Hepatic IR

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Introduction

The injury caused by hepatic ischemia-reperfusion (HIR) is a sign of impending liver damage and failure (Cour et al. 2011). Shock, trauma, hepatectomy, liver transplantation, and other surgical operations all inevitably result in HIR injury complications (Li et al. 2014; Zhaı et al. 2011). Hepatic ischemia-reperfusion injury causes liver damage mostly during the reperfusion phase, when blood reperfusion causes an innate immune response. Overproduction of reactive oxygen species, activation of Kupffer cells and other inflammatory cells, and calcium overload all contribute to the pathophysiological process of HIRI, which ultimately results in hepatocellular death (Teoh and Farrell, 2003). The regeneration of the liver is harmed by IRI. Hepatocyte destruction will result from the ischemia-induced lack of nourishment and oxygen in the early stages of liver IRI. The pathogenic processes of IRI have been widely researched (Almeida-Porada et al. 2010; Ishikawa et al. 2010; Kanazawa et al.2011), however the interactions between the signaling pathways involved in IRI are extremely complicated and poorly understood.

A promising method for tissue repair and regeneration is stem cell treatment (Trovato et al.2020). Numerous studies have demonstrated that exogenous stem cell injection or mobilization of endogenous stem cells can enhance tissue structural regeneration and functional recovery (Hu et al.2019; Jiao et al.2019)

Mesenchymal stem cells (MSCs) lower inflammatory and immune reactions. They can be used as bio-therapeutic tools to treat inflammatory illnesses (Ortiz et al.2007). MSCs can develop into cells that mimic hepatocytes, protect the liver from injury, diminish liver fibrosis, promote liver regeneration, and reduce inflammation (Chang et al. 2009; Bernardo and Fibbe, 2013).

In tissue engineering, adipose-derived mesenchymal stem cells (ADSCs), which serve as good progenitor cells for cell-based regenerative therapies, are often used. (Iaquinta et al. 2019). The benefits of adipose derived mesenchymal stem cells include plentiful supplies, simplicity of harvesting, less damaging fat storage for the body, low immunogenicity, and strong in vitro proliferative capacity (Li and Guo, 2018). The kidney (Lin et al. 2016), liver (Zhang et al. 2019), heart (Mazo et al.2012), and brain (Chung et al.2015) can all be protected against ischemia-reperfusion injury by ADSCs.

The cellular catabolic process known as autophagy is responsible for removing primarily damaged molecules and organelles by transporting them to lysosomes for destruction. The balance between the production, breakdown, and subsequent recycling of cellular products is maintained by autophagy (Rautou et al.2010).

The most popular marker for tracking autophagy is microtubule-associated protein-1 light chain 3-B (LC3B) .The exterior and inner autophagosomal membranes are integrated with lipidated LC3B (LC3B-II), allowing for the detection of various autophagic vesicle phases (Klionsky et al. 2012; Kabeya et al.2000) .

Numerous investigations have discovered that autophagy can be very selective (Mijaljica et al.2012). P62, one of the best-studied autophagy receptors, is involved in the autophagy-dependent removal of a variety of cargos, including bacteria and ubiquitinated protein aggregates. P62 is degraded by autophagy due to its interaction with LC3, and when autophagy is blocked, p62 positive aggregates form (Komatsu and Ichimura, 2010)

Therefore, the study's goal was to examine how ADSCs affected the autophagic process following hepatic ischemia/reperfusion.

Materials and Methods

Animals

This study was approved by the Technical Universal Verification local ethic commity (approved number: 0009/2022, Ankara, Turkey). 30 Wistar albino rat (200-220gr) were used. All animals were given seven days to adjust to the lab setting while having free access to water and regular laboratory food. Standard environmental conditions with a 12-hour light/dark cycle were used to house the animals.

Operative procedure

A ketamine cocktail consisting of ketamine (13 mg/mL), xylazine (2.6 mg/mL), and acepromazine (0.15 mg/mL) in 0.9% NaCl was injected intraperitoneally into rats for anesthesia. Under anesthesia, all surgical procedures were carried out.A midline incision was made to open the abdomen and the ligaments encircling each lobe were subsequently removed. A microvessel clip applied to the portal vein, hepatic artery, and bile duct supplying the liver's midline and left lateral lobes resulted in 70% partial liver ischemia. The vascular clip was loosened to start hepatic reperfusion after 40 minutes of partial hepatic ischemia. The ADSCs (1 x10^6 cells/kg) were injected into the liver parenchyma following 20 minutes of ischemia. Following the reperfusion, animals were sacrificed (Reza et al.2014).

Histological Examination

Standard procedures were followed to prepare the liver tissues for histological investigation after they were fixed in 4% paraformaldehyde. The Suzuki classification (Suzuki et al.1991), which is based on sinusoidal congestion, vacuolization of hepatocyte cytoplasm, and parenchymal necrosis, was used to grade hepatic IRI. The scores 0: none, 1: minimal, 2: mild, 3: moderate, and 4: severe, and necrosis as 0: none, 1: < 10%, 2: 30%, 3: 60%, and 4: >60%.

Immunohistochemistry Assay

The liver sections (5 μm) were deparaffinized and rehydrated by using xylene and graduated alcohols. Antigen retrieval took place in a microwave for 10 minutes at 96 °C (10 mM/L citrate buffer, pH: 6). Hydrogen peroxide at 3% (v/v) was used to suppress endogenous peroxidase for 15 minutes, followed by phosphate-buffered saline washing (PBS). After that, the slides were blocked with blocking serum (UltraV Block, ScyTek Laboratories, Utah, USA). The sections were treated with the primary antibodies LC3B, p62 and TGF-β for an overnight period at 4 °C. The tissue sections were incubated with

biotinylated anti-mouse secondary antibodies (LabVision, TM-015-BN) for 10 minutes at room temperature after followed by two PBS washes.Antigen-antibody complexes were fixed using a streptavidin-peroxidase complex (TP-060-HL; LabVision, Fremont, CA, USA) for 10 minutes after three PBS washes. DAB (3,3'-diaminobenzidine) was used to treat the sections after they were stained with Mayer's hematoxylin (Merck). After drying, the sections were mounted with Entellan (Mounting Medium, LabVision, TA-060-UG). They were observed and captured on camera using a light microscope (LeicaDM4000, Wetzlar, Germany). The immunohistochemistry analysis was conducted using H-score calculations (Akcan et al.2020).

Analyses of statistics

Expressions of immunocytochemistry were evaluated using the GraphPad Prism (Graphpad, San Diego, CA). Through the use of an ANOVA and Turkey's multiple comparison tests, the significance of the data between groups was investigated. The agreed point for statistical significance was $p < 0.05$.

Results

Histopathological Results

To determine ADSCs' protective effects in the rat liver IRI model, H&E staining was used to evaluate hepatocellular damage. The control and ADSCs animals' livers showed a typical lobular structure, a clean liver cord, and no indications of injury. The hepatic cell cord structure was disrupted in the Ischemia and I/R group rats, which led to hepatic sinus congestion, necrotic parancyme, and hepatocyte vacuolization. Additionally, the hepatic parenchyma of ischemia and I/R groups had inflammatory cell influx .The ADSC -treated group following I/R, showed reduced liver histopathologic injury, including portal inflammation, hepatocyte edema, cytoplasm deformation, and coagulation.

Immunohistochemistry Results

The immunoexpression of autophagic markers LC3B and p62 were investigated. The immunostaining of LC3B was increased in ischemia and I/R groups and decreased in the ADSCs administration. Compared with the control group (Figure 2A and B), apparent positive staining was observed in the

ıschemia and I/R groups (Figure 2D and E) and in the I/R + ADSC group (Figure 2F). The number of LC3B positive cells was notably decreased in IRI + ADSCs (Figure 2F) compared to those in the ischemia and I/R. In the ischemia and I/R groups, the expression of p62 tended to decline (Figure 3D and E). In contrast, ADSC therapy dramatically increased P62 protein expression (Figure 3F).

There was not any specific immunostaining of TGFβ in negative control, control and ADSC groups (Figure 4A, B and C). The staining of TGFβ was increased in ıschemia (Figure4D) and ischemia reperfusion groups (Figure 4D and E) and the staining intense was decreased with the treatment of ADSC (Figure 4F).

The H-SCORE analyzes of the immunoexpression of LC3B,p62 and TGFβ was given in Figure5.

Discussion

Clinical trials have examined at stem cell therapy as a potential treatment for a variety of inflammatory illnesses and liver disorders (Owen and Newsome, 2015). The effectiveness and mechanism of MSCs in preventing hepatic I/R injury were studied by Zheng et al. (2020). The effects of MSCs were investigated utilizing a mouse liver injury/recovery model and a hypoxia/reoxygenation model using L02 hepatocytes.These in vivo and in vitro I/R-induced hepatocellular apoptosis models were used to study the potential pathways of MSCs. MSCs also showed an improvement in hepatic damage and the capacity to control mitochondrial efficiency. Restored ATP generation, enhanced mitophagy, decreased buildup of fragmented mitochondria, and lower levels of excess mitochondrial reactive oxygen species served as proof of this.

Another study in the literature has investigated the function of the secretome produced by ADSCs in liver regeneration in small pigs. Serum and liver tissue samples were collected at 1, 3, and 7 days after surgery, and changes in tissue pathology, inflammatory markers in the serum, liver function, angiogenesis-related factors, and genes were evaluated. They discovered that ASC-secretome enhances liver regeneration and suppresses the inflammatory response induced by partial hepatectomy and ischemia-reperfusion in miniature pigs (Jiao et al.2021).

In the current study liver histology reviewed that ischemia reperfusion caused hepatic sinusoid congestion, vacuolization, and necrosis. Adipose-derived stem cells could alleviated the histological changes in liver after I/R.

The cytoplasmic elements are transported into lysosomes for destruction by autophagy, an intracellular degradation system (Seglen and Bohley, 1992). Biological process that maintains cell viability and homeostasis is autophagy, which recycles and reuses energy (Dikic and Elazar, 2018). On the other hand uncontrolled autophagy can result in the build up of autophagic vacuoles, which can result in cell death (Mizushima et al.2008), in extreme circumstances, such as acute organ injury or reperfusion injury (Bai et al. 2018). These findings also point to a potential novel strategy for improving the effects of IRI, which involves regulating the level of autophagy. (Bai et al. 2018; Ge et al. 2018; Zou et al. 2018).

By lowering the degree of hepatocyte autophagy, recent investigations have demonstrated that ADSCs transplantation can lessen the damage caused by HIRI paired with hepatectomy (Bai et al. 2018). Additionally, uncontrolled autophagy causes an increase of autophagy-related proteins, which causes hepatocytes to die in HIRI paired with hepatectomy injury (Bai et al. 2018; Ge et al. 2018)

According to studies, LC3 is a particular protein marker of autophagy that has been demonstrated to rise during ischaemia-reperfusion damage. When autophagy is triggered, a ubiquitin-like enzyme combines LC3A with phosphatidylethanolamine to form LC3B (Codogno and Meijer, 2005). The removal of the autophagic substrate requires two steps: first, the substrate must be identified as one that needs to be degraded by autophagy, and second, it must be transported to the autophagosomes. Whether autologous flow activation occurs or not depends on these two processes. The main element in controlling these actions is p62 because it is a specific autophagic receptor. This protein, which joins with ubiquitinated proteins to form the autophagosome and subsequently merges with lysosomes to produce autophagosomes to be eliminated, is a ubiquitin-binding protein that is implicated in cell signaling, oxidative stress, and autophagy (Kirkin and Mc Ewan,2009). As a regulator of the make-up of autophagosomes, p62 is destroyed during autophagy when it is connected to LC3B (Pankiv et al.2007). Yansong Ge et al. (2018) has investigated the efficacy of ADSCs in a porcine I/R model of laparoscopic hepatectomy. It was found that the ADSC treatment considerably reduced the serum levels of the enzymes aspartate aminotransferase, alanine aminotransferase, total bilirubin, and lactate dehydrogenase. Additionally, as critical autophagy markers Beclin1, ATG5, ATG12, and LC3II all showed decreased expression during phagophore development whereas P62 showed an increase.
IR surgery was performed on rats to measure liver damage and identify autophagy. Following ischemia and reperfusion, LC3B protein expression was significantly increased, and p62 expression was decreased according to immunohistochemical examination. The amount of hepatocyte autophagy was increased in the ıschemia and I/R groups by downregulating LC3B and upregulating P62 expression. Therefore we can say thata ADSCs inhibit excessive hepatic autophagy.

Rats receiving ADSC therapy were guarded against hepatic IRI. In line with earlier research, we found that ADSC-CM reduced hepatocyte autophagy, which improved the pathological and morphological alterations of the liver parenchyma in the porcine hepatic I/R combined with hepatectomy model.
The transforming growth factor-β (TGF-β) is a crucial myofibroblast activated mediator that causes stellate cells to release collagen fibers, which in turn causes liver fibrosis (Zou et al. 2019; Inagaki and Okazaki, 2007). TGF is the most potent fibrotic and pro-inflammatory factor, and it can also aggravate IR injury (Yang et al.2018). The effect of IR-induced liver damage on the TGF/Smad3 signaling pathway in vivo was examined by Li et al. (2021). The findings showed that TGF1, Smad3, and pSmad3 expression levels increased in the hepatic tissue of WT mice after IR injury.

In the current study , we examined TGF-beta protein expression. After an hepatic I/R injury, TGF-β immunoexpression was enhanced in the liver, and therapy with ADSCs decreased the upregulation of TGF-β.

Thus, we can conclude that ADSC therapy can protect the liver from damage caused by ischemia and reperfusion by lowering excessive autophagy.

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ORİJİNALLİK RAPORU

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