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ELAINE CRISTINA BEZERRA BASTOS

ANÁLISE DA BIOCOMPATIBILIDADE DA SOLUÇÃO DE CARNOY EM MODELO EXPERIMENTAL IN VIVO COM PEIXE-ZEBRA

Sobral – CE
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Linha de Pesquisa: Doenças Crônicas e Câncer
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DEDICATÓRIA

Ao meu filho amado, Antônio Neudimar Bezerra Bastos Costa, para que sirva de inspiração de perseverança e esforço.

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Chico Xavier

RESUMO

O complexo bucomaxilofacial é propenso a lesões, incluindo o ameloblastoma e o ceratocisto odontogênico, que, embora benignas, podem se manifestar de maneira agressiva, apresentando desafios em termos de recorrência. Esta pesquisa analisou a biocompatibilidade da Solução de Carnoy em um modelo experimental *in vivo* com peixe-zebra e *docking* molecular. Foi conduzido um estudo *in vivo* de natureza experimental e longitudinal, utilizando amostras independentes divididas aleatoriamente em grupos. O modelo animal escolhido foi o peixe- zebra (Danio rerio) adulto. Os peixes-zebra foram selecionados aleatoriamente e, em seguida, transferidos para uma esponja úmida para tratamento com administração por via oral (n=66) ou intraperitoneal (n=66) (20 μL da solução). As soluções de Carnoy com clorofórmio (grupo I), Carnoy sem clorofórmio (grupo II) com quatro níveis de concentração (100%, 50%, 25%, 10%) (n=8) ou controles (solução salina, n=4), foram colocados em copos individuais (250 mL) contendo 150 mL de água do aquário e permitidos a se recuperar, e avaliar as taxas de mortalidade ao longo de 24, 48, 72, e 96 horas. O *docking* molecular foi realizado a partir da construção de um complexo férrico e de sua interação com o receptor MDM2. A sobrevida global média foi calculada por meio de curvas de Kaplan-Meier, e a comparação entre os grupos foi realizada usando o teste de Log-Rank Mantel-Cox ($p<0,05$). A solução de Carnoy quando utilizada em concentração de 100% foi letal para todos os animais de ambos os grupos. A mesma taxa de letalidade foi evidenciada na solução de Carnoy com clorofórmio a 50%. A via de administração intraperitoneal apresentou menores taxas de letalidade, sendo evidenciada por sobrevivência dos animais por maior período. A toxicidade aumentou proporcionalmente a concentração havendo diferença estatisticamente significante entre as concentrações de cada grupo sem carnoy ($p=0.005$) e com carnoy ($p<0.005$). No geral, o tratamento com carnoy parece ser mais tóxico que o tratamento sem carnoy. O complexo férrico da solução de Carnoy evidenciou estabilidade e uma interação forte com o receptor MDM2. A acidez do pH favorece a ligação entre ambos. A presença do clorofórmio aumenta a toxicidade da solução e sua maior interação com o receptor MDM2, o que pode justificar a maior eficácia da formulação com clorofórmio. Mesmo em pequenas concentrações as soluções analisadas podem proporcionar grau de toxicidade ressaltando a importância do descarte correto dessas soluções no ambiente.

Palavras-chave: Peixe-Zebra; Solução de Carnoy; Teste de materiais.

ABSTRACT

The maxillofacial complex is prone to benign lesions, including ameloblastoma and odontogenic keratocyst, which can manifest aggressively, presenting challenges in terms of recurrence. This research analyzed the biocompatibility of Carnoy's Solution in an in vivo experimental model with zebrafish and molecular docking. An in vivo study of an experimental and longitudinal nature was conducted, using independent samples randomly divided into groups. The chosen animal model was the adult zebrafish (Danio rerio). The zebrafish were randomly selected and then transferred to a wet sponge for treatment, with oral administration (n=66) or intraperitoneal (n=66) (20 μL of the solution). The Carnoy's solutions with chloroform (group I), Carnoy without chloroform (group II) with four levels of concentration (100%, 50%, 25%, 10%) (n=8) or controls (saline solution, n=4), then they were placed in individual cups (250 mL) containing 150 mL of aquarium water and allowed to recover, and mortality rates were assessed over 24, 48, 72, and 96 hours. Molecular docking was performed from the construction of a ferric complex and its interaction with the MDM2 receptor. The overall average survival was calculated using Kaplan-Meier curves, and the comparison between the groups was performed using the Log-Rank Mantel-Cox test ($p<0.05$). The Carnoy solution when used at a concentration of 100% was lethal to all animals in both groups. The same lethality rate was evidenced in the Carnoy solution with chloroform at 50%. The intraperitoneal administration route showed lower lethality rates, being evidenced by the survival of the animals for a longer period. Toxicity increased proportionally to the concentration with a statistically significant difference between the concentrations of each Carnoy without chloroform group ($p=0.005$) and Carnoy with chloroform ($p<0.005$). Overall, Carnoy with chloroform treatment appears to be more toxic than Carnoy without chloroform treatment. The ferric complex of Carnoy's solution showed stability and a strong interaction with the MDM2 receptor. The acidity of the pH favors the bond between both. The presence of chloroform increases the toxicity of the solution and its greater interaction with the MDM2 receptor, which may justify the greater efficacy of the formulation with chloroform. Even at small concentrations, the analyzed solutions can provide a degree of toxicity, highlighting the importance of the correct disposal of these solutions in the environment.

Keywords: Zebra fish; Carnoy solution; Materials testing

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LISTA DE ABREVIATURAS E SIGLAS

CC	Solução de Carnoy com clorofórmio
CEUA	Comissão Ética no uso de animais
CONCEA	Conselho Nacional de Controle da Experimentação Animal
FDA	*Food and Drug Administration*
NUBEX	Núcleo de Biologia Experimental
SC	Solução de Carnoy sem clorofórmio
SPSS	*Statistical Package for the Social Sciences* - Pacote Estatístico para as Ciências Sociais
TGI	Trato gastrointestinal
PZa	Peixe Zebra adulto, *Danio rerio*

LISTA DE SÍMBOLOS

°C: Grau Celsius

g: Gramas

mL: Mililitros

µL: Microlitros

SUMÁRIO

1 INTRODUÇÃO

O complexo bucomaxilofacial pode ser sede para variedade de lesões, algumas de origem odontogênica e outras não odontogênicas, que, embora benignas, muitas vezes se manifestam de maneira agressiva e apresentam altas taxas de recorrência, como o ameloblastoma e o cisto odontogênico ceratocístico.[1,2] Quando confrontados com o diagnóstico de uma lesão agressiva, os planos de tratamento podem variar desde abordagens conservadoras, como a enucleação simples e curetagem mecânica, a procedimentos mais invasivos, como a ressecção.[3-5] No entanto, é importante notar que o uso de terapias conservadoras pode, às vezes, resultar em taxas elevadas de recorrência, enquanto as abordagens mais radicais podem causar comprometimento funcional e estético significativo.[1-3,6]

Solução de Carnoy (álcool absoluto, ácido acético glacial, cloreto férrico e clorofórmio) tem sido amplamente utilizada como agente cauterizante desde a década de 80 no tratamento de lesões mandibulares intra-ósseas. Seu princípio de ação é baseado na cauterização química que promove necrose superficial em estruturas ósseas.[6-9]. Estudos tem sido conduzidos para avaliar a eficácia da solução,[9] o efeito sobre substratos minerais humanos,[6] caracterização físico-química e reológica,[7] bem como possíveis modificações da solução com a inserção de agentes de viscosidade para aprimorar a aplicação clínica.[8]

No entanto, observam-se algumas lacunas acerca de possíveis mecanismos que ajudem a explicar os resultados clínicos advindos do uso da solução de Carnoy, incluindo o papel desempenhado pelos seus componentes químicos, informações relativas à sua biocompatibilidade, além da segurança de manejo e descarte. Nesse cenário, entende-se por biocompatibilidade como a capacidade de um material em desempenhar uma determinada função específica em um organismo vivo, sem promover uma resposta no hospedeiro que cause algum dano mensurável.[10]

Uma forma de avaliar esses critérios seria através de estudos *in vivo* com o peixe-zebra (*Danio rerio*),[11] bem como através do *docking* molecular.[12]

O modelo do peixe-zebra tem se mostrado um modelo alternativo devido à similaridade genética com os seres humanos (70-80%) e a viabilidade laboratorial em sua utilização.[11]

Por outro lado, o *docking* molecular é uma técnica essencial, em química computacional e biologia estrutural, que prevê a conformação de uma molécula em um sítio de ligação de uma proteína alvo a partir de modelos tridimensionais e algoritmos avançados para avaliar interações e energias de ligação. Sendo importante na identificação de compostos potenciais para alvos terapêuticos e no design de fármacos.[12-14]

Diante do exposto, o objetivo da presente pesquisa foi analisar a biocompatibilidade da solução de Carnoy em modelo experimental *in vivo* com peixe-zebra, e apresentar o *docking* molecular dessa solução. Até o presente momento, aparentemente, não existem trabalhos publicados que tenham realizado investigação semelhante.

2 MATERIAIS E MÉTODOS

2.1 Delineamento do estudo

Foi realizado um estudo *in vivo*, do tipo experimental longitudinal, de caráter analítico, com amostras independentes e divididas aleatoriamente entre os grupos. Estas consistiram no modelo animal peixe-zebra (*Danio rerio*) adulto (PZa). Os animais foram provenientes do Biotério do Núcleo de Biologia Experimental (NUBEX) da Universidade de Fortaleza (UNIFOR), seguindo-se a especificidade metodológica de cada protocolo experimental.

O presente trabalho 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 aprovado pela

Comissão de Ética no Uso de Animais da Universidade de Fortaleza (CEUA/UNIFOR) sob o número de protocolo 3919131217 (ID 000049) (Anexo 1).

2.2 Preparação das soluções e grupos experimentais

As formulações de Solução de Carnoy (SC) foram preparadas de acordo com o que é preconizado na literatura mundial. O Grupo I foi representado pela SC com clorofórmio, sendo preparada pelo mesmo farmacêutico treinado utilizando 6 mL de etanol absoluto, 3 mL de clorofórmio, 1 mL de ácido acético e 1 g de cloreto férrico hexahidratado ($FeCl_3.6H_2O$) onde se obteve aproximadamente 10mL da solução final da solução.[6-8] O Grupo II foi representado pela SC sem clorofórmio e, para tanto, o volume de 3 mL de clorofórmio será substituído por 3 mL de etanol absoluto.[6-8] O Grupo III tratou-se do grupo controle, sendo este representado pela solução salina 0,9%.

2.3 Protocolos experimentais – estudo *in vivo*

2.3.1 Peixe zebra *(Danio rerio)*

Foram utilizados peixes zebra (Danio rerio) selvagens, adultos, ambos os sexos, obtidos de um fornecedor comercial em Fortaleza, Ceará, Brasil.

Todos os protocolos seguiram estritamente as normas de cuidados com animais de laboratório preconizadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e serão submetidos à Comissão de Ética no Uso de Animais (CEUA) da Unifor.

Antes dos experimentos, grupos de PZa machos e fêmeas (140 a 150 animais), de tamanho uniforme de comprimento (3,5 ± 0,5 cm) e peso (0,5 ± 0,2 g) foram mantidos em aquário de vidro de 10 litros (30 x 15 x 20 cm), contendo água da torneira previamente tratada com anticloro (*ProtecPlus*®), aclimatizados por 24 horas no laboratório, a temperatura de 25±2

°C, pH 7,0 e fotoperíodo quase normal (14:10 h Claro/Escuro). Nenhum alimento foi fornecido

24 h antes ou durante os experimentos. No final do período experimental os peixes foram sacrificados com água gelada (5°C). No dia dos experimentos, os PZa dos grupos tratados e do controle foram acondicionados individualmente em becker (300 mL) contendo água do aquário (150 mL).[15]

2.3.2 Protocolo geral

No dia do experimento, os PZa foram selecionados aleatoriamente e, em seguida, transferidos para uma esponja úmida para tratamento com as soluções do estudo ou controles, por via oral ou intraperitoneal, em seguida foram colocados em copos individuais (250 mL) contendo 150 mL de água do aquário e permitidos a se recuperar. Para tratamentos orais, foi utilizada uma pipeta de volume variável de 20 µL, e os tratamentos intraperitoneais uma seringa de insulina de 0,5 ml. Como controle negativo, foi utilizado soro fisiológico 0,9% (20 µL).15,16

2.3 3 Toxicidade aguda para peixes-zebra adultos

O estudo foi realizado com base na adaptação da metodologia de Ferreira et al., 2020.16 Os PZa foram tratados por via intraoral (n=66) ou intraperitoneal (n=66) com 20 µL das soluções de Carnoy com clorofórmio (grupo I), Carnoy sem clorofórmio (grupo II) com quatro níveis de concentração (100%, 50%, 25%, 10%). Após 24, 48, 72 e 96 horas, os valores obtidos em relação ao número de PZa mortos foram registrados e submetidos à análise estatística.

2.4 Docking molecular

Com base nisso, este *docking* molecular investiga de forma computacional o potencial de interação da solução de Carnoy com a proteína MDM2, com base na função farmacológica que cada componente químico potencialmente executa no meio fisiológico.

De posse do espectro Raman da solução de Carnoy,[7] foi explorado o potencial de complexação do Ferro (FeCl2) presente na solução, e foi sugerido, com base na sua

reatividade em pH < 1 e na análise de seus orbitais moleculares, a formação de um complexo estável com 6 moléculas de clorofórmio (Figura 1).

2.4.1 Modelagem molecular

Para o *docking* molecular, foi simulada a ação do complexo férrico da solução de Carnoy ao interagir com a proteína MDM2, relacionada ao tumor presente in vivo. A estrutura tridimensional do complexo foi modelada com o software Avogadro 1.1.1,[21] respeitando toda a estereoquímica da molécula (Figura 2), e a estrutura da proteína foi obtida a partir do Protein Data Bank (PDB), com resolução de 2.1 angstroms (PDB ID: 5TRF) (disponível em: https://www.rcsb.org/structure/5TRF), obtida por métodos cristalográficos.[22]

2.4.2 Docking molecular

Para a simulação, foram utilizados os *softwares*: HEX 8.0.0,[23]que realiza esta ordenação de forma automática, e o PyMol v1.4.7,[24] que permite a investigação detalhada dos complexos formados, assim como medição de ligações, análise dos resíduos de aminoácidos envolvidos e observação do enovelamento dos sítios de interação. Para o encaixe, foi rotacionada, por toda a área do receptor, uma molécula do complexo férrico da solução de Carnoy, não considerando moléculas de água e explorando toda a superfície da proteína MDM2 (PDB ID: 5TRF).

Os parâmetros utilizados para o processo de *docking* foram: *Correlation type– Shape only*, *Calculation Device* - CPU, *Post-Processing* – DARS *Energies*, Número de Soluções - 50000, Modo FFT - 3D *fast lite*, *Grid Dimension* – 0.6, Faixa do receptor - 180 Receptor, Faixa do Ligante - 180, Faixa de Torção - 360, Faixa de Distância - 40.

2.5 Análise estatística

O tamanho da amostra adotada para os sub-grupos do experimento foi de 8 animais. Todos os dados foram tabulados no Microsoft Excel® e exportados para o programa de análises estatísticas *Statistical Packcage for the Social Sciences* (SPSS®) versão 20.0 para Windows®. Foi calculado a sobrevida global média na exposição dos peixes a solução através da via

intraoral por meio de curvas de kaplan-meier as quais foram comparadas usando o teste de Log-Rank Mantel-Cox ($p<0,05$). Os resultados foram expressos como valores médios $\pm$ erro padrão da média.

3 RESULTADOS

3.1 Estudo *in vivo*

O experimento foi realizado com 136 peixes, 4 deles foram destinados ao grupo controle (solução salina) e os demais foram distribuídos em dois grupos, sendo eles solução de Carnoy com clorofórmio (grupo I, n=66) e solução de Carnoy sem clorofórmio (grupo II, n=66). Cada um desses grupos foi dividido em quatro subgrupos com base na concentração da solução (n=8). Foram observadas 28 mortes na via de administração intraoral. A solução de Carnoy quando utilizada em concentração de 100% foi letal para todos os animais de ambos os grupos. A mesma taxa de letalidade foi evidenciada na solução de Carnoy com clorofórmio a 50%

(tabela 1).

A via de administração intraperitoneal apresentou menores taxas de letalidade, sendo evidenciada por sobrevivência dos animais por maior período. Entretanto, concentrações menores propiciaram um maior número de mortalidade tanto no grupo solução de Carnoy CC quanto no SC (tabela 2).

Os resultados da comparação da toxicidade baseada na sobrevida dos PZa frente a exposição a solução de Carnoy CC e SC, para diferentes doses do medicamento no período avaliado, mostram que o tratamento CC e SC foram menos tóxicos em doses menores não sendo observada diferença estatisticamente significante entre concentrações semelhantes em grupos diferentes. A toxicidade aumentou à medida que a concentração da dose aumentou independente da via de administração e do grupo estudado havendo diferença estatisticamente significante entre as concentrações de cada grupo SC ($p=0.005$) e CC ($p<0.005$). No geral, o tratamento CC parece ser mais tóxico que o tratamento SC (Tabela 3).

3.2 Docking molecular

Foi observado o encaixe do complexo férrico da solução de Carnoy em todos os 10 clusters de maior energia, gerados a partir do modelo matemático utilizado na procura de sítios de interação pelo *software*. Com base nestes clusters, foi possível sobrepor os 10 sítios de interação, e foi visualizada a sobreposição dos 5 mais energéticos. Isto sugere que dentro de 50 mil possibilidades prospectadas pelo *software*, as 10 mais energéticas foram selecionadas, e 5 estavam no mesmo sítio, uma reprodutibilidade química e espacial que indica fortemente que aquele é o melhor sítio de ligação, da proteína MDM2 ao complexo férrico da solução de Carnoy (Figura 03).

Em relação à interação química, é possível observar que a proteína MDM2 interage fortemente com o ligante, com ligações químicas que variam de 2,0 a 5,5 angstrons, e que vários aminoácidos da proteína propiciam encaixe estável do ligante (Figura 03), garantindo a ação biológica sugerida pelo que é observado *in vivo*.[25] Poucas repulsões eletrostáticas são observadas e os aminoácidos do receptor que interagem mais fortemente são: Arg65, Leu66, Tyr76, Ser78 e Asn79.

Os resíduos de aminoácidos envolvidos na interação com o complexo férrico da solução de Carnoy, sugerem diferentes tipos de ligações químicas, já que no cluster mais energético, as ligações mediram respectivamente: Arg 65 (5.5a), Leu 66 (4.0a), Tyr 76 (3.0a), Ser 78 (2.2a) e Asn79 (2.0a). Com base nestas medidas, o complexo mais estável apresenta ligações de hidrogênio, forças de van der waals e possíveis ligações iônicas, já que a molécula é dinâmica. O número de ligações também influi na análise energética dos clusters obtidos (Tabela 4).

O decréscimo brusco de energia de complexação evidencia que nos 5 primeiros clusters, que estão na mesma alça de enovelamento quaternário, a compatibilidade do complexo com a proteína é alta, o que sustenta a reprodutibilidade do sítio nos clusters de maior energia. Os valores também são significativos em relação a um complexo químico que contém apenas 6

moléculas de clorofórmio, o que sugere a interação de todos os íons reativos, com resíduos de aminoácidos e com íons H^+ presentes no meio (o que também acontece *in vivo* – pH<1).

4 DISCUSSÃO

A solução de Carnoy também desempenha um papel significativo no processo de diagnóstico do câncer colorretal, especialmente no que diz respeito à recuperação eficiente de linfonodos. Essa solução se revelou particularmente benéfica em cenários onde a recuperação de linfonodos é desafiadora, como após a quimiorradiação ou quando o número necessário de linfonodos para um estadiamento adequado é elevado. Dessa forma, a solução melhora a precisão do estadiamento, um fator crítico para determinar o prognóstico e o tratamento adequado proporcionando informações cruciais para o clínico de modo a evitar subestadiamento do câncer, garantindo que os pacientes recebam o tratamento adequado e melhorando suas perspectivas de sobrevivência.[30,31] Entretanto, não são descritos nos estudos[30,31] qual a forma de manejo dessa solução após sua utilização.

Apesar de ser amplamente utilizada como terapia adjuvante eficaz no tratamento de cistos odontogênicos que apresentam características de recidiva,[3-5,9,25,26] pouco se sabe sobre a biocompatibilidade da solução de Carnoy com e sem clorofórmio.

A presença de clorofórmio na solução aumenta a viscosidade e deixa o pH mais ácido em comparação com a solução de Carnoy modificada (sem clorofórmio).[8] Essa redução no pH pode estar associada a maior letalidade evidenciada da solução de Carnoy com clorofórmio, bem como ao maior potencial de ligação a proteína MDM2, conforme evidenciado no *docking* molecular.

Outro ponto a considerar é a maior capacidade de alteração tanto de matriz orgânica quanto inorgânica em substratos mineralizados humanos quando se compara a solução CC e SC.[6] O que pode justificar as maiores taxas de mortalidade nos animais do grupo solução de Carnoy com clorofórmio em ambas as vias de administração apesar de não haver diferença estatisticamente significante entre os grupos CC e SC. O que pode ser correlacionado

clinicamente com a ausência de diferença significativa nas taxas de recidiva das lesões como encontrado nos estudo de Sharma et al.,[27] e Karaca et al.[28] Justificando uma preferencia pela solução de Carnoy modificada no tratamento dessas lesões sem expor o paciente ao clorofórmio, a uma substância potencialmente carcinogênica, conforme a FDA (*Food and Drug Administration* – Agência Reguladora de Comida e Administração de Drogas) recomenda.[6,8]

A solução de Carnoy com ou sem clorofórmio promove aumento na concentração de íons Fe em amostras de substratos humanos calcificados. Esse achado provavelmente ocorreu devido à possibilidade de substituição do Ca na fórmula estequiométrica da hidroxiapatita por um cátion metálico, como o íon Fe.[6] Esse íon é fundamental na estabilização da solução e na capacidade de adesão aos receptores MDM2 conforme evidenciado no presente *docking* molecular. Sabe-se que o cloreto férrico possui um padrão ácido e atua promovendo a coagulação proteica. Como a solução de Carnoy apresentou efeito de aumento da concentração de Fe, é possível que tenha ocorrido uma substituição progressiva de Ca por Fe nos cristais de hidroxiapatita dentária e óssea, aumentando a concentração de Fe nos tecidos.[6] Portanto, o clorofórmio proporciona maior concentração de Fe, então pode-se esperar uma necrose coagulativa mais pronunciada, corroborando com as maiores taxas de mortalidade encontradas no grupo CC.

Estudos anteriores[6-8] demonstram que os íons ferro executam funções farmacológicas dentro do pH extremamente ácido, com provimento de íons hidrogênio (H^+) oriundos do ácido acético, força iônica em meio orgânico devido o etanol absoluto, e potencialmente pode ser associado ao clorofórmio e sua reatividade em meio fisiológico.

O ameloblastoma e o tumor odontógeno ceratocístico têm comportamento biológico semelhante devido à superexpressão das proteínas MDM2 e p53, que estão associadas à patogênese e oncogenese dessas lesões.[29] A expressão das proteínas MDM2 e p53 foi determinada imuno-histoquimicamente em 51 espécimes de cistos e tumores odontogênicos fixados em parafina[17] reforçando seu papel na etiopatogênese da doença. A ligação da solução de Carnoy com o receptor da proteína MDM2 de forma estável pode ser um fator

determinante para o princípio de ação da solução e seu papel na redução das taxas de recidiva dessa lesão quando utilizada de forma adjuvante ao tratamento.

Bloquear a interação proteína-proteína entre MDM2 e p53 tem sido considerada uma possibilidade de oferecer uma estratégia terapêutica contra neoplasias odontogênicas,[17] apesar dos potenciais riscos de seleção de tumores que abrigam mutações p53 e que escapam ao controle da proteína MDM2.[17-20]

O descarte de medicamentos[32] ou resíduos químicos[33] no meio ambiente tem sido foco de diversos estudos[32-37] em virtude dos prejuízos ambientais que essas substâncias podem provocar. A detecção de concentrações de traços de compostos farmacêuticos humanos em águas superficiais e subterrâneas continua a receber atenção considerável na literatura técnica e na imprensa popular.[37] Tal consideração é importante para prevenir contaminação do meio ambiente e da espécie humana, visto que a solução é tóxica, promove mortalidade considerável, mesmo em menores concentrações e utilizando a via intraoral.

A maioria das pessoas descarta medicamentos não utilizados ou vencidos no lixo doméstico ou despeja no vaso sanitário/pia.[32] O manejo incorreto dessas substâncias leva ao acúmulo de produtos farmacêuticos ativos em aterros municipais de resíduos sólidos, bem como levar a exposição potencial dos seres humanos a esses produtos químicos através da via da água potável e à biota aquática que está em águas superficiais que recebem esgoto doméstico tratado.[37] O que ressalta a importância do manejo desses resíduos.

Compreender as práticas atuais de eliminação de produtos farmacêuticos no ambiente e a percepção de risco associada a eles é fundamental para o desenvolvimento de políticas eficazes de gerenciamento de resíduos. Esse entendimento auxilia na promoção do descarte adequado, protegendo a saúde humana e o meio ambiente.[35,38] Compreender as causas e efeitos da percepção de risco dos produtos farmacêuticos no meio ambiente contribui para melhorar os processos de gerenciamento de resíduos e promover o descarte adequado, conforme confirmado pelos resultados de um estudo em uma amostra de 509 indivíduos em Portugal, Espanha e França.[38]

A compreensão da biocompatibilidade a partir de estudos *in sílico* e *in vivo*

contribuem para o entendimento da compreensão da ação da solução de Carnoy e da presença do clorofórmio em solução. Esse conhecimento pode ser a base para o desenvolvimento de novos fármacos ou princípios ativos para aumentar a taxa de sucesso dessa terapia adjuvante. A letalidade mesmo em baixas concentrações ressalta, também, o papel do descarte adequado desse material após sua utilização de modo a proteger a saúde humana e o meio ambiente.

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FIGURAS

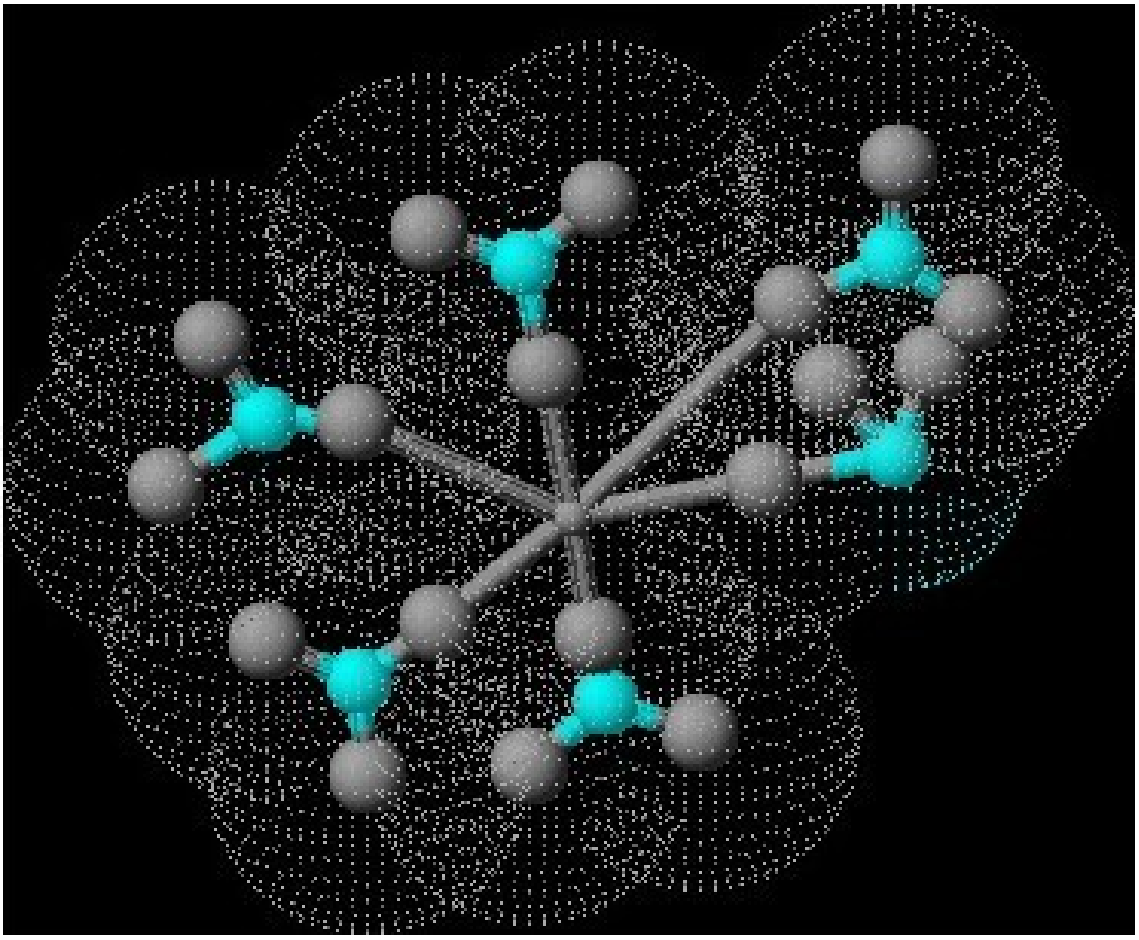

Figura 1: Complexo férrico com clorofórmio, modelado pela ferramenta computacional ACD/ChemSketch 5 Freeware 12.0, respeitando o orbital molecular, o raio atômico, os impedimentos estéricos e as cargas intrínsecas.

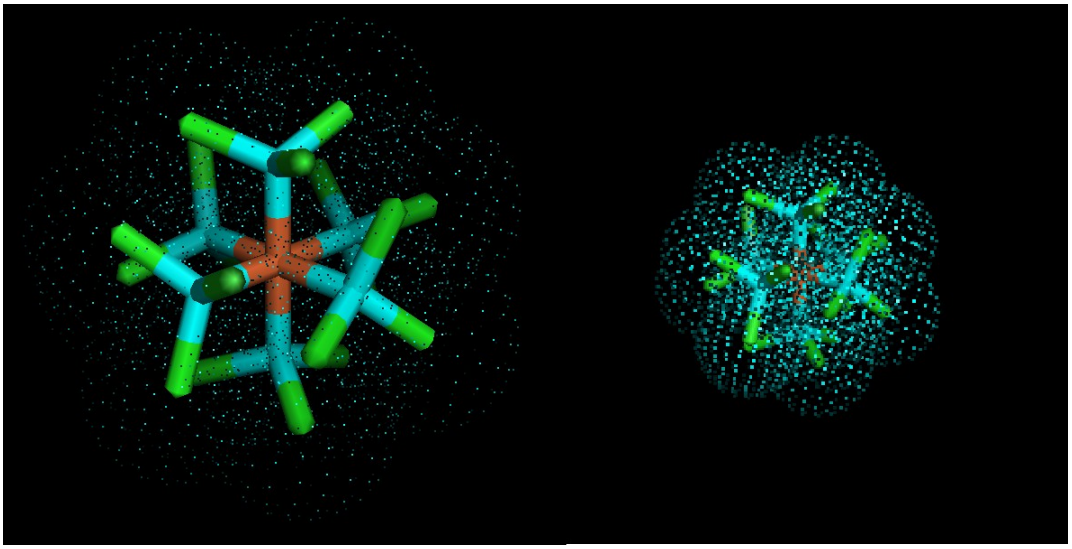

Figura 2: Modelagem do complexo FeCl2+ Clorofórmio, na sua representação em sticks. O Ferro (vermelho) permite um orbital molecular hexaédrico com 6 moléculas de Clorofórmio (ciano), expondo todos os seus íons reativos (verde). À direita, a representação da nuvem eletrônica de possíveis interações químicas.

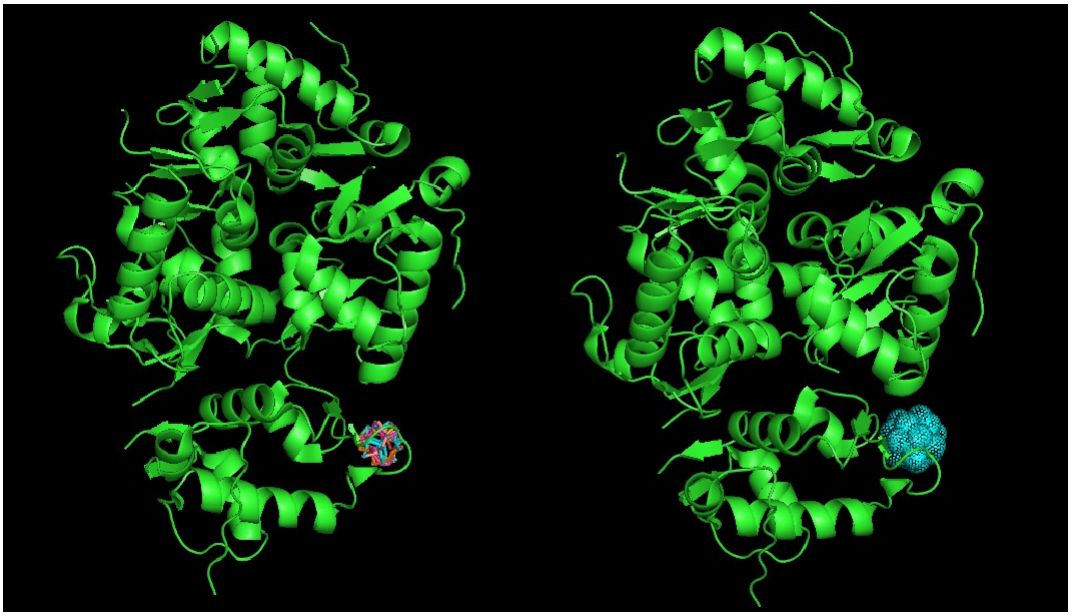

Figura 3: Localização espacial dos 5 clusters mais energéticos entre o complexo férrico e a proteína MDM2 (PDB ID: 5TRF), evidenciando alta reprodutibilidade e afinidade do sítio de ligação (à esquerda); Localização espacial do cluster mais energético, evidenciando complexação com a proteína (à direita).

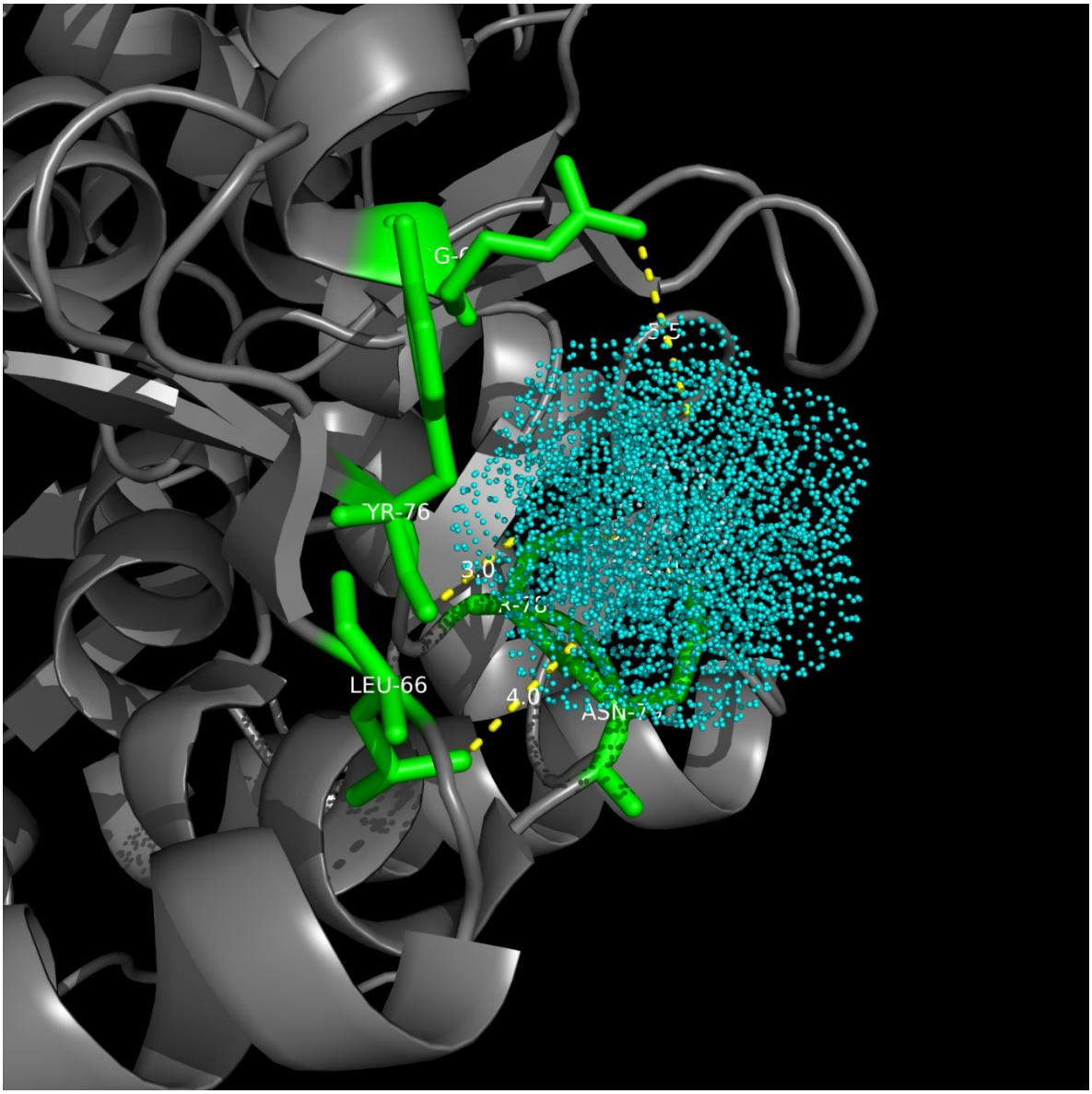

Figura 4: Interações químicas do complexo férrico com a proteína MDM2 (PDB ID: 5TRF). Evidenciando ligações químicas que variam de 2.0 a 5.5 angstrons, realizadas entre íons reativos do complexo de Carnoy com os resíduos de aminoácidos Arg65, Leu66, Tyr76, Ser78 e Asn79.

TABELAS

Tabela 1: Avaliação mortalidade dos peixes-zebra após administração via oral.

Concentração / Tempo	**Solução de Carnoy CC** 24h	48h	72h	96h	**Solução de Carnoy SC** 24h	48h	72h	96h
10%	2	0	0	0	0	1	0	0
25%	0	0	0	0	0	0	0	0
50%	8	0	0	0	1	0	0	0
100%	8	0	0	0	8	0	0	0

Carnoy SC: solução de Carnoy sem clorofórmio; Carnoy CC: solução de Carnoy com clorofórmio.

Tabela 2: Avaliação mortalidade dos peixes-zebra após injeção intraperitoneal.

Concentração / Tempo	**Solução de Carnoy CC** 24h	48h	72h	96h	**Solução de Carnoy SC** 24h	48h	72h	96h
10%	0	1	1	0	0	2	2	0
25%	3	1	0	0	1	1	1	0
50%	3	3	1	0	4	3	1	0
100%	5	3	0	0	4	3	0	0

Carnoy SC: solução de Carnoy sem clorofórmio; Carnoy CC: solução de Carnoy com clorofórmio.

Tabela 3: Sobrevida global de peixes-zebra considerando administração por via oral e via intraperitoneal.

Concentração	Carnoy SC	Carnoy CC	p-Valor
10%	2.80±0.33	2.80±0.51	1.000
25%	2.80±0.60	1.50±0.22	0.076
50%	1.63±0.26	1.60±0.40	0.951
100%	1.43±0.20	1.17±0.17	0.351
p-Valor	**0.005***	**<0.005***	

Carnoy SC: solução de Carnoy sem clorofórmio; Carnoy CC: solução de Carnoy com clorofórmio; *p<0,05, teste Log-Rank Mantel-Cox (média±EPM).

Tabela 4: Energia total obtida com a interação entre o complexo férrico presente na Solução de Carnoy e a proteína oncogênica MDM2 (Etotal – kcal/mol)

Cluster 01:	-825,73
Cluster 02:	-786,93
Cluster 03:	-648,62
Cluster 04:	-609,58
Cluster 05:	-598,99
Cluster 06:	-190,11
Cluster 07:	-183,66
Cluster 08:	-177,98
Cluster 09:	-174,81
Cluster 10:	-172,82

ANEXOS

ANEXO 1 – APROVAÇÃO NO COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE DE FORTALEZA (CEUA/UNIFOR)

FUNDAÇÃO EDSON QUEIROZ
UNIVERSIDADE DE FORTALEZA
ENSINANDO E APRENDENDO

Universidade de Fortaleza
Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "EVALUATION OF TOXICITY AND BIOCOMPATIBILITY OF CARNOY SOLUTION FOR USE IN BUCOMAXILLOFACIAL SURGERY: IN VIVO, IN VITRO, AND IN SILICO STUDY", protocolada sob o CEUA nº 3919131217 (ID 000049), sob a responsabilidade de **Francisco Samuel Rodrigues Carvalho** *e equipe; FÁBIO WILDSON GURGEL COSTA; ADRIANA ROLIM CAMPOS DE BARROS; FERNANDO ANDRÉ CAMPOS VIANA; PAULO GOBERLANIO DE BARROS SILVA* - 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 de Fortaleza (CEUA/UNIFOR) na reunião de 23/02/2018.

We certify that the proposal "EVALUATION OF TOXICITY AND BIOCOMPATIBILITY OF CARNOY SOLUTION FOR USE IN BUCOMAXILLOFACIAL SURGERY: IN VIVO, IN VITRO, AND IN SILICO STUDY", utilizing 60 Fishes (males and females), protocol number CEUA 3919131217 (ID 000049), under the responsibility of **Francisco Samuel Rodrigues Carvalho** *and team; FÁBIO WILDSON GURGEL COSTA; ADRIANA ROLIM CAMPOS DE BARROS; FERNANDO ANDRÉ CAMPOS VIANA; PAULO GOBERLANIO DE BARROS SILVA* - 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 Fortaleza's University (CEUA/UNIFOR) in the meeting of 02/23/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 04/2018 a 04/2019 Área: Medicina Veterinária

Origem: Não aplicável biotério
Espécie: Peixes sexo: Machos e Fêmeas idade: 1 a 480 horas Quantidade: 60
Linhagem: Danio rerio Peso: 1 a 10 g

Fortaleza, 06 de novembro de 2023

Prof. Dr. Ramon da Silva Raposo
Coordenador da Comissão de Ética no Uso de Animais
Universidade de Fortaleza

Prof. Dr. Saulo Ellery Santos
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade de Fortaleza

Avenida Washington Soares 1321, Bairro Edson Queiroz, CEP 60811-905 - Fortaleza/CE - tel: 55 (85) 3477-3122
Horário de atendimento: 2ª a 6ª das 8h ás 11:30 e 13:30 ás 17h : e-mail: ceua@unifor.br
CEUA N 3919131217

ANEXO 2 – NORMAS DA REVISTA ZEBRAFISH

Manuscript Submission Guidelines and Policies for Zebrafish

Last updated 7/20/2022 1:52:38 PM

Journal Information

Manuscript Submission Site: https://mc.manuscriptcentral.com/zebrafish
Editorial Office Contact: ekker.stephen@gmail.com
Support Contact: prosupport@liebertpub.com
Journal Model: Hybrid (Open Access Option)
Blinding: Single Blind
File formatting requirement stage: Upon submission
Instant Online Option (immediate publication of accepted version): No
Submission Fee: None
Average time to initial decision: 36 days

About the Journal

Zebrafish is a peer-reviewed journal focusing on research using zebrafish and other aquarium species including medaka, Fugu, and Xiphophorus as models for studies of vertebrate development, toxicology, and human disease. The Journal serves as a forum for articles discussing research on comparative genomics and evolution, the genetic analysis of embryogenesis and disease and the cellular and molecular mechanisms of cell growth, differentiation, and gene expression in these model species. The Journal publishes articles describing novel methods, tools, and experimental approaches using aquarium models.

Manuscript Types and Guidelines

Original Research Articles
3,000-word limit
Unstructured abstract of no more than 200 words
Maximum total of eight (8) figures and/or tables
Review Articles
8,000-word limit
Unstructured abstract of no more than 200 words
Maximum total of ten (10) figures and/or tables

Technofish-Previews

150-word limit
One (1) figure
Submit directly to Dr. David Kimelman

Technofish-Methods 750-word limit

Unstructured abstract of no more than 100 words
One (1) figure
Online supplementary material
Fish Around the World
2,500-word limit
Unstructured abstract of no more than 200 words
Maximum total of three (3) figures and/or tables
Letters to the Editor
500-word limit
May include one (1) figure OR table
Reference citations are identical in style to those of full original articles, but should not exceed five (5).
Protocol
The Protocol manuscript type is dedicated to supporting the awareness and publication of operating procedures for methodologies that reinforce key advances in the field. The stepby-step protocol provided in a Protocol Article is intended to establish peer-reviewed methodologies and enable technical improvements for specialists and non-specialists. The Protocol Article submission should describe a method that has already been used to produce results in a peer-reviewed original research article and should describe a technological or methodological update or advancement when compared to the “state-ofthe art” methodology.
Every submitted Protocol Article must provide data and compare the new process to

existing processes or identify gaps in prior related protocol publications.
4,000-word limit
350-word structured abstract
Composition: Introduction, Method, Experiment, Results, and Discussion
10 figures maximum
6 tables maximum
Word limits do NOT pertain to the abstract, disclosure statements, author contribution statements, funding information, acknowledgments, tables, figure legends, or references.

Description of Manuscript Types

Original Research Articles- Fish Haus, Edu Fish, and Evo Fish articles are included in this category.
TechnoFish Articles-
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Style Examples for Reference List:

Type of Reference	Punctuation and Order of Elements in Reference List
Journal article with 1-3 authors	Wang Q, Nambiar K, Wilson JM. Isolating natural adeno-associated viruses from primate tissues with a high-fidelity polymerase. Hum Gene Ther 2021;32(23-24):1439-1449; doi: 10.1089/hum.2021.055 [insert article-specific DOI if available].
Journal article with more than 3 authors	Pfister EL, DiNardo N, Mondo E, et al. Artificial miRNAs reduce human mutant Huntington throughout the striatum in a transgenic sheep model of Huntington's disease. Hum Gene Ther 2018;29(6):663–673; doi: 10.1089/hum.2017.199 [insert article-specific DOI if available].
Edited Book	Herzog RW, Zolotukhin S, (eds). A Guide to Human Gene Therapy. World Scientific Publishing Co. Pte. Ltd.: Singapore; 2010.
Chapter in an Edited Book	Nicklin SA, Baker AH. Adenoviral Vectors. In: A Guide to Human Gene Therapy. (Herzog RW, Zolotukhin S. eds.) World Scientific Publishing Co. Pte. Ltd.: Singapore; 2010; pp. 21-36.
Authored Book	Isaacson W. The Code Breaker: Jennifer Doudna, Gene Editing, and the Future of the Human

Race. Simon & Schuster: New York, NY; 2021.
Website
Last name, first/middle initial(s) of author(s) [if available]. U.S. Food and Drug Administration. What is Gene Therapy? Silver Spring, MD; 2018. Available from: https://www.fda.gov/vaccines-bloodbiologics/cellular-gene-therapy-products/what-gene-therapy [Last accessed: month/date/year].
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