérios da cisplatina (Figura 7). Além disso, a terapia com

MCP antagoniza eficientemente Gal-3 e Gal-9 no fígado, uma nova descoberta que terá utilidade em estudos futuros que buscam direcionar os efeitos da Gal-9.

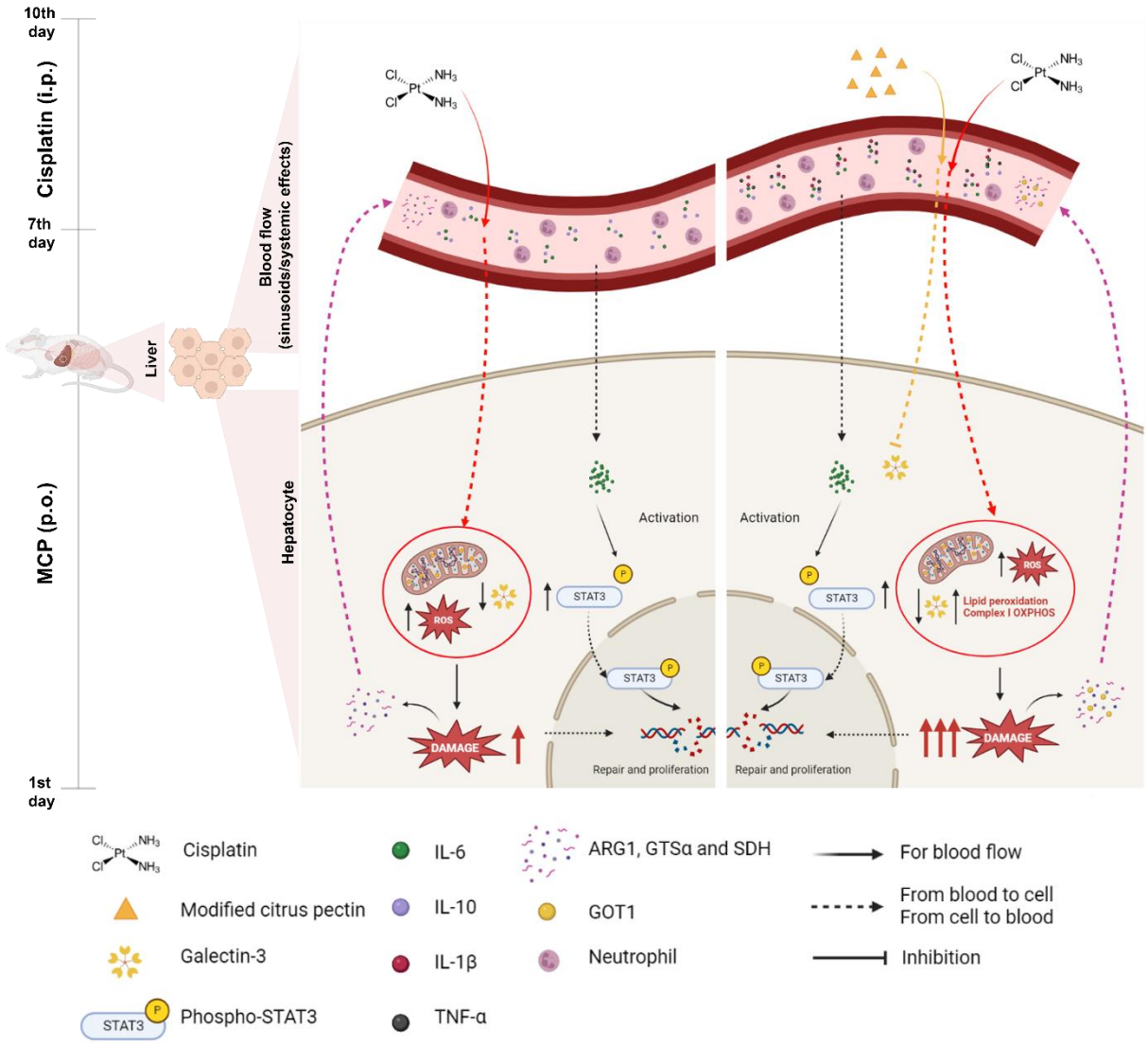


Figura 7. Efeitos do modelo de toxicidade hepática induzida pela cisplatina e do tratamento com MCP. A: A administração da cisplatina aumenta o influxo de neutrófilos periféricos e nos sinusoides hepáticos, bem como dos níveis de citocinas (IL-6, IL-10) e biomarcadores de lesão hepática (ARG1, GTSα e SDH). Nos hepatócitos, ativa a STAT3, aumenta a produção de ROS e diminui os níveis de Gal-3 na mitocôndria, efeitos associados a dano celular. **B:** O tratamento prévio com MCP além de não anular os efeitos da cisplatina, aumenta os níveis de outras

citocinas (IL-1β, TNF-α), GOT1, MDA (peroxidação lipídica) e do complexo I mitocondrial, elevando os danos celulares.

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ANEXO A – Aprovação do Comitê de Ética para o Uso de Animais

UNIFESP
UNIVERSIDADE FEDERAL DE SÃO PAULO

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "EFEITO DE INIBIÇÃO DA PROTEÍNA GALECTINA-3 NA TOXICIDADE HEPÁTICA INDUZIDA PELA CISPLATINA EM RATOS", protocolada sob o CEUA nº 5533211220 (ID 010408), sob a responsabilidade de **Cristiane Damas Gil** *e equipe; Diego Dias dos Santos; Gisela Rodrigues da Silva Sasso; Mauricio de Rosa Trotta; Cristiane Damas Gil* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de São Paulo (CEUA/UNIFESP) na reunião de 20/01/2021.

We certify that the proposal "EFFECT OF THE INHIBITION OF THE GALECTIN-3 PROTEIN ON HEPATIC TOXICITY INDUCED BY CISPLATIN IN RATS", utilizing 27 Heterogenics rats (27 males), protocol number CEUA 5533211220 (ID 010408), under the responsibility of **Cristiane Damas Gil** *and team; Diego Dias dos Santos; Gisela Rodrigues da Silva Sasso; Mauricio de Rosa Trotta; Cristiane Damas Gil* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Sao Paulo (CEUA/UNIFESP) in the meeting of 01/20/2021.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 04/2021 a 04/2023 Área: Histologia E Biologia Estrutural/morfologia E Genética

Origem:	CEDEME - Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	60 a 70 dias	N:	27
Linhagem:	WISTAR			Peso:	200 a 250 g		

Local do experimento: O modelo experimental será desenvolvido no Biotério do CEDEME. A eutanásia e coleta das amostras serão realizadas no laboratório de Histologia (LACIMI), Disciplina de Histologia e Biologia Estrutural, do Departamento de Morfologia e Genética.

São Paulo, 20 de janeiro de 2021

Profa. Dra. Monica Levy Andersen
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de São Paulo

Profa. Dra. Daniela Santoro Rosa
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de São Paulo

ANEXO B – Artigo publicados durante o período de Mestrado

Life Sciences 304 (2022) 120677

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Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

The role of annexin A1-derived peptide Ac_{2-26} on liver and kidney injuries induced by cisplatin in rats

Danilo B.M. Lucchi [a], Gisela R.S. Sasso [a], Letícia S. Sena [a], Diego D. Santos [c], Paulo C. Franco [a], Izabella Lice [a], Fernanda T. Borges [b], Sonia M. Oliani [c,d], Cristiane D. Gil [a,c,*]

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ARTICLE INFO

Keywords:
Hepatotoxicity
Inflammation
Nephrotoxicity
Neutrophil
Formyl peptide receptors

ABSTRACT

Aims: In this study we evaluated the effect of pharmacological treatment with AnxA1-derived peptide Ac_{2-26} in an experimental model of toxicity induced by cisplatin.

Main methods: Male rats were divided into Sham (control), Cisplatin (received intraperitoneal injections of 10 mg/kg/day of cisplatin for 3 days) and Ac_{2-26} (received intraperitoneal injections of 1 mg/kg/day of peptide, 15 min before cisplatin) groups.

Key findings: After 6 h of the last dose of cisplatin, an acute inflammatory response was observed characterized by a marked increase in the number of neutrophils and GM-CSF, IL-β, IL-6, IL-10 and TNF-α plasma levels. These findings were associated with increased AnxA1 protein levels in liver and kidneys, as well as positive AnxA1/Fpr2 circulating leukocytes. Treatment with Ac_{2-26} produced higher levels of GM-CSF, corroborating the high numbers of neutrophils, and the anti-inflammatory cytokine IL-4. Ac_{2-26} preserved the morphology of liver structures and increased Fpr1 expression, preventing the damage caused by cisplatin. In the kidneys, Ac_{2-26} caused downregulation of renal Fpr1 and Fpr2 levels and abrogated the increased levels of the CLU and KIM-1 biomarkers of kidney damage induced by cisplatin. However, no effect of peptide treatment was detected in cisplatin-induced kidney morphology injury.

Significance: Despite activation of the anti-inflammatory AnxA1/Fpr axis during cisplatin administration, treatment with Ac_{2-26} did not efficiently prevent its deleterious effects on the liver and kidneys.

1. Introduction

Cisplatin is a platinum-derived chemotherapy agent widely used in the treatment of solid tumors and leukemias [1,2]. Intracellularly, it acts by producing DNA inter- and intra-chain cross-links, causing damage to DNA, RNA, and proteins and, consequently, apoptotic or non-apoptotic cell death [1,2]. In addition, cisplatin induces oxidative stress by forming reactive oxygen species (ROS), such as hydroxyl and superoxide radicals that depend on cisplatin concentration and exposure time [3]. ROS are believed responsible for lipid peroxidation, depletion of sulfhydryl groups, and alteration of different signal transduction pathways, among other actions, which may also damage DNA [4]. Since the action of cisplatin is not selective for tumors, toxicity related to the use of this drug is common even when administered at standard doses.

In the kidney, when cisplatin is administered intravenously to patients with normal renal function, approximately 25 % of the dose is eliminated during the first 24 h, and up to 50 % in 5 days; it can last in the body for up to 28 days in overdose [2,5]. In this context, acute and

Abbreviations: AnxA1, Annexin A1; ARG1, Hepatic arginase 1; CLU, Clusterin; Fpr1, Formyl peptide receptor 1; Fpr2, Formyl peptide receptor 2; GM-CSF, Granulocyte-macrophage colony-stimulating factor; GOT1, Aspartate transaminase 1; GSTα, α-Glutathione S-transferase; IL, Interleukin; KIM-1, Renal injury molecule 1; ROS, Reactive oxygen species; SDH, Sorbitol dehydrogenase; TNF-α, Tumor necrosis factor-α.

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Galectin-3 is a key hepatoprotective molecule against the deleterious effect of cisplatin

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ARTICLE INFO

Keywords:
Hepatotoxicity
Inflammation
Lipid peroxidation
Mitochondria
Modified citrus pectin
Reactive oxygen species

ABSTRACT

Aims: Evaluate the role of galectin-3 in the liver using an acute model of cisplatin-induced toxicity.
Material and methods: Modified citrus pectin (MCP) treatment was used to inhibit galectin-3. Rats were distributed into four groups: SHAM, CIS, MCP and MCP + CIS. On days 1–7, animals were treated by oral gavage with 100 mg/kg/day of MCP (MCP and MCP + CIS groups). On days 8, 9 and 10, animals received intraperitoneal injection of 10 mg/kg/day of cisplatin (CIS and MCP + CIS groups) or saline (SHAM and MCP groups).
Key findings: Cisplatin administration caused a marked increase in hepatic leukocyte influx and liver degeneration, and promoted reactive oxygen species production and STAT3 activation in hepatocytes. Plasma levels of cytokines (IL-6, IL-10), and hepatic toxicity biomarkers (hepatic arginase 1, α-glutathione S-transferase, sorbitol dehydrogenase) were also elevated. Decreased galectin-3 levels in the livers of animals in the MCP + CIS group were also associated with increased hepatic levels of malondialdehyde and mitochondrial respiratory complex I. Animals in the MCP + CIS group also exhibited increased plasma levels of IL-1β, TNF-α, and aspartate transaminase 1. Furthermore, MCP therapy efficiently antagonized hepatic galectin-9 in liver, but not galectin-1, the latter of which was increased.
Significance: Reduction of the endogenous levels of galectin-3 in hepatocytes favors the process of cell death and increases oxidative stress in the acute model of cisplatin-induced toxicity.

1. Introduction

Galectin-3 (Gal-3) is a chimera-type β-galactoside-binding lectin characterized by a carbohydrate recognition domain (CRD) linked to another N-terminal domain, the latter of which can form oligomers [1,2]. This lectin acts as a pleiotropic mediator with numerous exerted intra- and extracellular functions [1,3]. Among the various roles described for Gal-3, those related to the inflammatory response, tumorigenesis, and innate and acquired immunity are the most well-understood [3,4]. In inflammatory processes, Gal-3 predominantly acts as a pro-inflammatory cytokine. For example, it activates mast cells and basophils [5], facilitates eosinophil migration [6,7], induces cytokine release by monocytes [8], promotes chemotaxis [9] and enables phagocytosis by macrophages in vitro [10]. Gal-3 also plays an important role in apoptosis, which is a fundamental component of the tissue-repair phase of inflammatory response [2]. Notably, Gal-3 can have either pro- or anti-apoptotic roles depending on its location and activation state, as well as the cell type in question [11–13].

Different experimental hepatotoxicity models have shown an essential role for Gal-3 in oxidative stress, injury, and hepatic fibrosis [14–16]. For example, in mouse models, acetaminophen administration increased levels of inducible nitric oxide synthase, IL-1β, macrophage

Abbreviations: ARG1, hepatic arginase 1;; CIS, cisplatin; GOT1, aspartate transaminase 1; GSTα, α-glutathione S-transferase; IL, interleukin; MCP, modified citrus pectin; MDA, malondialdehyde; ROS, reactive oxygen species; SDH, sorbitol dehydrogenase; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α..

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RESEARCH ARTICLE

Cell Biology International WILEY

Bisphenol A disruption promotes mammary tumor microenvironment via phenotypic cell polarization and inflammatory response

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Abstract

Inflammation in the established tumor microenvironment (TME) is often associated with a poor prognosis of breast cancer. Bisphenol A (BPA) is an endocrine-disrupting chemical that acts as inflammatory promoter and tumoral facilitator in mammary tissue. Previous studies demonstrated the onset of mammary carcinogenesis at aging when BPA exposure occurred in windows of development/susceptibility. We aim to investigate the inflammatory repercussions of BPA in TME in mammary gland (MG) during neoplastic development in aging. Female Mongolian gerbils were exposed to low (50 μg/kg) or high BPA (5000 μg/kg) doses during pregnancy and lactation. They were euthanized at 18 months of age (aging) and the MG were collected for inflammatory markers and histopathological analysis. Contrarily to control MG, BPA induced carcinogenic development mediated by COX-2 and p-STAT3 expression. BPA was also able to promote macrophage and mast cell (MC) polarization in tumoral phenotype, evidenced by pathways for recruitment and activation of these inflammatory cells and tissue invasiveness triggered by tumor necrosis factor-alpha and transforming growth factor-beta 1 (TGF-β1). Increase of tumor-associated macrophages, M1 (CD68 + iNOS+) and M2 (CD163+) expressing pro-tumoral mediators and metalloproteases was observed; this aspect greatly contributed to stromal remodeling and invasion of neoplastic cells. In addition, the MC population drastically increased in BPA-exposed MG. Tryptase-positive MCs increased in disrupted MG and expressed TGF-β1, contributing to EMT process during carcinogenesis mediated by BPA. BPA exposure interfered in inflammatory response by releasing and enhancing the expression of mediators that contribute to tumor growth and recruitment of inflammatory cells that promote a malignant profile.

KEYWORDS
bisphenol A, mast cells, TGF-β1, TNF-α, tumor-associated macrophages

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ANEXO C – Ata da defesa pública da Dissertação de Mestrado

UNIVERSIDADE ESTADUAL PAULISTA
Câmpus de São José do Rio Preto

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE DIEGO DIAS DOS SANTOS, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIAS, DO INSTITUTO DE BIOCIÊNCIAS, LETRAS E CIÊNCIAS EXATAS - CÂMPUS DE SÃO JOSÉ DO RIO PRETO.

Aos 19 dias do mês de abril do ano de 2023, às 14:00 horas, por meio de Videoconferência, realizou- se a defesa de DISSERTAÇÃO DE MESTRADO de DIEGO DIAS DOS SANTOS, intitulada **Efeito de inibição da proteína galectina-3 na toxicidade hepática induzida pela cisplatina em ratos**. A Comissão Examinadora foi constituida pelos seguintes membros: Profa.Dra. CRISTIANE DAMAS GIL (Orientador(a) - Participação Virtual) do(a) Departamento de Morfologia e Genética / Universidade Federal de São Paulo/UNIFESP, Profa. Dra. ANA PAULA GIROL (Participação Virtual) do(a) Laboratório Multidisciplinar / Centro Universitário Padre Albino/UNIFIPA, Profa. Dra. VANESSA MOREIRA (Participação Virtual) do(a) Departamento de Farmacologia / Universidade Federal de São Paulo. Após a exposição pelo mestrando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final:_ _ _ _ _ _ _ _ _ _ _ _ _ . Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Profa. Dra. CRISTIANE DAMAS GIL