Original Paper

Intravenous Anesthetics Enhance the Ability of Human Bone Marrow-Derived Mesenchymal Stem Cells to Alleviate Hepatic Ischemia-Reperfusion Injury in a Receptor-Dependent Manner

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Key Words

Intravenous anesthetics • Human bone marrow-derived mesenchymal stem cells • Hepatic ischemia-reperfusion injury • NF-κB

Abstract

Background/Aims: The degree of hepatic ischemia-reperfusion injury (HIRI) is highly relevant to the incidence of postoperative liver failure and mortality. Bone marrow-derived mesenchymal stem cells (BM-MSCs) have been shown to migrate to the liver and restore the damaged liver. Intravenous anesthetics are commonly used in the perioperative period; however, it is not yet known whether they have an influence on the functions of BM-MSCs and eventually affect the recovery of HIRI. **Methods:** A rat model of HIRI and a hypoxia-reoxygenation (H/R) model using L02 cells were generated, and human BM-MSCs (hBM-MSCs) were injected through the portal vein or co-cultured with L02 cells in a Transwell system, respectively. Three intravenous anesthetics, namely, dexmedetomidine, midazolam, and propofol, were given as pretreatments to hBM-MSCs. Quantitative real-time PCR for growth factors (HGF, FGF, VEGF, and IGF) and a migration assay were used to detect the paracrine and migration abilities of hBM-MSCs. NF-κB expression was detected using an immunofluorescence method. Furthermore, three receptor inhibitors, namely, yohimbine, PK11195, and bicuculline, were given to explore whether the three anesthetics worked in a receptor-dependent manner. **Results:** Preconditioning with dexmedetomidine and midazolam, but not propofol, enhanced the efficacy of hBM-MSCs in HIRI. Dexmedetomidine and midazolam, but not propofol, changed the paracrine spectrum and NF-κB p65 nuclear translocation of hBM-MSCs co-cultured with L02 cells after H/R injury. All three anesthetics enhanced the migration ability of hBM-MSCs when cultured in L02 H/R conditioned medium. However, the addition of receptor antagonists resulted in an opposite tendency. **Conclusions:** The intravenous anesthetics dexmedetomidine and midazolam J. Feng and W. Yao contributed equally to this work.

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enhanced the liver protective effects of hBM-MSCs during HIRI more effectively than propofol, by binding with their receptors and regulating the paracrine effect, migration ability, and NF- κ B p65 nuclear translocation of hBM-MSCs.

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Introduction

Hepatic ischemia-reperfusion injury (HIRI) is a major clinical problem encountered during liver resection surgery and liver transplantation, which results in a higher incidence of postoperative liver failure and mortality [1-3]. Thus, it is crucial to alleviate HIRI. Although many studies have been performed, protective measures are still lacking [4].

Mesenchymal stem cells (MSCs), originally derived from bone, are widely used in the clinical setting due to their multi-potency, self-renewal, and immunomodulation abilities [5]. For example, both experimental and clinical trials have demonstrated the benefits of MSCs in ischemia-reperfusion injury and liver diseases [6, 7]. Research suggests that the differentiation of MSCs into hepatocyte-like cells, immunomodulation, and secretome contribute to their effect [7]. Except for the allogeneic transplantation of MSCs, MSCs that reside in bone marrow (BM-MSCs) can also be mobilized in the circulation to migrate to injured sites after liver damage [8]. These findings suggest that BM-MSCs could possibly participate in liver recovery after HIRI.

Intravenous anesthetics are used frequently during the perioperative period, among which, dexmedetomidine, midazolam, and propofol could be taken as representative examples. By binding with the α_2 adrenoceptor, benzodiazepine receptor, and gamma-aminobutyric acid-A (GABAA) receptor, respectively, they exert sedative effects in various clinical situations, such as preoperative antianxiety, anesthesia adjunct, and sedation in intensive care units [9-11]. Besides, additional protective effects of these anesthetics have been demonstrated. However, there is no research focusing on whether these drugs would affect the functions of BM-MSCs.

Considering the findings mentioned above, we hypothesized that dexmedetomidine, midazolam, and propofol would exert an influence on BM-MSCs and eventually have an impact on the outcome of HIRI. Hence, we generated *in vivo* and *in vitro* models to compare their effects on the ability of human BM-MSC (hBM-MSCs) to alleviate HIRI, and explored the underlying mechanisms. Insights acquired from the current study would provide a foundation for the selection of sedatives during the perioperative period in patients suffering from HIRI.

Materials and Methods

Animals and grouping

Animal experiments were conducted in compliance with the National Institutes of Health criteria for the care and use of laboratory animals in research. The study was approved by the Laboratory Animal Care Committee of Sun Yat-sen University (China). Specific-pathogen-free adult male Sprague-Dawley rats weighing 180-230 g were obtained from Guangzhou University of Chinese Medicine (China). According to a random number table, the animals were assigned randomly to 11 groups ($n = 4$ per group).

Establishment of a rat model of HIRI

Rats starved for 12 h were anaesthetized with inhalational isoflurane and the abdominal cavity was opened. The hepatic artery, portal vein, and bile duct to the left and median lobes of the liver were dissociated and clamped by an atraumatic vascular clamp in order to generate a 70% hepatic ischemia model. A piece of wet gauze was used to cover the abdomen to avoid fluid loss. After 60 min, reperfusion (for 6 or 24 h) was initiated by removing the clamp, and the abdomen was closed. The rats in the sham operated group underwent the same procedures, except for clamping.

Assessment of liver damage

Aspartate transaminase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured in serum samples with an automatic biochemistry analyzer (7600-020/7170A; Hitachi,

Japan). Small liver specimens obtained from the left or median lobe were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin.

Cell culture

hBM-MSCs were kindly provided by Professor A.P. Xiang (Center for Stem Cell Biology and Tissue Engineering, Sun Yat-sen University) and cultured in Dulbecco's modified Eagle's medium (DMEM, 1000 mg/L D-glucose) supplemented with 10% fetal bovine serum (FBS) and 2 ng/mL basic fibroblast growth factor. L02 cells (cell line of normal human hepatocytes) were purchased from Sun Yat-sen University and cultured in DMEM (1000 mg/L D-glucose) supplemented with 10% FBS. Both kinds of cells were incubated in a 37°C chamber with 5% CO₂.

Pretreatments of hBM-MSCs

When hBM-MSCs approached approximately 50% confluence, 2.4 µL dexmedetomidine, midazolam, and propofol was added to 1 mL conditioned medium to achieve a final concentration of 1 µM, 1.5 µM, and 10 µM, respectively. In order to block the receptors of the intravenous anesthetics, 2 µL yohimbine, PK11195, and bicuculline was added to 1 mL conditioned medium at 15 min before the addition of the intravenous anesthetics, achieving a final concentration of 10 µM, 3 µM, and 50 µM, respectively. For the hBM-MSCs in the control group, the drugs were replaced with the same volume of phosphate-buffered saline (PBS). The culture plates were returned to the incubator for 24 h. To prevent the sedatives from affecting the subsequent experiments, the hBM-MSCs were washed twice with PBS before they were trypsinized.

Hypoxia-reoxygenation injury model using L02 cells and their co-cultivation with hBM-MSCs

When L02 cells approached approximately 60% confluence in 6-well plates, they were incubated in a starvation medium (DMEM without D-glucose) and placed in a humidified hypoxia (1% O₂ + 5% CO₂) incubator (Galaxy 48R; Eppendorf, Germany) for 4 h. Then, D-glucose and FBS were added to make a normal conditioned medium, and L02 cells underwent reoxygenation (20% O₂ + 5% CO₂) for 4 h. After the establishment of the hypoxia-reoxygenation (H/R) model, L02 cells were co-cultured in Transwell inserts containing hBM-MSCs in DMEM (1000 mg/L D-glucose) supplemented with 10% FBS under normoxia for 24 h.

Cell counting kit-8 and LDH assays

After co-cultivation, the conditioned medium was harvested and centrifuged, and the supernatant was used to estimate the release of LDH by an Epoch microplate reader (Gene, USA) following the manufacturer's protocol (Cytotoxicity Detection Kit; Roche, USA). The Transwell inserts were removed and L02 cells were washed twice with PBS. Then, 1 mL PBS and 100 µL reagent were added to each well of the plate, which was put in normoxic incubator for 2.5 h. A standard curve was generated for the number of surviving cells, and optical density was also plotted according to the manufacturer's instructions (KGA317; Keygen, China). Optical density was detected by a microplate reader, and the number of surviving L02 cells was calculated according to the standard curve. The results of the L02 surviving fraction and LDH level were standardized by the normal group (as 1) in the Fig.

Quantitative real-time PCR

The mRNA levels of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), adrenoceptor alpha 2C (ADRA2), peripheral benzodiazepine receptor (PBR), and GABAA β3 receptor (GABAAR) were determined by quantitative real-time PCR (qRT-PCR) amplification using SYBR Green I Master (Roche) followed by detection in Step One equipment (Light Cycler 480; Roche). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference. All primers were human-specific and their sequences were as follows:

HGF forward 5'-CACG AACACAGCTTTTGCC-3',
HGF reverse 5'-AACTCTCCCCATTGCAGGTC-3';
VEGF forward 5'-ACTCGCGTTGCAAGATGTGA-3',
VEGF reverse 5'-GCAGCGTGGTTTCTGTATCG-3';
FGF forward 5'-CAAGCGGCTGTACTGCAAAA-3',
FGF reverse 5'-TAGCTTGATGTGAGGGTCGC-3';

IGF forward 5'-GACCCAGAAGTATCAGCCCC-3',
IGF reverse 5'-CAGGTAACGTCGAGAGCA-3';
ADRA2 forward 5'-GTTCTGCTCTGCTGGTTC-3',
ADRA2 reverse 5'-TCCTGGTTGAAGACCGTGTA-3';
PBR forward 5'-AGGGTCTCCGCTGGTACGCC-3',
PBR reverse 5'-TGGGGCAACCTCTGAAGCTC-3';
GABAAR forward 5'-CAGAATCACCACGACAGCAG-3',
GABAAR reverse 5'-CTTTCCACTCCGGTAACAGC-3';
GAPDH forward 5'-CGACTTCAACAGCAACTCCCACTCTTCC-3',
GAPDH reverse 5'-TGGGTGGTCCAGGGTTTCTTACTCCTT-3'.

hBM-MSC migration assay

After the establishment of the L02 cell H/R injury model, conditioned medium was harvested and centrifuged, and the supernatant was stored at -80°C as hypoxia-reoxygenation conditioned medium (HR-CM). The upper chamber of a Transwell was seeded with 5.0×10^4 hBM-MSCs (at 5.0×10^5 cells/mL) and 600 µL chemoattractant was added to the bottom chamber. For the experimental groups, HR-CM was used. FBS-free DMEM (1000 mg/L D-glucose) and DMEM (1000 mg/L D-glucose) supplemented with 40% FBS was used as a negative and positive control, respectively. After incubation at 37°C for 10 h, the cells were fixed and those on the upper part of the membrane were wiped off. Cells migrating through the membrane were stained with crystal violet and counted for statistical analysis.

Immunofluorescence analysis

hBM-MSCs were seeded on glass coverslips in a 24-well plate. When the cells reached approximately 80% confluence, the conditioned medium was removed and 500 µL HR-CM was added to each well. After different durations of stimulation, immunofluorescence analysis was carried out by removing the residual reagent from each well and post-washing with PBS for each step. The cells were fixed for 15 min at room temperature in 4% paraformaldehyde. Nonspecific binding sites were blocked with blocking solution (PBS with 5% goat serum and 0.3% Triton X-100) for 60 min. The cells were incubated with an anti-nuclear factor kappa B (NF-κB) p65 rabbit monoclonal antibody (1:400; #8242; Cell Signaling Technology, USA) overnight and goat anti-rabbit IgG (1:400; Molecular Probes, USA) for 1.5 h in antibody diluent (PBS with 1% bovine serum albumin and 0.3% Triton X-100). After incubation with 4',6-diamidino-2-phenylindole (1:2000; KGA215; Keygen) in antibody diluent for 1 min, the coverslips were covered with immunomount medium (DAKO, USA) and observed under an inverted fluorescence microscope (IX71; Olympus, Japan).

Statistical analysis

All data are presented as the mean $\pm$ standard error of the mean (SEM), and statistical analysis was performed by SPSS 16.0. One-way analysis of variance and Tukey's *post hoc* comparisons were used for multiple comparisons among groups. A two-tailed *P*-value of less than 0.05 was set as the significance level.

Results

Preconditioning with dexmedetomidine and midazolam, but not propofol, enhanced the efficacy of hBM-MSCs to ameliorate HIRI in rats

To compare the ameliorative efficacy of hBM-MSCs in HIRI within groups of different pretreatments (normal or preconditioned with 1 µM dexmedetomidine/1.5 µM midazolam/10 µM propofol for 24 h), animal experiments were carried out. *In vivo*, a Sprague-Dawley rat model of 70% HIRI was used, and hBM-MSCs were injected through the portal vein at the onset of reperfusion. Fig. 1D shows that hBM-MSCs significantly alleviated congestion in the hepatic sinusoid, vacuolization of hepatocytes, and disorder of hepatic lobules caused by HIRI, whereas pretreatment with the intravenous anesthetics did not lead to a significant difference in the pathologic damage of the liver (Fig. 1E). However, in the rats that underwent 6 h of reperfusion, preconditioning with dexmedetomidine and midazolam,

Fig. 1. Preconditioning with dexmedetomidine and midazolam, but not propofol, enhanced the efficacy of hBM-MSCs in a HIR rat model. HIR group: rats were used to generate a 70% hepatic ischemia model. Sham group: rats underwent the same procedures as the HIR group, except for clamping. HIR+MSC group: normal hBM-MSCs were injected through the portal vein at the onset of reperfusion. HIR+Dex/Mid/Pro MSC group: hBM-MSCs (preconditioned with dexmedetomidine/midazolam/propofol) were injected through the portal vein at the onset of reperfusion.

Preconditioning with dexmedetomidine and midazolam, but not propofol, enhanced the efficacy of hBM-MSCs in the rat HIRI model. (A, B, and C) Serum levels of AST, ALT, and LDH. (D) Liver damage in rats receiving hBM-MSCs after HIRI (reperfusion 6 h) (hematoxylin-eosin staining, original magnification $\times 400$). (E) Comparison of liver histologic injury scoring. The data are presented as the mean $\pm$ SEM, $n = 4$ independent experiments. * $p < 0.05$ vs. sham group; # $p < 0.05$ vs. HIR group; \$ $p < 0.05$ vs. control group.

Fig. 2. Preconditioning with dexmedetomidine and midazolam, but not propofol, changed the paracrine spectrum of hBM-MSCs co-cultured with L02 cells after H/R injury, thus enhancing their efficacy. Con/Dex/Mid/Pro-MSC group: L02 cells underwent H/R injury and were co-cultured with normal hBM-MSCs or hBM-MSCs preconditioned with dexmedetomidine/midazolam/propofol. NOR group: normal L02 cells. H/R group: L02 cells underwent H/R injury. HR+MSC group: co-cultivation of L02 cells that underwent H/R injury and hBM-MSCs. Con group: normal hBM-MSCs. Dex/Mid/Pro: hBM-MSCs preconditioned with dexmedetomidine/midazolam/propofol. Preconditioning with dexmedetomidine and midazolam,

but not propofol, changed the paracrine spectrum of hBM-MSCs co-cultured with L02 cells (cell line of normal human hepatocytes) after H/R injury, thus enhancing their efficacy. (A) After establishment of the H/R model, L02 cells were co-cultured with hBM-MSCs preconditioned with anesthetics in a Transwell system. (B) mRNA expression of growth factors (FGF, HGF, IGF, and VEGF) in hBM-MSCs after co-cultivation. (C) Levels of LDH in co-culture conditioned medium. (D) Survival of L02 cells co-cultured with hBM-MSCs after H/R injury. The data are presented as the mean $\pm$ SEM, $n = 5$ independent experiments. * $p < 0.05$.

but not propofol, resulted in lower serum levels of LDH, AST, and ALT (Fig. 1A-C), which are typical serological indicators of liver damage.

Preconditioning with dexmedetomidine and midazolam, but not propofol, changed the paracrine spectrum of hBM-MSCs co-cultured with L02 cells after H/R injury, thus enhancing their efficacy

In vitro, an H/R model using hepatocyte L02 cells was established, in which hBM-MSCs were co-cultured within a Transwell system (Fig. 2A). Fig. 2C-D illustrate that dexmedetomidine and midazolam increased the survival of L02 cells and reduced the release of LDH into the conditioned medium, but there was no significant difference between normal hBM-MSCs and those preconditioned with propofol. Since the factors secreted by hBM-MSCs, including FGF, HGF, IGF, and VEGF, contribute to the recovery of liver damage, we used qRT-PCR to quantify the mRNA expression of growth factors in hBM-MSCs after co-cultivation. Fig. 2B shows that dexmedetomidine increased the mRNA expression of FGF, HGF, IGF, and VEGF. In addition, midazolam increased the expression of FGF, IGF, and VEGF. However, propofol had no effect on the expression of growth factors.

Receptor blockers of dexmedetomidine and midazolam prevented them from enhancing the efficacy of hBM-MSCs

Dexmedetomidine, midazolam, and propofol have an impact on the central nervous system by binding with the α_2 adrenoceptor, benzodiazepine receptor, and GABAA receptor, respectively. Thus, we determined the mRNA expression of these receptors via qRT-PCR and found that they are also expressed by hBM-MSCs (Fig. 3A). However, whether the effects of dexmedetomidine, midazolam, and propofol on hBM-MSCs were mediated by receptor-related activity remains unknown. Thus, yohimbine (inhibitor of the dexmedetomidine receptor), PK11195 (inhibitor of the midazolam receptor), and bicuculline (inhibitor of the propofol receptor) were added to the conditioned medium of hBM-MSCs at 15 min before the addition of the intravenous anesthetics (Fig. 3B). Fig. 3G-H show that the addition of yohimbine and PK11195 led to a lower efficacy of hBM-MSCs preconditioned with dexmedetomidine or midazolam, respectively, which was manifested as a smaller surviving fraction of L02 cells and increased LDH release into the conditioned medium. There was no significant difference between the efficacy of hBM-MSCs with or without GABAA receptor blocker treatment.

Fig. 3. Receptor blockers of dexmedetomidine and midazolam prevented them from enhancing the efficacy of hBM-MSCs. Dex/Mid/Pro-MSC group: L02 cells underwent H/R injury and were co-cultured with hBM-MSCs preconditioned with dexmedetomidine/midazolam/propofol. Con group: only anesthetics were used to pretreat hBM-MSCs. Yoh/PK/Bicu group: yohimbine/PK11195/bicuculline were added to the conditioned medium of hBM-MSCs at 15 min before the addition of dexmedetomidine/midazolam/propofol. The addition of receptor blockers (yohimbine, PK11195, and bicuculline, respectively) led to a reduced efficacy of dexmedetomidine/midazolam-preconditioned hBM-MSCs, but had no significant effect on propofol-preconditioned hBM-MSCs. (A, C-F) mRNA expression in hBM-MSCs. Gene expression was standardized to GAPDH. (A) Normal hBM-MSCs. GABAAR: GABAA receptor; PBR: peripheral benzodiazepine receptor; ADRA2: α_2 adrenoceptor. (C-F) hBM-MSCs under different treatments. (B) Receptor blockers were added prior to the anesthetics. (G) Levels of LDH in conditioned medium. (H) Survival of L02 cells co-cultured with hBM-MSCs after H/R injury. The data are presented as the mean $\pm$ SEM, n = 5 independent experiments. *p<0.05.

It is mentioned above that changing the paracrine spectrum may be a potential mechanism by which intravenous anesthetics affect hBM-MSCs. As blocking their receptors appeared to account for the inhibited function of the selected sedatives upon the therapeutic effect of hBM-MSCs, there is a possibility that these receptors are related to how the drugs influence the paracrine ability of hBM-MSCs. Therefore, we quantified growth factor levels in the groups treated with the receptor blockers. Fig. 3C-F show that the addition of yohimbine and PK11195 reduced the levels of the growth factors (FGF, HGF, IGF, and VEGF) secreted by hBM-MSCs preconditioned with dexmedetomidine or midazolam, but bicuculline had no significant impact on propofol-preconditioned hBM-MSCs.

Preconditioning with dexmedetomidine, midazolam, or propofol enhanced the migration ability of hBM-MSCs under stimulation with L02 HR-CM, which was related to their receptors

It has been proven that the migration of hBM-MSCs toward impaired organs is beneficial to their repair; thus, migration was assayed using Transwell chambers with 8- μ m-pore membranes (Fig. 4A). After incubation, there was an increased amount of hBM-MSCs passing through the membrane under stimulation with HR-CM, while only a few hBM-MSCs were detectable when serum-free medium was used, suggesting that HIRI could induce the migration of hBM-MSCs. Additionally, preconditioning with dexmedetomidine, midazolam, or propofol led to a dramatic increase in the number of crystal violet-stained hBM-MSCs, while the addition of the receptor antagonists resulted in an opposite tendency. The positive controls consisted of hBM-MSCs in conditioned medium with 40% FBS, and a large number of these cells migrated (Fig. 4B-C).

Preconditioning with dexmedetomidine and midazolam, but not propofol, regulated the translocation of NF- κ B p65 in hBM-MSCs under stimulation with HR-CM

To determine the role of NF- κ B in the above process, we observed the immunofluorescence signal of NF- κ B in hBM-MSCs (normal or pretreated with dexmedetomidine/midazolam/

Fig. 4. Preconditioning with dexmedetomidine, midazolam, or propofol enhanced the migration ability of hBM-MSCs under stimulation with L02 HR-CM, which was related to their receptors. Neg/Pos/Con group: normal hBM-MSCs were exposed to FBS-free conditioned medium/conditioned medium with 40% FBS/HR-CM. Dex/Mid/Pro group: hBM-MSCs were preconditioned with dexmedetomidine/midazolam/propofol. Yoh/PK/Bicu group: yohimbine/PK11195/bicuculline were added to the conditioned medium of hBM-MSCs at 15 min before the addition of dexmedetomidine/midazolam/propofol. Preconditioning with dexmedetomidine, midazolam, or propofol enhances the migration ability of hBM-MSCs under stimulation with L02

HR-CM. All three receptor blockers (yohimbine, PK11195, and bicuculline) resulted in a smaller number of hBM-MSCs migrating through the Transwell membrane. (A) hBM-MSCs under different pretreatments were exposed to chemoattraction (FBS-free conditioned medium [negative control], conditioned medium with 40% FBS [positive control], or HR-CM) in a Transwell chamber. (B) hBM-MSCs that had migrated toward the stimulus (lower chamber) by passing through a membrane filter (8 μ m) were stained with crystal violet (original magnification $\times 100$). Representative images from 5 independent experiments are shown. (C) Stained cells from a minimum of 5 fields of view (100 $\times$) for 3 replicates were counted, and the data are expressed as the average number of migrated cells. ** $p < 0.01$.

Fig. 5. Preconditioning with dexmedetomidine and midazolam, but not propofol, regulated the translocation of NF- κ B p65 in hBM-MSCs under stimulation with HR-CM. Con group: hBM-MSCs were stimulated with DMEM (1000 mg/L D-glucose). HR-CM group: normal hBM-MSCs were stimulated with HR-CM. HR-CM+Dex/Mid/Pro group: hBM-MSCs preconditioned with dexmedetomidine/midazolam/propofol were stimulated with HR-CM. hBM-MSCs under different pretreatments were stimulated with HR-CM, and NF- κ B p65 localization was detected by immunofluorescence. Original magnification: 400 $\times$. Representative images from 5 independent experiments are shown. Images observed after 15 min of stimulation are shown.

propofol for 24 h) after different durations (1 min, 5 min, 10 min, 15 min, 30 min, 45 min, and 1 h) of HR-CM stimulation. Preconditioning hBM-MSCs with dexmedetomidine or midazolam reduced the translocation of NF- κ B p65, while pretreatment with propofol did not have an effect (Fig. 5 after 15 min stimulation).

Discussion

Hepatic segment resection and liver transplantation is the most effective treatment for malignant liver tumors and end-stage liver disease. However, HIRI is a major cause of mortality and morbidity after liver surgery [12]. Fortunately, BM-MSCs are recruited and migrate toward the injured liver, contributing to its recovery [8]. Whether intravenous anesthetics applied during the perioperative period could alter the efficacy of hBM-MSCs remains unclear.

Previous research has shown that dexmedetomidine protects against injury/reperfusion-induced intestinal injury, partly by inhibiting the inflammatory response and intestinal mucosal epithelial apoptosis via α_2 adrenoceptor activation [13]. Midazolam can also provide a strong neuroprotective effect in fetal rat brain in an ischemia-reperfusion model by preventing lipid peroxidation and mitochondrial damage [14]. In addition, propofol alleviates HIRI via BRG1-mediated Nrf2/HO-1 transcriptional activation [15]. To exclude the reported direct effects of dexmedetomidine, midazolam, and propofol on HIRI, they were used only to pretreat hBM-MSCs in our investigation. *In vivo*, a 70% HIRI rat model was used. A previous study showed that liver damage is the most severe at both 6 h and 24 h after reperfusion, and then tissue injury recovers gradually [16]. Therefore, we used 6 and 24 h after reperfusion as time points to estimate liver injury. Given that the deprivation and restoration of blood supply leads to HIRI [17], a hypoxia-reoxygenation model of L02 cells was generated *in vitro* by regulating the components of conditioned medium and oxygen

concentration in an incubator. Our results showed that dexmedetomidine and midazolam could improve the efficacy of hBM-MSCs, whereas propofol showed no such effect.

No effective long-term engraftment of MSCs has been reported [18], but the transplantation of conditioned medium has been shown to promote wound repair [19–22], implying that the paracrine effects of MSCs have a significant impact on their efficacy. Additionally, the more MSCs that migrate to an injured site, the better. Thus, we focused on whether sedatives could regulate the paracrine and migration abilities of hBM-MSCs in follow-up experiments. The secretome of hBM-MSCs contains diverse chemokines and cytokines, including FGF, HGF, IGF, and VEGF, which have been shown to be beneficial for the recovery of various tissues, liver diseases included [23–28]. Hence, we used them as indicators for the paracrine ability of hBM-MSCs. We found that dexmedetomidine and midazolam could improve the paracrine and migration abilities of hBM-MSCs, while propofol improved only their migration ability.

Since the $\alpha 2$ adrenoceptor, peripheral benzodiazepine receptor, and GABAA receptor are expressed on human MSCs [29–32], we hypothesized that the influence of the sedatives on hBM-MSCs might be associated with their receptors. In the present study, the expression of $\alpha 2$ -adrenoceptor, benzodiazepine receptor, and GABAA receptor on hBM-MSCs was confirmed by qRT-PCR. To validate their roles in the functions of hBM-MSCs, receptor blockers were added at 15 min before the addition of the anesthetics. Accordingly, our results indicated that the improved paracrine and migration abilities of hBM-MSCs fostered by dexmedetomidine or midazolam were inversely affected by respective receptor blockade, illustrating the importance of receptor-mediated signaling in this process.

Another question remains as to which signaling pathway participates after the binding of the anesthetics and their receptors. It is reported that NF- κ B regulates the survival and secretion profile of MSCs [33–35]. For instance, NF- κ B contributes to the production of growth factors (VEGF, FGF2, HGF, and IGF-1) by human MSCs under stimulation with TNF- α , LPS, or hypoxia [36]. In addition, in a hypoxic environment, the decreased activation of NF- κ B contributes to a better outcome for myocardial infarction [37, 38]. Thus, we hypothesized that NF- κ B also participates in the ability of hBM-MSCs to alleviate HIRI. To determine the role of NF- κ B in this process, we observed the immunofluorescence signals of NF- κ B p65 in hBM-MSCs (normal or pretreated with dexmedetomidine/midazolam/propofol for 24 h) at different time points of HR-CM stimulation. Preconditioning hBM-MSCs with dexmedetomidine and midazolam was found to reduce the translocation of NF- κ B p65, while pretreatment with propofol had no effect. Afterward, the decreased activation of NF- κ B resulted in a different paracrine spectrum (Fig. 6). However, more experiments are needed to confirm further the role of NF- κ B in regulating the functions of hBM-MSCs.

In conclusion, hBM-MSCs alleviated HIRI, on which various anesthetics-pretreatments had different influences. Dexmedetomidine and midazolam improved the efficacy of hBM-MSCs, whereas propofol had no effect. The underlying mechanisms may be related to the receptors of the anesthetics and regulation of the paracrine and migration abilities of hBM-MSCs. Our investigation is original and represents a new consideration in the choice of anesthetics during the perioperative period with the aim of a better outcome for patients suffering from HIRI.

Fig. 6. Proposed signaling pathway for the ability of hBM-MSCs to alleviate HIRI. Preconditioning with dexmedetomidine and midazolam, but not propofol, regulated the translocation of NF- κ B p65 in hBM-MSCs under stimulation with HR-CM in a receptor-dependent manner. The decreased activation of NF- κ B, which interferes with the transcription of nuclear DNA, resulted in a different paracrine spectrum, that is, increased expression of FGF, HGF, IGF, and VEGF.

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Disclosure Statement

The authors declare that they have no competing interests.

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