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O TRATAMENTO COM O RESVERATROL REVERTE O PRIAPISMO EM CAMUNDONGOS TRANSGÊNICOS PARA A ANEMIA FALCIFORME

Bragança Paulista
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RESUMO

O priapismo constitui uma importante complicação clínica em homens com anemia falciforme, com prevalência estimada entre 30% e 45%, sendo que cerca de 30% dos pacientes evoluem para disfunção erétil permanente. Os episódios recorrentes de priapismo demandam atendimentos frequentes em serviços de emergência e comprometem significativamente a qualidade de vida, afetando o bem-estar físico, psicológico e social dos pacientes. Apesar da alta incidência, as terapias disponíveis são voltadas apenas para o manejo agudo das crises, não havendo estratégias eficazes de prevenção. Do ponto de vista fisiopatológico, o priapismo na anemia falciforme está associado à diminuição da biodisponibilidade de óxido nítrico (NO) e ao aumento do estresse oxidativo. Evidências experimentais indicam que os corpos cavernosos de indivíduos e de camundongos com anemia falciforme apresentam produção exacerbada de espécies reativas de oxigênio (EROs), atribuída à maior expressão da NADPH oxidase e à desacoplagem da eNOS, resultando na formação de ânion superóxido (O_2^-) e consequente inativação do NO. O resveratrol, um polifenol natural presente *em Polygonum cuspidatum*, uvas e amendoins, é conhecido por suas propriedades antioxidantes, anti-inflamatórias e vasoprotetoras. Assim, este estudo teve como objetivo avaliar se o tratamento com resveratrol é capaz de reverter o fenótipo de priapismo em camundongos transgênicos para anemia falciforme, investigando seus efeitos sobre a via NO–GMPc–PDE5 e sobre a enzima pró-oxidante NADPH oxidase 2. Camundongos machos selvagens (C57BL/6) e transgênicos para anemia falciforme foram tratados com resveratrol (100 mg/kg/dia, por gavagem) ou veículo durante duas semanas. O tratamento com resveratrol promoveu redução da expressão de NADPH Oxidase 2, diminuição dos marcadores de estresse oxidativo (4-hidroxinonenal e 3-nitrotirosina) e restauração da sinalização endotelial mediada por NO. Além disso, normalizou a expressão de eNOS e PDE5, aumentou os níveis teciduais de GMPc e reduziu as respostas de relaxamento exacerbadas do corpo cavernoso induzidas por acetilcolina, nitroprussiato de sódio e estimulação elétrica de campo. Esses efeitos foram observados exclusivamente nos camundongos falciformes. Em conjunto, os resultados indicam que o resveratrol atenua o estresse oxidativo e restaura a via NO–GMPc–PDE5, revertendo as alterações vasculares associadas ao fenótipo de priapismo na anemia falciforme. Esses achados sugerem que o resveratrol se configura como um potencial agente terapêutico para a restauração da função erétil e a prevenção de episódios recorrentes de priapismo em pacientes com anemia falciforme.

Palavras-chaves: Priapismo. Anemia Falciforme. Disfunção erétil. Resveratrol. Estresse oxidativo.

ABSTRACT

Priapism represents a major clinical complication in men with sickle cell disease (SCD), with an estimated prevalence ranging from 30% to 45%, and approximately 30% of these patients progressing to permanent erectile dysfunction. Recurrent episodes of priapism often require frequent visits to emergency departments and markedly impair quality of life, affecting patients' physical, psychological, and social well-being. Despite its high incidence, currently available therapies focus solely on acute management, with no effective preventive strategies. From a pathophysiological perspective, priapism in SCD is associated with reduced nitric oxide (NO) bioavailability and increased oxidative stress. Experimental evidence indicates that the corpora cavernosa of both humans and transgenic mice with SCD exhibit excessive production of reactive oxygen species (EROs), attributed to upregulated NADPH oxidase activity and uncoupled endothelial nitric oxide synthase (eNOS). These alterations promote superoxide anion (O_2^-) formation and consequent NO inactivation, resulting in endothelial dysfunction and impaired cavernosal regulation. Resveratrol, a natural polyphenol found in *Polygonum cuspidatum*, grapes, and peanuts, is well recognized for its antioxidant, anti-inflammatory, and vasoprotective properties. Therefore, this study aimed to evaluate whether resveratrol treatment can reverse the priapism phenotype in transgenic SCD mice, by investigating its effects on the NO–cGMP–PDE5 signaling pathway and the pro-oxidant enzyme NADPH oxidase 2. Male wild-type (C57BL/6) and SCD transgenic mice were treated with resveratrol (100 mg/kg/day, by gavage) or vehicle for two weeks. Resveratrol treatment resulted in reduced NADPH oxidase 2 expression, decreased oxidative stress markers (4-hydroxynonenal and 3-nitrotyrosine), and restoration of endothelial NO-mediated signaling. Furthermore, it normalized eNOS and PDE5 expression, increased tissue cGMP levels, and attenuated the exaggerated relaxation responses of cavernosal smooth muscle induced by acetylcholine, sodium nitroprusside, and electrical field stimulation. These effects were observed exclusively in SCD mice. Taken together, the findings demonstrate that resveratrol mitigates oxidative stress and restores the NO–cGMP–PDE5 pathway, thereby reversing the vascular alterations associated with the priapism phenotype in SCD. These results suggest that resveratrol may represent a promising therapeutic agent for the restoration of erectile function and prevention of recurrent priapism in patients with sickle cell disease.

Keywords: Priapism. Sickle Cell Anemia. Erectile Dysfunction. Resveratrol. Oxidative Stress.

LISTA DE SÍMBOLOS E ABREVIAÇÕES

eNOS - Óxido Nítrico Sintase Endotelial

EROs - Espécies Reativas de Oxigênio

GMPc - Monofosfato Cíclico de Guanosina

HbF - Hemoglobina Fetal

NADPH oxidase - Oxidase de Nicotinamida Adenina Dinucleotídeo Fosfato

NO- Óxido Nítrico

PDE5 - Fosfodiesterase tipo 5

SUMÁRIO

1. INTRODUÇÃO

A anemia falciforme corresponde a uma doença hereditária resultante de uma modificação na hemoglobina caracterizada por alterações estruturais na cadeia beta da hemoglobina, resultando na produção da hemoglobina S, uma forma anormal da proteína. Essa mutação modifica a morfologia dos eritrócitos, que adquirem formato de foice, o que justifica a denominação “falciforme” (ALVAIA et al., 2020).

Essa doença afeta milhões de pessoas em todo o mundo e está relacionada a diversas complicações clínicas decorrentes da vaso-oclusão, da hipóxia e do estresse oxidativo crônico. Entre essas complicações, destaca-se o priapismo isquêmico recorrente, uma condição caracterizada por ereção peniana prolongada e persistente, geralmente dolorosa, sem estímulo sexual, com duração superior a quatro horas. Esse tipo de priapismo é o mais frequente e clinicamente relevante, sendo o subtipo classicamente associado à anemia falciforme (JOICE; LIU; BURNETT, 2021; SALONIA et al., 2014).

O priapismo isquêmico constitui uma emergência médica urológica, exigindo intervenção em até 4 a 6 horas, geralmente por meio da aspiração e irrigação dos corpos cavernosos, associada à injeção intracavernosa de agentes simpatomiméticos. Quando essas medidas falham, pode ser necessária intervenção cirúrgica, e, em casos graves, o implante de prótese peniana é considerado (SALONIA et al., 2014).

Essa condição representa um importante problema clínico em homens com anemia falciforme. Os episódios recorrentes de priapismo resultam em sofrimento físico, impacto psicológico e prejuízo à qualidade de vida, comprometendo o bem-estar pessoal, familiar e social. Do ponto de vista fisiopatológico, o priapismo isquêmico provoca modificações estruturais nos corpos cavernosos, como fibrose peniana irreversível e prejuízos às células endoteliais e musculares lisas, culminando em disfunção erétil (JOICE; LIU; BURNETT, 2021). Estudos epidemiológicos mostram que 30% a 45% dos homens com anemia falciforme apresentam episódios de priapismo isquêmico, e cerca de 30% deles evoluem para disfunção erétil permanente (ADEYOJU et al., 2002).

Apesar da frequência e da gravidade do quadro, as terapias disponíveis concentram-se apenas no manejo agudo, sem estratégias efetivas de prevenção (JOICE; LIU; BURNETT,

2021). Dessa forma, há uma necessidade urgente de novas abordagens terapêuticas que atuem sobre os mecanismos moleculares subjacentes ao priapismo, visando prevenir sua ocorrência e recorrência.

Estudos experimentais indicam que o priapismo está associado à desregulação da via de sinalização óxido nítrico–guanosina monofosfato cíclico–fosfodiesterase tipo 5 (NO–GMPc–PDE5) nos corpos cavernosos, comprometendo o equilíbrio entre os mecanismos de ereção e detumescência. Nesses casos, observa-se redução sustentada da expressão da enzima óxido nítrico sintase endotelial (eNOS), o que compromete a produção basal de óxido nítrico (NO) e, consequentemente, a ativação fisiológica da via NO–GMPc. Essa hipoatividade crônica leva à diminuição da expressão da PDE5, enzima responsável pela degradação do GMPc. Como resultado, há perda do controle homeostático da sinalização cavernosa, de modo que, durante estímulos que promovem liberação aguda de NO, ocorre acúmulo excessivo de GMPc e relaxamento exacerbado e prolongado do músculo liso peniano, caracterizando o fenótipo de priapismo (PEREIRA et al., 2024; PINHEIRO et al., 2022).

Além disso, a anemia falciforme agrava o estresse oxidativo, levando ao aumento da produção de espécies reativas de oxigênio (EROs) e redução da capacidade antioxidante tecidual. O excesso de ânion superóxido reage rapidamente com o NO, formando peroxinitrito e diminuindo sua biodisponibilidade, o que contribui para a desregulação da via NO–GMPc–PDE5. Esse ambiente pró-oxidante contribui para lesão endotelial, inflamação e fibrose cavernosa, mecanismos que participam do desenvolvimento e manutenção do fenótipo de priapismo na anemia falciforme (PINHEIRO et al., 2022).

Diante deste cenário, torna-se indispensável investigar substâncias com potencial antioxidante e vasoprotetor capazes de reverter o estresse oxidativo e restaurar a função endotelial. O resveratrol (3,5,4'-trihidroxiestilbeno), um polifenol natural encontrado em uvas, amendoins e frutas vermelhas, é amplamente reconhecido por suas propriedades antioxidantes, anti-inflamatórias e cardioprotetoras (PETROVSKI; GURUSAMY; DAS, 2011; BURNS et al., 2002). Estudos pré-clínicos demonstraram que o resveratrol melhora a função endotelial, aumenta a expressão de eNOS, eleva os níveis de GMPc e diminui a produção de espécies reativas de oxigênio (EROs) no corpo cavernoso, promovendo melhor

relaxamento da musculatura lisa peniana e preservação da função erétil (MURAT et al., 2016; YAZIR et al., 2018; YU et al., 2013).

Com base nessas evidências, pressupõe-se que o tratamento com resveratrol é capaz de reverter o fenótipo de priapismo em camundongos transgênicos para anemia falciforme, por meio da restauração da via NO–GMPc–PDE5 e da redução da atividade da enzima pró-oxidante NADPH oxidase 2, atenuando o estresse oxidativo e restabelecendo a função endotelial. Assim, o presente estudo teve como objetivo avaliar o efeito farmacológico do resveratrol sobre as alterações da função erétil associadas à disfunção endotelial e ao estresse oxidativo em camundongos transgênicos para anemia falciforme.

1.1. Mecanismos Moleculares da Ereção Peniana: A Importância do Óxido Nítrico

O NO constitui o principal neurotransmissor em um sistema de transdução de sinais que atua no pênis para regular a resposta erétil. As enzimas responsáveis pela formação do NO são as óxido nítrico sintases, presentes nas fibras nervosas não adrenérgicas não colinérgicas (NANC) nitrérgicas e nas células endoteliais (eNOS). Essas enzimas catalisam a conversão da L-arginina em NO e L-citrulina (FORSTERMANN; SESSA 2012).

No rato, camundongo e no homem, a Óxido Nítrico Sintase Neuronal está localizada no plexo pélvico, nos nervos cavernosos e seus terminais nervosos no tecido erétil, além dos nervos penianos dorsais e dos plexos nervosos na adventícia de artérias cavernosa e dorsal (BURNETT et al., 1992; MIZUSAWA et al., 2001). Além disso, a acetilcolina induz a liberação endotelial de NO no tecido peniano (SAENZ DE TEJADA et al., 1988). A despolarização do nervo cavernoso provoca vasodilatação das artérias helicinais, aumentando o fluxo sanguíneo e, consequentemente, a força de cisalhamento sobre o endotélio sinusoidal. Esse aumento na força de cisalhamento estimula a via de sinalização envolvendo a fosfatidilinositol 3-quinase e a proteína quinase B, levando à fosforilação e ativação da eNOS. A ativação da eNOS resulta em maior produção de NO pelo endotélio (HURT et al. 2002, 2012). Portanto, a regulação da função erétil não é mediada exclusivamente pelo NO proveniente das fibras nitrérgicas, mas também envolve a produção de NO endotelial induzida pelo aumento do fluxo sanguíneo.

Quando liberado pelas fibras nitrérgicas ou pelo endotélio, o NO alcança as células musculares lisas adjacentes e liga-se ao seu receptor fisiológico intracelular, a guanilato ciclase solúvel. Essa ligação ocorre diretamente no grupo heme, formando um complexo heme-ferro-nitrosil. A interação do NO promove a quebra da ligação entre a histidina axial e o ferro, resultando em uma mudança conformacional que ativa guanilato ciclase solúvel (LUCAS et al., 2000; EVGENOV et al., 2006). A ativação da enzima pelo NO leva à conversão de guanosina trifosfato no segundo mensageiro GMPc. O aumento dos níveis de GMPc ativa a proteína quinase dependente de GMPc, que promove a redução da concentração intracelular de cálcio. Isso causa o relaxamento da musculatura lisa e, consequentemente, a ereção peniana. O GMPc é hidrolisado a GMP pela ação da PDE5, encerrando, assim, a resposta erétil (ANDERSSON, 2011).

1.2. Anemia falciforme e Priapismo

A anemia falciforme constitui uma hemoglobinopatia hereditária de alta relevância epidemiológica, configurando um importante problema de saúde pública nível mundial. Estima-se que, em 2010, cerca de 312 mil recém-nascidos foram afetados pela doença e 5,4 milhões apresentaram o traço falciforme (PIEL et al., 2013). No Brasil, dados do Programa Nacional de Triagem Neonatal indicam que entre 60 a 100 mil pessoas são afetadas pela doença (Ministério da Saúde, 2022).

A mutação pontual no códon 6 do gene da β-globina, promove a substituição do ácido glutâmico por valina, resultando na formação da hemoglobina S e provocando alterações estruturais nas hemácias, que desencadeiam fenômenos de vaso-oclusão, hipóxia e estresse oxidativo. Essas alterações são responsáveis por múltiplas manifestações clínicas, entre as quais se destaca o priapismo isquêmico, uma das complicações urológicas mais graves e incapacitantes da doença (KATO et al., 2018; JOICE; LIU; BURNETT, 2021).

O priapismo na anemia falciforme caracteriza-se por ereção peniana prolongada e dolorosa, sem estímulo sexual, decorrente de obstrução do fluxo sanguíneo e consequente hipóxia tecidual nos corpos cavernosos (BURNETT et al., 2014). Essa condição pode ocorrer de forma aguda ou recorrente, sendo o tipo isquêmico a forma predominante e fisiopatologicamente associada à anemia falciforme. A prevalência varia de 3,6% em adolescentes a até 42% em adultos (ALVAIA et al., 2020).

A duração das crises é um fator prognóstico determinante: episódios prolongados resultam em fibrose cavernosa, lesão endotelial irreversível e disfunção erétil irreversível (JOICE; LIU; BURNETT, 2021; MUSICKI; BURNETT, 2020). O tratamento deve ser iniciado nas primeiras quatro a seis horas e envolve medidas como oxigenação, hidratação, transfusão, aspiração peniana e administração intracavernosa de agentes simpaticomiméticos. Em casos refratários, indica-se intervenção cirúrgica. Evitar novos episódios é crucial para a preservação da função erétil e para a melhoria da qualidade de vida dos pacientes, reforçando a importância de estudos que busquem novas estratégias terapêuticas de caráter preventivo.

1.3. Disfunção da Via do Óxido Nítrico na Fisiopatologia do Priapismo Associado à Anemia Falciforme

Estudos demonstram que, na anemia falciforme, o priapismo pode ser iniciado por um bloqueio do fluxo venoso causado pelas interações entre hemácias falcizadas e diferentes tipos celulares, impedindo, assim, a drenagem venosa dos espaços sinusoidais dos corpos cavernosos e resultando em isquemia tecidual devido ao processo vaso-oclusivo. Paradoxalmente, a redução da biodisponibilidade do NO endotelial nos corpos cavernosos é considerada um dos principais elementos que desencadeiam o priapismo na doença falciforme (SILVA et al., 2016b; KATO, 2012; GLADWIN; SACHDEV, 2012). Na anemia falciforme, a diminuição da biodisponibilidade de NO está associada à hemólise intravascular, ao aumento do estresse oxidativo e à elevação da atividade enzimática da arginase, o que causa redução do substrato L-arginina (KATO; STEINBERG; GLADWIN, 2017; GLADWIN; SACHDEV, 2012).

Dados experimentais apontam que a redução da biodisponibilidade basal de NO endotelial/GMPc resulta em diminuição compensatória da atividade e expressão da PDE5 no corpo cavernoso, prejudicando, assim, o sistema de controle da função erétil (BIVALACQUA et al., 2012; SILVA et al., 2016b; CHAMPION et al., 2005). Assim, a produção neuronal de NO pela Óxido Nítrico Sintase Neuronal em resposta a um estímulo erétil in vivo resulta no acúmulo de GMPc nas células musculares lisas do corpo cavernoso, que passam a apresentar relaxamento exacerbado. A ereção peniana permanece (priapismo) porque o GMPc não é degradado de forma adequada pela PDE5 (CHAMPION et al., 2005;

OLUJOHUNGBE; BURNETT, 2013). Estudos anteriores sugerem que a normalização dos níveis basais de NO/GMPc pode restaurar a expressão e atividade da PDE5 e, consequentemente, o controle regulatório da ereção peniana (SILVA et al., 2016b; BIVALACQUA et al., 2013; LAGODA et al., 2014). De fato, análises funcionais *in vitro* indicam o aumento do relaxamento nitrérgico e do relaxamento dependente e independente do endotélio em corpos cavernosos de camundongos transgênicos para anemia falciforme ("Berkeley" e "Townes"), assim como redução na expressão da PDE5 (SILVA et al., 2016a SILVA et al., 2016b; CHAMPION et al., 2005; CLAUDINO et al., 2009).

Outro fator que pode contribuir para o maior relaxamento do músculo liso cavernoso no priapismo é a elevação dos níveis de adenosina no pênis, a qual promove um estado cronicamente relaxado nos corpos cavernosos (BIVALACQUA et al., 2010; NING et al., 2014). A diminuição da atividade e expressão da via da Rho-quinase (mediador contrátil) foi identificada em corpos cavernosos de homens e de camundongos com anemia falciforme (LAGODA et al., 2013; BIVALACQUA et al., 2010). Dessa forma, dois mecanismos moleculares distintos, porém complementares, parecem atuar de maneira sinérgica no priapismo associado à anemia falciforme: o aumento do relaxamento do músculo liso mediado por GMPc e adenosina, e a redução dos efeitos contráteis da via RhoA/Rho-quinase. Esse duplo desequilíbrio entre as vias relaxantes e contráteis perpetua a perda do controle tônico dos corpos cavernosos, caracterizando o quadro fisiopatológico do priapismo (BIVALACQUA et al., 2010; NING et al., 2014; LAGODA et al., 2013).

1.4. Estresse Oxidativo/Nitrosativo na Fisiopatologia do Priapismo Associado à Anemia Falciforme

O estresse oxidativo e nitrosativo desempenha um papel significativo no desenvolvimento do priapismo associado à anemia falciforme. Os corpos cavernosos de homens e camundongos com anemia falciforme apresentam níveis elevados de EROs, atribuídos principalmente ao aumento da expressão da isoforma NADPH oxidase-2 e ao desacoplamento da eNOS (SILVA et al., 2016a; BIVALACQUA et al., 2013; MUSICKI et al., 2011; LAGODA et al., 2013).

As principais fontes de EROs nas células incluem a cadeia de transporte de elétrons mitocondrial, a NADPH oxidase, a xantina oxidase, as enzimas do citocromo P450 e a eNOS

desacoplada. Dentre essas fontes, as NADPH oxidases destacam-se por serem a única família de enzimas cuja função primária e exclusiva é a produção de EROs (CASAS et al., 2020).

As NADPH oxidases são complexos enzimáticos transmembranares compostos por uma subunidade catalítica e proteínas reguladoras de membrana e citosólicas, como p47phox e p22phox. Até o momento, sete membros da família NADPH Oxidase foram identificados: NADPH Oxidase 1, NADPH Oxidase 2 (também conhecida como gp91phox), NADPH Oxidase 3, NADPH Oxidase 4, NADPH Oxidase 5, Dual Oxidase 1 e Dual Oxidase 2. Suas estruturas e mecanismos de ativação têm sido amplamente estudados. Em humanos, todas as sete enzimas são expressas, e cada isoforma NADPH Oxidase apresenta expressão tecidual específica, diferentes mecanismos regulatórios e produz tipos específicos de EROs. Em contraste, camundongos e ratos expressam as isoformas NADPH Oxidase 1 a NADPH Oxidase 4 e Dual Oxidase, mas não possuem a NADPH Oxidase 5 (SCHRÖDER, 2020).

O aumento da expressão de NADPH Oxidase 2 nos corpos cavernosos na anemia falciforme leva a uma produção excessiva de superóxido (O_2^-), contribuindo para o estresse oxidativo. Além disso, o desacoplamento da eNOS ocorre quando a enzima, em vez de produzir NO, gera superóxido, agravando ainda mais o estresse oxidativo. Uma das consequências desse aumento é a neutralização do NO pelas altas concentrações de O_2^-, formando peroxinitrito, um radical nitrogenado com alta capacidade oxidante (GLADWIN; SACHDEV, 2012). Isso reduz a biodisponibilidade de NO, comprometendo a via NO-GMPc-PDE5, essencial para o controle da função erétil.

Marcadores de estresse oxidativo e nitrosativo, como 4-hidroxinonenal e 3-Nitrotirosina, estão elevados no tecido erétil de camundongos e pacientes com anemia falciforme. Esses marcadores indicam danos oxidativos a lipídios, proteínas e ácido desoxirribonucleico, afetando a integridade estrutural e funcional das células musculares lisas e endoteliais (PEREIRA et al., 2022). Portanto, intervenções terapêuticas direcionadas à redução do estresse oxidativo podem contribuir de forma significativa para o manejo clínico e a prevenção do priapismo nesses pacientes.

1.5. Resveratrol

O resveratrol, um polifenol encontrado naturalmente em uvas, vinho tinto e frutas vermelhas, tem sido amplamente estudado por suas propriedades antioxidantes, anti-inflamatórias e cardioprotetoras. Evidências científicas indicam que esse composto contribui para a prevenção e o tratamento de diversas doenças cardiovasculares, incluindo aterosclerose, hipertensão, isquemia/reperfusão, insuficiência cardíaca e distúrbios metabólicos associados, como diabetes e obesidade, além de exercer efeitos benéficos relacionados ao processo de envelhecimento (PETROVSKI; GURUSAMY; DAS, 2011; BURNS et al., 2002). Essa substância é produzida por plantas em resposta a estresses ambientais, atuando como um mecanismo de defesa contra patógenos e radiação ultravioleta.

Estudos pré-clínicos demonstram que o resveratrol apresenta efeitos protetores em vários modelos de doenças, incluindo enfermidades cardiovasculares, diabetes, neoplasias e desordens neurodegenerativas (BAUR et al., 2006; XIA et al., 2017). Tais benefícios são atribuídos às suas propriedades antioxidantes, anti-inflamatórias, antiapoptóticas e vasoprotetoras. O resveratrol pode modular diversas vias de sinalização celular, influenciando processos como inflamação, proliferação celular e resposta ao estresse oxidativo.

Diversos estudos experimentais demonstram que o resveratrol exerce uma atividade antioxidante expressiva, capaz de atenuar o estresse oxidativo e proteger os tecidos contra danos celulares em diferentes modelos animais (QIN et al., 2012; TAYLOR et al., 2006). Ele é capaz de neutralizar espécies reativas de oxigênio (EROs) e de nitrogênio, protegendo as células contra danos oxidativos. O resveratrol ainda estimula a expressão de enzimas antioxidantes endógenas, como a superóxido dismutase, catalase e glutationa peroxidase, fortalecendo os mecanismos antioxidantes naturais do organismo.

Estudos anteriores demonstraram que o resveratrol tem a importante capacidade de induzir o aumento da Hemoglobina Fetal (HbF), uma propriedade valiosa para o tratamento da anemia falciforme (RODRIGUE et al., 2001; FIBACH et al., 2012). A indução de HbF pode inibir a polimerização da hemoglobina S, reduzindo a deformação das hemácias e, consequentemente, a ocorrência de eventos vaso-oclusivos. Dessa forma, o resveratrol pode contribuir para a melhora dos sintomas e complicações associados à anemia falciforme.

Em estudos experimentais com modelos de disfunção erétil, o resveratrol demonstrou potencial em restaurar a função erétil por meio da melhoria da função endotelial, evidenciada pelo aumento da expressão da enzima eNOS**,** pela elevação dos níveis de GMPc e pela redução da geração de espécies reativas de oxigênio (EROs) no tecido cavernoso. Esses mecanismos favorecem o relaxamento da musculatura lisa peniana, promovendo vasodilatação e facilitando o processo de ereção (MURAT et al., 2016; YAZIR et al., 2018; YU et al., 2013).

Considerando esse conjunto de evidências, o resveratrol se destaca como um potencial agente terapêutico no tratamento do priapismo associado à anemia falciforme, uma vez que sua capacidade de induzir o aumento da HbF, reduzir o estresse oxidativo e melhorar a função endotelial pode atuar de maneira sinérgica sobre os mecanismos fisiopatológicos envolvidos nessa condição, configurando uma abordagem multifacetada e promissora para o manejo da doença.

2. OBJETIVOS

2.1 Objetivos gerais

Avaliar o efeito farmacológico do tratamento com resveratrol na restauração da função erétil alterada pela redução da biodisponibilidade de NO e ao estresse oxidativo em camundongos transgênicos para a anemia falciforme.

2.2 Objetivos Específicos

(I) Determinar parâmetros hematológicos nos camundongos controles e falciformes tratados com o resveratrol ou veículo;

(II) Avaliar o relaxamento do músculo liso de corpos cavernosos induzido pela estimulação da via NO-GMPc nos camundongos controles e falciformes tratados com o resveratrol ou veículo;

(III) Quantificar a expressão gênica da eNOS e PDE5 nos corpos cavernosos dos camundongos controles e falciformes tratados com o resveratrol ou veículo;

(IV) Mensurar os níveis de GMPc nos corpos cavernosos dos camundongos controles e falciformes tratados com o resveratrol ou veículo;

(V) Avaliar os principais marcadores de estresse oxidativo e nitrosativo nos corpos cavernosos nos camundongos controles e falciformes tratados com o resveratrol ou veículo.

3. CAPÍTULO 1: publicado

Título do artigo: *Resveratrol Attenuates the Priapism Phenotype in Sickle Cell Mice by Restroring NO-cGMP-PDE5 Signaling and Reducing NADPH Oxidase 2 Expression*

Referência do artigo: SPLENDORE, C. O.; SILVEIRA, T. H. R.; PEREIRA, D. A.; BOSSARINO, B. P.; GONÇALVES DE OLIVEIRA, M.; CALMASINI, F. B.; BURNETT, A. L.; COSTA, F. F.; SILVA, F. H. *Resveratrol attenuates the priapism phenotype in sickle cell mice by restoring NO-cGMP-PDE5 signaling and reducing NADPH oxidase 2 expression*. *Frontiers in Pharmacology*, v. 16, 30 abr. 2025. DOI: 10.3389/fphar.2025.1551533.

O objetivo deste estudo foi avaliar se o resveratrol é capaz de reverter o fenótipo de priapismo em camundongos transgênicos para anemia falciforme, investigando seus efeitos sobre a via NO–GMPc–PDE5 e sobre a enzima pró-oxidante NADPH oxidase 2. Camundongos machos selvagens (C57BL/6) e transgênicos para anemia falciforme foram tratados com resveratrol (100 mg/kg/dia, por gavagem) ou veículo durante duas semanas. O tratamento com resveratrol promoveu redução da expressão de NADPH Oxidase 2, diminuição dos marcadores de estresse oxidativo e restauração da sinalização endotelial mediada pelo NO. Além disso, normalizou a expressão de eNOS e PDE5, aumentou os níveis de GMPc e reduziu as respostas de relaxamento exacerbadas do corpo cavernoso induzidas por acetilcolina, nitroprussiato de sódio e estimulação elétrica de campo. Esses efeitos foram observados exclusivamente nos camundongos falciformes. Em conjunto, esses achados indicam que o resveratrol atenua o estresse oxidativo e restaura a via NO–GMPc–PDE5, revertendo as alterações vasculares associadas ao priapismo na anemia falciforme e configurando-se como um potencial agente terapêutico para a restauração da função erétil nesses pacientes.

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Resveratrol attenuates the priapism phenotype in sickle cell mice by restoring NO-cGMP-PDE5 signaling and reducing NADPH oxidase 2 expression

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The pathogenesis of priapism in sickle cell disease (SCD) is closely linked to oxidative stress and reduced bioavailability of nitric oxide (NO) in penile tissue. Resveratrol, a potent natural antioxidant, has demonstrated protective effects in various vascular disorders. To evaluate the therapeutic effects of resveratrol on priapism, oxidative stress markers, and NO-cGMP signaling in the penile tissue of transgenic SCD mice. Male wild-type (C57BL/6) and transgenic SCD mice were treated with resveratrol (100 mg/kg/day, gavage) or vehicle for 2 weeks. Functional studies were conducted on CC strips mounted in organ baths to assess relaxation responses to acetylcholine (ACh), sodium nitroprusside (SNP), and nitrergic stimulation (electrical field stimulation, EFS). The oxidative stress markers (NOX-2, 4-HNE, and 3-NT), cGMP levels, and the mRNA expression of endothelial nitric oxide synthase (eNOS) and phosphodiesterase type 5 (PDE5) were evaluated. Resveratrol treatment decreased exaggerated ACh-, SNP-, and EFS-induced relaxation responses in SCD mice. It also reduced oxidative stress markers (NOX-2, 4-HNE, and 3-NT) and normalized eNOS and PDE5 mRNA expression in the CC of SCD mice. Additionally, cGMP levels in the CC were significantly increased by resveratrol treatment. These effects were specific to SCD mice and not observed in wild-type mice. In conclusion, resveratrol reduces oxidative stress and restores NO-cGMP signaling in the penile tissue, reducing the exaggerated cavernosal relaxation characteristic of priapism in SCD. These findings highlight resveratrol as a promising therapeutic candidate for managing priapism in patients with SCD.

KEYWORDS

3-nitrotyrosine, cGMP, hemoglobin, reactive oxygen species, corpus cavernosum

1 Introduction

Sickle cell disease (SCD) is the most common genetic disease in the world, with an estimated 515,000 babies born annually in Africa, predominantly in sub-Saharan Africa, with 50%–90% dying before adulthood (Nkya et al., 2024). The condition is characterized by the presence of mutant hemoglobin S (HbS), which, under low oxygen conditions, promotes the polymerization of HbS and consequent deformation of erythrocytes into a sheet-like shape in the capillary beds (Kato et al., 2018). This morphological inheritance compromises efficient oxygen transport and predisposes patients to recurrent episodes of hemolysis and vaso-occlusive events. These manifestations have resulted in a wide range of acute and chronic complications, among which priapism stands out for its significant impact on quality of life (Kavanagh et al., 2022).

Ischemic priapism is a medical condition characterized by a prolonged and often painful erection of the penis that typically lasts for more than 4 h, regardless of sexual stimulation (Bivalacqua et al., 2022). Research encompassing adult males with SCD indicates a priapism prevalence of 32.6%, with the majority (74%) experiencing stuttering episodes, generally resolving within 4 h (Idris et al., 2020). Prolonged and untreated priapism can cause irreversible damage to penile tissue, including necrosis and fibrosis, culminating in irreversible erectile dysfunction (Salonia et al., 2014). Despite being recognized as a prevalent complication of SCD, the therapeutic options remain suboptimal, underscoring a need for more effective treatment modalities. Current clinical strategies, such as the use of low-dose sildenafil, have shown promise in reducing the frequency of recurrent priapism episodes in men with SCD without affecting normal erectile function (Burnett et al., 2006b; Burnett et al., 2006a; Burnett et al., 2014).

The primary molecular defect contributing to priapism in SCD is the diminished bioavailability of nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) in the penis (Musicki and Burnett, 2020; Pereira et al., 2024a). This condition is attributed to diminished endothelial nitric oxide synthase (eNOS) activity along with increased reactive oxygen species (ROS), which readily neutralize NO (Champion et al., 2005; Musicki et al., 2011; Musicki et al., 2014; Musicki et al., 2018; Musicki et al., 2020; Bivalacqua et al., 2013; Silva et al., 2016a). The heightened oxidative stress observed in SCD penile tissue is linked with an upregulation of NOX-2 NADPH oxidase expression and the uncoupling of eNOS (Musicki et al., 2011; Musicki et al., 2014; Bivalacqua et al., 2013; Silva et al., 2016b; Pereira et al., 2022). Impaired signaling downstream from NO in SCD is linked with a diminished phosphodiesterase type 5 (PDE5) regulatory function, stemming from the lack of the cGMP-dependent feedback control mechanism. This deficiency leads to unchecked accumulation of cGMP following neurostimulation within the penile tissue, ultimately resulting in priapism (Champion et al., 2005; Lagoda et al., 2013; Silva et al., 2016b).

Resveratrol (3,5,4′-trihydroxystilbene) is a natural phytoalexin with potent antioxidant properties, commonly found in foods such as peanuts, grapes, and berries (Baur et al., 2006). Numerous studies have demonstrated the therapeutic potential of resveratrol in protecting against a range of diseases, including diabetes, cancer, hypertension, and cardiovascular disease, through its anti-hypertensive, cardioprotective, and endothelial protective effects (Bonnefont-Rousselot, 2016). In models of erectile dysfunction, resveratrol improved erectile function by improving endothelial function associated with increased eNOS expression, increased cGMP levels, and reduced production of ROS in the corpus cavernosum (Fukuhara et al., 2011; Sener et al., 2018; Yazir et al., 2018; Yu et al., 2022).

Given that oxidative stress impairs NO bioavailability and is implicated in the pathogenesis of priapism, we hypothesized that resveratrol treatment may reverse the oxidative stress and the priapism phenotype from transgenic SCD mice. Therefore, we have undertaken functional and molecular evaluations in this study to evaluate the therapeutic potential of resveratrol on priapism and the associated oxidative stress markers in penile tissue of SCD mice.

2 Materials and methods

2.1 Animals and treatment

All experimental procedures were approved by the Ethics Committee for the Use of Experimental Animals of the University of Campinas (UNICAMP). Three-to five-month-old wild-type (WT, C57BL/6) and Townes transgenic SCD mice were used. The animals were obtained from Jackson Laboratories (Bar Harbor, ME, United States) and bred and characterized at the Multidisciplinary Center for the Investigation of Biological Science in Laboratory Animals at UNICAMP. The homozygous Townes SCD model is a knock-in strain in which the murine α-globin genes are replaced with human α-globin genes, and the β-globin genes are replaced with human Aγ and βS (sickle) globin genes (Wu et al., 2006).

Mice were treated with resveratrol (100 mg/kg/day) or vehicle (water), administered once daily by oral gavage for 2 weeks. The gavage volume was adjusted to 10 μL/gof body weight.

2.2 Hematological parameters

Whole blood was collected by intracardiac puncture from mice anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), using EDTA-coated vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, United States). Complete blood counts were performed within 30–60 min of collection using the Sysmex XN-3000™ hematology analyzer (Sysmex, Kobe, Japan). Following tissue collection, animals were euthanized by an overdose of isoflurane (12%).

2.3 Functional studies in cavernosal strips and concentration-response curves

CC tissues were collected from mice anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). All efforts were made to minimize animal suffering. After tissue collection, animals were euthanized by an overdose of isoflurane (12%). CC from mice were mounted in a 5-mL organ system containing Krebs-Henseleit solution at 37°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). Changes in isometric force were recorded using a strip

myograph for isometric force recording (Model 610M, Denmark) coupled with an acquisition system (PowerLab 8/30, LabChart 7, ADInstruments, Sydney-NSW, Australia). At the start of the experiments, the resting tension was set to 2.5 mN. The corpus cavernosum strips were given a 60-minute equilibration period, during which the bathing medium was changed every 15 min. Cumulative concentration-response curves were constructed for both the muscarinic agonist acetylcholine (ACh, 10^{-9} to 10^{-5} M) and the NO-donor compound sodium nitroprusside (SNP; 10^{-9} to 3×10^{-4} M) in tissue strips pre-contracted with phenylephrine (3×10^{-6} to 10^{-5} M). EC_{50} values are presented as the negative logarithm (pEC_{50}), and calculated by a fitting concentration–response relationship to a sigmoidal model of the form log-concentrations vs. response using the GraphPad Software (GraphPad Software, San Diego, CA, United States) (Pereira et al., 2024b).

2.4 Electrical-field stimulation (EFS) in corpus cavernosum strips

EFS was applied to the cavernosal tissues positioned between two platinum electrodes, connected to a Grass S88 stimulator (Astro-Med Industrial Park, RI, United States). The EFS was performed at a voltage of 50 V, with a pulse width of 1 m and trains of stimuli that lasted 10 s at varying frequencies. The frequency-response relationships were investigated using supra-maximum voltage in all electrically stimulated preparations. In order to study the nitrergic cavernosal relaxations, tissues were pretreated with guanethidine (3×10^{-5} M; to deplete the catecholamine stores of adrenergic fibers) and atropine (10^{-6} M; to produce muscarinic receptor antagonism) prior to pre-contraction with phenylephrine (3×10^{-6} to 10^{-5} M). When a stable contraction level was attained, a series of EFS-induced relaxations were constructed (2–32 Hz). Data were calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%.

2.5 Western blot analysis

Total penile homogenates (50 μg total protein) were run on 4%–20% Tris-HCl gels (Bio-Rad Laboratories, Hercules, CA, United States), transferred to a polyvinylidene fluoride membrane, and incubated overnight at 4°C with the following antibodies: monoclonal anti-NOX-2 (1:1,000; BD Transduction Laboratories, catalog number 611414, San Diego, CA, United States), monoclonal anti-3-NT (1:1,000, Abcam, catalog number ab7048, Cambridge, MA), polyclonal anti-4-HNE antibody (1:1,000, catalog number ab46545, Abcam). Quantified densitometry results were normalized to GAPDH.

2.6 Real time reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted with Trizol Reagent (Invitrogen Corp., Carlsbad, Ca, United States) from mouse corpus cavernosum samples. Three microgram RNA samples were incubated with 1U DNaseI (Invitrogen, Rockville, MD, United States) for 15 min at room temperature (RT) and ethylenediaminetetraacetic acid was added to a final concentration of 2 mM to stop the reaction. The DNaseI enzyme was subsequently inactivated by incubation at 65°C for 5 min. DNaseI-treated RNA samples were then reverse transcribed with Superscript III and RNaseOut (Invitrogen Corp., Carlsbad, Ca, United States) for 50 min at 50°C, and 15 min at 70°C. cDNA samples were quantified using a Nanodrop spectrophotometer (ND-1000; Nanodrop Technologies Inc., Wilmington, DE, United States). Primers were designed using the PrimerExpressTM program (Applied Biosystems, Foster City, CA, USA) (Table 1). The ideal concentration of use was determined for each pair of primer and the amplification efficiency was calculated according to the equation $E^{(-1/slope)}$, to confirm the accuracy and reproducibility of the reactions. Amplification specificity was verified by running a dissociation protocol. qRT-PCRs were performed in duplicate, using 6 μL SYBR Green Master Mix (Applied Biosystems), 10 ng cDNA and ideal quantities of each primer in a final volume of 12 μL. Samples were run in MicroAmp Optical 96-well plates (Applied Biosystems) in a 7,500 Fast Real Time PCR System (Applied Biosystems). Gene expression data were normalized using the geNorm method, based on the geometric mean of two validated reference genes (β-actin and GAPDH) (Vandesompele et al., 2002). Results are expressed as relative mRNA expression levels.

2.7 Statistical analysis

The statistical analysis was performed using the GraphPad Prism program (GraphPad Software Inc., San Diego, CA, United States). The data was presented as the mean ± standard error of mean (S.E.M.) of N experiments. To compare the statistical significance of the results, a one-way analysis of variance (ANOVA) was used, and the Tukey method was applied as a post-test. A P value of less than 0.05 was considered statistically significant.

3 Results

3.1 Treatment with resveratrol did not change hematological parameters in mice

The hematological parameters shown in Figure 1 demonstrate that mice with SCD have severe anemia, evidenced by a reduction in the number of red blood cells, hematocrit values, and hemoglobin levels when compared to the WT group. Additionally, fetal hemoglobin levels were significantly higher in the SCD group than in the WT group (Figure 1D). Treatment with resveratrol did not result in any changes in the evaluated hematological parameters (Figures 1A–D).

3.2 Resveratrol reduces increased ACh-induced cavernosal relaxation in SCD mice

The addition of phenylephrine (3×10^{-6} to 10^{-5} M) to the tissue bath caused submaximal contractions of cavernosal segments that did not significantly differ between groups (0.41 ± 0.02, 0.40 ± 0.03,

TABLE 1 Sequences and ideal concentrations for the primers used in qRT-PCR.

Gene	Primer sequence	Concentration
NOS3 – F *NOS3*– R	5′-CCCAGGAGAGATCCACCTCA-3′ 5′-CAGACACCGTAGTGCAGAGGG-3′	150 nM
PDE5A – F *PDE5A* – R	5′-GGAAATGGTGGGACCTTCACT-3′ 5′-AAGAACAATACCACAGAATGCCA-3′	150 nM
ACTB – F *ACTB* – R	5′-ACTGCCGCATCCTCTTCCT-3′ 5′-GAACCGCTCGTTGCCAATA-3′	70 nM
GAPDH – F *GAPDH* – R	5′-TGCACCACCAACTGCTTA-3′ 5′-GGATGCAGGGATGATGTTC-3′	70 nM

F, foward; R, reverse; eNOS, endothelial nitric oxide synthase; PDE5, phosphodiesterase type 5.

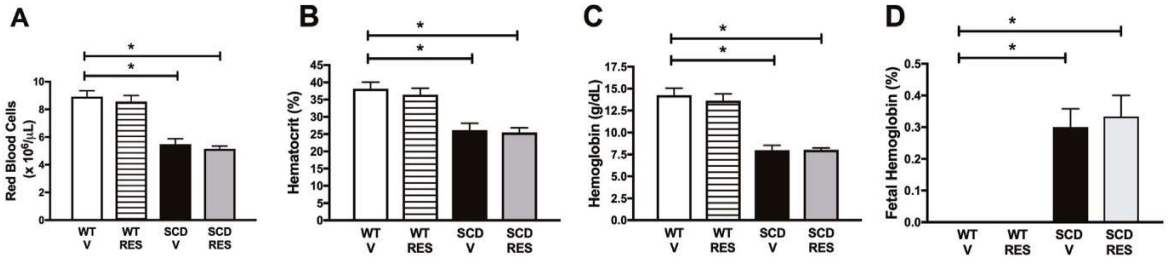

FIGURE 1
Hematological parameters in WT, SCD mice treated with vehicle (V) or resveratrol (RES). **(A)** Erythrocytes, **(B)** hematocrit, **(C)** total hemoglobin, **(D)** fetal hemoglobin. Data represent the means ± SEM of 10 animals. *P < 0.05 compared to control group.

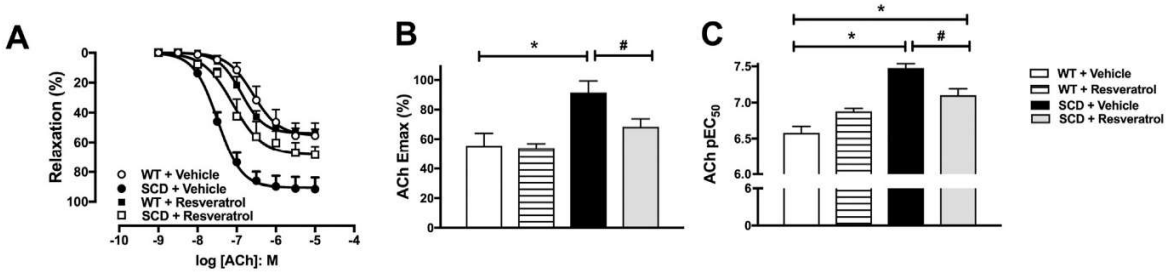

FIGURE 2
(A) Concentration-effect curves for acetylcholine (ACh) in corpus cavernosum of WT and SCD mice treated with resveratrol. **(B,C)** ACh maximum response (Emax) and potency (pEC_{50}) values, respectively. Data represent the means ± SEM of 5 animals. *P < 0.05 compared to vehicle-control group; #P < 0.05 compared to SCD-Vehicle.

0.42 ± 0.03, and 0.43 ± 0.03 mN for WT + vehicle, WT + resveratrol, SCD + vehicle, and SCD + resveratrol groups, respectively; n = 15).

Endothelium-dependent relaxation was assessed by constructing concentration-effect curves for ACh (1 nM - 10 μM) in CC of mice pre-contracted with phenylephrine (3–10 μM) (Figure 2A). ACh potency (pEC_{50}) and maximal response (E_{max}) values were significantly higher (P < 0.05) in the CC of SCD mice compared to WT mice (Figures 2B,C). Treatment with resveratrol significantly (P < 0.05) reduced ACh potency and E_{max} in the CC of the SCD group (Figures 2B,C). However, neither the potency (Figure 2B) nor the Emax (Figure 2C) of ACh were altered in the CC of WT mice treated with resveratrol.

3.3 Resveratrol reduces increased SNP-induced cavernosal relaxation in SCD mice

Endothelium-independent relaxation was assessed by constructing concentration-effect curves to SNP (10 nM –300 μM) in CC of mice pre-contracted with phenylephrine (3–10 μM) (Figure 3A). SNP pEC_{50} and E_{max}

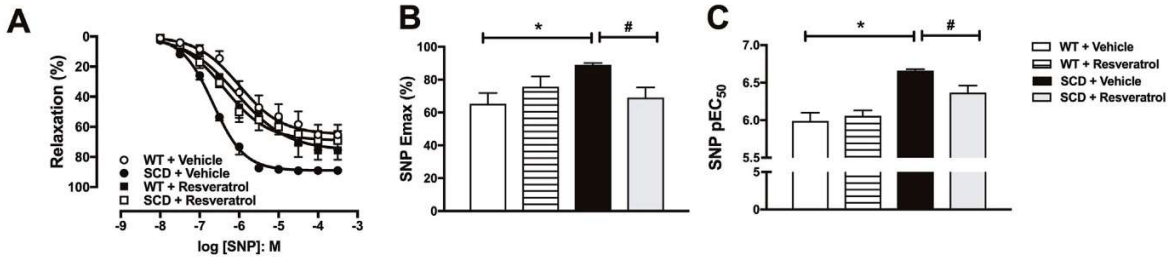

FIGURE 3
(A) Concentration-effect curves for sodium nitroprusside (SNP) in corpus cavernosum of WT and SCD mice treated with resveratrol. **(B,C)** SNP maximum response (E_{max}) and potency (pEC_{50}) values, respectively. Data represent the means ± SEM of 5 animals. *P < 0.05 compared to vehicle-control group; #P < 0.05 compared to SCD-Vehicle.

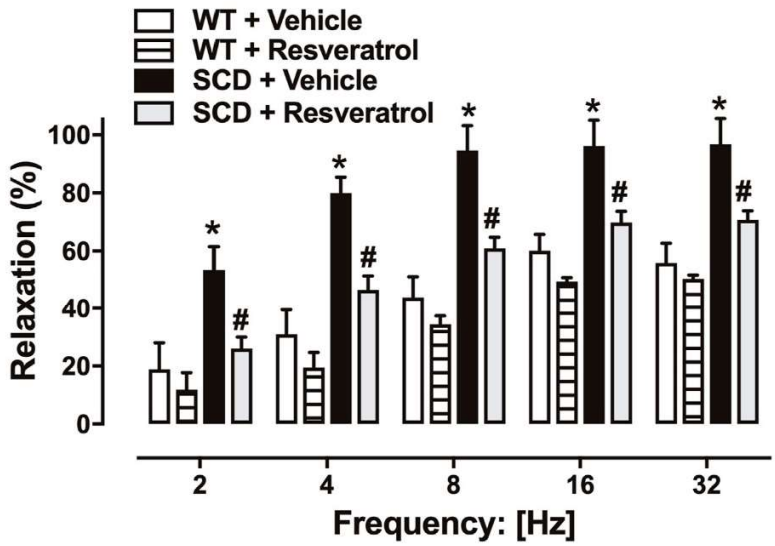

FIGURE 4
Relaxations of corpus cavernosum induced by EFS (2–32 Hz) in corpus cavernosum of WT and SCD mice treated with resveratrol or vehicle. Data represent the means ± SEM of 5 animals. *P < 0.05 compared to Control-Vehicle group; #P < 0.05 compared to SCD-Vehicle.

values were significantly higher (P < 0.05) in the CC of SCD mice compared to WT mice (Figures 3B,C). Treatment with resveratrol fully normalized (P < 0.05) the values of E_{max} (Figure 3B) and pEC_{50} (Figure 3C) of the SNP in CC of the SCD group to values similar to those of the WT. Neither the pEC_{50} (Figure 3B) nor the Emax of SNP (Figure 3C) were altered in the CC of WT mice treated with resveratrol.

3.4 Resveratrol reduces increased nitrergic relaxation-induced cavernosal relaxation in SCD mice

EFS induced frequency-dependent relaxations (2–32 Hz) in CC in all groups. Nitrergic relaxations were significantly greater in the CC of SCD mice at all studied frequencies (Figure 4). Treatment with the compound resveratrol normalized nitrergic relaxation in the SCD group but did not change it in the control group (Figure 4).

3.5 Resveratrol normalized the expression of eNOS and PDE5 mRNA in the corpus cavernosum of SCD mice

Expression of eNOS and PDE5 mRNA was significantly lower in the SCD group compared to the WT group (Figures 5A,B). Treatment with resveratrol normalized eNOS and PDE5 mRNA expression in the CC of the SCD group, but did not change it in the WT group (Figures 5A,B).

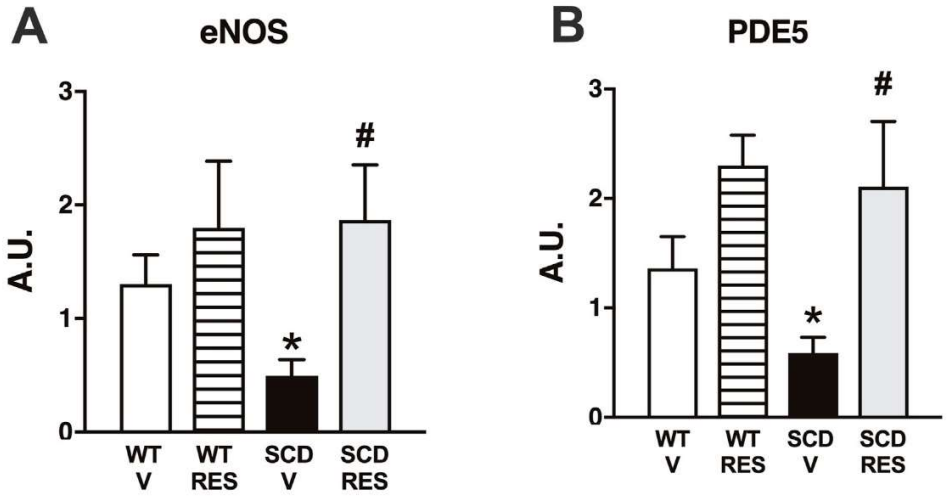

FIGURE 5
Expression of eNOS **(A)** and PDE5 **(B)** mRNA in corpus cavernosum of WT and SCD mice treated with resveratrol. Data are presented as mean ± SEM (n = 5–6) of arbitrary units (a.u.). *P < 0.05 compared with control group; #P < 0.05 compared to SCD-Vehicle. V, vehicle. RES, resveratrol.

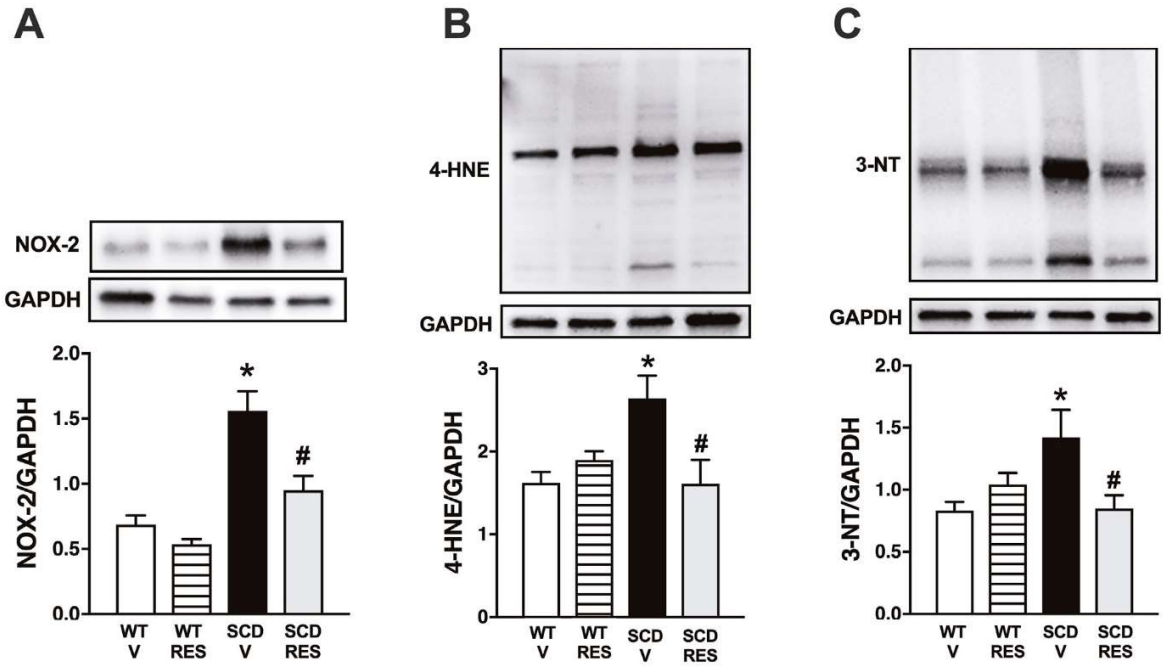

FIGURE 6
NOX-2 **(A)**, 4-HNE **(B)** and 3-NT **(C)** protein expression in CC from WT and SCD mice treated with resveratrol. Data represent the means ± SEM of 4-5 animals. *P < 0.05 compared with control group; #P < 0.05 compared to SCD-Vehicle.

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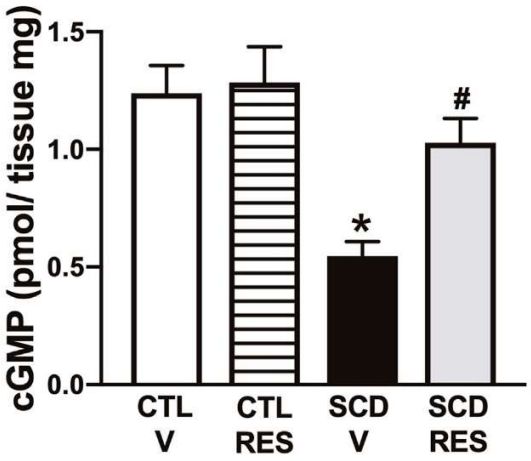

FIGURE 7
Basal GMP contents of penis from WT and SCD mice treated with resveratrol. Data represent the mean ± S.E.M. for 6 mice in each group. P < 0.05 compared with control group; #P < 0.05 compared to SCD-Vehicle. V, vehicle. RES, resveratrol.

3.6 Treatment with resveratrol normalized NOX-2 protein expression

NOX-2 protein expression was 129% higher ($P < 0.05$) in CC from the SCD group compared to the WT group (Figure 6A). Treatment with resveratrol normalized the NOX-2 protein expression in the CC of the SCD group, but did not modify it in the WT group.

3.7 Treatment with resveratrol normalized the protein expression of markers of oxidative stress and nitrosative stress in the corpus cavernosum of the sickle cell group

Protein expression of 4-HNE (Figure 6A) and 3-NT (Figure 6B) was 62% and 73% higher ($p < 0.05$) in the CC of the SCD group compared to the control group, respectively. Treatment with resveratrol normalized the protein expression of 3-NT and 4-HNE in the CC of the SCD group, but did not modify it in the WT group (Figures 6A,B).

3.8 Treatment with resveratrol increased cGMP levels in the corpus cavernosum of the sickle cell group

The basal cGMP content in the erectile tissue was 56.4% lower ($P < 0.05$) in penises of SCD mice compared with WT-vehicle mice (Figure 7). Resveratrol treatment increased ($P < 0.05$) the cGMP levels in the penis of the SCD group, but did not modify it in the control group (Figure 7).

4 Discussion

The present study demonstrates that resveratrol exerts significant therapeutic effects on the penile tissue of SCD mice, primarily by modulating key pathways associated with oxidative stress and NO-cGMP signaling. Despite the severe anemia and altered hematological parameters characteristic of SCD, resveratrol treatment did not influence these systemic markers. Notably, resveratrol effectively reduced the exaggerated cavernosal relaxation responses to endothelium-dependent (ACh), endothelium-independent (SNP), and nitrergic (EFS) stimuli in SCD mice, indicating a restoration of normal penile smooth muscle function. This normalization was further supported by the upregulation of eNOS and PDE5 mRNA expression and the reduction of oxidative stress markers (NOX-2, 4-HNE, and 3-NT) in the corpus cavernosum. Additionally, the observed increase in cGMP levels in the erectile tissue of resveratrol-treated SCD mice underscores its role in maintaining the balance required for normal erectile function.

Penile erection is initiated by the relaxation of the corpus cavernosum smooth muscle (Burnett, 2006). The NO-cGMP signaling pathway plays a crucial role in this process, acting as the main inducer of erectile tissue relaxation (Andersson, 2011). The production of NO, which occurs in the endothelial cells and nitrergic fibers in the penis, is catalyzed by the enzymes eNOS and nNOS (Burnett, 2006). Upon production, NO activates sGC in the smooth muscle, converting GTP to cGMP and leading to the stimulation of cGMP-dependent protein (PKG), culminating in the relaxation of CC smooth muscle cells (Andersson, 2011). The duration of the erectile response is determined by the quick conversion of cGMP to 5′GMP by the enzyme PDE5 (Lin et al., 2002). In additional, cGMP has a crucial role in the regulation of PDE5 gene expression in the smooth muscle of the CC (Lin et al., 2002). Notably, the penile tissue of mice with SCD exhibits a reduction in PDE5 expression, which is attributed to the diminished basal bioavailability of NO-cGMP due to decreased eNOS expression and increased oxidative stress (Bivalacqua et al., 2013; Silva et al., 2016b; Musicki et al., 2018; 2020; Pereira et al., 2022). Consistent with previous research, our findings demonstrate that the priapism phenotype in SCD mice is characterized by enhanced smooth muscle relaxation of the CC in response to NO-cGMP pathway stimulants such as ACh, SNP, and EFS, attributed to reduced cGMP degradation by PDE5, resulting in prolonged and exaggerated penile erections (Silva et al., 2016b; Musicki et al., 2020; Pereira et al., 2022; Pinheiro et al., 2022).

An effective treatment strategy for priapism involves addressing the underlying pathophysiological mechanisms, particularly by enhancing the expression of eNOS. Numerous studies have shown that resveratrol can increase eNOS expression in both blood vessels and the corpora cavernosa, positioning it as a promising therapeutic candidate (Das et al., 2005; Arunachalam et al., 2010; Pektaş et al., 2015; Yazir et al., 2018; Feng et al., 2019; Li et al., 2019; Tasatargil et al., 2019). In our study, SCD mice exhibited reduced eNOS expression in the corpus cavernosum, an alteration that was effectively reversed by resveratrol treatment. This normalization of eNOS expression was accompanied by an upregulation of PDE5. Based on these findings, we propose that

the restoration of eNOS function may directly influence PDE5 levels. Consequently, resveratrol treatment led to a significant reduction in the exaggerated cavernosal relaxation induced by NO-cGMP pathway stimuli (ACh, SNP, and EFS), which is consistent with the increase in PDE5 expression. These findings underscore the potential of resveratrol to correct the molecular imbalances contributing to priapism, offering a targeted approach to managing this condition.

SCD is associated with oxidative stress, which is characterized by an imbalance between the production and elimination of ROS (Sies et al., 2024). This results in oxidative stress in the penis of SCD patients (Lagoda et al., 2013) and mice (Musicki et al., 2012; Musicki et al., 2020; Silva et al., 2016b; Pereira et al., 2022). NOX-2 (aka gp91phox), a well-known NADPH oxidase isoform, is expressed in various cell types, including endothelial cells, and is a major source of superoxide anion in vascular tissues (Frey et al., 2009). In SCD mice, the upregulation of NOX-2 plays a crucial role in the pathophysiology of priapism (Lagoda et al., 2013; Lagoda et al., 2014; Musicki et al., 2014; Silva et al., 2016b; Pereira et al., 2022). This is because the excess of superoxide anion, which are produced by an upregulation of NOX-2, can lead to oxidative stress and subsequent lipid peroxidation. Lipid peroxidation is the process by which polyunsaturated fatty acids in cellular membranes are attacked by free radicals, causing cell membrane damage and the production of reactive aldehydes such as 4-HNE (Yang et al., 2003). 4-HNE is a highly reactive aldehyde that can modify proteins and other cellular components, leading to cellular damage and dysfunction (Yang et al., 2003). 4-HNE is widely used as an indicator of oxidative stress and its elevated levels are associated with priapism in SCD (Musicki et al., 2014; Pereira et al., 2022). Additionally, excessive superoxide anion concentration can react with NO, generating peroxynitrite, which is a powerful oxidant and cytotoxic agent that can lead to increased production of 3-nitrotyrosine (Radi, 2013). 3-nitrotyrosine is a stable marker of protein modification by peroxynitrite and is used as an indicator of oxidative stress and its elevated levels of 3-NT are associated with priapism in SCD (Lagoda et al., 2014; Silva et al., 2016b; Pereira et al., 2022). Resveratrol has been demonstrated to improve vascular and erectile function by reducing oxidative stress, which is achieved through the downregulation of the expression or activity of NOX-2 (Murat et al., 2016; Pierre et al., 2022), reduction of 4-HNE (Kaneko et al., 2011; Mattison et al., 2014) and 3-NT. Our study demonstrated that resveratrol treatment effectively reduced the elevated NOX-2 protein expression in the penile tissues of SCD mice. Decreased 4-HNE and 3-NT protein expression by resveratrol treatment is likely associated with lower superoxide anion production by NADPH oxidase in smooth muscle cavernosal cells of SCD. This reduction in oxidative stress likely resulted from lower superoxide anion production by NADPH oxidase, thereby improving the bioavailability of NO-cGMP and contributing to the normalization of PDE5 expression in the penile tissue of SCD mice.

Hydroxyurea was the first drug approved for the treatment of SCD, which increases the production of fetal hemoglobin within erythrocytes, thereby reducing the formation of HbS polymers and improving patient outcomes (Steinberg et al., 2003). Fetal hemoglobin is composed of two gamma chains and two alpha chains ($\alpha 2\gamma 2$), and previous studies have shown that resveratrol is an inducer of gamma-globin chains, leading to increased HbF synthesis in cultured erythroid progenitor cells from SCD patients (Fibach et al., 2012). However, in our study, we found that treatment with resveratrol did not change HbF expression, number of red blood cells and hematocrit of SCD mice. These findings suggest that the beneficial effects of resveratrol on the penile tissue of SCD mice are not related to improvements in hematological parameters.

One limitation of our study is that we did not evaluate the effects of resveratrol on endogenous antioxidant defense systems, such as superoxide dismutase, catalase, or glutathione peroxidase (Truong et al., 2018). Resveratrol is known to modulate these enzymatic pathways, which may also contribute to the observed reduction in oxidative stress. Therefore, we cannot exclude the possibility that the antioxidant effects of resveratrol in SCD penile tissue are partially mediated by the upregulation of intrinsic antioxidant responses, in addition to the suppression of NOX-2 expression. Furthermore, plasma and penile tissue concentrations of resveratrol were not assessed in the present study. Future investigations should include pharmacokinetic analyses to determine the systemic and local concentrations achieved under the experimental conditions, and to better correlate exposure with the observed biological effects. Additionally, we did not investigate the acute effects of resveratrol in isolated tissue preparations. Studies evaluating the impact of resveratrol applied directly to the bath at physiologically relevant concentrations could help elucidate potential rapid, non-genomic effects on cavernosal reactivity and further clarify its pharmacological profile.

5 Conclusion

In summary, our study showed that resveratrol treatment reduces excessive cavernosal relaxation in SCD mice induced by the stimulation of the NO-cGMP pathway due to the normalization of PDE5 expression. Additionally, resveratrol treatment increased the cGMP bioavailability due to the downregulation of markers of oxidative stress, such as NOX-2, 3-NT, and 4-HNE, as well as the increased expression of eNOS in the penis of the SND group. Furthermore, the benefits of resveratrol appear to be localized to the erectile tissue, as the treatment did not alter systemic hematological parameters, including HbF levels. Overall, our results suggest that resveratrol may have therapeutic potential to modulate the priapism phenotype in patients with SCD, although further *in vivo* studies are required to confirm this effect.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Ethics Committee for the Use of Experimental Animals of the University of Campinas. The study

was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CS: Formal Analysis, Writing – original draft, Writing – review and editing. TS: Formal Analysis, Investigation, Writing – original draft, Writing – review and editing. DP: Formal Analysis, Investigation, Writing – original draft, Writing – review and editing. BB: Formal Analysis, Investigation, Writing – original draft, Writing – review and editing. MdO: Writing – original draft, Writing – review and editing. FaC: Writing – original draft, Writing – review and editing. AB: Writing – original draft, Writing – review and editing. FeC: Writing – original draft, Writing – review and editing. FS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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4. CAPÍTULO 2: publicado

Título do artigo: Resveratrol and Its Nitric Oxide-Donor Hybrid as na Emerging Therapy *for Oxidative-Stress-Driven Priapism in Sickle Cel Disease.*

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O objetivo deste artigo de revisão foi descrever os mecanismos que relacionam o estresse oxidativo e a desregulação do NO na fisiopatologia do priapismo associado à anemia falciforme, com ênfase na via de sinalização NO–GMPc–PDE5. Além disso, foram analisadas evidências pré-clínicas que sustentam o potencial terapêutico do resveratrol e de seus derivados híbridos doadores de NO como candidatos emergentes para o tratamento dessa condição. O artigo também discute a possibilidade de associação do resveratrol às terapias atualmente disponíveis e os desafios translacionais que ainda precisam ser superados para a aplicação clínica. Em conjunto, as evidências apresentadas reforçam o resveratrol como uma abordagem promissora para o tratamento do priapismo mediado pelo estresse oxidativo na anemia falciforme e destacam perspectivas relevantes para investigações futuras.

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Review

Resveratrol and Its Nitric Oxide–Donor Hybrid as an Emerging Therapy for Oxidative-Stress-Driven Priapism in Sickle Cell Disease

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Abstract

Priapism is a frequent and debilitating complication in patients with sickle cell disease (SCD), characterized by recurrent ischemic episodes that can culminate in fibrosis of the erectile tissue and irreversible erectile dysfunction. Despite significant advancements in the management of acute episodes, current therapies remain largely ineffective in preventing recurrences, emphasizing the need for novel strategies that target the underlying pathophysiology. This narrative review describes the mechanistic links between oxidative stress and nitric oxide (NO) dysregulation in the pathogenesis of SCD-associated priapism, with a particular focus on the NO–cyclic guanosine monophosphate (cGMP)–phosphodiesterase type 5 (PDE5) signaling axis. We analyze preclinical evidence supporting resveratrol, a natural polyphenolic compound, as well as its NO-donor hybrid derivatives, as emerging therapeutic candidates. Additionally, we discuss the potential of combining resveratrol with current treatment approaches, and address the translational challenges that must be overcome to move from preclinical data to clinical application. Taken together, the evidence presented in this review supports resveratrol-based therapies as a promising approach for oxidative-stress-driven priapism in SCD and delineates critical perspectives for their further investigation.

Keywords: 3-nitrotyrosine; cGMP; hemoglobin; reactive oxygen species; corpus cavernosum

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1. Introduction

Sickle cell disease (SCD) is the most common hereditary hemoglobinopathy, affecting millions of individuals worldwide. Caused by a single point mutation in the β-globin gene, SCD results in the production of hemoglobin S (HbS), an abnormal variant that polymerizes under deoxygenated conditions. This intracellular polymerization increases red blood cell (RBC) rigidity and fragility, distorting the characteristic biconcave morphology into sickle-shaped forms. These pathologically altered erythrocytes exhibit reduced deformability, promoting chronic hemolysis, endothelial dysfunction, microvascular occlusion, and cumulative organ damage, hallmarks that contribute to substantial morbidity and premature mortality in affected individuals [1].

Ischemic priapism is a well-recognized complication of SCD, often associated with significant physical and psychological morbidity. Characterized by a persistent penile erection unrelated to sexual arousal, ischemic priapism affects up to 48% of male patients with SCD, particularly during adolescence and early adulthood [2,3]. The ischemic form,

which is the most common in this context, results from impaired venous outflow and blood stasis within the corpora cavernosa, leading to prolonged and often painful episodes. Recurrent episodes known as stuttering priapism consist of frequent, painful erections lasting less than four hours and may lead to fibrosis of the erectile tissue and irreversible erectile dysfunction, severely compromising reproductive health and quality of life [3]. Although acute episodes can be managed with self-administered intracavernosal injections of α-adrenergic sympathomimetics, such as phenylephrine, this pharmacological intervention does not constitute a true preventive strategy, as it does not address the underlying pathophysiology of SCD-associated priapism [3,4].

Historically, priapism in SCD was largely attributed to mechanical venous occlusion driven by interactions among sickled erythrocytes, endothelial cells, leukocytes, and platelets. However, recent advances have revealed a more nuanced pathophysiology, implicating dysregulation of the molecular signaling pathways that govern penile smooth muscle tone [5]. Central to this dysfunction is the aberrant over-relaxation of the corpus cavernosum smooth muscle, mediated by disturbances in nitric oxide (NO) signaling and exacerbated by oxidative stress [6].

Oxidative stress plays a pivotal role in the pathophysiology of priapism in SCD, marked by excessive production of reactive oxygen species (ROS) and diminished antioxidant defenses. This redox imbalance aggravates hemolysis-induced vascular injury and reduces endothelial NO bioavailability in the corpus cavernosum [7–10]. The resulting impairment of NO–cyclic guanosine monophosphate (cGMP) signaling is a key driver of priapism in SCD [5]. Preclinical evidence demonstrates that strategies aimed at restoring redox homeostasis, enhancing endothelial NO signaling, and normalizing phosphodiesterase type 5 (PDE5) expression effectively attenuate the priapism phenotype, highlighting oxidative stress as a therapeutic target [9–13].

In this context, resveratrol (3,5,4′-trihydroxystilbene), a polyphenol present in grapes, berries, and peanuts, has emerged as a promising therapeutic candidate. By enhancing endothelial nitric oxide synthase (eNOS) activity, reducing oxidative stress, and improving NO bioavailability, resveratrol directly addresses the molecular drivers of priapism in SCD [14–18]. Recent preclinical findings demonstrated that resveratrol treatment attenuated the priapism phenotype in transgenic SCD mice by restoring NO–cGMP signaling and downregulating NADPH oxidase 2 expression [19]. Furthermore, hybrid compounds that combine resveratrol with nitric oxide–donor moieties have been developed to amplify these effects [20,21]. This review explores the mechanistic links between oxidative stress and priapism in SCD and highlights resveratrol and its NO-donor hybrids as emerging therapeutic strategies.

2. Molecular Mechanisms of Penile Erection

A penile erection results from a complex interplay of vascular, neural, and hormonal factors [22]. Among the several pathways involved, the NO-cGMP-PDE5 signaling cascade represents the principal molecular axis mediating penile erection (Figure 1). Two critical NO synthase (NOS) enzymes exist in the penis: endothelial (eNOS) and neuronal (nNOS). These enzymes are pivotal in starting and sustaining an erection, catalyzing the transformation of L-arginine into L-citrulline and NO [23]. Upon sexual stimulation, NO diffuses freely across cell membranes and activates sGC in adjacent cavernosal smooth muscle cells. Activated sGC catalyzes the conversion of guanosine-5′-triphosphate (GTP) into cGMP, leading to a rapid increase in intracellular cGMP levels. cGMP is as a second messenger that acitivates cGMP-dependent protein kinase (PKG), which influences various proteins that mediate muscle relaxation, such as myosin light chain phosphatase and potassium channels [22]. Collectively, these molecular events culminate in cavernosal smooth muscle relaxation,

arterial dilation, and sinusoidal blood filling, which together generate penile rigidity. The duration and magnitude of the erectile response are regulated by PDE5, a cGMP-specific phosphodiesterase abundantly expressed in cavernosal smooth muscle. PDE5 hydrolyzes cGMP into its inactive form, 5′-GMP, thereby terminating the NO-mediated signal and penile erection. This finely tuned balance between cGMP synthesis by sGC and degradation by PDE5 ensures the transient nature of penile erection [23].

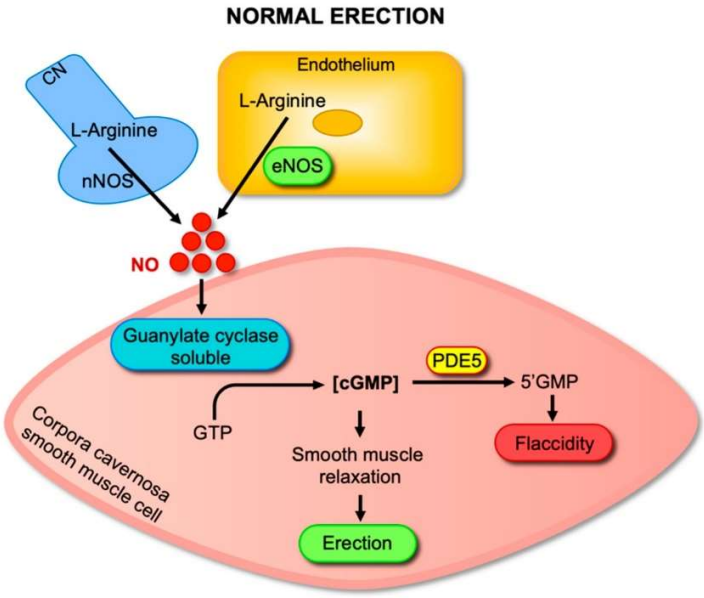

Figure 1. NO–cGMP–PDE5 signaling pathway in penile erection. Sexual stimulation activates neuronal nitric oxide synthase (nNOS) in cavernosal nerves and endothelial nitric oxide synthase (eNOS) in endothelial cells, leading to the conversion of L-arginine into nitric oxide (NO). NO diffuses into cavernosal smooth muscle cells and stimulates soluble guanylate cyclase (sGC), which catalyzes the conversion of guanosine-5′-triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). Increased cGMP levels activate downstream targets, resulting in corpus cavernosum smooth muscle relaxation, arterial dilation, and penile erection. Phosphodiesterase type 5 (PDE5) terminates this signal by degrading cGMP into 5′-GMP, thereby returning the penis to flaccidity.

3. Pathophysiology of SCD-Associated Priapism: Role of Oxidative Stress and Nitric Oxide Dysregulation

Experimental and clinical evidence has established that priapism in SCD is primarily driven by dysregulation of the NO–cGMP–PDE5 signaling axis [6,24,25]. Reduced NO bioavailability in the corpus cavernosum of SCD patients and animal models leads to chronically diminished basal cGMP levels [9,11–13,24]. Because PDE5 expression is positively regulated by intracellular cGMP concentrations, reduced basal levels result in marked downregulation of PDE5 protein in cavernous smooth muscle cells [26] (Figure 2A). As a result, upon sexual or nocturnal stimuli that transiently increase NO, cGMP accumulates abnormally, triggering exaggerated smooth muscle relaxation and initiating prolonged, unregulated penile erection [5] (Figure 2B).

These pathophysiological features have been extensively characterized in transgenic SCD mouse models, including both the Berkeley and Townes strains, which exhibit reduced PDE5 expression and heightened erectile responses [9,24,27]. Functional ex vivo studies have demonstrated augmented nitrergic relaxation following electrical field stimulation, as well as enhanced endothelium-dependent (acetylcholine-induced) and endothelium-independent (NO donor-mediated) relaxation of corpus cavernosum strips [9–11,13]. Con-

sistently, in vivo assessments demonstrate elevated intracavernosal pressure (ICP) in response to cavernous nerve stimulation in SCD mice, corroborating the hyperresponsiveness of erectile tissue in this context [11,13,28].

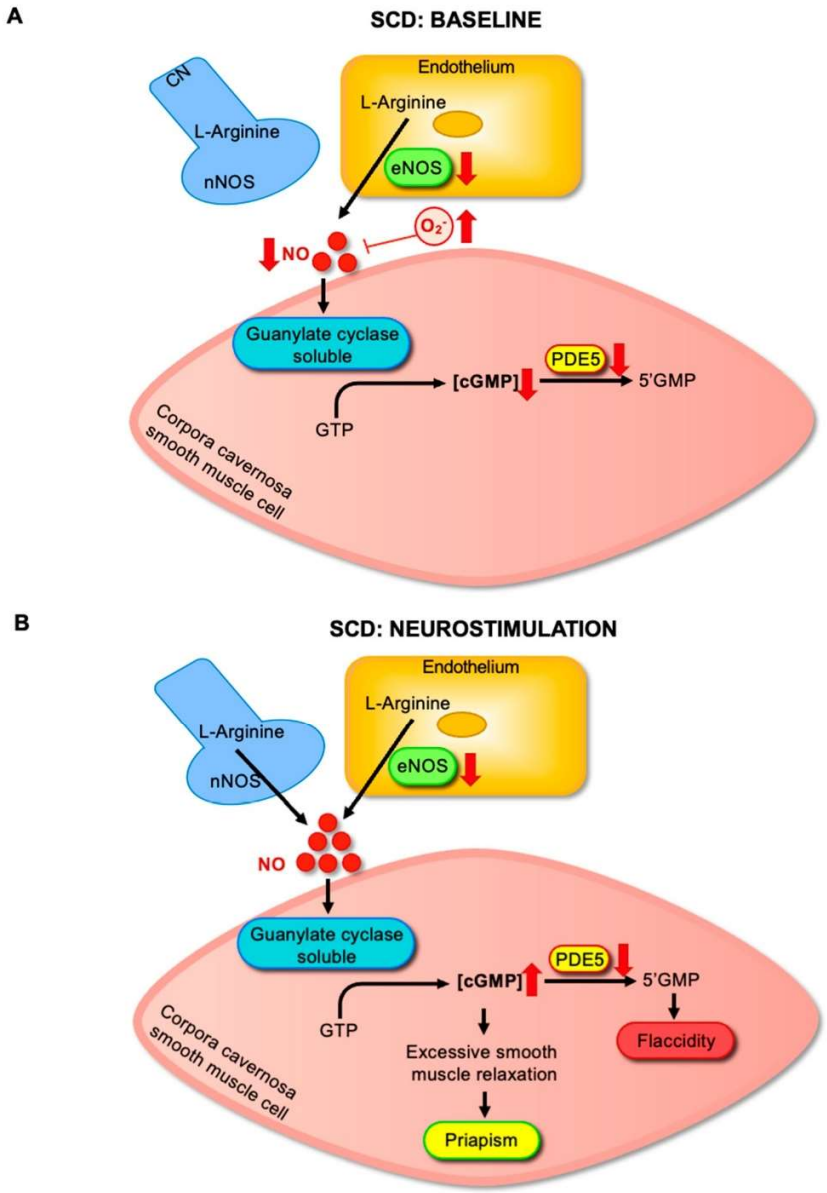

Figure 2. (**A**) Altered NO–cGMP–PDE5 signaling in sickle cell disease (SCD) at baseline. In SCD, reduced endothelial nitric oxide synthase (eNOS) activity and increased oxidative stress, driven primarily by elevated superoxide (O_2^-) levels, and diminished NO bioavailability. Lower NO levels impair soluble guanylate cyclase (sGC) activation and limit cGMP production in cavernosal smooth muscle cells. Reduced cGMP levels subsequently lead to the downregulation of PDE5 expression and activity. (**B**) Dysregulated NO–cGMP–PDE5 signaling during neurostimulation in sickle cell disease (SCD). Upon neurostimulation, neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) generate NO, which activates soluble guanylate cyclase (sGC) and increases cGMP production in cavernosal smooth muscle cells. However, in SCD, the downregulation of PDE5 impairs cGMP degradation, leading to its excessive accumulation. This imbalance drives sustained relaxation of cavernosal smooth muscle, predisposing individuals to prolonged erections and the development of priapism.

A key pathophysiological determinant of priapism in the penile tissue of individuals with SCD is reduced NO bioavailability due to the accumulation of ROS [6]. Multiple mechanisms contribute to oxidative stress in SCD penile tissue. A major factor is the chronic intravascular hemolysis characteristic of SCD, which leads to elevated levels of cell-free hemoglobin in the plasma [29]. This hemoglobin binds and inactivates NO, thus reducing NO bioavailability [10,30]. In parallel, enzymatic sources of ROS are upregulated, particularly xanthine oxidase and NADPH oxidase [8,9,19]. Xanthine oxidase catalyzes the sequential oxidation of hypoxanthine and xanthine to uric acid, generating superoxide anion as a byproduct [31]. This superoxide rapidly reacts with NO to form peroxynitrite, a highly reactive nitrogen species that further depletes NO and promotes oxidative injury in penile tissues [32].

Among enzymatic sources, NADPH oxidase significantly contributes to priapism through its superoxide production in SCD. In particular, the NOX2 isoform, which requires the assembly of membrane-bound subunits (gp91^{phox} and p22^{phox}) and cytosolic subunits (p47^{phox}, p67^{phox}, p40^{phox}, Rac1/Rac2). Increased expression of gp91^{phox}, p47^{phox}, and p67^{phox} has been consistently reported in penile tissue from both SCD patients and mouse models, contributing to sustained superoxide production [8,10,11,13,25,33].

Another important contributor to oxidative stress in the penile tissue of individuals with SCD is uncoupled eNOS [8]. Under physiological conditions, eNOS generates NO to maintain vascular homeostasis. However, in the oxidative environment characteristic of SCD, eNOS becomes uncoupled and begins producing superoxide instead of NO, further amplifying ROS levels [5,8]. This shift exacerbates formation of peroxynitrite, which triggering lipid peroxidation and protein nitration, which together compromise membrane integrity and disrupt intracellular signaling pathways [32]. Elevated levels of 3-nitrotyrosine (3-NT), a stable end-product of peroxynitrite-mediated protein modification, have been consistently detected in the corpus cavernosum of SCD mice, serving as a biomarker of nitrosative stress and molecular dysfunction [10,11,19,25].

Building upon these established mechanisms, further molecular alterations have been identified that contribute to priapism in SCD. Opiorphin, an endogenous pentapeptide that inhibits neutral endopeptidase activity, is upregulated in SCD and has been implicated in the prolongation of smooth muscle relaxation through enhanced pro-erectile signaling [34]. Adenosine is markedly elevated in the penile tissue of SCD mice and contributes to priapism by promoting smooth muscle relaxation via A_2B receptor-mediated cAMP signaling [35]. Impairments in the RhoA/Rho-kinase pathway, which normally facilitates detumescence through contractile signaling, have also been observed in SCD mice, shifting the balance toward persistent smooth muscle relaxation [36]. Moreover, decreased expression of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, indicates compromised sympathetic neurotransmission, further impairing detumescence mechanisms [4].

Collectively, these findings illustrate the complex and interrelated mechanisms underlying priapism in SCD. The combined effects of oxidative stress, reduced NO signaling, impaired contractile pathways, and dysregulated neuromodulatory control create a pathological state that favors recurrent, unregulated penile erections. These mechanistic insights highlight the urgent need for therapeutic approaches that target not only NO-cGMP-PDE5 dysregulation but also the broader redox and neuromodulatory landscape of SCD-associated priapism.

4. Pharmacological Profile of Resveratrol

Resveratrol is a naturally occurring polyphenolic stilbene predominantly found in grapes, berries, peanuts, and several medicinal plants. It has been extensively studied due to

its broad spectrum of biological activities, including potent antioxidant, anti-inflammatory, anti-cancer, and cardioprotective effects [15,37]. Its antioxidant activity primarily derives from its chemical structure, which allows it to effectively scavenge ROS, thereby preventing oxidative damage to lipids, proteins, and nucleic acids [38,39].

At the cellular and molecular levels, resveratrol exerts antioxidant effects by activating nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates antioxidant response elements (ARE) and upregulates the expression and activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase [40–42]. Furthermore, resveratrol decreases both gene and protein expression of NADPH oxidase isoforms, particularly NOX2, a major enzymatic source of reactive oxygen species (ROS) in vascular tissues under pathophysiological conditions [43–45].

In addition to its antioxidant properties, resveratrol also modulates endothelial function. It is known to upregulate eNOS expression and activity, enhancing NO bioavailability and improving endothelial-dependent vasodilation in animals and humans [46–49]. This protective vascular action of resveratrol is largely mediated via the activation of sirtuin-1 (SIRT1), a NAD^+-dependent protein deacetylase [37]. SIRT1 activation by resveratrol promotes eNOS deacetylation and subsequent activation, leading to increased NO production and attenuation of oxidative-stress-induced endothelial dysfunction [50].

Resveratrol also exerts anti-inflammatory effects by inhibiting the nuclear factor-kappa B (NF-κB) signaling pathway, leading to reduced expression of pro-inflammatory cytokines and adhesion molecules such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) [51–53]. Collectively, these actions contribute to decreased vascular inflammation, reduced leukocyte infiltration, and improved expression function.

In addition, resveratrol exhibits potent anti-proliferative effects on vascular smooth muscle cells by targeting key signaling pathways involved in vascular remodeling. It suppresses mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), and transforming growth factor-beta (TGF-β) cascades, which are implicated in pathological vascular remodeling [54–56].

Taken together, these antioxidant, anti-inflammatory, endothelial-protective, and anti-remodeling effects provide a mechanistic rationale for the use of resveratrol in vascular conditions characterized by oxidative stress and endothelial dysfunction. In SCD, where redox imbalance, NO deficiency, and altered vascular reactivity converge to promote priapism, these properties may hold particular therapeutic promise.

5. Evaluation of Resveratrol and Its Nitric Oxide–Donor Hybrid in SCD-Associated Priapism

5.1. Evaluation of Resveratrol Monotherapy

The therapeutic potential of resveratrol in SCD-associated priapism has been thoroughly investigated using transgenic mouse models that replicate key pathophysiological features of the human condition. These include chronic intravascular hemolysis, oxidative stress, and dysregulation of NO signaling within the corpus cavernosum. In a recent study, male SCD mice (Townes model) received oral resveratrol at a dose of 100 mg/kg/day for 14 consecutive days, and the effects were evaluated through both functional and molecular endpoints [19].

Ex vivo experiments using isolated corpus cavernosum strips mounted in organ bath chambers revealed that vehicle-treated SCD mice displayed enhanced relaxation responses to acetylcholine, sodium nitroprusside, and electrical field stimulation. These findings were consistent with the exaggerated cavernosal relaxation characteristic of the priapism phenotype [9–12]. Resveratrol treatment significantly attenuated these responses, indicating

restoration of regulatory control over cavernosal smooth muscle tone. In wild-type controls, resveratrol did not alter cavernosal reactivity, supporting a disease-specific pharmacological effect rather than generalized vascular inhibition [19].

Mechanistic analysis showed that resveratrol reestablished important elements of the NO–cGMP–PDE5 pathway. Untreated SCD mice exhibited downregulation of eNOS and PDE5, along with reduced tissue cGMP levels. These molecular alterations reflect a dysfunctional signaling environment that predisposes to unregulated smooth muscle relaxation. Resveratrol treatment normalized eNOS and PDE5 gene expression and led to a significant increase in cavernosal cGMP content. These changes indicate improved NO production and enhanced downstream signaling efficacy [19] (Figure 3).

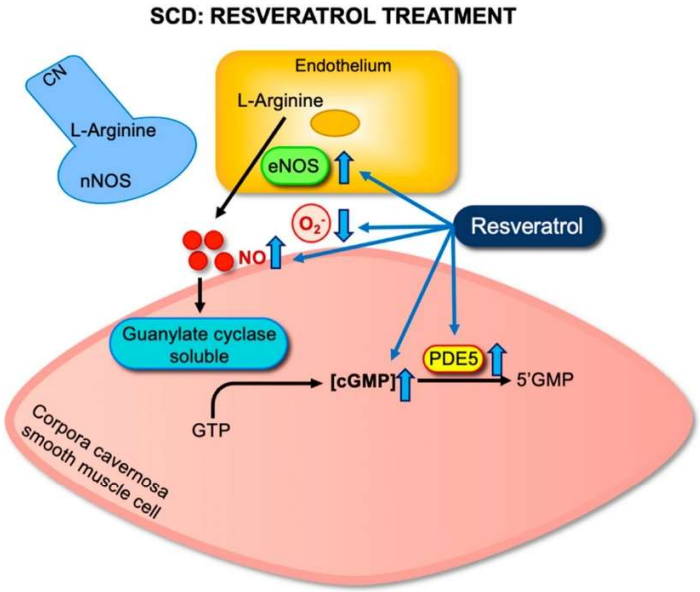

Figure 3. Effects of resveratrol on NO–cGMP–PDE5 signaling in sickle cell disease (SCD). Resveratrol enhances endothelial nitric oxide synthase (eNOS) expression and reduces oxidative stress, thereby increasing NO bioavailability. Improved NO signaling promotes soluble guanylate cyclase (sGC) activation and cGMP synthesis in cavernosal smooth muscle cells. Additionally, resveratrol upregulates phosphodiesterase type 5 (PDE5), restoring cGMP degradation. Together, these effects rebalance the NO–cGMP–PDE5 pathway and normalize cavernosal smooth muscle tone, potentially preventing priapism in SCD.

In parallel, resveratrol effectively reduced oxidative stress within penile tissue. Treatment led to a marked decrease in the expression of NOX2, a catalytic subunit of NADPH oxidase, which is a primary enzymatic source of ROS in this context. Furthermore, levels of oxidative stress markers, including 4-hydroxynonenal and 3-NT, were significantly decreased following resveratrol administration. These findings indicate that resveratrol enhances NO bioavailability by stimulating its synthesis and preventing its oxidative inactivation [19].

It is important to note that resveratrol treatment did not significantly modify systemic hematological parameters such as hemoglobin concentration, hematocrit, or red blood cell counts [19]. This observation reinforces the tissue-selective effect of resveratrol and suggests that its vascular benefits are achieved without altering the underlying hematological status of the SCD model, an important consideration for translational applications.

Overall, these preclinical findings demonstrate that resveratrol reverses key functional and molecular abnormalities associated with priapism in SCD. By restoring NO signaling, reducing oxidative stress, and normalizing cavernosal responsiveness, resveratrol repre-

sents a promising targeted pharmacological strategy for this debilitating complication. Clinical studies are now warranted to define its therapeutic potential and establish its efficacy in patients with SCD-related priapism.

5.2. Evaluation of Resveratrol–NO Donor Hybrids (RVT-FxMe)

To expand the therapeutic potential of resveratrol and enhance its pharmacodynamic properties, hybrid compounds incorporating NO donor functionalities have been developed. One such compound, I-4-(4-(4-methoxystyryl) phenoxy)-3-methyl-1,2,5-oxadiazole 2-N-oxide (RVT-FxMe or compound **10a**), was synthesized by chemically linking a NO-donating moiety to resveratrol, with the rationale of simultaneously targeting oxidative stress and NO deficiency, which are two central mechanisms in the pathophysiology of priapism in SCD [20,21]. This dual-action design integrates NO donation with the intrinsic antioxidant profile of resveratrol, thereby addressing the two core mechanisms driving priapism in SCD.

RVT-FxMe was evaluated in two murine models that exhibit the priapism phenotype: transgenic SCD mice and eNOS-deficient ($eNOS^{-/-}$) mice [21]. Both models display exaggerated cavernosal relaxation with downregulated PDE5 protein expression. However, while the $eNOS^{-/-}$ model represents a non-hemolytic state characterized by isolated NO deficiency, the SCD model involves a multifactorial pathophysiology, including hemolysis, oxidative stress, NO inactivation, and endothelial dysfunction [21,27]. These contrasting mechanisms provide a relevant framework for evaluating the therapeutic performance of RVT-FxMe under distinct pathophysiological conditions.

Mice were treated orally with RVT-FxMe (25 mg/kg/day) for 14 days. In $eNOS^{-/-}$ mice, RVT-FxMe significantly attenuated the exaggerated cavernosal relaxation responses to SNP and EFS. These findings suggest that exogenous NO delivery via the hybrid compound is sufficient to restore downstream signaling in a model characterized by impaired NO synthesis. The effectiveness of RVT-FxMe in this context demonstrates its capacity to normalize sGC–cGMP–PDE5 signaling.

In contrast, RVT-FxMe failed to reverse the exaggerated relaxation responses in SCD mice [21]. Despite its structural similarity to resveratrol and its NO-releasing capacity, the hybrid compound did not improve functional or molecular parameters associated with priapism in this hemolytic model. This discrepancy is likely due to the high concentrations of circulating cell-free hemoglobin in SCD, which bind and inactivate NO before it reaches its smooth muscle targets. Consequently, the NO released by RVT-FxMe is rapidly scavenged, limiting its ability to restore cGMP signaling and regulate cavernosal tone.

An additional factor to consider is the difference in dosing between the two compounds. Whereas resveratrol was administered at 100 mg/kg/day, RVT-FxMe was tested at a quarter of that dose (25 mg/kg/day). Although this lower dose was chosen to reflect the presumed increased potency of the hybrid, it may have been insufficient to overcome the biochemical barriers presented by the SCD microenvironment. Dose-escalation studies are required to determine whether higher concentrations of RVT-FxMe can achieve therapeutic effects comparable to resveratrol monotherapy.

Beyond its effects on penile vascular function, RVT-FxMe has demonstrated additional pharmacological advantages compared to native resveratrol. Originally developed as part of a series of RVT derivatives (10a–i) aimed at treating SCD symptoms more broadly, RVT-FxMe was identified as the most potent in several biological assays. Unlike resveratrol, RVT-FxMe was capable of releasing NO, with NO release levels reaching up to 26.3%. In a murine model of acetic acid-induced visceral pain, RVT-FxMe produced a significant antinociceptive effect, offering up to 37.3% protection, the highest among the series. In vitro studies using LPS-stimulated macrophages showed that RVT-FxMe reduced

TNF-α levels by 41.1–64.3% across tested concentrations (3.13–12.5 μM), a more robust anti-inflammatory effect than that observed with resveratrol. Moreover, both RVT-FxMe and resveratrol doubled gamma-globin (γG+γA) chain production in $CD34^+$ hematopoietic cells compared to vehicle, indicating potential utility in modifying the hematological profile of SCD. RVT-FxMe did not exhibit mutagenicity or membrane-disruptive activity in safety assays. These findings suggest that RVT-FxMe may offer systemic therapeutic benefits through combined anti-inflammatory, analgesic, and gamma-globin-inducing properties, supporting its continued exploration as a multifunctional therapeutic candidate for SCD.

6. Potential Therapeutic Role of Resveratrol Combined with Current Treatments

The therapeutic potential of resveratrol in managing priapism associated with SCD could be significantly enhanced by combining it with current treatment options. Currently approved therapies for SCD include hydroxyurea, L-glutamine, and crizanlizumab, each targeting different aspects of the disease pathophysiology. However, these treatments primarily focus on reducing hemolysis, inflammation, and vaso-occlusive episodes, with limited direct impact on the specific oxidative and nitrosative stress mechanisms underlying priapism.

Preclinical findings have demonstrated the limited ability of hydroxyurea to directly correct cavernosal smooth muscle dysregulation in SCD. In a study using transgenic SCD and eNOS-deficient mice, hydroxyurea administered intraperitoneally for three weeks did not reverse the priapism phenotype stress [27]. These results indicate that hydroxyurea does not restore NO–cGMP signaling at the tissue level, likely due to persistent NO scavenging by free hemoglobin and ongoing oxidative stress [27]. By contrast, a clinical trial demonstrated a significant reduction in priapism frequency after a median of 10 months of hydroxyurea therapy [57]. This discrepancy likely reflects the systemic hematological benefits of long-term therapy in humans, including reduced hemolysis and lower circulating levels of cell-free hemoglobin, which were not reproduced in the short-term animal protocol. These upstream effects may secondarily improve endothelial function and smooth muscle homeostasis in the penis.

Together, these findings suggest that while hydroxyurea may not directly normalize molecular signaling pathways involved in priapism at the penile level, its systemic effects can indirectly reduce the occurrence and severity of priapism episodes. This reinforces the potential value of adjunctive agents like resveratrol that act locally to restore redox balance and NO bioactivity within the corpus cavernosum. Combining systemic hematologic correction with local vascular modulation may offer additive or synergistic benefits.

L-glutamine is the first FDA-approved antioxidant therapy for SCD, shown to reduce the frequency of vaso-occlusive crises by improving the redox potential of sickled erythrocytes through increased NAD synthesis [58]. Although its systemic antioxidant effects are clinically relevant, its impact on localized vascular complications, such as priapism, remains unexplored. In contrast, resveratrol exerts both systemic and tissue-level antioxidant effects, including direct modulation of endothelial NO bioavailability. These complementary mechanisms suggest that resveratrol could enhance the benefits of L-glutamine, and future studies should evaluate their combined potential to provide broader vascular protection in SCD.

Despite prior positive findings from the SUSTAIN trial, the recent STAND phase 3 study failed to demonstrate significant efficacy of crizanlizumab over placebo in reducing the frequency of vaso-occlusive crises, although its safety profile was confirmed [59,60]. Following these disappointing results, crizanlizumab was withdrawn from the European market in 2023 and subsequently from the Brazilian market. These outcomes clearly

demonstrate the limitations of anti-adhesive monotherapy and reinforce the urgent need for alternative or combinatorial strategies targeting multiple pathophysiological mechanisms.

Collectively, integrating resveratrol with established SCD therapies offers a promising strategy, particularly for complications such as priapism that are driven by local oxidative and endothelial dysfunction. By complementing the systemic effects of current treatments, resveratrol may help normalize tissue-specific redox signaling and restore NO bioavailability in the penis. Translational studies combining pharmacokinetic, molecular, and functional endpoints are needed to confirm these potential synergistic effects and support future clinical applications.

7. Clinical Evidence and Translational Challenges

Although most evidence supporting the therapeutic potential of resveratrol comes from preclinical models, an increasing number of clinical studies have examined its effects on vascular health [61]. These trials consistently report that resveratrol is well tolerated at doses up to 1 g/day, with only mild gastrointestinal symptoms, such as flatulence, nausea, or diarrhea, observed at higher doses (2.5–5 g/day) [61–64]. This favorable safety profile supports its continued investigation as an adjunctive therapeutic agent for vascular dysfunction.

The clinical efficacy of resveratrol remains inconsistent. Randomized controlled trials have reported variable effects on endothelial and vascular biomarkers, often without a clear dose–response relationship [61]. This variability may reflect differences in study design, participant characteristics, treatment duration, formulation, and the absence of standardized outcome measures. Moreover, the clinical effects reported are frequently modest when compared to those observed in animal models [61].

A central barrier to clinical translation is the poor pharmacokinetic profile of resveratrol. Although trans-resveratrol demonstrates high oral absorption, its systemic bioavailability is extremely limited due to rapid and extensive first-pass metabolism. Sulfate and glucuronide conjugation of phenolic hydroxyl groups are the primary metabolic routes, with intestinal and hepatic sulfation representing a critical rate-limiting step [65]. The half-life of trans-resveratrol ranges from 1 to 3 h after a single dose and extends to 2 to 5 h following repeated administration [66]. Circadian variation has also been observed, with higher bioavailability occurring after morning intake [66]. Despite low plasma levels of the parent compound, the biological activity of its conjugated metabolites remains poorly characterized and should be addressed in future studies.

Beyond systemic pharmacokinetics, tissue distribution and accumulation are important to therapeutic efficacy but remain underexplored. Plasma concentrations may not adequately reflect local bioactivity at target sites. Preclinical evidence has shown that resveratrol metabolites can accumulate in myocardial tissue in a dose- and time-dependent manner, with levels correlating to functional hemodynamic outcomes in diabetic rats [67]. Whether similar tissue-level bioavailability is achieved in the corpus cavernosum remains unknown. Quantifying local concentrations and pharmacodynamic responses at the site of action is essential for understanding therapeutic mechanisms and optimizing dosage strategies.

To address these limitations, multiple strategies have been proposed, including nanoformulations, liposomal carriers, and structural modifications such as hybridization with NO donor groups. These approaches aim to enhance oral bioavailability, improve tissue penetration, and reduce metabolic degradation. However, their effectiveness in improving site-specific drug delivery remains to be validated.

8. Conclusions

Resveratrol represents a promising therapeutic approach for oxidative-stress-mediated priapism in SCD, acting through the restoration of NO bioavailability, reduction in oxidative stress, and normalization of cavernosal smooth muscle tone. Although NO-donor hybrids have not yet demonstrated efficacy under hemolytic conditions, their development demonstrates the need for optimized delivery strategies. The favorable safety profile of resveratrol supports its continued investigation as an adjunct to existing therapies for this debilitating complication.

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5. CONCLUSÃO

O tratamento com resveratrol reverteu o aumento do relaxamento do músculo liso dos corpos cavernosos em camundongos com anemia falciforme, induzido pela estimulação da via NO-GMPc devido à normalização da expressão de PDE5. Além disso, o tratamento com resveratrol aumentou a biodisponibilidade de NO por meio da redução do estresse oxidativo e do aumento da expressão de eNOS no pênis dos camundongos com anemia falciforme. No entanto, o tratamento com resveratrol não modificou os parâmetros hematológicos ou a expressão de HbF. O presente estudo posiciona o resveratrol como um promissor candidato terapêutico para o tratamento do priapismo em pacientes com anemia falciforme.

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ANEXOS

Anexo I - Parecer do CEUA

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Priapismo e disfunção miccional na anemia falciforme: fisiopatologia e novos candidatos a fármacos**, registrada com o nº **4702-1/2017**, sob a responsabilidade de **Prof. Dr. Fernando Ferreira Costa e Fabio Henrique Silva**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas** - CEUA/UNICAMP, em **27 de outubro de 2017**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	**01/10/2017-01/10/2019**
Vigência da autorização para manipulação animal:	**27/10/2017-01/10/2019**
Espécie / linhagem/ raça:	**Camundongo transgênico / Hbatm1(HBA)TowHbbtm2(HBG1,HBB*)Tow/Hbbtm3(HBG1,HBB)Tow/J**
No. de animais:	**90**
Idade/Peso:	**03 meses / 30g**
Sexo:	**machos**
Espécie / linhagem/ raça:	Camundongo Knockout / B6.129P2-Nos3tm1Unc/J
No. de animais:	**120**
Idade/Peso:	**03 meses / 30g**
Sexo:	**machos**
Origem:	**CEMIB/UNICAMP**
Biotério onde serão mantidos os animais:	**Biotério do Laboratório de Biologia Molecular e Hemostasia, HEMOCENTRO/UNICAMP**

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao **IBAMA**, **SISBIO** ou **CIBio** e é **restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, 27 de outubro de 2017.

Prof. Dr. Wagner José Fávaro
Presidente

Fátima Alonso
Secretária Executiva

IMPORTANTE: **Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.**