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MINIREVIEWS

Therapeutic potential of elafibranor in alcohol-associated liver disease: Insights into macrophage modulation and fibrosis reduction

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Abstract

Alcohol-associated liver disease (ALD) is a major global health concern, contributing to liver injury, morbidity, and mortality. Elafibranor (EFN), a dual peroxisome proliferator-activated receptor α/δ agonist, has shown promise as a therapeutic candidate in preclinical studies. EFN reduces liver fibrosis by inhibiting lipid accumulation, apoptosis, and inflammatory pathways (LPS/TLR4/NF-κB), while enhancing autophagy and antioxidant responses. It also improves intestinal barrier function and modulates gut microbiota, reducing endotoxin-producing bacteria and increasing beneficial species. By strengthening the intestinal barrier and suppressing pro-inflammatory mediators like tumor necrosis factor-alpha and interleukin-6, EFN mitigates hepatic stellate cell activation and fibrogenic signaling. Macrophages play a central role in ALD progression, and EFN's ability to modulate macrophage activity further highlights its anti-inflammatory properties. This review emphasizes EFN's dual-targeted approach, addressing both hepatic and intestinal dysfunctions, distinguishing it from conventional ALD treatments. While preclinical results are promising, EFN remains under clinical investigation, with ongoing trials evaluating its safety and efficacy. Future research should focus on elucidating EFN's molecular mechanisms and advancing its clinical application to establish its therapeutic potential in human populations. EFN represents a novel, comprehensive strategy for ALD management, targeting

both liver and gut pathologies.

Key Words: Alcohol-associated liver disease; Elafibranor; Peroxisome proliferator-activated receptor α/δ agonist; Macrophages; Liver fibrosis; Inflammatory responses

Core Tip: Macrophages play a central role in the progression of alcohol-associated liver disease (ALD), from initial liver injury to advanced stages like cirrhosis. Elafibranor (EFN), a dual peroxisome proliferator-activated receptor α/δ agonist, offers a promising therapeutic approach by reducing liver fibrosis, inhibiting macrophage activation, and suppressing TLR4/NF-κB inflammatory pathways. Additionally, EFN strengthens intestinal barrier function, addressing key drivers of ALD. With its dual anti-inflammatory and fibrosis-reducing effects, EFN holds potential for ALD and other liver diseases characterized by chronic inflammation and fibrosis. Further research is needed to validate its clinical applications.

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INTRODUCTION

Alcohol-associated liver disease (ALD) encompasses a spectrum of conditions induced by excessive alcohol consumption, ranging from hepatic steatosis to more severe stages such as alcoholic hepatitis (AH) and alcohol-associated cirrhosis[1]. ALD is a major global health challenge, contributing to approximately 2 million liver-related deaths annually, including those from cirrhosis and hepatocellular carcinoma (HCC). The prevalence of decompensated cirrhosis has notably increased, from 1.1 million cases in 1990 to 2.5 million in 2017[2]. Despite its widespread impact, no Food and Drug Administration (FDA)-approved therapies specifically target liver fibrosis in ALD[3]. The World Health Organization estimates that chronic alcohol consumption accounts for nearly 50% of liver-related deaths, with HCC being a major consequence of ALD progression. This highlights the urgent need for novel therapeutic strategies, as there are currently no FDA-approved treatments for ALD-related fibrosis or cirrhosis. ALD pathogenesis is driven by oxidative stress, lipid peroxidation, and chronic inflammation, which promote disease progression from steatosis to cirrhosis and HCC. Lipid peroxidation is particularly critical, generating reactive aldehydes that exacerbate hepatocyte injury and fibrosis[4]. Recent bibliometric analyses have emphasized the increasing research focus on lipid peroxidation in ALD and its therapeutic implications[5]. Moreover, the gut-liver axis and intestinal microbiota dysbiosis play central roles in ALD progression. Chronic alcohol consumption disrupts gut barrier integrity, allowing bacterial endotoxins to enter the liver and activate inflammation *via* the LPS/TLR4 pathway[6]. Modulating gut microbiota and inflammation has emerged as a promising therapeutic approach for ALD management[7].

Macrophages, particularly pro-inflammatory M1 macrophages, are key mediators of ALD progression. Their persistent activation drives liver inflammation in early disease stages, highlighting the potential of macrophage-targeted therapies for ALD treatment[8,9]. Elafibranor (EFN), a dual peroxisome proliferator-activated receptor (PPAR)α/δ agonist, has demonstrated significant therapeutic potential in ALD by modulating key metabolic and inflammatory pathways[10,11]. EFN mitigates liver fibrosis by suppressing lipid accumulation and hepatocellular apoptosis while enhancing autophagic flux and antioxidant defense mechanisms[11]. Additionally, EFN downregulates the LPS/TLR4/NF-κB pathway and strengthens intestinal barrier integrity, thereby alleviating ALD progression[10,12]. It also induces autophagy in intestinal epithelial cells, reduces intestinal apoptosis, and modulates gut microbiota composition, contributing to gut-liver axis homeostasis[11,13]. These effects collectively support EFN's potential as a comprehensive treatment for ALD. Given the pivotal role of PPARs in hepatic metabolism and inflammation, comparative analyses with other PPAR modulators, such as pan-PPAR agonists (*e.g.*, lanifibranor) and selective PPARγ agonists (*e.g.*, pioglitazone), are warranted. Such studies could elucidate EFN's mechanistic advantages and provide a broader perspective on the clinical relevance of PPAR-targeted strategies in mitigating ALD pathogenesis (Figure 1) [12,14].

METHODOLOGY OF DATA COLLECTION

The data presented in this review were collected through a comprehensive search of peer-reviewed articles and preclinical studies using PubMed, Scopus, and Web of Science databases. Key search terms included "Elafibranor", "ALD", "PPAR agonists", "macrophage modulation" and "intestinal barrier". Only studies published in the last five years were prioritized to ensure up-to-date findings. Inclusion criteria focused on research examining the therapeutic potential of EFN in ALD and its mechanisms of action. The primary study for this review is "Elafibranor interrupts adipose

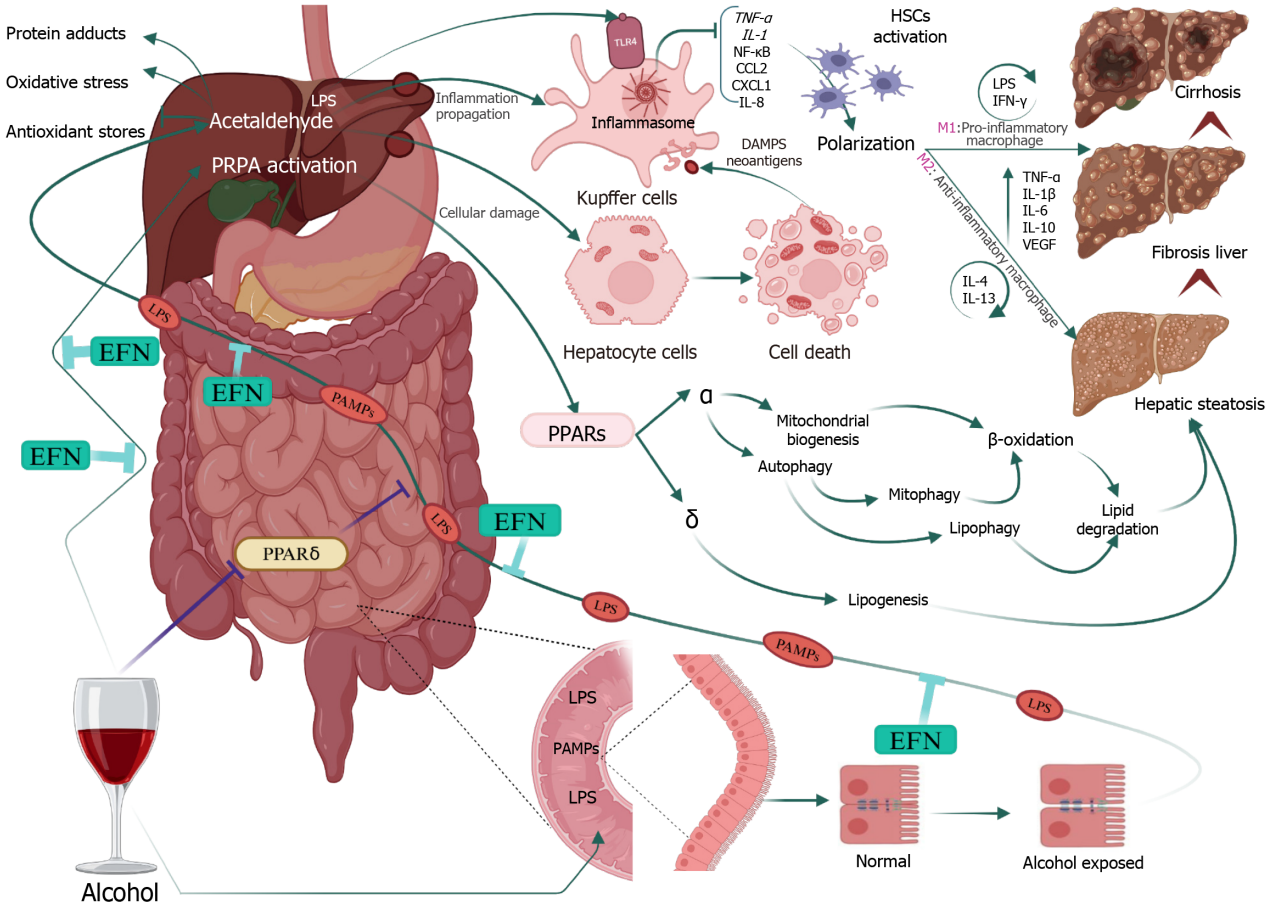

Figure 1 Mechanistic pathways of elafibranor in alcohol-associated liver disease. Elafibranor (EFN) exerts its therapeutic effects through dual activation of peroxisome proliferator-activated receptor (PPAR)α and PPARδ. PPARα activation reduces oxidative stress and inflammation by modulating Kupffer cell polarization and suppressing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Concurrently, PPARδ activation enhances mitochondrial biogenesis, β-oxidation, and lipid metabolism, counteracting hepatic steatosis. EFN also strengthens the gut barrier by limiting lipopolysaccharides and pathogen-associated molecular patterns translocation, thereby reducing systemic inflammation and liver injury. Moreover, EFN inhibits hepatic stellate cell activation, thereby attenuating fibrosis progression. These multifaceted actions position EFN as a promising therapeutic strategy for alcohol-associated liver disease, addressing both metabolic dysfunction and immune-mediated fibrosis. EFN: Elafibranor; ALD: Alcohol-associated liver disease; TNF-α: Tumor necrosis factor-alpha; IL: Interleukin; PPAR: Peroxisome proliferator-activated receptor; LPS: Lipopolysaccharides; HSC: Hepatic stellate cells.

dysfunction-mediated gut and liver injury in mice with alcoholic steatohepatitis".

CURRENT STATUS OF CLINICAL RESEARCH ON EFN

Despite promising preclinical findings, the clinical application of EFN in ALD remains under investigation. While its efficacy has been demonstrated in clinical trials for non-alcoholic steatohepatitis (NASH), data on its effects in ALD patients are still limited. Current research focuses on evaluating its ability to mitigate alcohol-induced hepatic fibrosis and inflammation, with early-phase studies assessing its safety and pharmacokinetics. Further randomized controlled trials are essential to establish its long-term benefits and determine optimal dosing regimens. Beyond its role in NASH, EFN has also shown potential in treating primary biliary cholangitis (PBC). A 2023 clinical study reported that EFN treatment led to significant biochemical improvements in cholestatic markers among PBC patients, reinforcing its broader therapeutic potential in liver diseases[15].

THE ROLE OF MACROPHAGES IN ALD PATHOGENESIS

Macrophages play a pivotal role in the progression of ALD, contributing to both inflammation and fibrosis at various stages of the disease, including AH and cirrhosis[16]. Macrophage polarization plays a critical role in determining the severity of ALD. The pro-inflammatory M1 phenotype secretes cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6, which exacerbate hepatic injury and fibrogenesis. Conversely, the M2 phenotype is associated with the secretion of IL-10 and transforming growth factor-beta (TGF-β), promoting tissue repair and fibrosis resolution. However, an imbalance between these two phenotypes in ALD leads to chronic inflammation and progressive

liver damage. "Macrophage Polarization in Liver Diseases: Mechanisms and Therapeutic Targets" Hepatology, 2024[17]. Among them, Kupffer cells, the resident liver macrophages, are particularly responsive to alcohol and its metabolites. Upon activation, they release pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, intensifying hepatic inflammation and accelerating ALD progression[9,18,19]. Beyond their inflammatory function, macrophages also drive liver fibrosis, a hallmark of ALD. By secreting profibrotic mediators, they activate hepatic stellate cells (HSCs), promoting excessive extracellular matrix deposition, tissue scarring, and hepatic dysfunction[20,21]. Additionally, macrophages are key players in the gut-liver axis, a crucial pathway linking alcohol consumption to liver damage. Chronic alcohol intake compromises intestinal barrier integrity, allowing bacterial endotoxins like lipopolysaccharides (LPS) to translocate into the liver *via* the portal circulation. Upon recognizing these microbial components through pattern recognition receptors such as TLRs, macrophages trigger NF-κB signaling, amplifying cytokine and chemokine production, and exacerbating hepatic injury[18,22-24]. Given their central role in ALD pathogenesis, targeting macrophage activity represents a promising therapeutic approach. Emerging evidence suggests that EFN modulates macrophage activation by regulating the TLR4/NF-κB pathway, leading to reduced pro-inflammatory cytokine secretion. This immunoregulatory function positions EFN as a potential therapeutic agent in ALD, distinct from its established role in NASH[25].

EFN'S IMPACT ON MACROPHAGE ACTIVITY

EFN, a dual agonist of PPARα and PPARδ, exhibits potent anti-inflammatory properties that may be beneficial in the treatment of ALD. Unlike traditional fibrates, which primarily target lipid metabolism through PPARα activation, EFN also modulates inflammatory pathways *via* PPARδ activation. This positions EFN as a promising candidate for addressing both metabolic and inflammatory disturbances in ALD[5,10,12,26]. Recent studies have demonstrated that EFN significantly reduces macrophage activation in ALD models. Notably, the infiltration of F4/80□ macrophages, a marker of macrophage activation, was markedly diminished following EFN treatment. This reduction was accompanied by a decrease in hepatic mRNA expression of LPS binding protein, TLR4, and its co-receptor CD14 key components of the LPS recognition complex. Consequently, EFN suppressed TLR4-mediated inflammatory signaling, as evidenced by reduced IκBα degradation and diminished NF-κB phosphorylation[12,27-29]. Additionally, EFN treatment led to a notable decrease in hepatic pro-inflammatory cytokines, including TNF-α, IL-6, IL-1β, and CCL2 key drivers of inflammation and fibrosis in ALD. These findings underscore EFN's ability to mitigate liver inflammation and its progression toward fibrosis. Unlike conventional ALD treatments, EFN exerts direct effects on macrophages and modulates inflammation at multiple levels. Furthermore, EFN enhances intestinal barrier integrity, a benefit rarely observed in other ALD treatments[12,19,30]. Emerging evidence highlights EFN's role in suppressing the LPS/TLR4/NF-κB signaling pathway, thereby reducing hepatic inflammation. A recent study demonstrated that EFN administration significantly reduced NF-κB activation and pro-inflammatory cytokine levels in a mouse model of ALD, reinforcing its potential as a macrophage-targeted therapy[12]. However, further clinical investigations are necessary to validate its efficacy and explore its broader applications in inflammatory liver diseases[12].

MOLECULAR MECHANISMS OF EFN ACTION

EFN is a dual agonist of peroxisome proliferator-activated receptors (PPARα and PPARδ) that shows promise as a therapeutic agent for ALD by targeting key metabolic, inflammatory, and cellular pathways. PPARs are nuclear receptors involved in lipid metabolism, inflammation regulation, glucose homeostasis, and cellular differentiation, making EFN's dual activation profile particularly effective against the complex pathology of ALD[12,31,32]. EFN activates PPARα enhances lipid metabolism by stimulating fatty acid oxidation (β-oxidation) and lipolysis, reducing hepatic lipid accumulation, a hallmark of ALD. Furthermore, PPARα activation promotes autophagy, a critical process for clearing damaged organelles and lipid droplets, thereby reducing oxidative stress and lipotoxicity. This effect is complemented by the upregulation of antioxidant enzymes such as superoxide dismutase and catalase, further mitigating oxidative damage and inflammation in the liver[32,33]. Beyond its role in lipid metabolism, EFN has been shown to modulate glucose homeostasis and hepatic inflammation. Activation of PPARα/δ by EFN not only enhances fatty acid oxidation but also suppresses hepatic glucose output by downregulating phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. This dual action prevents hyperglycemia-induced oxidative stress, a key factor in ALD progression. Furthermore, EFN inhibits NF-κB signaling, thereby reducing TNF-α and IL-6 expression, ultimately mitigating hepatic inflammation[13]. PPARδ activation by EFN contributes to maintaining intestinal barrier integrity, which plays a crucial role in reducing endotoxin translocation from the gut to the liver. EFN upregulates tight junction proteins such as occludin and claudin-1, which enhance intestinal barrier function, and reduces apoptosis in intestinal epithelial cells by modulating pro- and anti-apoptotic proteins. Additionally, EFN positively influences gut microbiota composition, reducing systemic inflammation and its impact on the liver[13,34]. EFN also exerts direct anti-inflammatory effects by modulating macrophage activity. It suppresses pro-inflammatory M1 macrophage markers (*e.g.*, TNF-α, IL-1β, NOS2) through inhibition of the TLR4/NF-κB pathway while promoting an anti-inflammatory M2 macrophage phenotype. This shift reduces hepatic inflammation and fibrosis by lowering pro-inflammatory cytokines (*e.g.*, TNF-α, IL-6, IL-1β) and increasing anti-inflammatory cytokines such as IL-10. EFN further suppresses Kupffer cell activation, limiting their contribution to liver inflammation and fibrosis[35,36]. Beyond its effects on macrophages, EFN inhibits HSC activation, thereby reducing extracellular matrix deposition and liver fibrosis. EFN further modulates extracellular matrix (ECM) remodeling by regulating the balance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), promoting ECM degradation and

reducing fibrogenesis[37,38]. By downregulating MMP-2 and MMP-9 activity, EFN facilitates the breakdown of excessive collagen deposition, thereby decreasing liver stiffness and improving hepatic architecture[39]. Additionally, EFN suppresses lysyl oxidase, an enzyme involved in ECM cross-linking, further contributing to fibrosis resolution[40]. These effects collectively highlight EFN's potential as a therapeutic agent in reversing fibrosis and restoring liver homeostasis. It modulates MMP and their inhibitors (TIMPs), playing a role in extracellular matrix remodeling. EFN also suppresses the expression of LPS binding protein and CD14, attenuating the LPS/TLR4 signaling pathway and reducing inflammatory responses in ALD[12,35,37,38]. In addition to macrophage modulation, EFN exerts anti-fibrotic effects by targeting HSCs. Activated HSCs are the primary source of ECM deposition, leading to hepatic fibrosis. EFN inhibits HSC activation by downregulating TGF-β1 and collagen I/III expression, thereby reducing ECM accumulation. Moreover, EFN induces apoptosis in activated HSCs, preventing the persistence of fibrogenic signaling in the liver[39]. Furthermore, PPARα activation induces fibroblast growth factor 21, a hormone with anti-inflammatory and metabolic regulatory properties, adding another layer to EFN's therapeutic effects. By integrating these mechanisms, EFN effectively addresses the metabolic, inflammatory, and fibrotic aspects of ALD[35,40]. In conclusion, EFN's dual activation of PPARα and PPARδ allows it to target multiple facets of ALD pathology. Its ability to enhance lipid metabolism, reduce oxidative stress, restore intestinal barrier function, modulate macrophage phenotypes, and inhibit fibrosis underscores its potential as a novel therapeutic strategy for ALD and other inflammatory liver disorders[13,31].

EFN modulates inflammatory cytokines such as TNF-α and IL-10, which play crucial roles in immune regulation. TNF-α, a pro-inflammatory cytokine, is downregulated through inhibition of the TLR4/NF-κB signaling pathway, reducing hepatic inflammation. Conversely, IL-10, an anti-inflammatory cytokine, is upregulated, promoting M2 macrophage polarization and tissue repair. These effects suggest a broader immunomodulatory role for EFN beyond ALD, particularly in cancer-associated inflammation. Recent studies have highlighted the tumor microenvironment's influence on T cell exhaustion, with novel CD8+ markers identified in breast cancer models[41]. Given EFN's impact on immune regulation, future studies should explore its potential in modulating T cell function in cancer settings (Table 1).

IMPLICATIONS FOR ALD TREATMENT

EFN, a dual agonist of PPARα and PPARδ, has emerged as a promising therapeutic option for ALD by modulating macrophage activity. Through the activation of PPARs, EFN inhibits the TLR4/NF-κB signaling pathway, which plays a critical role in hepatic and intestinal inflammation. This inhibition reduces the overall inflammatory response in both the liver and gut, helping to prevent liver fibrosis progression and promote liver regeneration. Additionally, the combined effects of EFN on both the liver and intestinal barrier function underscore its potential as a holistic treatment for ALD, addressing multiple disease mechanisms and offering a comprehensive therapeutic approach[11,38,42].

HYPOTHESIS ON THE ROLE OF EFN IN ALD TREATMENT

Recent evidence suggests that EFN, as a dual PPARα/δ agonist, may exert therapeutic effects in ALD beyond its metabolic benefits. Emerging hypotheses indicate that EFN could modulate the hepatic immune response by influencing macrophage polarization and reducing pro-inflammatory cytokine secretion. Moreover, its impact on gut barrier integrity suggests a potential role in mitigating endotoxemia-induced liver injury. These insights open promising avenues for investigating EFN as a broad-spectrum therapeutic strategy in ALD.

FUTURE DIRECTIONS

The current study highlights the therapeutic potential of EFN in treating ALD, particularly through its modulation of macrophage activity. However, further research is necessary to elucidate its precise molecular mechanisms and long-term effects. Key directions for future studies include.

Macrophage and HSC interactions

Investigating the direct effects of EFN on isolated macrophages and HSC will help clarify its impact on fibrotic pathways in ALD.

Bile acid metabolism and liver-gut axis

Understanding how EFN influences bile acid metabolism and its crosstalk with the gut microbiome could provide insights into its broader metabolic benefits.

Comparative efficacy across ALD subtypes

Since ALD presents with heterogeneity in disease progression, it is crucial to study EFN's differential effects in various ALD subgroups (*e.g.*, early-stage steatosis vs. advanced fibrosis).

Table 1 Mechanisms of elafibranor in alcohol-associated liver disease

Mechanism	Target/effect	Benefit in ALD	Translational implications
PPARα activation	Stimulates fatty acid breakdown (lipolysis) and β-oxidation	Reduces hepatic steatosis	Potential target for reducing liver fat accumulation in ALD patients
	Promotes autophagy	Mitigates oxidative stress	Enhances cellular repair mechanisms in ALD-related damage
	Upregulates antioxidant genes	Maintains cellular homeostasis	May prevent disease progression and hepatocyte injury
		Reduces hepatic injury and inflammation	Could serve as a therapeutic strategy to limit liver damage
PPARδ activation	Enhances tight junction protein expression (intestinal barrier integrity)	Reduces intestinal permeability	Prevents endotoxin leakage, reducing inflammation in ALD
	Promotes intestinal epithelial cell autophagy	Prevents endotoxin translocation	Protects gut-liver axis and limits systemic inflammation
	Reduces intestinal apoptosis	Maintains intestinal barrier function	Prevents gut-derived inflammation in ALD pathogenesis
		Reduces inflammation and systemic effects	Could lower systemic complications associated with ALD
Macrophage activity	Reduces M1 macrophage markers (pro-inflammatory)	Suppresses TLR4/NF-κB signaling	Decreases inflammation-driven liver damage
	Promotes M2 macrophage marker (anti-inflammatory)	Shifts macrophage phenotype to anti-inflammatory state	Could be harnessed for immunomodulation in ALD therapy
	Reduces hepatic pro-inflammatory cytokine expression	Attenuates liver fibrosis	Potential strategy to prevent fibrosis progression
Other mechanisms	Decreases LPS binding protein and CD14 expression (reduces LPS signaling)	Reduces inflammation	Targets gut-derived inflammation, a key factor in ALD
	Influences bile acid metabolism	Potentially reduces hepatic lipid accumulation	May improve metabolic balance in ALD patients
	Regulates Kupffer cell activity	Mitigates liver damage and fibrosis	Modulating Kupffer cells could be a therapeutic approach for ALD
	Induces fibroblast growth factor 21 expression (anti-inflammatory and metabolic regulation)	Further modulates inflammatory responses and metabolic processes	Could be explored for systemic metabolic benefits in ALD

ALD: Alcohol-associated liver disease; LPS: Lipopolysaccharides; PPAR: Peroxisome proliferator-activated receptor.

Clinical validation and translational research

Future clinical trials should focus on validating the efficacy of EFN in ALD patients with advanced fibrosis.

PROPOSED CLINICAL TRIALS AND EXPERIMENTAL APPROACHES FOR EFN VALIDATION

To establish the clinical efficacy and safety of EFN, we propose the following experimental setups

Randomized controlled trial: A multicenter, double-blind, placebo-controlled randomized controlled trial enrolling ALD patient at different disease stages to assess EFN's impact on fibrosis regression, inflammatory cytokine levels (*e.g.*, TNF-α, IL-6), and intestinal barrier integrity over 12 to 24 months.

Patient-derived xenograft models: These models can provide a more physiologically relevant platform to evaluate EFN's effects on liver fibrosis and macrophage polarization, bridging the gap between preclinical and clinical research. High-throughput single-cell RNA sequencing (scRNA-seq) can be used to gain deeper insights into EFN's cellular effects.

Combination therapy studies: Investigating the synergistic effects of EFN with farnesoid X receptor (FXR) agonists or other anti-fibrotic agents to determine potential combination treatments that enhance fibrosis resolution and hepatic homeostasis.

Biomarker-based response assessment: Implementing transcriptomic and metabolomic analyses to track PPARα/δ target genes, oxidative stress markers, bile acid metabolism, and gut microbiota composition, providing insights into EFN's mechanisms of action and personalized treatment strategies.

These studies will contribute to defining EFN's clinical relevance, optimizing its therapeutic application, and addressing current knowledge gaps regarding its long-term safety and efficacy in ALD patients.

A major limitation in current research is the lack of patient-derived xenograft (PDX) models that accurately mimic human ALD pathology. Although preclinical studies have demonstrated EFN's therapeutic potential, significant limitations exist in translating these findings to human pathology. Murine models of ALD exhibit fundamental differences in hepatic metabolism, immune system responses, and fibrosis progression compared to humans, which may affect EFN's observed efficacy. For instance, differences in HSC activation and inflammatory cytokine signaling can influence the extent of fibrosis resolution.

To overcome these limitations, PDX models offer a promising alternative by preserving human-like liver architecture, stromal interactions, and disease-specific genetic alterations. PDX models have been widely used in oncology and other liver diseases to evaluate drug efficacy under human-relevant conditions. Previous studies, such as "Comparing volatile and intravenous anesthetics in a mouse model of breast cancer metastasis, 2018" have demonstrated the advantages of xenograft models in mimicking human disease progression and drug responses. Incorporating PDX models in future EFN research could enhance our understanding of its therapeutic effects on human hepatic fibrosis and inflammation, leading to more reliable translational outcomes. Additionally, implementing high-throughput scRNA-seq in these models will provide deeper insights into cellular heterogeneity and EFN's specific effects on immune and hepatic cell populations.

Further studies should also explore combining EFN with anti-fibrotic agents such as FXR agonists, which may offer synergistic benefits in reducing liver fibrosis[43,44].

Beyond ALD

Potential applications in other liver diseases: Given its efficacy in NASH and PBC, EFN may also offer therapeutic value in other metabolic liver diseases, including cholestatic disorders and fibrosis-related conditions, warranting further investigation[11,20,27].

LONG-TERM SAFETY AND EFFICACY OF EFN

Despite promising preclinical and clinical findings, concerns regarding the long-term safety of EFN remain. Chronic activation of PPARα/δ signaling pathways may lead to unintended metabolic consequences, necessitating further research into its prolonged effects on lipid metabolism, insulin sensitivity, and cardiovascular health. Additionally, interindividual variability in response to EFN suggests personalized treatment approaches are needed. Future research should prioritize large-scale, multi-center clinical trials with extended follow-up periods to establish a robust safety profile and optimize patient selection criteria.

CONCLUSION

EFN represents a promising therapeutic candidate for ALD due to its dual action on hepatic inflammation and gut barrier function. By inhibiting macrophage activation and the TLR4/NF-κB signaling pathway, EFN effectively reduces hepatic inflammation and fibrosis. Additionally, its role in strengthening gut barrier integrity helps prevent endotoxin translocation, a critical factor in ALD progression. Beyond ALD, EFN has demonstrated efficacy in other liver disorders such as NASH and PBC, highlighting its broader therapeutic potential. However, further research is essential to fully characterize its molecular mechanisms, evaluate its long-term safety, and determine its clinical applicability across different subtypes of liver disease.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Bioactivity of dressings based on platelet-rich plasma and Platelet-rich fibrin for tissue regeneration in animal model

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Abstract

BACKGROUND

Skin wounds are common injuries that affect quality of life and incur high costs. A considerable portion of healthcare resources in Western countries is allocated to wound treatment, mainly using mechanical, biological, or artificial dressings. Biological and artificial dressings, such as hydrogels, are preferred for their biocompatibility. Platelet concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), stand out for accelerating tissue repair and minimizing risks of allergies and rejection. This study developed PRF and PRP-based dressings to treat skin wounds in an animal model, evaluating their functionality and efficiency in accelerating the tissue repair process.

AIM

To develop wound dressings based on platelet concentrates and evaluating their efficiency in treating skin wounds in Wistar rats

METHODS
Wistar rats, both male and female, were subjected to the creation of a skin wound, distributed into groups (n = 64/group), and treated with Carbopol (negative control); PRP + Carbopol; PRF + Carbopol; or PRF + $CaCl_2$ + Carbopol, on days zero (D0), D3, D7, D14, and D21. PRP and PRF were obtained only from male rats. On D3, D7, D14, and D21, the wounds were analyzed for area, contraction rate, and histopathology of the tissue repair process.

RESULTS
The PRF-based dressing was more effective in accelerating wound closure early in the tissue repair process (up to D7), while PRF + $CaCl_2$ seemed to delay the process, as wound closure was not complete by D21. Regarding macroscopic parameters, animals treated with PRF + $CaCl_2$ showed significantly more crusting (necrosis) early in the repair process (D3). In terms of histopathological parameters, the PRF group exhibited significant collagenization at the later stages of the repair process (D14 and D21). By D21, fibroblast proliferation and inflammatory infiltration were higher in the PRP group. Animals treated with PRF + $CaCl_2$ experienced a more pronounced inflammatory response up to D7, which diminished from D14 onwards.

CONCLUSION
The PRF-based dressing was effective in accelerating the closure of cutaneous wounds in Wistar rats early in the process and in aiding tissue repair at the later stages.

Key Words: Skin wound; Murine model; Platelet-rich fibrin; Platelet-rich plasma; Tissue repair

Core Tip: This study compares the effects of dressings made from platelet concentrates platelet-rich fibrin (PRF), platelet-rich plasma, and PRF activated with $CaCl_2$ - on accelerating the tissue repair process and closing cutaneous wounds in Wistar rats. The results indicated that the PRF dressing accelerated wound closure and tissue repair.

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INTRODUCTION

Skin wounds are prevalent discontinuity lesions in the global population, significantly impacting the quality of life of affected individuals and the economy, both due to the scientific investment in treatments and the high cost of clinical management[1]. Regarding wound treatment costs, it is estimated that about 1% of healthcare resources in Western countries are allocated for this purpose[2].

Dressings are the most common method for wound treatment, whether mechanical, biological, or artificial. In general, they offer significant benefits, such as protecting the wound from external agents, good biocompatibility, and easy application. However, because they require frequent changes, mechanical dressings can cause abrasion of the lesion, which may progress to ischemia and necrosis. Thus, biological dressings can be more advantageous due to their biocompatibility and biodegradability, while artificial dressings, such as hydrogels, are advantageous because of their porous structure, resulting in better fluid adsorption and oxygen exchange[3].

Despite advances in treatments, the resources for wound coverage that can accelerate the tissue repair process are still scarce, and the cost-benefit ratio must be considered to make them viable. In this context, platelet concentrates offer unique advantages for their use as a therapeutic method. Platelet concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), are used in various fields of medicine and dentistry to optimize the tissue repair process by concentrating and providing a prolonged release of growth factors and other autologous molecules[4,5].

Other advantages are that, being a by-product for autologous use, there is a considerable reduction in triggering allergic reactions or rejection and in the transmission of diseases *via* the parenteral route, besides being a resource that is quickly and easily obtained. Thus, this study aimed to develop PRP and PRF-based wound coverings for the treatment of excisional skin wounds in an animal model and to evaluate the functionality and efficiency of these dressings in accelerating the tissue repair process.

MATERIALS AND METHODS

Animals and ethics

This study was approved by the Animal Use Ethics Committee of the Universidade Federal do Paraná (Approval 1499/R.O. 11/2022). The experiments were conducted on Wistar strain rats (*Rattus norvegicus*). For the preparation of platelet concentrates PRP and PRF, healthy male rats over 90 days old and weighing over 500 g were used. For the skin wound experiments, male and female rats, aged 90 to 120 days and weighing 250 to 350 g, were used. The animals were kept under controlled conditions of temperature (22 ± 2.0 °C), a 12-hour light/dark cycle, and received pelletized feed and water ad libitum. They underwent an adaptive handling period of 15 days before the start of the experiments.

Obtaining platelet concentrates

The animals were anesthetized with ketamine/xylazine (90/9 mg/kg; i.p.), and 5 mL of whole blood was collected *via* cardiac puncture using a 10 mL syringe and a 22G1" needle. For obtaining PRF, the protocol described by Dohan *et al*[6] with modifications was used. The whole blood was subjected to a single centrifugation at 400 G for 10 minutes in 10 mL polypropylene tubes, ensuring that the interval between puncture and centrifugation did not exceed 60 seconds. For obtaining PRP, the protocol described by Borges[7] with modifications was used. The whole blood was centrifuged at 300 G for 10 minutes in a tube containing 3.2% (w/v) sodium citrate. The plasma was transferred to a Falcon tube and centrifuged at 640 G for 10 minutes. The upper 2/3 was considered platelet-poor plasma and discarded, and the final 1/3 was used as platelet concentrate-PRP. The PRP was collected and incubated with 1.7 mmol/L $CaCl_2$ (calcium chloride) at 37 °C for 30 minutes.

Preparation of PRP and PRF-based wound dressings

To facilitate the application of platelet concentrates and allow for prolonged contact with the skin wound, they were incorporated into a Carbopol 940 hydrogel. Carbopol 940 is an acrylic polymer that forms a hydrogel with biological adhesion and prolonged residence time when applied topically to the skin[8]. Separately, the platelet concentrates were incorporated into a 0.75% (w/v) Carbopol 940 gel in sterile 0.9% (w/v) NaCl solution. Three types of dressings were prepared: (1) PRP + Carbopol 940; (2) PRF + Carbopol 940; and (3) Activated PRF + Carbopol 940. The latter was obtained by adding 1.7 mmol/L $CaCl_2$ followed by incorporation into the Carbopol 940 gel and incubation at 37 °C for 30 minutes before use.

Excisional skin wounds

The animals were sedated with isoflurane 3%-4% for induction and 1.5%-2% for maintenance, in 100% oxygen. Subsequently, the dorsal region of the animal, 2 cm from the base of the neck, was shaved. Local antisepsis was performed with 10% (w/v) chlorhexidine digluconate, and local anesthesia was administered with 5 mg/kg lidocaine hydrochloride (s.c.). Using a 1.5 cm diameter metal punch and a scalpel, a circular wound was created. The skin was completely removed down to the muscular fascia, and the wound was cleaned with 0.9% (w/v) NaCl solution and gauze.

Experimental groups and treatments

The animals were randomly divided into four experimental groups (n = 64/group): Carbopol 940 0.75% (w/v) (negative control), PRP + Carbopol 940 0.75% (w/v) (PRP), PRF + Carbopol 940 0.75% (w/v) (PRF), and PRF + $CaCl_2$ + Carbopol 940 0.75% (w/v) (PRF + $CaCl_2$). Each animal received a topical application of the respective treatment (300 µL) once a day on the day of wound creation (D0), 3 (D3), 7 (D7), 14 (D14), and 21 (D21) days after wound creation. All treatments were prepared on the day of each application, and the wounds were pre-cleaned and debrided with 0.9% (w/v) NaCl solution and gauze before the treatment application.

Macroscopic evaluation, area, and contraction rate of skin wounds

On days 3, 7, 14, and 21, 16 animals from each group were randomly euthanized by overdose of isoflurane, followed by visual and morphometric evaluation of the wounds. The wounds were photographed, and the lesion area was calculated using ImageJ® software (National Institutes of Health). The macroscopic analysis was performed using parameters adapted from the Bates-Jensen Wound Assessment Tool, as described by Harris *et al*[9]. The evaluated parameters included wound appearance (regarding necrosis), type of exudate, wound bed (regarding granulation tissue), and epithelialization (regarding the covered area). All parameters were assessed using a tissue damage score (1 to 5). The wound contraction rate was calculated using the equation [% contraction = 100 (Wo-Wi)/Wo], where Wo = initial wound area and Wi = wound area on the observed experimental day, as described by Al-Watban and Andres[10] and Oliveira *et al*[11].

Microscopic evaluation of skin wounds

Histological analysis was performed by removing a biopsy of the injured skin area with 0.5 cm margins on D3, D7, D14, and D21. The samples were fixed in 3.7% buffered formalin (v/v) to prepare histological slides, which were stained with hematoxylin-eosin (HE) according to Garro *et al*[12], and Masson's trichrome (MT) according to Coleman[13], with modifications, to evaluate the organization and maturation of collagen fibers. A blinded pathologist performed the histopathological analysis, assigning scores according to the protocols by Harris *et al*[9]. For the quantitative evaluation of collagenization intensity, digitized images of the TM-stained histological sections were analyzed using ImageJ 1.54 g software (National Institutes of Health). Three regions of each histological section, with the same area (cm²), were

analyzed for all experimental groups on D3, D7, D14, and D21.

Statistical analysis

The mean values obtained for each group of animals in each experiment were calculated and analyzed using one-way analysis of variance with GraphPad Prism 9.5.0 software. Tukey's test was used to compare the groups within each treatment. Results with $P < 0.05$ were considered significant.

RESULTS

Area and contraction rate of the skin wounds

The analysis of the skin wound area in animals treated with Carbopol, PRF, PRF + $CaCl_2$, and PRP was conducted on D3, D7, D14, and D21. On D3, a significant reduction in wound area was observed in animals treated with PRF compared to the other groups. On D7, animals treated with PRF continued to show a significant reduction in wound area compared to the other groups. However, the PRP-treated group showed a significant reduction in wound area compared to the PRF + $CaCl_2$ group, indicating increased efficiency of PRP in accelerating tissue repair from D3 to D7. On D14 and D21, no significant differences were observed between the experimental groups. By D21, the control group animals and most of the animals in the PRF and PRP groups had no remaining wound area, while most animals treated with PRF + $CaCl_2$ still had a wound area (Figure 1A). A visual representation of the wound areas during the experiment is shown in Figure 1B.

Regarding the contraction rate of the skin wounds in animals treated with PRF, there was a linear increase from D3 to D14. On D7, the contraction rate approached 50% in the PRF group, compared to less than 25% in the other groups. On D3 and D7, the contraction rate in the PRF-treated group was significantly higher than in the negative control, PRF + $CaCl_2$, and PRP groups. Additionally, on D7, the PRP group showed a significantly higher contraction rate than the PRF + $CaCl_2$ group. From D14 onwards, no significant differences were observed between the experimental groups, and all groups had a contraction rate greater than 90%. On D21, the negative control group had a contraction rate of 100%, followed by most animals in the PRF and PRP groups. Animals treated with PRF + $CaCl_2$ had a contraction rate between 97.6% and 100%, corroborating the wound area reduction data for the same period (Figure 1C).

Macroscopic evaluation of skin wounds

The analysis of macroscopic parameters of the skin wounds showed that regarding wound appearance, all experimental groups on D3, D7, D14, and D21 exhibited either a thick crust firmly adhered, covering 75% to 100% of the wound (score 1), or a soft and adhered crust covering 50% to 75% of the wound (score 2). A significant difference was observed only on D3, indicating that the PRF + $CaCl_2$ group had a more pronounced parameter (Figure 2A). For the parameter "type of exudate", on D3, no exudate (score 1) was observed in any experimental group (100% of animals). On D7, it was observed that in all groups, some animals exhibited serosanguinous exudate (score 2). By D14 and D21, there was no exudate present in any of the experimental groups (100% of animals). There was no significant difference observed for this parameter (Figure 2B).

The reepithelialization score measures the area of the wound with new epithelial formation, and this score decreases with increased reepithelialization. Although no statistical difference was observed between the PRF, PRF + $CaCl_2$, PRP, and Carbopol groups on any experimental day, the reepithelialization process progressed towards tissue repair. On D3 and D7, all groups showed high scores, indicating less than 25% (score 5) or 25 to 50% (score 4) of the wound area reepithelialized. By D7, all experimental groups (100% of animals) exhibited 75 to less than 100% of the wound area reepithelialized (score 2), and on D21, the groups presented animals with scores of 2 and 1 (100% of the wound reepithelialized), with a predominance of the latter except for the PRF + $CaCl_2$ group, in which the majority of animals still did not have 100% of the wound reepithelialized (Figure 2C).

Histopathology of skin wounds

Microscopic analysis of the skin wounds indicated that on D3, all experimental groups showed less than 25% of the wound area with new blood vessel formation, with no statistical difference between the groups. From D 3 to D7, there was an increase in new vessel formation. By D7, the PRF + $CaCl_2$ group exhibited significantly lower neoangiogenesis (scores 2 and 3 - new vessels in 25%-50% and 50%-75% of the wound area, respectively) compared to the Carbopol group (scores 3 and 4; 4 = new vessels in 100% of the wound area). By D21, the PRF + $CaCl_2$ group showed significantly higher neoangiogenesis compared to both the Carbopol group (scores 2 and 3) and the PRF group (with a predominance of score 3), with most animals exhibiting 100% of the wound area with new vessels (score 4) (Figure 3A).

Regarding fibroblast proliferation, on D3, most animals across all experimental groups exhibited less than 25% of the wound area with fibroblasts, with no statistical differences between the groups. From D14, fibroblast proliferation increased and generally remained high until D21. On D14, fibroblast proliferation was significantly higher in the PRF + $CaCl_2$ group, with 50%-75% or 100% of the wound area containing fibroblasts (scores 3 and 4, respectively), compared to the Carbopol group, in which animals had 25%-50% (score 2) or 50%-75% (score 3) of the wound area with fibroblasts. By D21, fibroblast proliferation in the PRF + $CaCl_2$ group (scores 3 and 4) remained significantly higher than in the Carbopol group (scores 2 and 3) and the PRF group (scores 2 and 3), while the PRP group (scores 3 and 4) showed significantly higher fibroblast proliferation compared to the PRF group (Figure 3B).

The presence of inflammatory infiltrate in the wound area was significantly higher in the PRF + $CaCl_2$ group compared to the PRF group on D3 and D7. On D3, animals in the PRF + $CaCl_2$ group had inflammatory infiltrate in 100% of the

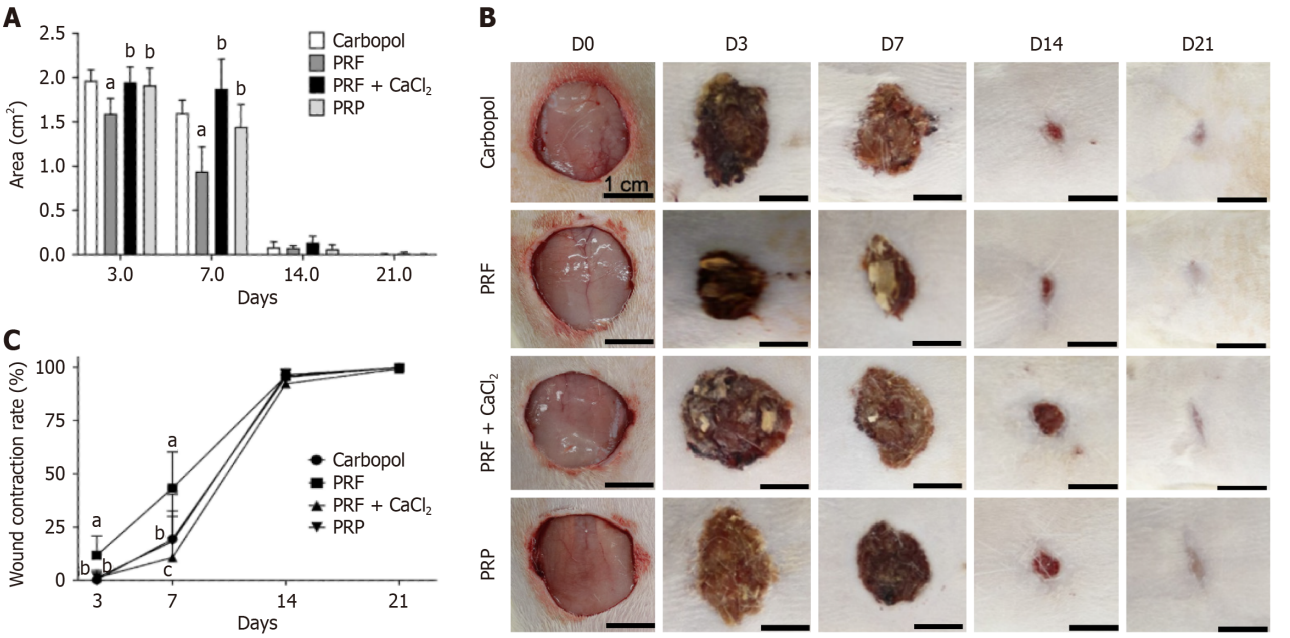

Figure 1 Evolution of skin wound closure on D3, D7, D14, and D21. A: Skin wound area (cm²); B: Representative image of the visual wound area; C: Wound contraction rate. Results are presented as the mean ± SEM for 8 animals per group. [a]*P* < 0.05 *vs* the Carbopol group. [b]*P* < 0.05 *vs* the platelet-rich fibrin group. [c]*P* < 0.05 *vs* the platelet-rich fibrin + $CaCl_2$ group. One-way analysis of variance followed by Tukey's test. Error bars: Standard error of the mean. Bar = indicates image scale (1 cm). PRF: Platelet-rich fibrin; PRP: Platelet-rich plasma.

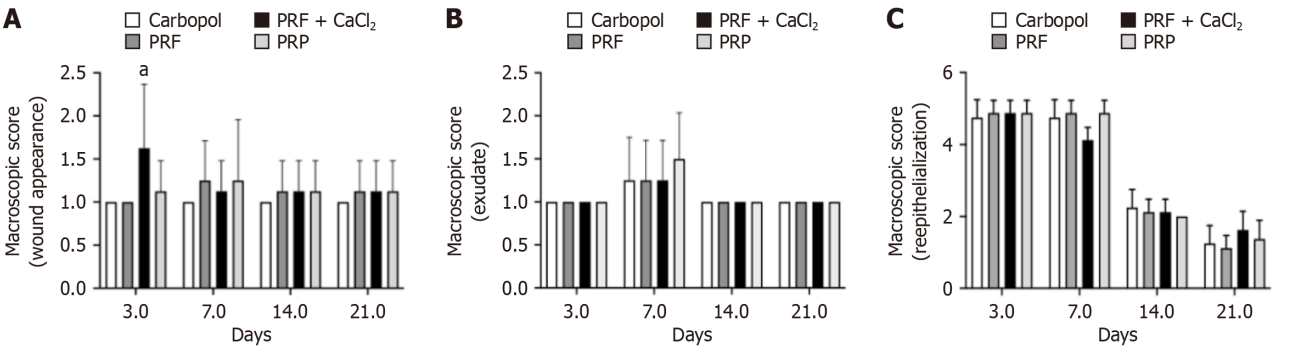

Figure 2 Macroscopic analysis of skin wounds on D3, D7, D14, and D21. A: Wound appearance; B: Type of exudate; C: Reepithelialization. Results are presented as the mean ± SEM for 8 animals per group. [a]*P* < 0.05 *vs* the Carbopol and platelet-rich fibrin groups. One-way analysis of variance followed by Tukey's test. Error bars: Standard error of the mean. Bar = indicates image scale (1 cm). PRF: Platelet-rich fibrin; PRP: Platelet-rich plasma.

wound area (score 4), while the PRF group had inflammatory infiltrate in 50%-75% (score 3) or 100% of the wound area. By D7, most animals in the PRF + $CaCl_2$ group exhibited inflammatory infiltrate in 50%-75% of the wound area, whereas the PRF group had infiltrate in 25%-50% (score 2) or 50%-75% (score 3) of the wound area. On D14 and D21, the PRF + $CaCl_2$ group had a smaller area with inflammatory infiltrate compared to the PRF group, with a significant difference on D14. On D14, the PRF + $CaCl_2$ group had inflammatory infiltrate in 25%-50% of the wound area, whereas the PRF group had inflammatory infiltrate in 50%-75% of the wound area. Overall, the inflammatory infiltrate decreased over time (D3-D21), except for the PRP group, which on D21 showed a significantly larger area of inflammatory infiltrate compared to the Carbopol, PRF, and PRF + $CaCl_2$ groups (Figure 3C).

Regarding the presence of collagen (collagenization), on D3, it was either absent or observed in less than 25% of the wound area across all experimental groups, with no statistical differences between them. From D3 to D7, the presence of collagen increased, and starting from D7, there was a slight trend of increasing collagenization until D21. On D14, higher collagenization was observed in the PRF group (50%-75% of the wound area-score 3), PRF + $CaCl_2$ group (25%-50%-score 2, and 50%-75%), and PRP group (50%-75%), compared to the Carbopol group (25%-50%), with this difference being significant. By D21, collagenization in the PRF group had increased, observed in 100% (score 4) or 50%-75% of the wound area. Collagenization in this group was significantly higher compared to the Carbopol group (less than 25% or 25%-50% of the wound area) and the PRP group (25%-50% or 50%-75%). Significant differences were also observed between the PRF and PRP groups, and between the PRF + $CaCl_2$ (25%-50% or 50%-75%) and Carbopol groups (Figure 3D). Representative histological images of the skin wounds from the experimental groups during the repair process are shown in Figure 4.

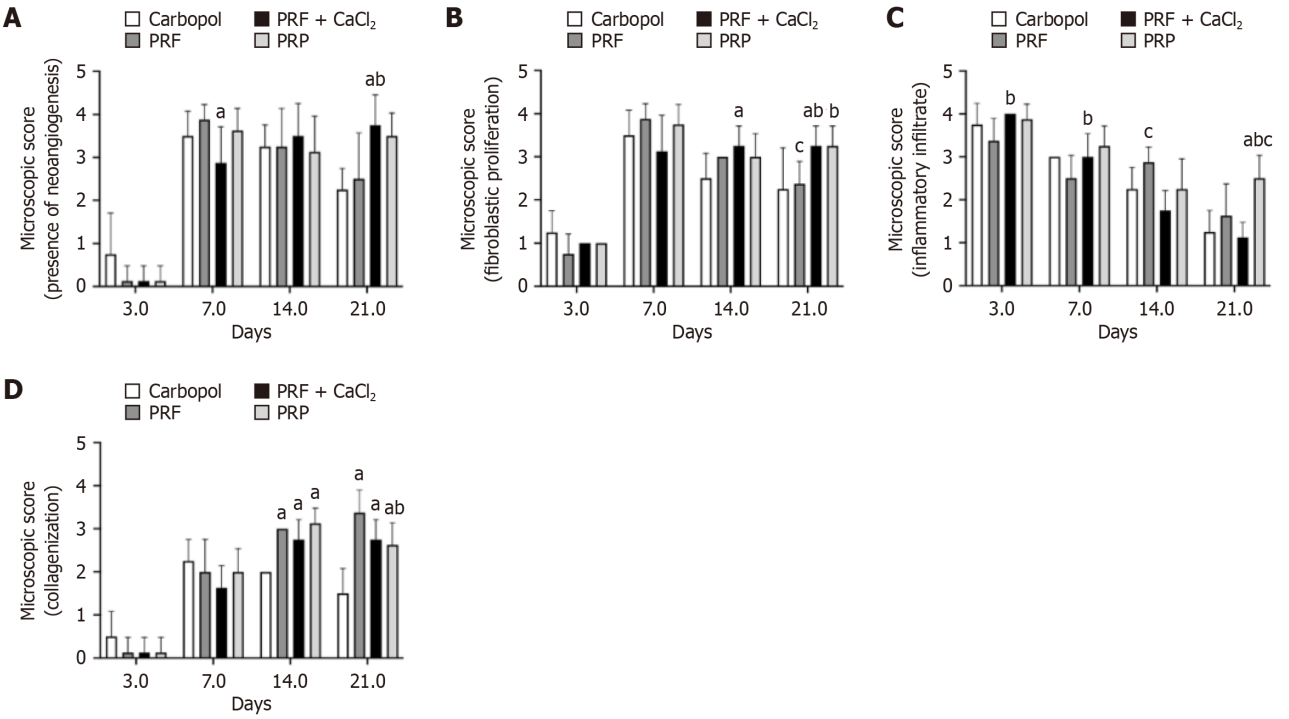

Figure 3 Microscopic analysis of skin wounds on D3, D7, D14, and D21. A: Presence of neoangiogenesis; B: Fibroblast proliferation; C: Inflammatory infiltrate; D: Collagenization. Results are presented as the mean ± SEM for 8 animals per group. $^{a}P < 0.05$ *vs* the Carbopol group. $^{b}P < 0.05$ *vs* the platelet-rich fibrin group. $^{c}P < 0.05$ *vs* the platelet-rich fibrin + $CaCl_2$ group. One-way analysis of variance followed by Tukey's test. Error bars: Standard error of the mean. Bar = indicates image scale (1 cm). PRF: Platelet-rich fibrin; PRP: Platelet-rich plasma.

MT stains collagen blue, distinguishing it from cellular elements, which are stained red. The analysis of blue staining intensity was conducted for the Carbopol group, PRF group, PRF + $CaCl_2$ group, and PRP group on D3, D7, D14, and D21. On D3, the staining intensity in all groups was significantly higher compared to the Carbopol group, with the PRF group showing higher collagen content than the PRP group, and the PRP group showing higher content than the PRF + $CaCl_2$ group. From D3 to D7, the blue staining intensity did not seem to increase for the PRF + $CaCl_2$ group, while in the other groups, the staining intensity increased, indicating that the collagen amount was significantly higher in these groups compared to the PRF + $CaCl_2$ group. No significant differences in blue staining intensity were observed between the experimental groups on D14. By D21, collagen intensity appeared to have increased in all experimental groups, being significantly higher in the PRF group compared to the Carbopol and PRP groups (Figure 5).

DISCUSSION

Inflammation is a fundamental process for tissue regeneration; however, excessive inflammation can impair the development of subsequent phases, such as proliferation and tissue remodeling[14]. Previous studies have shown improvements in the healing process with the use of platelet concentrates, and the beneficial effect appears to be associated with a reduction in inflammation, leading to decreased vascular permeability and edema[15]. Additionally, they stimulate the secretion of growth factors that induce cellular proliferation of fibroblasts, osteoblasts, endothelial cells, epithelial cells, and leukocyte migration[16-18].There is evidence showing that PRF has a more significant role in wound healing compared to PRP. PRF provides a continuous and gradual release of growth factors, facilitating more effective clonal expansion and cellular differentiation[19]. Horgos *et al*[20] demonstrated that patients treated with PRF injections showed a substantial reduction in wound size, which consequently led to a decrease in injury-associated hyperalgesia. PRP combined with $CaCl_2$ results in a more efficient release of growth factors due to more complete platelet activation [21]. In this study, we used $CaCl_2$ in combination with PRF to determine if calcium chloride could positively influence platelet activity. The enhanced inflammatory response caused by the addition of $CaCl_2$, especially in the initial days, may be linked to the modulation of the immune response through calcium-sensitive receptors, inducing a response *via* the inflammasome complex and activation of the NF-κB pathway[22], which could accelerate the inflammatory phase. In this study, PRF was more effective compared to the Carbopol (control) group in the initial days following wound creation, particularly in terms of wound closure. The efficacy of PRF in the healing process appears to be linked to its architecture, forming a dense fibrin network that captures growth factors secreted by activated platelets, concentrating them at the injury site and facilitating the creation of a microenvironment conducive to tissue repair[23]. The macroscopic analysis of skin wounds revealed the appearance of the wound, with a thick crust firmly adhered or a soft and adhered crust covering the wound. In terms of the type of exudate, on D3, a small exudate was present on groups, probably due to the polarization of the immune response. However, by D7, some animals in all groups showed serosanguinous exudate. The

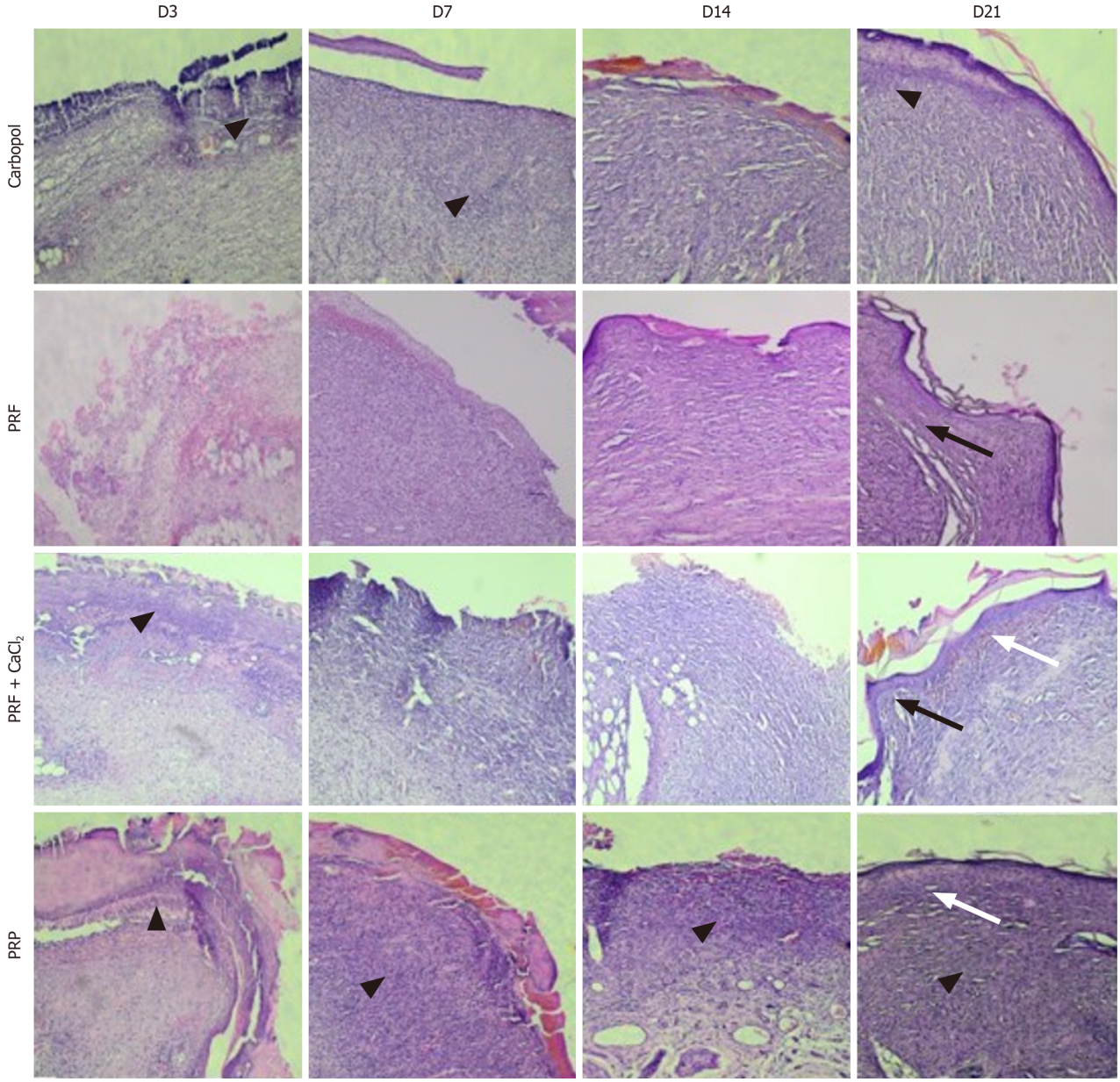

Figure 4 Representative image of the skin wound healing process. Photomicrograph of histological sections of skin wounds from carbopol, platelet-rich fibrin (PRF), PRF + $CaCl_2$, and platelet-rich plasma groups, at D3, D7, D14, and D21. Hematoxylin and eosin staining, 100 × magnification. Black arrow: neovascularization; Write arrow: fibroblast proliferation; Arrowhead: inflammatory cells. PRF: Platelet-rich fibrin; PRP: Platelet-rich plasma.

reepithelialization score measures the area of the wound with new epithelial formation, and this score decreases with increased reepithelialization; there was no difference on this parameter. The data from this study suggest an increase in the inflammatory process associated with PRF (D14) and PRP (D21), which could be related to the neoangiogenesis inherent to the wound healing process[24]. Both *in vitro* and *in vivo* studies indicate that PRP and PRF are involved in inducing angiogenesis[25,26]. These platelet concentrates, and consequently their granules, including growth factors, show a high capacity to stimulate endothelial proliferation in a coordinated manner, restoring local microvasculature and enabling an optimal blood supply for the recovery process[27,28]. The group of animals treated with PRF + $CaCl_2$ maintained neovascularization until the end of the experiment (Day 21); this could be related to the delayed resolution of the wound, prolonging the natural course of tissue repair. Despite the proven efficacy of PRP in the tissue repair process [8,29], this concentrate did not produce significant results compared to PRF. This may be associated with the pronounced inflammatory process and the local concentration of $CaCl_2$. In this study, we used the methodology described by Lima IN [30], which employs 1.7 mmol/L $CaCl_2$ to activate PRP, a widely used approach in humans. The literature shows a lack of standardization regarding the concentration of $CaCl_2$ used for activating platelet concentrates, with variations including 20 mmol/L[31], 22.8 mmol/L[32], and 901 mmol/L[33]. As described by Chan *et al*[34], dysregulation of an inflammatory response mediated by T helper 2 (Th2) lymphocytes under excessive conditions can trigger pathological fibrosis with excessive extracellular matrix deposition. In our experiments, we observed a significant increase in collagen deposition and fibroblast proliferation starting from the 14th day. Ridiandries *et al*[35] stated that an unbalanced response from these mediators tends to prolong the inflammatory phases and impair the natural course of healing. Collagen production is a

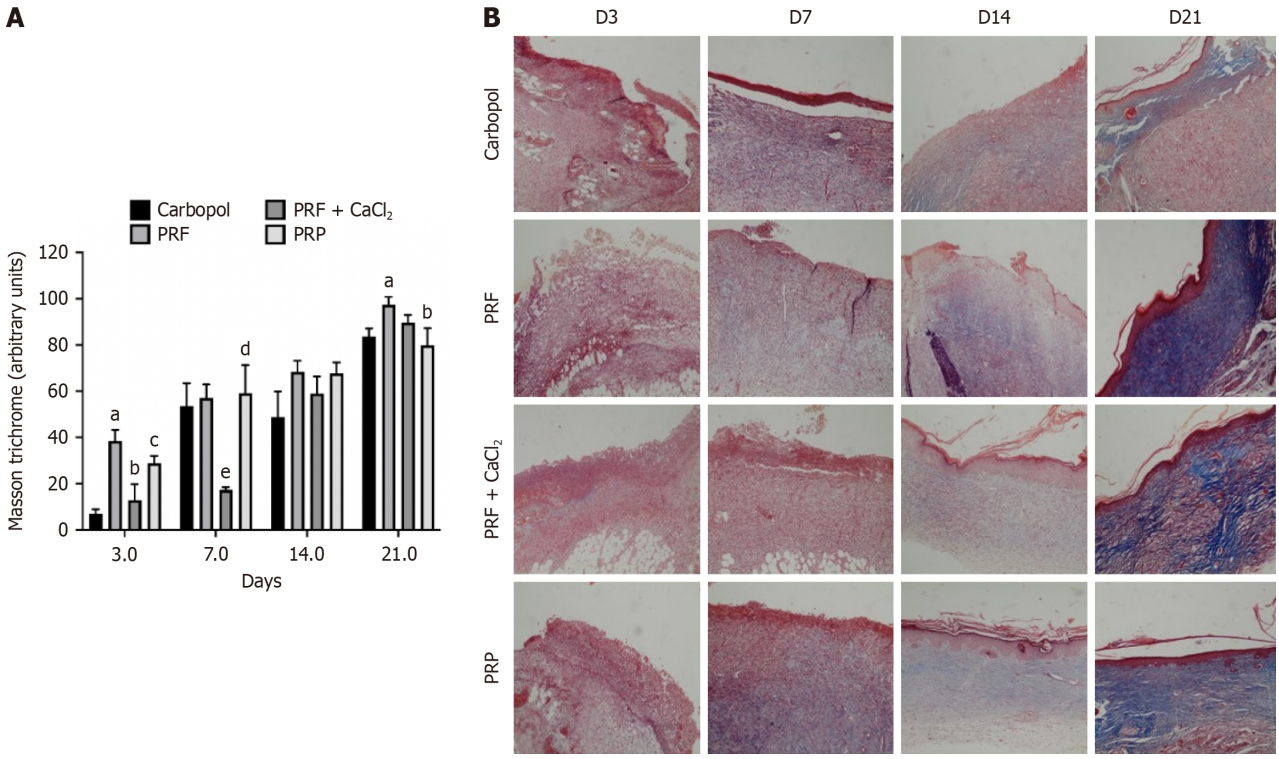

Figure 5 Analysis of collagenization in skin wounds. A: Inference of collagen amount (collagenization); B: Photomicrograph of histological sections of skin wounds from Carbopol, platelet-rich fibrin (PRF), PRF + $CaCl_2$, and platelet-rich plasma groups, at D3, D7, D14, and D21. TM staining, 100 × magnification. [a]*P* < 0.05 *vs* the Carbopol group. [b]*P* < 0.05 *vs* the platelet-rich fibrin group. [c]*P* < 0.05 *vs* compared to the Carbopol and platelet-rich fibrin + $CaCl_2$ groups. [d]*P* < 0.05 *vs* the platelet-rich fibrin + $CaCl_2$ group. [e]*P* < 0.05 *vs* the Carbopol and platelet-rich fibrin groups. One-way analysis of variance followed by Tukey's test. Error bars: Standard error of the mean. PRF: Platelet-rich fibrin; PRP: Platelet-rich plasma.

crucial factor in wound healing, as it provides strength to the tissue. During tissue regeneration, fibroblasts deposit type III collagen, which over time loses water and reorganizes, interweaving to form the more resilient type I collagen[36,37]. In this study, the experimental groups treated with platelet concentrates showed greater collagen deposition compared to the control group. Previous studies have demonstrated the efficacy of PRF in a rabbit model of induced periodontitis, concluding that PRF application is associated with improved collagen density, as evidenced by MT staining at 14 days [38]. According to the results obtained in this study, PRF appears to be effective in negatively modulating the inflammatory phase of the repair process and stimulating neoangiogenesis. Regarding PRP, further studies are needed to elucidate the influence of different concentrations of $CaCl_2$ in its activation. $CaCl_2$ leads to immediate platelet activation, which releases their granules, enhancing the healing response in the initial days of the process[39,40].

CONCLUSION

This study suggests that PRF-based dressing is a good approach in the initial stages of healing. Wound treatment with PRF significantly affected the size of the lesion, the rate of retraction and allowed better organization of collagen through HE and TM stains. However the group treated with PRF + $CaCl_2$ and PRP demonstrated higher fibroblast proliferation and inflammatory infiltration, which probably culminated in a delay in the healing process. Therefore, PRF can be considered a promising therapeutic approach in wound treatment. Furthermore, the murine model study provides a solid basis for future clinical investigations in humans. However new research is necessary to fully understand the mechanism of action and the mediators involved in PRF and PRP efficacy and safety in clinical trials.

FOOTNOTES

Author contributions: Sá-Oliveira JA, Geraldo MV, Marques M and Luiz RM performed the *in vivo* experiments; Lima IN and De Souza TC carried out the pilot tests; Sá-Oliveira JA performed histology; Sá-Oliveira JA and Cestari FK analyze histology; Sá-Oliveira JA, Zarpelon-Schutz AC and Teixeira KN wrote manuscript; Zarpelon-Schutz AC and Teixeira KN interpreted the data, performed the critical analysis of the results and coordinated the study. All authors approved the final version of the manuscript.

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