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Original Article

Multipotent Stromal Stem Cell Approach in Alleviating Autophagy Beclin-1/XBP-1/STAT5A/PTEN Signaling Pathways in Novodrin-induced Liver Dysfunction

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Abstract

Background and objective: Bone marrow-derived mesenchymal stem cells (MSCs), possess the unique ability of self-renewal and development into specialized cells, and long-lived cells with specific metabolic needs. It has been demonstrated that autophagy is essential for MSC differentiation, quiescence, activation, and self-renewal. The present study aims to elucidate how autophagy influences bone marrow-derived MSC post-novodrin-prompted liver dysfunction.

Methods: Hepatic dysfunction was induced in rats using novodrin (100 mg/kg, subcutaneously), which was divided into two doses for two alternative days, followed by the treatment with 100 µL of intravenous injection of allogeneic MSCs (5×10^6).

Results: A month preceding MSC therapy, a marked decline in liver function biomarkers, including alanine aminotransferase and aspartate aminotransferase, was observed, in addition to the significant decrease in oxidative stress biomarker, lipid peroxide. Meanwhile, novodrin significantly elevated the gene expression of cell survival biomarkers, including signal transducer and activator of transcription, phosphatidylinositol-3-kinase, and serine/threonine kinase-1, in addition to the concomitant increase in oncogenic biomarker, phosphatase and tensin homolog, and this was reversed post-MSC implantation. Furthermore, the autophagy biomarkers, including Beclin-1 and X-box binding protein 1, were restored post-MSC implantation. Moreover, the MSCs labeled with the PKH26 red fluorescent dye were sown into the injured liver tissue, which presented with hepatic tissues with a nearly normal architecture as confirmed through histopathological examination.

Conclusion: The present study demonstrated that autophagy is essential for bone marrow-derived MSC in novodrin-induced liver dysfunction.

Keywords: Mesenchymal stem cells; Hepatic injury; Phosphatidylinositol-3-kinase; Signal transducer and activator of transcription 5A; Phosphatase and tensin homolog.

Abbreviations: AKT1, AKT serine/threonine kinase 1; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; ANOVA, analysis of variance; AST, aspartate aminotransferase; Bcl-2, B-cell leukemia/lymphoma-2; BM-MSCs, bone marrow-derived mesenchymal stem cells; DMEM, Dulbecco's modified Eagle's medium; HPCs, hepatic progenitor cells; LDH, lactate dehydrogenase; LPO, lipid peroxide; MDA, hepatic malondialdehyde; MI, myocardial infarction; MSCs, mesenchymal stem cells; p-AKT, phosphorylated AKT; PI3K, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of the mean; STAT5A, signal transducer and activator of transcription 5A; XBP-1, X-box binding protein 1.

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Introduction

Autophagy is a basic cell survival mechanism that allows cells to respond to metabolic stress by degrading and recycling intracellular components, in order to generate macromolecular precursors and energy. The autophagy system is essential for the development, maintenance of cellular and tissue homeostasis, immunity, and disease prevention in humans. Autophagy defects have been linked to cancer, neurodegeneration, muscle and cardiac disease, infectious disease, and aging. Although autophagy has once been considered to have a passive quality control and general housekeeping function, new data has revealed that this is an active process that regulates the metabolic condition of cells. Adult stem cells have unique metabolic requirements, since these are long-lived cells with the ability to self-renew and develop into specialized cells throughout the body. Autophagy has key functions in stem cell quiescence, activation, differentiation

and self-renewal. Furthermore, autophagy is a crucial regulator of stem cell activity. Impaired autophagy in stem cells contributes to degenerative illness, aging, and the formation of cancer stem cells. Thus, the use of autophagy as a regenerative medicine technique can improve stem cell activity and stem cell-based therapeutics.[1]

Right heart ventricular dysfunction would consecutively lead to abnormal liver function.[1] Even in the absence of concomitant heart illness, chronic liver diseases can have an impact on cardiac function. Therefore, improving cardiac dysfunction is essential to improve hepatic dysfunction. A severe increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels would lead to cardiac ischemic hepatitis within 5–10 days of onset.[2]

The synthetic catecholamine, novodrin, can induce infarct-like lesions in animal models. Novodrin overdose can affect the liver's metabolism by disrupting the equilibrium between its oxidant and antioxidant levels. The production of harmful reactive oxygen species (ROS), such as $OH^{\bullet}$, $O_2^{\bullet}$ and H_2O_2, can damage the liver. Novodrin is an α-adrenergic agonist and synthetic catecholamine, and it was previously noted that this can cause severe myocardial dysfunction, which results in hepatic injury. Furthermore, novodrin can rapidly oxidize, and the oxidative byproducts are the cause of hepatic dysfunction.[3]

Beclin-1 is important for mammalian autophagy. Furthermore, Beclin-1 is a type III phosphatidylinositol 3-kinase complex component necessary for the production of autophagic vesicles. Human melanoma, colon, ovarian, liver and brain malignancies have been linked to abnormal Beclin-1 expression.[4]

Both the liver and the large intestine consistently express X-box binding protein 1 (XBP-1). XBP-1 possesses specific RNA polymerase II-enzyme binding activity, and promotes DNA-binding transcription factor and PI3K regulatory subunit binding. This has a vital role in several functions, including lipid homeostasis, and cellular response to hexose stimulation, in addition to controlling the macromolecule metabolism. Furthermore, this can affect a number of activities, including the cellular response to the formation of the exocrine system, the leukemia inhibitory factor, and the positive control of the proteasomal protein catabolic process. XBP1 deficit triggers liver pyroptosis by limiting mitophagy activation of mtDNA/cGAS/STING signaling in macrophages, supplying potential therapeutic targets for liver injury. According to a study conducted XBP-1 can be utilized to examine inflammatory bowel illness and liver dysfunction.[5]

Mesenchymal stem cells (MSCs) have the ability to clonally differentiate into adipocytes, chondrocytes, hepatocytes and osteoblasts.[6] Furthermore, MSCs are suitable for transplantation, contributing to its multi-lineage potential capacity, and preventing immune system recognition after transplantation and facile multiplication.[7,8]

Liver transplantation has been regarded as the sole curative option for late-stage liver injury.[9] However, liver transplantation is not considered a practical alternative, due to its limitations, the danger of immunological rejection, and the dependence on donor supply. Consequently, investigations are being conducted in a range of therapeutic models, including stem cell transplantation.

MSCs are present in various tissues, including umbilical cord, bone marrow, placenta, adipocyte, adult, and embryonic tissues.[10] Due to its significant therapeutic potential, MSCs have gained great consideration in the arena of regenerative medication. A modest amount of aspirated bone marrow can be used to separate and cultivate bone marrow-derived mesenchymal stem cells (BM-MSCs) without reducing its potency. Numerous researches have revealed that BM-MSCs can develop into tissues that resemble hepatocytes. These cells interact with different immune cells, have minimal inherent immunogenicity, and may modulate immunological responses, overall increasing the safety of employing MSCs in liver therapy.[6] By performing the functional action of mature hepatocytes, which are engaged in supportive functions necessary for regenerative therapy practice, MSCs can enhance liver function.[11]

The present study aims to assess the liver dysfunction correlated with myocardial infarction (MI) following novodrin hepatotoxicity. In addition, the present study was expanded to compare the autophagy genomic pathways in rats that received MSCs as treatment.

Materials and methods

Chemicals

Novodrin was obtained from Sigma-Aldrich (St. Louis, MO, USA). The kits were purchased from Randox (Antrim, UK). The reverse transcription-polymerase chain reaction (RT-PCR) kits were supplied by Qiagen (USA). All other chemicals had the highest analytical grade.

Preparation of bone marrow-derived mesenchymal stem cells from rats

Male, white, albino 6-week-old rats were used to collect the bone marrow by flushing the tibiae and femur with 10% fetal bovine serum supplemented with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL). Then, the isolated nucleated cells were re-suspended in full culture media (GIBCO/BRL), which contained 1% penicillin-streptomycin. First-passage cultures were the names given to the ensuing cultures.[6]

Labeling of MSCs with PKH26

The PKH26 Red Fluorescent Cell Linker Kit was obtained from Sigma-Aldrich (Saint Louis, MO, USA) for the labeling of MSCs. Intravenously, the cells were administered via the tail vein of rats. Then, the cells in the hepatic tissues were traced after a month using a fluorescent microscope.

Animals

Male, Wistar Albino rats (150–170 g, 4–5 months old) were used for the present study. The central animal facility housed the animals in polypropylene cages, and was regulated at a standard temperature of 26–30°C and a relative humidity of 40–60%. Water and rat pellets were provided for the rats at all times (VRK Laboratory Animal Feed, Maharashtra, India). Before the experiment began, the animals were bred for a week, in order to allow these animals to become acclimated to the laboratory environment. The experiment was approved by the Animal Ethics Committee of the National Research Center. The National Research Center's Animal Care and Use Committee's ethical guidelines and rules were rigorously followed throughout all animal care and treatment processes (19068).

Experimental design

After acclimation, the animals were divided into three groups, with eight rats in each group: control group, the animals received cell culture media; novodrin-intoxicated group, the animals were intoxicated with novodrin by performing two consecutive subcutaneous injections of novodrin (100 mg/kg) for two days[12]; MSC group, the animals that received novodrin were administered a single 100 µL intravenous injection of MSCs from a cell solution,

Table 1. Sequences of the forward and reverse primers used in the present study

Gene	Primer
STAT5A	5′-GGG ACA GCC TTT CCT ACT ACC-3′ 5′-GAT CTG CGC AAA AGT CCT GT-3
PI3K	5′-CCA GAC CCT CAC ACT CAG ATCA-3′ 5′-TCC GCT TGG TGG TTT GCT A-3′
PTEN	5′-GGA ACT CCA ACA AGG GAG CA-3′ 5′-TTC GGG GTC GGA AGA CCT TA-3′
AKT1	5′-CAT GAA GAG AAG ACA CTG ACC ATG GAAA-3′ 3′-TGG ATA GAG GCT AAG TGT AGA CAC G-5′
Beclin-1	5′-GGGCTTGGGGTCAGACTGTCTT-3′ 3′-AAGAAGCGGAATCCACCAGA-3′
XBP-1	5′-CCTTGTAGTTGAGAACCAGG-3′ 5′-GGGCTTGGTATATATGTGG-3′
ß-actin	5′-TGG AGT CTA CTG GCG TCT T-3′ 5′-TGT CAT ATT TCT CGT GGT TCA-3

AKT1, AKT serine/threonine kinase 1; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog; STAT5A, signal transducer and activator of transcription 5A; XBP-1, X-box binding protein 1.

which comprised 5×10^6 allogeneic rat MSCs at the time of boost.[6]

It is noteworthy that the chosen dose for novodrin has already been shown to be the most potent in causing liver damage.

Blood sampling and liver tissue preparation

Post-carbon dioxide anesthesia in all groups, blood samples were collected from each animal by puncturing the sublingual vein, and placing the samples in sterilized tubes. Then, these samples were centrifuged to separate the serum, and stored at 80°C for subsequent analysis. In addition, the liver tissue was carefully removed at the same time. In order to separate the cell debris, the homogenate was centrifuged at 3,000 rpm for 10 minutes at 4°C. Then, the supernatant was stored at −80°C for the subsequent RT-PCR analysis for phosphatidylinositol-3-kinase (PI3K), AKT serine/threonine kinase 1 (AKT1), STAT5A, Beclin, XBP-1 and PTEN.

Measured parameters

Serum alanine aminotransferase and aspartate aminotransferase activity

According to Reitman and Frankel, the ALT and AST activity was spectrophotometrically calculated using kits provided by Randox (AS2359; Antrim, UK).[13]

Hepatic malondialdehyde (MDA) determination

The MDA level was determined using Randox kits (AS2359; Antrim, UK), according to the manufacturer's instructions.[14]

RT-PCR determination for PI3K, AKT1, STAT5A, PTEN, Beclin-1 and XBP-1

The liver tissues were homogenized using β-mercaptoethanol and the QIAzol lysis reagent. Then, the one-step extraction kit was used, according to the manufacturer's instructions, in order to separate the total RNA. Afterward, the complementary DNA was synthesized using the SuperScript Choice System (Life Technologies, Breda, the Netherlands). Table 1 presents the primers used for the RT-PCR analysis.[15]

Determination of phosphatidylinositol-3,4,5-trisphosphate (PIP3) and phosphorylated AKT (p-AKT) by enzyme-linked immunosorbent assay

The PIP3 and p-AKT protein expression was determined using an enzyme-linked immunosorbent assay kit (R&D systems, MN, USA), according to the manufacturer's instructions. Then, quantitative sandwich enzyme immunoassay was used to evaluate the assays. Next, the microplate was pre-coated with specific antibodies. Then, the immobilized antibody that bound to PIP3 and p-AKT was added, and the wells were supplemented with the enzyme-linked secondary antibody specific for PIP3 and p-AKT. Afterward, the absorbance was determined at 450 nm.

Histopathological examination

For sectioning, samples with paraffin-embedded material were processed. Then, hematoxylin and eosin were used to stain the slides, and a light microscope was utilized to view the results.[16]

Statistical analysis

The data was presented in mean ± standard error of the mean (SEM). GraphPad Instat 3 (Graphpad Software Inc., San Diego, CA, USA) was used for the statistical analysis. SPSS 16 was used to analyze the data by one-way analysis of variance (ANOVA), followed by post hoc Tukey's test. A p-value of <0.05 was considered statistically significant.

Results

Seeding of MSCs labeled with PKH26 red fluorescent dye in liver tissues

After the transplantation to rats, MSCs stained with the PKH26 red fluorescent dye in liver tissues presented with an intense red autofluorescence, indicating that these were sown into the liver tissue to replace the injured hepatocytes (Fig. 1).

Inhibition of novodrin-induced hepatotoxicity

Compared to the controls, novodrin intoxication considerably increased serum ALT and AST levels (Fig. 2). However, the liver enzyme levels were significantly lower in groups that received stem cells, when compared to the novodrin-intoxicated group, suggesting the potential therapeutic effect of MSCs on hepatic damage (Fig. 2).

Modulation of oxidative stress biomarkers

A state of oxidative stress induced by the novodrin intoxication was demonstrated by the increase in MDA levels when compared to the controls (Fig. 3). However, these MDA levels became significantly lower after the administration of stem cells, when compared to the novodrin-intoxicated group. Evidently, compared to the novodrin-intoxicated group, the stem cell regimen significantly lowered the MDA levels by approximately four times.

Impact of MSCs on the hepatic mRNA gene expression of PI3K, AKT1, STAT5A, and PTEN

Novodrin intoxication induced the increase in expression of genes for PI3K, AKT1, STAT5A, and PTEN by approximately 25, 20, and 30 times, respectively, when compared to the controls, according to the data in Figures 4 and 5. However, this was considerably downregulated in rats that received stem cell treatment. In addi-

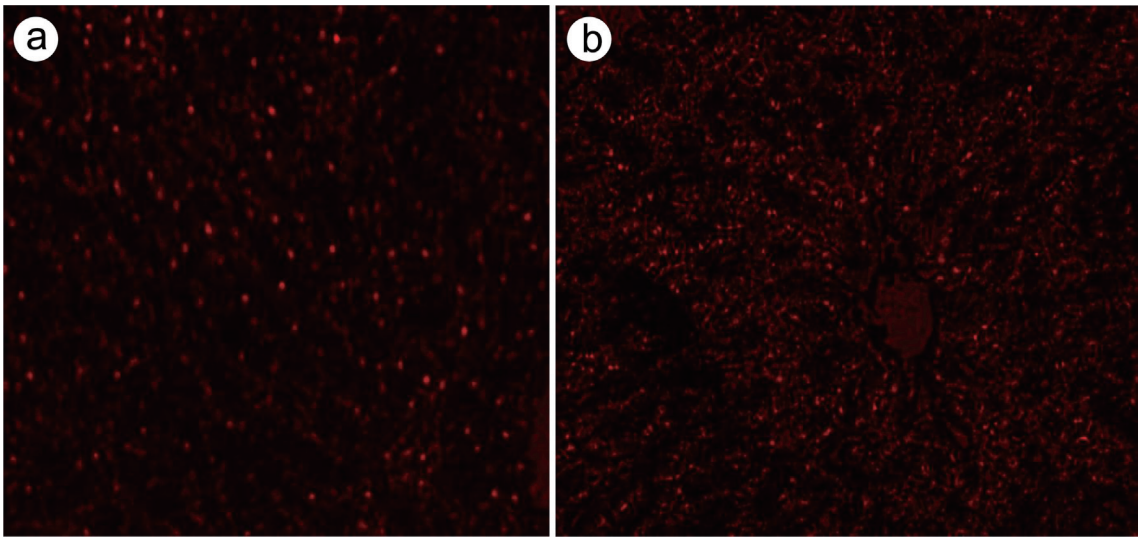

Fig. 1. The PKH26-labeled MSCs were sown into the injured liver tissue. (a) The post-recognition of MSCs labeled with PKH26 red fluorescent dye in the whole liver tissue. (b) The fluorescence imaging revealed that the MSCs dispersed along the liver's central vein. MSCs, mesenchymal stem cells.

tion, compared to novodrin, the stem cell regimen significantly reduced the expression of these genes.

Impact of MSCs on the hepatic protein expression of PIP3 and p-AKT

Novodrin induced the protein expression of PIP3 and p-AKT to increase by approximately 750% and 480%, respectively, when compared to the controls, according to the data in Table 2. However, rats that received stem cell treatment did not present a significant change in these values.

Impact of MSCs on the hepatic mRNA gene expression of autophagy biomarkers

The novodrin intoxication downregulated the expression of autophagy biomarkers, including Beclin-1 and XBP-1, by approximately 1.6 and 3.2 folds, respectively, when compared to the controls, according to data in Figures 6 and 7. However, this was considerably upregulated in rats that received stem cell treatment. In addition, when compared to novodrin, the stem cell regimen significantly increased the expression of these genes.

Histopathological examination

As shown in Figure 8, the control groups presented normal hepatocytes arranged in thin plates, the novodrin-intoxicated group presented with cirrhotic hepatocytes, and the MSC group presented with hepatic tissues with a nearly normal architecture.

Heatmap for the different gene expression levels

Figure 9 presents the heatmap for the different gene expression levels, and its correlations: yellow represents a high score, while blue represents a low score.

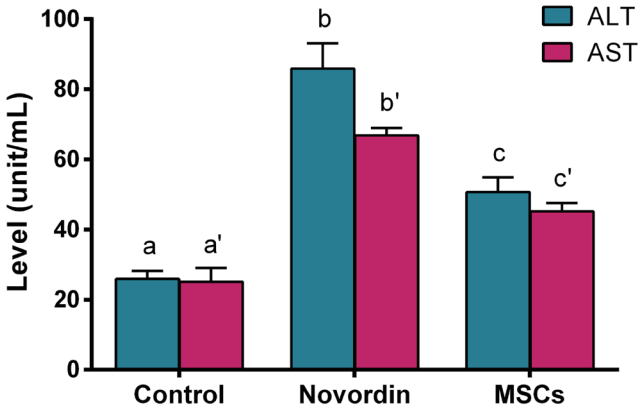

Fig. 2. Impact of MSCs on ALT and AST at post-novordin intoxication. The results were presented in mean ± standard error of the mean (SEM, n = 10), $p < 0.05$. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. b, b', c and c' represent highly significant from a and a' (the negative control group). ALT, alanine aminotransferase; AST, aspartate aminotransferase; MSCs, mesenchymal stem cells; SEM, standard error of the mean.

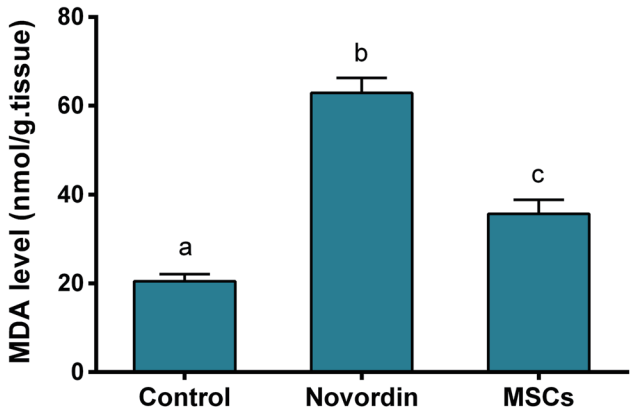

Fig. 3. Impact of MSCs on the hepatic MDA level post-novordin intoxication. The data were expressed in mean ± standard error of the mean (SEM, n = 10), $p < 0.05$. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. b and c represent highly significant from a (the negative control group). MDA, hepatic malondialdehyde; MSCs, mesenchymal stem cells; SEM, standard error of the mean.

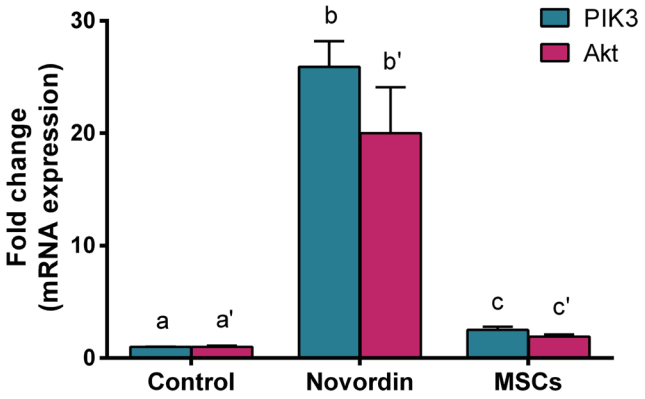

Fig. 4. Impact of MSCs on the hepatic mRNA gene expression of PI3K and AKT1 post-novordin intoxication. The results were presented in mean ± standard error of the mean (SEM, n = 8), p < 0.05. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. b, b', c and c' represent highly significant from a and a' (the negative control group). β-actin was used as the reference standard. AKT1, AKT serine/threonine kinase 1; MSCs, mesenchymal stem cells; PI3K, phosphatidylinositol-3-kinase; SEM, standard error of the mean.

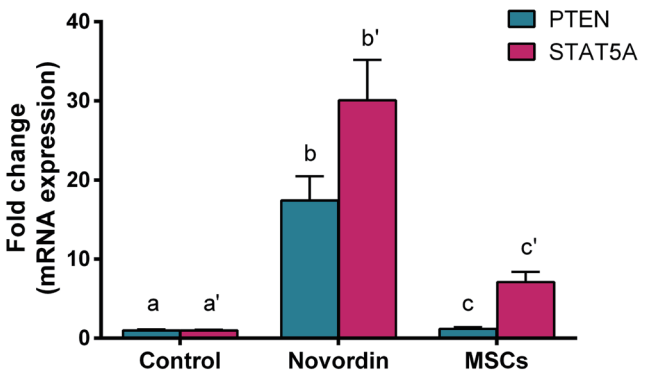

Fig. 5. Impact of MSCs on the hepatic mRNA gene expression of PTEN and STAT5A post-novordin intoxication. The results were presented in fold change (n = 8), p < 0.05. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. b, b', c and c' represent highly significant from a and a' (the negative control group). B-actin was used as the reference standard. MSCs, mesenchymal stem cells; PTEN, phosphatase and tensin homolog; STAT5A, signal transducer and activator of transcription 5A.

Discussion

Autophagy is a highly conserved cellular mechanism that sequesters damaged or toxic cytoplasmic components in autophagosomes, which eventually fuse with the lysosome for destruction. Due to the potential to self-replicate and give rise to any specialized cell type, MSCs have become extremely significant resources for cell-based medicinal interventions, and these have opened various interesting avenues for research on human disease. It has been proposed that autophagy is essential for the preservation of cellular homeostasis in MSCs, and the regulation of MSC self-renewal and differentiation.[6]

Furthermore, autophagy is a highly conserved procedure regulated by complicated signaling networks, including the AKT/PKB system, the PI3K inositol pathway, the p52 route, endoplasmic reticulum stress, and AMP-activated protein kinase (AMPK) and ROS production. Furthermore, autophagy is induced when the AMPK pathway is activated. The AMPK pathway is activated by metabolic stress, culminating in the phosphorylation of p27, which is a cyclin-dependent kinase inhibitor. The phosphorylation of p27 improves its stability, and this allows cells to survive the growth factor withdrawal through autophagy.[6]

In addition, the autophagy process has been recently identified

Table 2. Impact of MSCs on hepatic p-AKT and PIP3 protein expression at post-novordin intoxication

Group/ Parameter	Control	Novordin	MSCs
p-AKT	0.500 ± 0.002[a]	2.400 ± 0.004[b]	1.800 ± 0.001[b]
PIP3	0.200 ± 0.001[a]	1.500 ± 0.003[b]	1.100 ± 0.002[b]

The results were represented in mean ± standard error of the mean (SEM, n = 8), p < 0.05. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. MSCs, mesenchymal stem cells; P-AKT, phosphorylated AKT; PIP3, phosphatidylinositol-3,4,5-trisphosphate; SEM, standard error of the mean.

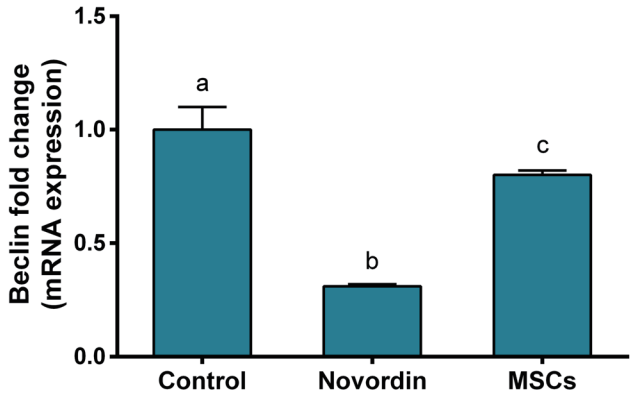

Fig. 6. Impact of MSCs on the hepatic mRNA gene expression of Beclin-1 post-novordin intoxication. The results were presented in fold change (n = 8), p < 0.05. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. b and c represent highly significant from a (the negative control group). β-actin was used as the reference standard. MSCs, mesenchymal stem cells.

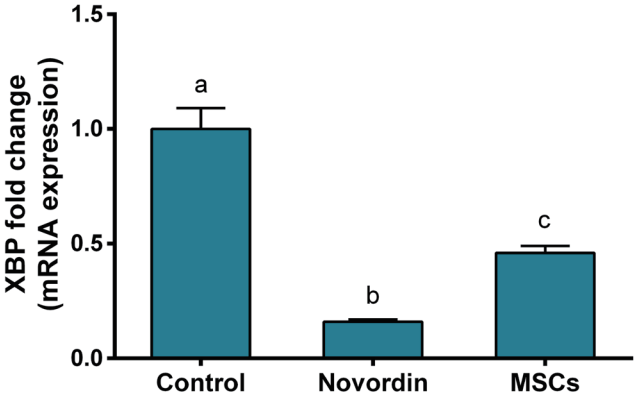

Fig. 7. Impact of MSCs on the hepatic mRNA gene expression of XBP-1 post-novordin intoxication. The results were presented in fold change (n = 8), p < 0.05. The groups labeled with different letters reflect the statistical significance, when compared to the negative control. b and c represent highly significant from a (the negative control group). B-actin was used as the reference standard. MSCs, mesenchymal stem cells; XBP-1, X-box binding protein 1.

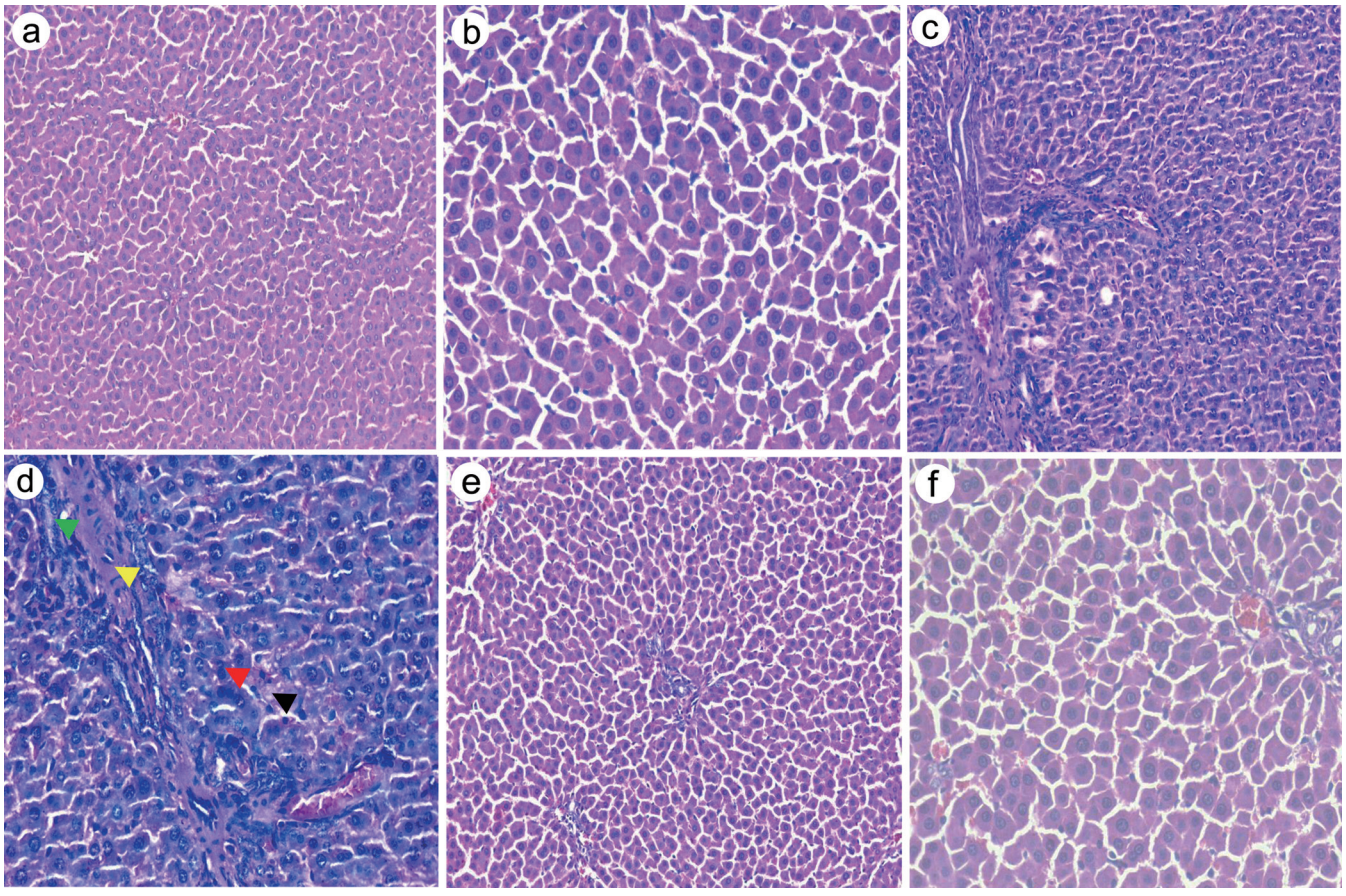

Fig. 8. Histopathological examination. (a and b) control group, the hepatic tissue presented with normal hepatocytes arranged in thin plates (H&E: ×200 and ×400); (c and d) novodrin-intoxicated group, there was partial loss in architecture with a thick portal tract (yellow arrow), dilated bile ducts (green arrow) and liver cells with mild hydropic degeneration, an area of necrosis (black arrow), and binucleated nuclei (red arrow) (H&E: ×200 and ×400); (e and f) MSC group, the liver section presented with hepatic lobules, which consisted of the central vein surrounded by normal hepatocytes, and preserved architecture and liver cells arranged in thin plates (H&E: ×200 and ×400). MSCs, mesenchymal stem cells.

as the fundamental mechanism by which MSCs achieve its precise morphology and function by controlling the protein turnover. Recent research has revealed that autophagy activation is required for MSC self-renewal and differentiation.[17] Furthermore, autophagy can efficiently transport sets of adhesion molecules, transcription factors, or released factors, in response to environment stimulation and hormone activation, and all of these are critical for stem cell self-renewal and differentiation.[17]

Previous research indicated that the liver's regenerating ability is primarily due to resident hepatic progenitor cells (HPCs), which are defined as cells that give rise to both biliary epithelial cells and hepatocytes at the post-liver injury. The role of autophagy in hepatocyte regulation has been extensively investigated in liver regeneration,[6] and the maintenance of metabolic balance in the liver. It has been observed that the knockdown of the critical autophagy gene Beclin-1 reduced the clonogenic and proliferative capacity of HPCs. Furthermore, it has been demonstrated that autophagy deficiency enhances the accumulation of damaged mitochondria and mitochondrial ROS, and blocks the homologous recombination pathway for DNA damage repair in HPCs.[10] These results demonstrate that autophagy plays an indispensable role in MSC-associated expansion. In the present study, comparisons were conducted to investigate the effectiveness of transplanted MSCs in reducing rat liver injury induced by novodrin, and the potential autophagy pathways involved.

In the present study, novodrin increased the ALT and AST levels, when compared to the controls. As indicated by the novodrin-induced hepatic injury and plasma membrane leakiness, the elevated liver biomarkers were linked to the loss of functional integrity of the liver cell membrane, and cellular leakage. In previous research, carbon tetrachloride was employed to establish the liver cirrhosis model,[18] and the BM-MSC group presented with significantly lower levels of ALT, alkaline phosphatase, LDH, AST, and liver function indicators (bilirubin, albumin, and total protein), when compared to the carbon tetrachloride-treated group. This result is consistent with the results of other studies, which reported that transplanted MSCs may considerably reduce liver injury by restoring blood-liver function biomarkers and liver enzyme levels.[10,19]

It may be considered that the novodrin-prompted lipid peroxide (LPO) and production of ROS are responsible for the hepatic membrane injury, and leakage of LDH, ALT, and AST in serum.[12] Oxidative stress, which results from the increase in free radical production and decreases in antioxidant levels in hepatocytes, is a crucial factor in the development of hepatic disorders, such as necrosis. The production of harmful ROS, such as $O_2^{\bullet}$, H_2O_2 and $OH^{\bullet}$, would damage the hepatocytes. Hepatotoxicity has been linked to

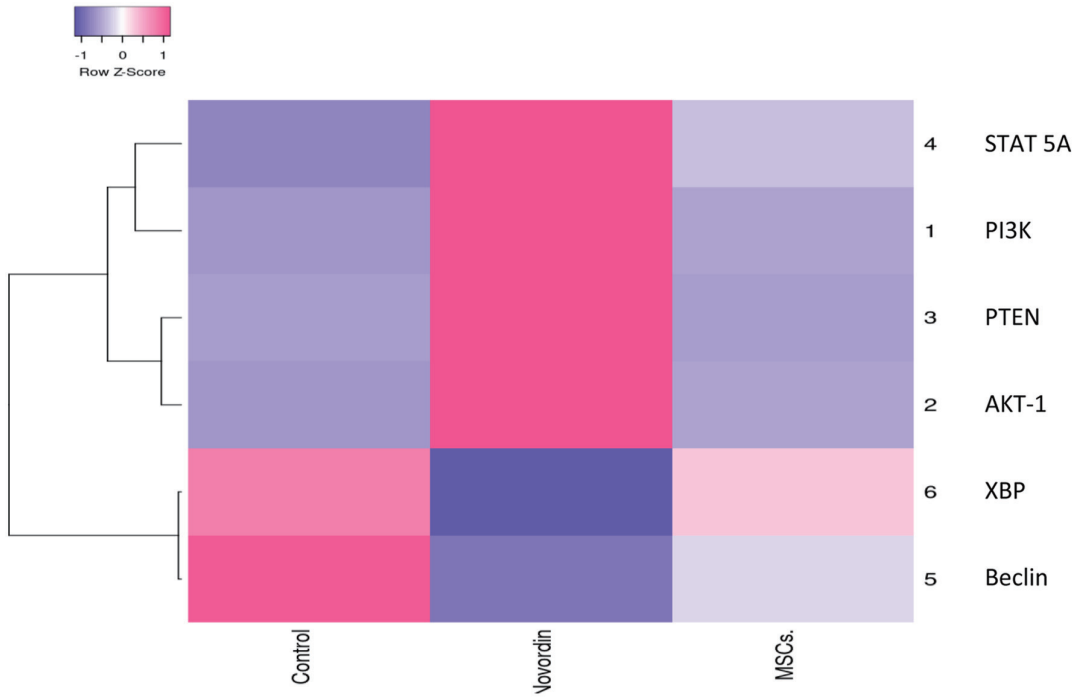

Fig. 9. The heatmap presents the different gene expression levels and its correlation: red represents a high score, while blue represents a low score. AKT1, AKT serine/threonine kinase 1; MSCs, mesenchymal stem cells; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog; STAT, signal transducer and activator of transcription; XBP-1, X-box binding protein 1.

the β-adrenergic agonist, novodrin, which rapidly goes through auto-oxidation, and it was proposed that alterations in hepatocytes are caused by the oxidative byproducts of catecholamines.[15]

In this regard, the present study revealed that the novodrin intoxication significantly increased the hepatic MDA level, demonstrating that the liver of novodrin-intoxicated rats experienced severe oxidative stress. A well-known mechanism of cellular damage is lipid peroxidation, which is a marker for oxidative stress that contributes to MI and is linked to liver dysfunction. Novodrin-intoxicated rats with MI have been shown to present with LPO-mediated liver injury.[20]

Previous studies have reported that hepatic dysfunction, inflammatory cascades, and histological alterations, as the result of novodrin injections, markedly decreased the glutathione, superoxide dismutase and catalase activity, and enhanced the vascular responsiveness to different catecholamines. The pathogenic stage that makes liver tissue more vulnerable to oxidative injury appears to be the increase in lipid peroxidation. Novodrin quinone metabolites may cause oxidative stress by interacting with antioxidant enzymes to synthesize $O_2^{\bullet}$ and other ROS.[15]

The stem cell transplantation led to a substantial decrease in MDA levels, as a result of the increase in hepatic antioxidants and glutathione levels. Kumar *et al.* reported that the main molecular pathway behind the curative impact of MSCs in hepatotoxicity is the antioxidant and ROS quenching properties.[21–23]

Novodrin significantly upregulated the PTEN, STAT5A, PI3K and AKT1 genes, and these were modulated by the post-stem cell treatment. Meanwhile, MSCs elevated the PIP3 and p-AKT protein expression. This was highlighted by the study conducted by Chen *et al.*, which revealed that BM-MSCs resemble immune cells, and react to various stress signals induced by injured areas at post-inflammation, similar to the case of hepatic and autoimmune illnesses.[24] The ability of MSCs to discriminate into hepatocytes, and the immune-modulatory and antioxidant capabilities are all factors that may contribute to its therapeutic index in liver injury.[25] For its capacity for multipotent discrimination, self-regeneration and low immunogenicity, MSCs exhibit a great prospective for therapeutic utility.[26] Numerous reports have revealed how MSCs can diminish liver injury.[6,10]

PIP3 is produced by a lipid kinase, which is known as PI3K. Trisphosphate is a secondary messenger and is a vital AKT1 plasma membrane transporter, which is activated and phosphorylated. According to the studies conducted by Wang *et al.* and Záleák *et al.*, the activation of AKT1 is essential for cell survival and proliferation.[27,28] MSCs release a range of physiologically active chemicals, including growth factors, chemokines and cytokines, and together, this is referred to as the MSC secretome, which in physiopathological circumstances, is similar to ischemia. Recently, the idea of the MSC secretome has been considered to be crucial to mechanisms that involve repair.[29] The role of the PI3K/AKT pathway in MSC survival was previously reported through the overexpression of certain pathway-specific genes. Rat heart transplantation can result in improved MSC survival, when AKT1 is overexpressed in the transplanted cells. Furthermore, anti-apoptotic protein, B-cell leukemia/lymphoma-2 (Bcl-2), was upregulated in MSCs that overexpressed AKT1, while Bax, which is a pro-apoptotic protein, decreased. It has been demonstrated that MSC survival in MI therapy increased after overexpressing Bcl-2.[30] Cell apoptosis may be impacted by statins (STAT) through lowering anti-apoptotic biomarkers Bcl-xL and Bcl-2, and increasing the activity of pro-apoptotic biomarkers, caspase 3, 8 and 9, Bax and Bad, and some tumors have been treated using these effects.[31,32] In general, the PI3K/AKT pathway functions as a survival mechanism that regulates cell division, migration, proliferation and apoptosis. The results for the overexpression of several pathway-specific genes present the role of the PI3K/AKT pathway

in MSC survival.[33]

BM-MSCs may lower acute liver inflammation, and its subsequent hepatocyte damage by secreting various soluble compounds and trophic factors, such as growth factors, some specific cytokines, and chemokines, which have significant therapeutic roles in regenerative medicine.[34] Furthermore, MSCs control the production of inflammatory cytokines, which produce hepatoprotection and inflammatory biomarkers. Moreover, in addition to limiting cell death, inflammation and fibrosis in injured tissues, these promote tissue regeneration and angiogenesis.[35,36]

The novodrin intoxication significantly downregulated the gene expression of autophagy biomarkers, including Beclin-1 and XBP-1, when compared to the controls. However, these biomarkers were considerably upregulated in rats that received stem cell treatment.

Recent research revealed that autophagy, which is a conserved cellular response to stress, plays a part in human malignancies. Beclin-1, which is a crucial autophagic gene, has been reported to have an aberrant expression in a number of human malignancies. Furthermore, a previous study determined the correlation between Beclin-1, and cell apoptosis and proliferation or prognosis in liver carcinoma. Hepatic carcinoma pathophysiology and development are linked to diminished autophagy. The Bax and Beclin-1 expression in hepatic carcinoma have a synergistic impact in reducing the growth, invasion, metastasis, and angiogenesis of a disease. The expression of Beclin-1 can be a useful prognostic indicator for hepatocellular carcinoma.

XBP-1 is a transcription factor from the cAMP-response element binding protein/Cyclic AMP-dependent transcription factor family, which is highly expressed in hepatocellular carcinomas. Furthermore, XBP-1 is necessary for the growth of the liver. XBP-1 deficient mice presented with hypoplastic fetal livers, and the diminished hematopoiesis induced anemia, which led to mortality. However, there were no cell-autonomous differentiation deficits in XBP-1-deficient hematopoietic progenitors. Instead, the hepatocyte formation was substantially influenced by two factors: the high rate of apoptosis, and the decreased rate of growth. Thus, XBP-1 is a vital transcription factor required for the proliferation of hepatocytes.[37] XBP-1 knockdown may cause organ-specific inflammation, offering a mechanistic explanation for the onset of pro-inflammatory illnesses.[5]

Conclusion

In conclusion, BM-MSC transplantation may reduce liver tissue damage due to regulating autophagy through Beclin-1/XBP-1/STAT5A/PTEN Signaling Pathways. In light of this, the unique approach adopted in the present study for the utilization of BM-MSCs may be a useful approach for the treatment of liver injury in regenerative medicine.

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Conflict of interest

The authors declare no conflict of interests.

Author contributions

MOK: Perceived and designed the experiments; shared in experimenting; analyzed (biochemical parameters and RT-PCR gene expression) and interpreted the data; wrote the paper. RMAM: Performed the experiment; Analyzed (biochemical parameters and RT-PCR gene expression) and interpreted the data and statistical analysis.

Data availability

The data are available upon reasonable request.

Ethics statement

The experiment was approved by the Animal Ethics Committee of the National Research Center. The National Research Center's Animal Care and Use Committee's ethical guidelines and rules were rigorously followed throughout all animal care and treatment processes (19068).

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