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Sufentanil preconditioning reduces hepatic ischemia-reperfusion injury in rats by regulating ferroptosis through ATF3

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Abstract

Objective To examine the correlation between the therapeutic efficacy of sufentanil preconditioning on hepatic ischemia-reperfusion injury (HIRI)-induced ferroptosis and the activation of activating transcription factor 3 (ATF3), as well as to elucidate the underlying mechanisms involved.

Methods Eighteen Sprague-Dawley (SD) male rats were randomly assigned to three groups: a sham operation group, an ischemia-reperfusion injury group, and a sufentanil pretreatment group. Serum transaminase levels, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were measured to assess liver function. Liver injury was evaluated using hematoxylin and eosin staining. Ferroptosis was assessed by measuring iron content, glutathione peroxidase 4 (GPX4) expression levels, and mitochondrial morphological changes in hepatocytes. The relationship between the protective effects of sufentanil and ATF3 was investigated through immunohistochemistry, Western blotting, and quantitative real-time polymerase chain reaction (qRT-PCR).

Results Sufentanil pretreatment was found to ameliorate liver tissue damage, attenuate the inflammatory response, and mitigate ferroptosis in the context of HIRI. Furthermore, Sufentanil enhanced the expression levels of ATF3 while concurrently reducing iron content within liver tissue.

Conclusion Sufentanil preconditioning may mitigate ferroptosis associated with HIRI through the upregulation of ATF3 expression. This protective mechanism may be linked to a concomitant reduction in iron levels.

Keywords Activating transcription factor 3, Ferroptosis, Liver, Ischemia-reperfusion injury, Sufentanil

Background

Liver diseases, including hepatocellular carcinoma and cirrhosis, remain among the leading causes of mortality worldwide. Surgical interventions such as hepatectomy are commonly employed to manage these conditions [1]. To reduce intraoperative hemorrhage during liver resections and related procedures, various vascular occlusion techniques are utilized; however, these approaches can result in hepatic ischemia–reperfusion injury (HIRI) [2]. HIRI is a major contributor to postoperative liver dysfunction and may progress to liver failure [3]. Despite extensive research, the complex pathophysiological mechanisms underlying HIRI remain incompletely understood [4]. Therefore, continued investigation into the mechanisms of HIRI, the identification of novel therapeutic targets, and strategies to improve clinical outcomes are of critical importance.

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Ferroptosis, a newly recognized form of regulated cell death characterized by iron dependency and lipid peroxidation, has been implicated in the pathogenesis of ischemia–reperfusion (I/R) injury across multiple organs [5]. Recent studies suggest that ferroptosis plays a pivotal role in the development of HIRI [6], highlighting it as a potential therapeutic target. However, current research into the suppression of ferroptosis in the context of HIRI is limited, and its regulatory mechanisms remain to be fully elucidated.

Our prior bioinformatics analysis indicated a significant association between activated transcription factor 3 (ATF3) and HIRI-induced ferroptosis, thereby establishing a theoretical foundation for this investigation [7]. Previous research has suggested that ATF3 may serve as a potential target for mitigating I/R-induced organ dysfunction [8]. Furthermore, ATF3 has been implicated in the pathophysiology of I/R injury across various organs, including the kidney, brain, and heart [9–11]. Nevertheless, the specific role of ATF3 in HIRI-induced ferroptosis remains unreported.

Drug pretreatment is a widely accepted approach for the prevention and treatment of HIRI [12]. Sufentanil, a commonly utilized anesthetic in clinical settings, has been demonstrated in numerous studies to exert a protective effect against HIRI [13]. ZOU et al. have identified that sufentanil can inhibit ferroptosis induced by HIRI via the HIF-1α/KCNQ1OT1 axis, both in vitro and in vivo [14]. Given that sufentanil can mitigate ferroptosis associated with HIRI, thus providing hepatic protection, and considering that ATF3 is closely linked to ferroptosis in HIRI, we propose that sufentanil pretreatment may attenuate HIRI by inhibiting ferroptosis through the modulation of ATF3.

This study aims to develop a rat model of HIRI to investigate the impact of sufentanil pretreatment on liver damage and ferroptosis induced by HIRI. Additionally, it seeks to elucidate the relationship between these effects and ATF3, thereby providing both theoretical and experimental foundations for potential therapeutic strategies for HIRI.

Materials and methods

Animals and surgical procedure

The experimental protocol and procedures were approved by the Medical Ethics Committee of Chengde Medical College (Approval No. CYFYLL2023119). A total of eighteen clean-grade, healthy, 2-month-old male Sprague-Dawley (SD) rats, each weighing between 220 and 240 g, were procured from Beijing Huafukang Biotechnology Co., Ltd. (License No. SCXK [Beijing] 2019-0008). The rats were housed in the Hebei Key Laboratory of Panvascular Diseases, where they were provided with ad libitum access to food and water. The housing conditions were maintained at a temperature of 21–23 °C, a relative humidity of 45%–55%, and a controlled light-dark cycle of 12 h each. The rats were randomly assigned to three groups, each consisting of six rats: the sham operation group (Sham group), the hepatic ischemia-reperfusion injury group (I/R group), and the HIRI with sufentanil treatment group (I/R + SF group). Prior to the experimental procedures, the rats were subjected to a 12-hour fasting period with access to water only.

Following established protocols, the hepatic ischemia-reperfusion model was induced as described in the literature [15]. Specifically, the rats were anesthetized with pentobarbital (50 mg/kg) to create a 70% hepatic ischemia-reperfusion model. The rats were then positioned supine on the operating table, and the surgical area was prepared and disinfected. A midline abdominal incision was made to expose the hepatic hilum. Subsequently, the hepatic hilum structure was occluded to the left and middle lobes using a non-invasive vascular clamp. The surgical incision was covered with moistened gauze to prevent dehydration. Following 45 min of inducing 70% hepatic ischemia, the non-invasive vascular clamp was removed to restore blood flow and simulate reperfusion, after which the abdominal wall cavity was sutured. After a 24-hour reperfusion period, whole blood was collected via retro-orbital puncture, and liver samples were harvested post-euthanasia for subsequent experimental analysis. The Sham group underwent the same surgical procedure without the induction of vascular obstruction. In the I/R + SF group, sufentanil (AB40401321 Hubei Yichang Renfu Pharmaceutical Co., Ltd.) was administered intraperitoneally at a dosage of 3 μg/kg 30 min prior to laparotomy [13]. The remaining groups received an equivalent volume of normal saline at the corresponding time point.The Ethics Committee of the Affiliated Hospital of Chengde Medical College approved the study protocol(CYFYLL2023119).

Quantification of liver injury

The extent of hepatocyte injury was assessed by quantifying serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Blood samples were obtained from the cavity, and serum was isolated following centrifugation at 3000 × g for 10 min. ALT and AST activity assay kits were procured from Beijing Box Biotechnology Co., Ltd. (Batch No. : AKAM019M, AKAM006M, Beijing Box Biotech Co., Ltd.).

Hematoxylin-eosin staining (HE)

Liver tissue sections were fixed in 4% formalin and subsequently embedded in paraffin. The paraffin-embedded tissue samples were sectioned into four-micrometer-thick slices. These sections underwent deparaffinization in

xylene followed by rehydration through a graded ethanol series. The sections were then stained with hematoxylin and eosin (H&E), dehydrated again in a graded ethanol series, and cleared in xylene. The stained slides were examined using an optical microscope (BX60-32FB3-E01, Japan) at magnifications of ×200 and ×400.

Immunohistochemical staining

ATF3 (1 : 50, batch no. : DF3110, Affinity, USA) and liver tissue sections were incubated overnight at 4 °C. Subsequently, the samples were washed three times with PBS buffer and then incubated with a peroxidase-conjugated secondary antibody (Batch No. : PV-6000, Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) at 37 °C for 60 min. The peroxidase conjugate was then stained using a DAB kit (Batch No. : ZLI-9018, Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.), and images were captured with an optical microscope (BX60-32FB3-E01).

Transmission electron microscope (TEM)

To determine the sampling site, fresh liver tissue was utilized, and tissue samples of 1 mm^3 were obtained. Following the excision of these small tissue blocks in vitro, they were promptly placed into an EP tube containing electron microscope fixative and maintained at 4 °C. The samples were then rinsed three times with 0.1 M phosphate buffer (PB) at pH 7.4, with each rinse lasting 15 min. Subsequently, the fixed tissue underwent post-fixation, dehydration, and was ultimately embedded for ultrathin sectioning. Subsequently, the samples were examined using a HT7800/HT7700 transmission electron microscope (Hitachi, Japan) following staining with uranyl acetate saturated alcohol solution and lead citrate solution, and image analysis was performed.

Determination of iron content

Intracellular iron concentrations were quantified utilizing the Lipid Iron Assay Kit (Batch No. : DOJINDO I291, Beijing Beiren Chemical Technology Co., Ltd.) in accordance with the manufacturer's protocol. Absorbance at 593 nm, indicative of intracellular iron, was then measured using a spectrophotometer.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted with Trizol (15596-026, Thermo Fisher, USA) and reverse transcribed into cDNA using a reverse transcription kit (Batch No. :ZS-M14003-50T, Genentech). The reverse transcription reaction solution was prepared using 5 × Fast Plus RT Master Mix, 20 × Oligo dt (25) & Random Primer, and RNase Free H2O according to the instructions, followed by 50 ° C 5 min (first strand cDNA synthesis). 95 ° C 1 min (inactivated MuLV). Then, a 20 μl PCR reaction system was prepared according to the instructions of the third-generation ZAPA SYBR Green qPCR premix (2 ×) (Batch No. :ZS-M13002, Genentech). The qPCR system was set as follows: 2 × ZAPA3G SYBR Green qPCR Mix 10μL, forward primer 0.8μL, reverse primer 0.8μL, cDNA 2μL, ddH2O 6.4μL; the qPCR was performed in a real-time fluorescent PCR machine (Batch No. :COBAS Z480,Genentech) using the following cycles: 95 ° C for 5 min, followed by 40 cycles at 95 ° C for 10 s and 60 ° C for 20 s. The experiment was repeated three times. Primers were synthesized by Thermo (Table 1). The relative expression of target genes was calculated by the $2-\Delta\Delta CT$ method. $\Delta\Delta CT = Ctexperiment - Ctcontrol$, $\Delta CT = Cttarget - reference$.

Western blot

Total proteins were isolated from each group and quantified using the BCA Quantification Kit (P1511). Subsequently, 30 μg of total protein were loaded and electrophoresed on a 12% FuturePAGE pre-made gel at a constant voltage of 160 V in a double-stable electrophoresis apparatus (Bio-Rad, USA) for 45 min, then transferred to a 0.22 μm polyvinylidene fluoride (PVDF) membrane, and blocked with 5% skim milk at room temperature for 2 h. The membrane was incubated with mouse anti-ATF3 (1 ： 1 000, lot number : P18847, Shanghai Aibimat Pharmaceutical Technology Co., Ltd.), rabbit anti-GAPDH (1 ： 5 000, lot number : ET1601-4, Hangzhou Huaan Biotechnology Co., Ltd.) and rabbit anti-GPX4 (1 ： 5 000, lot number : ET1706-45, Hangzhou Hua 'an Biotechnology Co., Ltd.) overnight at 4°C. The next day, the PVDF membrane was rewarmed at room temperature for 15 minutes, washed three times with TBST, once for 10 minutes, and then incubated with goat anti-mouse HRP secondary antibody (1 ： 5 000, lot number : AS003, Wuhan Abbott Biotechnology Co., Ltd.) or goat anti-rabbit HRP secondary antibody (Batch No. : S1002-100, 1 : 5 000, Ruipat Biotechnology Co., Ltd.) at room temperature for 1 hour. PVDF was washed three times with TBST, 10 minutes each, and developed with a chemiluminescence detection system (C300,30100). The relative expression of the protein was analyzed by ImageJ-win64 software. The experiment was repeated three times.

Statistical methods

The data are presented as mean ± standard deviation. Statistical analyses were conducted using GraphPad Prism version 9.5. Group comparisons were performed using one-way ANOVA, with statistical significance set at $p < 0.05$.

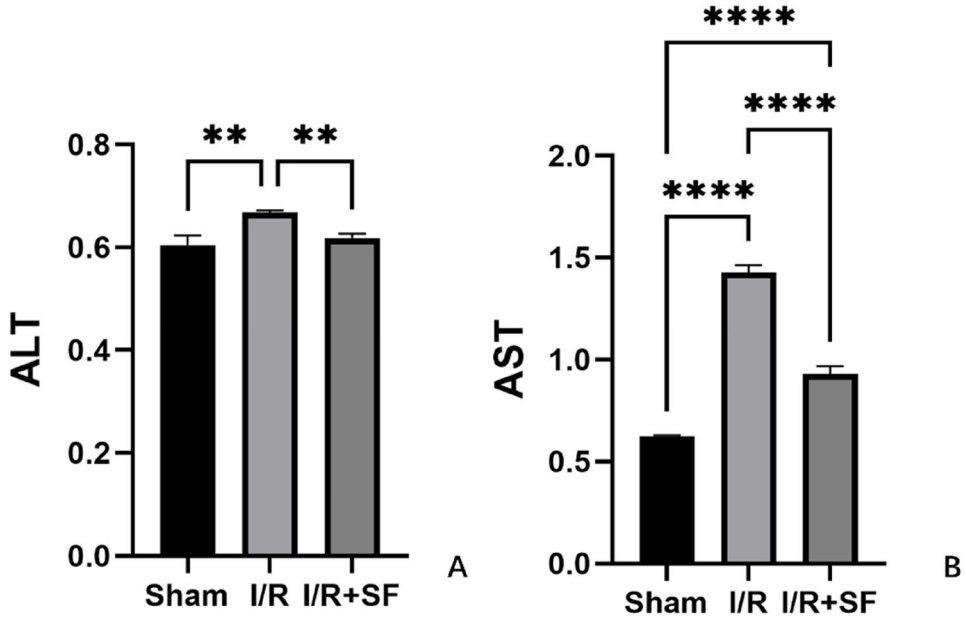

Fig. 1 Sufentanil alleviates hepatocyte injury; **A**: ALT; **B**: AST.$n = 6$/group, U/ml, * * $p < 0.01$, * * * * $p < 0.0001$

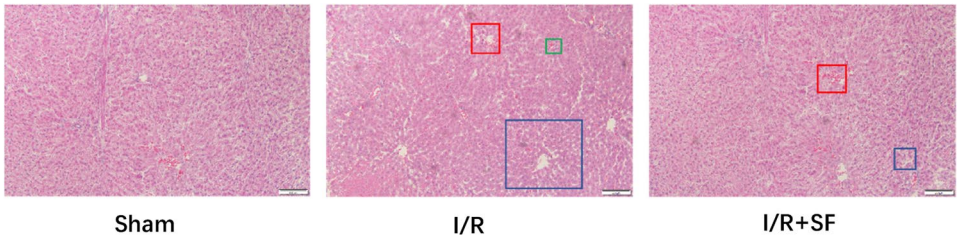

Fig. 2 Pathological manifestations of liver tissue injury: Within the blue frame, the hepatic sinus injury is characterized by an indistinct structure of the hepatic lobule and dilation of the hepatic sinus. The red frame indicates hepatic sinus congestion, while the green box highlights the infiltration of inflammatory cells(original magnification × 200)

Results

Effects of sufentanil preconditioning on HIRI-induced liver injury

The serum levels of ALT and AST in rats from each experimental group were measured, with the results presented in Fig. 1. The ALT and AST levels in the I/R group were significantly elevated compared to the Sham group (0.668 ± 0.004 vs. 0.604 ± 0.019, $p < 0.01$; 1.427 ± 0.037 vs. 0.624 ± 0.004, $p < 0.0001$). However, pretreatment with sufentanil in the I/R + SF group significantly attenuated these increases (0.618 ± 0.009 vs. 0.668 ± 0.004, $p < 0.01$; 0.930 ± 0.037 vs. 1.427 ± 0.037, $p < 0.0001$). These findings suggest that sufentanil exerts a protective effect against liver damage induced by HIRI.

Effect of sufentanil preconditioning on liver pathology induced by HIRI

By examining the liver pathological sections across different groups, it was observed that the liver section structure of the Sham group conformed to expected norms. Conversely, the I/R + SF group exhibited a reduction in alterations such as hepatic sinus injury and congestive inflammatory cell infiltration, which were prominent in the I/R group. This indicates that sufentanil pretreatment ameliorated HIRI and reversed the associated histological changes (Fig. 2).

The expression of ATF3 under immunohistochemical staining

As illustrated in Fig. 3, the expression of ATF3 in the liver tissue of sham-operated rats was prominent. Conversely, the expression of ATF3 in the I/R group was significantly reduced ($p < 0.0001$). Notably, the expression of ATF3 was significantly elevated in rats pretreated with sufentanil compared to those in the I/R group. ($p < 0.01$)

Effects of sufentanil preconditioning on HIRI-induced ferroptosis (morphological aspect)

We conducted an examination of rat liver tissue across different experimental groups using transmission electron microscopy. As illustrated in Fig. 4, the hepatocytes in the Sham group exhibited slight edema, with

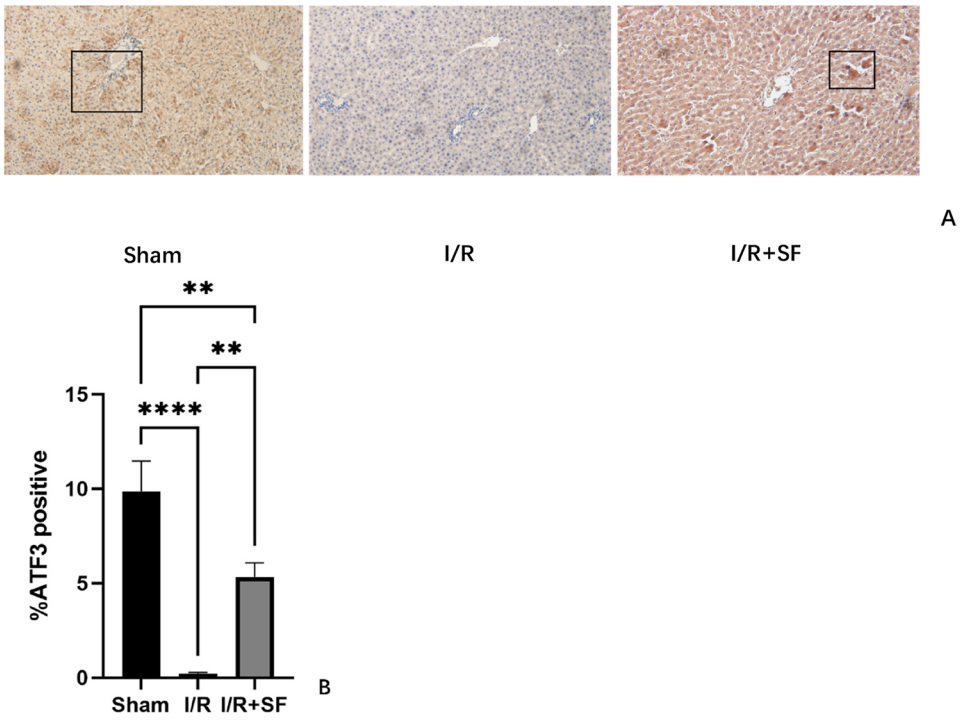

Fig. 3 **A**: Immunohistochemical results of ATF3: The positive signal exhibited a brownish-yellow hue, while the black box indicated ATF3 positive expression. **B**: The area of ATF3 coloration in each group was quantified. * * $P < 0.01$, * * * * $P < 0.0001$

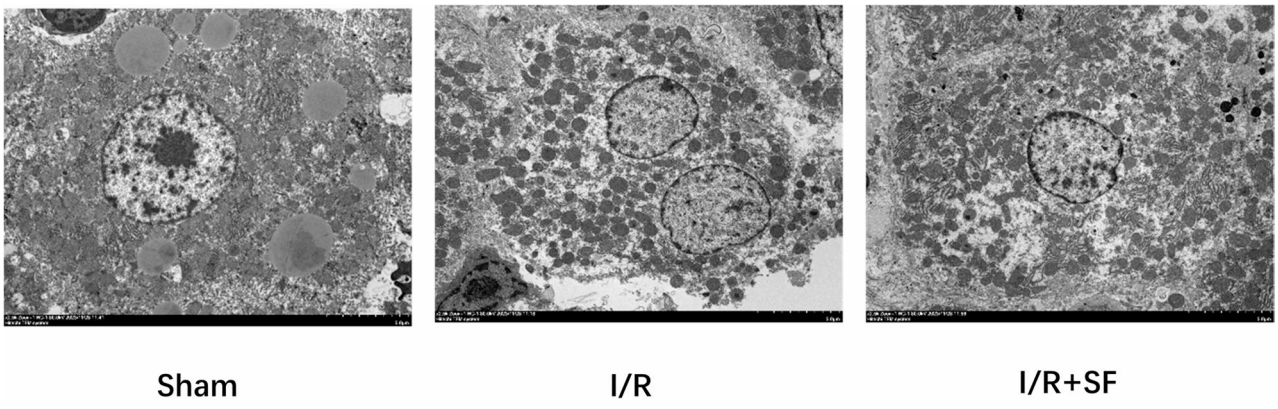

Fig. 4 Transmission electron microscopy of liver tissues (original magnification × 2500)

the majority of intracellular organelles maintaining their structural integrity. In contrast, the hepatocytes in the I/R group displayed moderate damage. Specifically, the cell membranes were partially compromised, and a significant proportion of mitochondria appeared markedly condensed and reduced in size. Additionally, the mitochondrial matrix was densely packed, exhibited high electron density, and the cristae were notably widened. Following pretreatment with sufentanil, there was a reduction in hepatic cellular damage. The cell membrane exhibited partial damage, while intracellular organelles, including mitochondria, were predominantly slightly condensed. Additionally, the matrix within the mitochondrial membrane appeared concentrated. These findings indicate that sufentanil pretreatment exerts a protective effect against HIRI by mitigating ferroptosis.

Effect of sufentanil pretreatment on iron content in hepatocytes

As shown in Fig. 5, compared with the Sham group, the iron content in the liver tissue of the I/R group was significantly increased ($p < 0.0001$). Combined with the changes in the transmission electron microscope of the I/R group, the occurrence of ferroptosis in the liver tissue was further verified. After sufentanil pretreatment, the iron content in the liver tissue decreased ($p < 0.05$). This

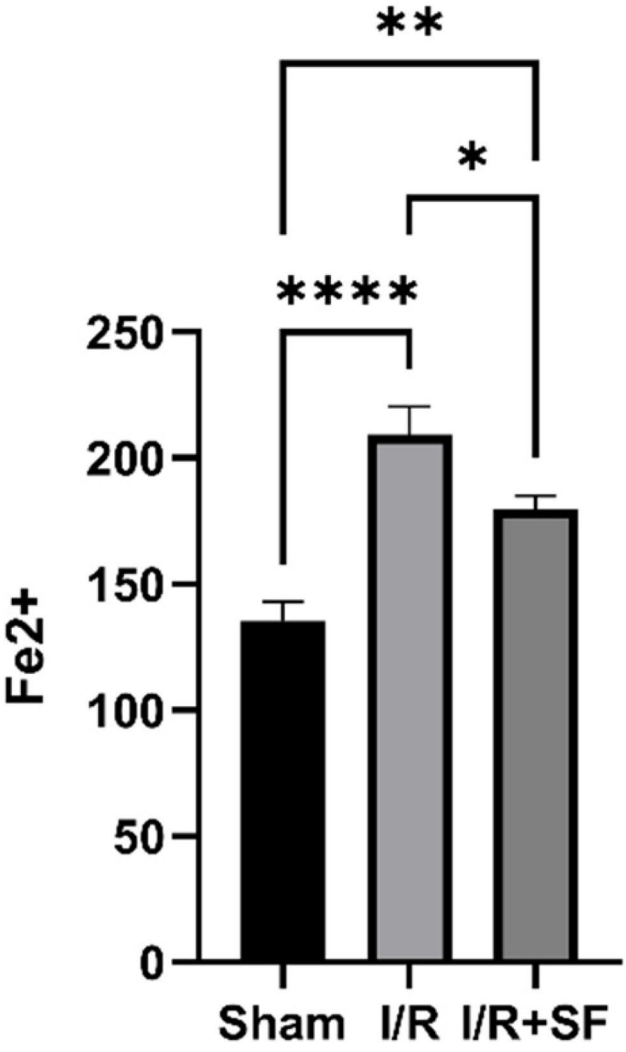

Fig. 5 Quantification of iron content in hepatocytes of rats in each group. * $p < 0.05$, ** $p < 0.01$, * * * * $p < 0.0001$

also proves the inhibitory effect of sufentanil on ferroptosis in HIRI.

Effect of sufentanil preconditioning on the expression of ATF3 and GPX4 in liver tissue

Western blot analysis demonstrated that the expression levels of ATF3 and GPX4 in the I/R group were significantly reduced compared to the sham group ($p < 0.001$, Fig. 6). Following pretreatment with sufentanil, there was a significant increase in the expression levels of ATF3 and GPX4 in liver tissue ($p < 0.05$). Additionally, the RT-PCR results for ATF3 corroborated the Western blot findings (Fig. 7A). These results support the hypothesis that sufentanil preconditioning mitigates ferroptosis in HIRI, and this protective effect may be associated with ATF3.

Discussion

The aforementioned experimental results indicate that: sufentanil pretreatment was found to ameliorate liver tissue damage, attenuate the inflammatory response, and mitigate ferroptosis in the context of HIRI. Furthermore, Sufentanil enhanced the expression levels of ATF3 while concurrently reducing iron content within liver tissue.

During liver surgeries, such as hepatectomy, the implementation of hepatic blood flow occlusion inevitably induces HIRI, which is the primary cause of postoperative liver damage [16]. Given that liver cancer is among the most prevalent malignant tumors globally, [17] hepatectomy remains a principal treatment modality. Consequently, identifying strategies to prevent HIRI during surgical procedures and enhance patient prognosis holds substantial clinical significance. Ferroptosis, a recently identified form of iron-dependent cell death, may offer new insights into these preventative strategies [18]. The involvement of ATF3 in various pathological processes has been increasingly recognized, positioning it as a potential therapeutic target for conditions such as I/R injury [19]. Research indicates that ATF3 can modulate ferroptosis in I/R injury,[20] and it has been identified as a crucial factor in HIRI [21]. Nevertheless, the specific role of ATF3 in HIRI-induced ferroptosis remains largely unexplored. Sufentanil, an opioid analgesic commonly utilized in clinical settings, has demonstrated efficacy in reducing the inflammatory response and hepatocyte apoptosis associated with HIRI in certain animal studies [22].

ALT and AST are critical biomarkers frequently employed in the clinical assessment of hepatic function. Under physiological conditions, the serum concentrations of these enzymes are minimal. However, hepatocellular injury results in the release of ALT and AST into the bloodstream, thereby elevating their serum levels. Consequently, elevated ALT and AST levels serve as indicators of hepatic dysfunction. When combined with histopathological analysis of liver tissue using hematoxylin and eosin (HE) staining, these biomarkers facilitate a more comprehensive and intuitive evaluation of the extent of hepatic tissue damage. In this study, it was observed that, in comparison to the Sham group, the levels of ALT and AST were significantly elevated in the I/R group. Additionally, histopathological analysis revealed pathological alterations in the liver tissue of the I/R group, thereby confirming the successful establishment of the HIRI model. Notably, pretreatment with sufentanil in the I/R group markedly mitigated the elevation of ALT and AST levels induced by HIRI and attenuated the extent of liver.

HIRI represents a highly intricate pathophysiological process, wherein ferroptosis constitutes a critical pathogenic mechanism implicated in the injury of multiple organs due to I/R. The inhibition of ferroptosis has

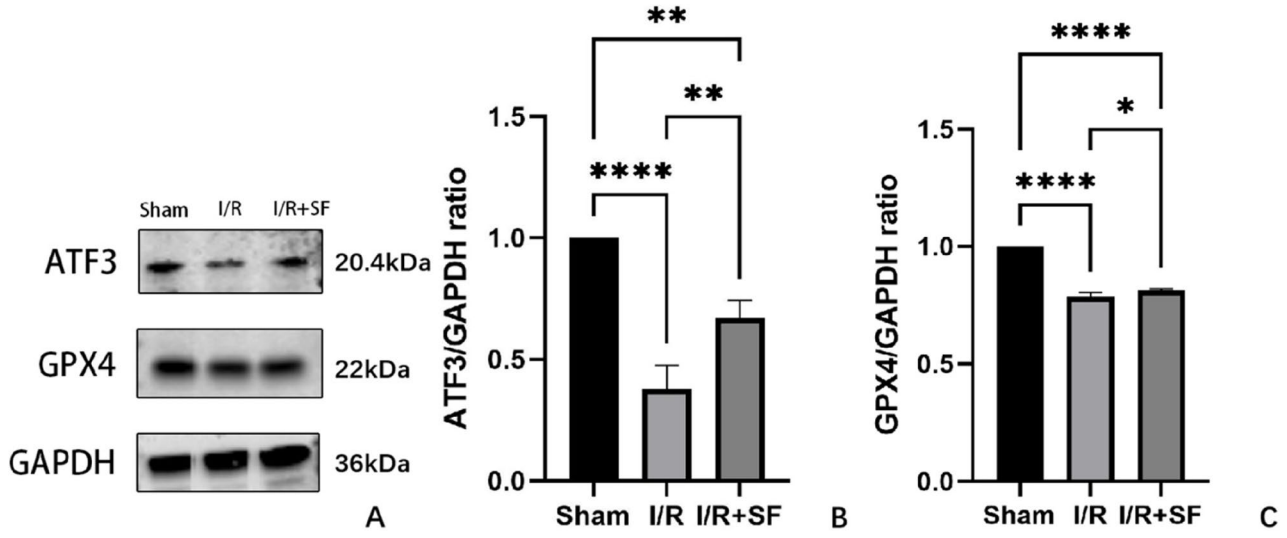

Fig. 6 Western blotting was used to detect the expression of ATF3 and GPX4 in the liver tissues of rats in each group.**A**: Western blotting results; **B**: Quantitative analysis of the ratio of ATF3 to GAPDH; **C**: Quantitative analysis of the ratio of GPX4 to GAPDH. * $p < 0.05$, * * $p < 0.01$, * * * * $p < 0.0001$

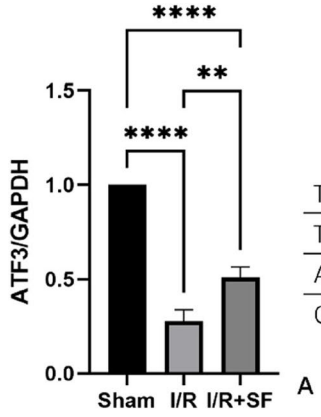

Table 1	Forward primer	Reverse primer
Target DNA		
ATF3	5′-GGCAGGAGCATCCTTTGTCT-3′	5′-TACCCTGGCCATTGGACAAC-3′
GAPDH	5′-GGCTCATGACCACAGTCCAT-3′	5′-ACATTGGGGGTAGGAACACG-3′

Fig. 7 The expression of ATF3 in liver tissue of rats in each group was detected by qRT-PCR.A: qRT-PCR results; Table 1 is the amplified sequence of ATF3 and GAPDH primers. * * $p < 0.01$, * * * * $p < 0.0001$

emerged as a novel therapeutic strategy for addressing conditions such as I/R injury [23]. Recent studies have demonstrated that Maresin Conjugates in Tissue Regeneration 1 (MCTR1) mitigates hepatocyte ferroptosis by upregulating nuclear factor erythroid 2-related factor 2 (NRF2) expression, thereby ameliorating liver injury induced by ischemia-reperfusion [24]. Qi et al. [25] demonstrated that dimethyl fumarate exerts a specific regulatory influence on ferroptosis, as evidenced by both in vivo and in vitro experiments, and confers a protective effect against HIRI through this mechanism. Morphologically, ferroptosis is characterized by a reduction in mitochondrial volume and an increase in bilayer membrane density. Biochemically, it is indicated by an elevation in intracellular Fe^{2+} levels [26].

In this study, we quantified the iron content in hepatic cells of rats across different experimental groups and examined the morphological alterations in mitochondria using transmission electron microscopy. Our findings revealed that the iron content in the I/R group was significantly elevated compared to the Sham group. Additionally, the majority of mitochondria in the I/R group exhibited pronounced pyknosis, reduced size, concentrated matrix within the membrane, and increased electron density. In contrast, mitochondria in the Sham group largely maintained structural integrity. These observations suggest that ferroptosis is implicated in HIRI. Following pretreatment with sufentanil, the iron content was significantly reduced compared to the I/R group, and the morphological changes indicative of ferroptosis observed in the I/R group were notably reversed. The protective role of glutathione peroxidase 4 (GPX4) against ferroptosis, by mitigating oxidative stress-induced cell death, has been well-documented and is inversely correlated with ferroptosis. Our study corroborated these findings. Specifically, the expression level of GPX4

in the I/R group was significantly lower than that in the Sham group, further substantiating the occurrence of ferroptosis. Following pretreatment with sufentanil, this alteration was reversed. These findings indicate that the protective effect of sufentanil preconditioning on HIRI may be mediated through the regulation of ferroptosis.

ATF3 is a member of the ATF/CREB transcription factor family [27]. Numerous domestic and international studies have demonstrated that ATF3 plays a crucial role in ischemia-reperfusion (IR) injury in various organs, including the heart, brain, and kidneys. Wang et al.[28]discovered that tobacco glycosides can significantly reduce renal cell apoptosis following ischemia-reperfusion and exert a protective effect on glomerular filtration rate, a phenomenon associated with ATF3 [9]. Recent research indicates that ATF3 confers protection against myocardial ischemia-reperfusion-induced ferroptosis by promoting the transcription of the ferroptosis-related gene FANCD2 and GPX4 [20]. In this experiment, we quantified the expression levels of ATF3 in various groups of rats using Western blot and RT-qPCR techniques. The results indicated that, relative to the Sham group, the expression level of ATF3 decreased following HIRI, suggesting a potential negative correlation between ATF3 expression and HIRI. Furthermore, pretreatment with sufentanil resulted in a significant increase in ATF3 expression compared to the I/R group. Notably, the expression pattern of GPX4 exhibited a similar trend to that of ATF3.Nevertheless, this observation contrasts with the trend in iron content, indicating that the protective effect of sufentanil on HIRI ferroptosis may be associated with ATF3. This protective mechanism might be linked to a reduction in iron content, presenting an additional avenue for investigating the role of sufentanil in mitigating ferroptosis in HIRI. Regrettably, the precise regulatory mechanisms by which ATF3 influences ferroptosis to alleviate HIRI remain undetermined and warrant further research and exploration.

However, this study also has some limitations. First, we should add ferroptosis agonists and ferroptosis inhibitors to the experimental group to prove that sufentanil pretreatment to reduce HIRI is closely related to ferroptosis. In addition, we should also use ATF3 inhibitors or ATF3 gene knockout rats for experiments to prove that the inhibitory effect of sufentanil on HIRI ferroptosis is achieved through ATF3. Ultimately, it is advisable to incorporate additional ferroptosis verification indicators to enhance the robustness of the experimental findings. The molecular mechanism of the anti-ferroptosis effect of ATF3 is still unknown, and further research and exploration are needed. Although sufentanil significantly reduces HIRI, there is still a long way to go before it enters the clinical research stage, and more in-depth research and exploration are needed.

In summary, this study initially investigated the potential association between the protective effects of sufentanil on ferroptosis in HIRI and the activation of ATF3. The experimental findings suggest that sufentanil preconditioning may confer protection against HIRI-induced ferroptosis by upregulating ATF3 expression, potentially through the reduction of intracellular iron content. Additionally, these results propose an alternative therapeutic strategy for the management of HIRI.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12871-025-03568-z.

Supplementary Material 1.

Acknowledgements
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Authors' contributions
Z.H.F. wrote the main manuscript text; designed and implemented experimental research; collected data and analyzed and interpreted data. Y.F.B. collected data; and conducted statistical analysis. Y.Y.N. conducted statistical analysis. H.J.L. collected data. H.Q. collected data. J.L.L. reviewed the content of the article, and obtained research funding.

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Data availability
The datasets used or analyzed during this study can be made available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The experimental protocol and procedures were approved by the Medical Ethics Committee of Chengde Medical College (Approval No. CYFYLL2023119).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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