Human umbilical cord blood-derived mesenchymal stem cells improve glucose homeostasis in rats with liver cirrhosis

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Abstract. Disturbance of glucose metabolism is a common feature in liver cirrhosis which is associated with insulin resistance and is an established risk factor for disease progression and survival in patients with chronic liver diseases. We investigated whether umbilical cord blood-derived mesenchymal stem cells (HMSCs) have an effect on the expression of molecules responsible for glucose utilization and hepatic gluconeogenesis, focusing on the insulin signaling pathway in rats with liver cirrhosis. Rats received a dose of CCl_4 (100 μ l/100 g 4:1 in corn oil) thrice-weekly. HMSCs were infused at 4 weeks after induction of liver cirrhosis by CCl₄. Infusion of HMSCs improved insulin resistance which was associated with increased glucose levels and decreased insulin sensitivity in CCl₄-induced cirrhotic rats. HMSCs increased activities in the proximal part of the insulin signaling cascade, as evidenced by increased expression of key enzymes such as phosphatidylinositol-3-kinase (PI 3-kinase), protein kinase B (PKB), protein kinase C- ζ (PKC- ζ), and the decrease of glycogen synthase kinase 3 (GSK-3) compared to CCl₄-induced liver cirrhotic rats. We also observed that glucose-6-phosphatase (G-6-P) and phosphoenolpyruvate kinase (PEPCK), two hepatic enzymes involved in gluconeogenesis were strongly decreased over 40-50% after infusion of HMSCs. Taken together, our results showed that HMSCs could improve insulin resistance in CCl₄-induced liver cirrhosis, thereby contributing to glucose homeostasis.

Introduction

The liver plays a pivotal role in nutrient and hormone metabolism. In physiological conditions, hepatocytes are also the main site of hepatic glucose metabolism. Glucose intolerance is well-

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established feature of advanced liver cirrhosis that may result from excess glucose production and impaired glucose utilization (1,2). These phenomena are closely associated with insulin resistance (3). Insulin regulates blood glucose primarily by stimulating glucose uptake into liver, muscle, and adipose tissue and by inhibiting hepatic glucose production. In type 2 diabetes, most of the intracellular actions of insulin are reduced or absent (4,5), yet the lesions underlying insulin resistance is not clear. To find ways to solve insulin resistance, research has focused on the insulin-regulated signaling pathway that normally mediate glucose production or glucose utilization and that connect insulin receptor (IR) to the proteins that directly mediate each action of insulin. The IR is localized not only on the surface of the hepatocytes, but also on the other liver cells. The insulin binding to IRs causes the autophosphorylation of the receptor on intracellular tyrosine residues, followed by the activity phosphatidylinositol 3-kinase (PI 3-kinase). Next, PI 3-kinase activates protein kinase B (PKB) or protein kinase C- ζ (PKC- ζ), which are PI 3-kinase downstream substrates (6). Also, the activation of PKB leads to GSK-3 phosphorylation and activates glycogen synthase (7,8). In addition, insulin regulates glucose-6-phosphatase (G-6-P) and phosphoenolpyruvate kinase (PEPCK), insulinresponsive proteins (9,10). Numerous studies have suggested that the inability of insulin to regulate the rate-controlling gluconeo-genesis enzymes such as G-6-P and PEPCK may contribute to glucose intolerance (11,12).

In the past 10 years, there have been substantial developments in stem cell research and the transplantation of these cells now holds great promise for regenerative medicine. Among stem cells, human umbilical cord blood (HUCB) has emerged as an attractive tool for cell-based therapy. HUCB are capable of differentiating into cells of different connective tissue lineages, such as bone, cartilage, adipose, and islets tissues (13-15). Mesenchymal stem cells (MSCs) are highly proliferating and they have been demonstrated to have potential not only for the various cells of mesodermal origin, but also for ectodermal neural cells and endodermal hepatic cells (16-18). The most common source of MSCs is bone marrow (BM), but aspirating BM from a patient is an invasive and uncomfortable procedure. In addition, it has been demonstrated that the number and differentiating potential of BM-derived MSCs decreased with age (19). Therefore, HUCB is of significant value as good source of MSCs. Indeed, it was

Key words: umbilical cord bloods, mesenchymal stem cell, insulin resistance, phosphatidylinositol-3-kinase, glucose-6-phosphatase, glycogen synthase kinase 3

reported that HUCB-derived MSC (HMSCs) have multilineage differentiation activity rather than BM-derived MSC (20). Recently, numerous studies have ascribed not only potent tissue regeneration and repair but also anti-inflamatory effects of HMSCs in various diseases such as kidney disease, lung injury, hypoxia-induced brain injury, and liver cirrhosis (21-24). Especially, HMSCs have been explored both as modulators of the immune response in type I diabetes and as potential sources of insulin-positive cells (25-27). However, most HMSC studies on diabetes have focused on insulin resistance induced from pancreas abnormality. There have been no reports on the effect of HMSCs on insulin sensitivity/ resistance and impaired glucose homeostasis induced from liver cirrhosis.

This study was undertaken to investigate whether HMSCs have an effect on decreased cellular glucose uptake and increased glucose production caused by insulin resistance in CCl_4 -induced liver cirrhotic rats. To test these effect of HMSCs, we investigated expression of PI 3-kinase, PKB, PKC- ζ , GSK-3, G-6-P and PEPCK, which are known insulinmediated molecules.

Materials and methods

HMSC preparation. HUCB samples were harvested (Seoul Cord Blood Bank, Seoul, Korea) from term and pre-term deliveries at the time of birth with the mothers' consent. Blood samples were processed within 24 h from collection. The mononuclear cells were separated from HUCB using Ficoll-PaqueTM PLUS (Amersham Bioscience, Uppsala, Sweden) and were suspended in culture medium (DMEM; Gibco, Grand Island, NY) containing 15% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 M L-glutamine and 1 M sodium pyruvate. The cells were then seeded at a density of 1x10⁶ cells/cm² in culture flasks. After 7 days of culture, suspended cells were removed and adherent cells were additionally cultured (MSCs). MSCs were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide, with a change of culture medium every 3-4 days. Approximately 50-60% of confluent cells were detached with 0.1% trypsin-EDTA and replaced at a density of 2x10³ cells/cm² in culture flasks. To label cells with CM-1,1'-dioctadecyl-3,3,3'-testramethylindo-carbocyanine perchloride (CM-DiI; Invitrogen, Carlsbad, CA), 1 µg of CM-DiI per microliter of dimethylsulfoxide (DMSO; Sigma, Canton, MA) stock solution was added to HMSCs suspensions at 1x10⁶ cell/ml in Plasmalyte A (Baxter) to yield a final concentration of 1 μ g of CM-DiI solution/ml. This suspension was incubated at 37°C for 5 min, then at 4°C for 15 min with occasional mixing and the HMSCs labeled with CM-DiI were washed 3 times.

 CCl_4 -induced liver cirrhosis model. Male Sprague-Dawley rats (6-week old, 180-200 g) were used for the experiments. These animals were housed in an air-conditioned room at 25°C with a 12 h dark/light cycle. All animals received human care during the study with unlimited access to chow and water. Liver cirrhosis was induced by intraperitoneal (i.p.) administration of CCl₄ (4:1 olive oil) at a dose of 0.1 ml/100 g body weight thrice-weekly. The same volume of olive oil alone was used as control. Liver cirrhosis was determined by sacrificing 3-6 rats with histopathology per week. After 8 weeks, CCl_4 -induced liver cirrhosis was identified using morphologic method by special pathologist. Eighty rats were randomly divided at 8 weeks into four groups: HMSCs/CCl₄ (n=20); saline/CCl₄ (n=20), HMSCs was infused a dose of 1x10⁶ cells labeled with CM-DiI and saline was infused the same volume per rat by tail vein. Blank controls, HMSCs/olive (n=20) and saline/olive (n=20), were designed to infuse saline and HMSCs of the same volume at 8 weeks after olive injection. At 4 weeks after infusion of HMSCs, rats were sacrificed and venous blood was collected.

Pathology. Liver samples were fixed in 10% buffered formaldehyde solution, processed by the paraffin slice technique. Sections of 4 μ m thick were stained with hematoxylin and eosin (H&E) staining for routine histology, and Masson's trichrome (MT) staining for collagen. For H&E staining, sections were stained with hematoxylin for 3 min, washed, and stained with 0.5% eosin for 3 min. After an additional washing step with water, the slides were dehydrated in 70, 96, and 100% ethanol, and then in xylene. The degree of liver damage was examined in a blind manner by a pathologist under a light microscope (Olympus, Hamburg, Germany).

Oral glucose tolerance test. At 4 weeks after HMSCs infusion in CCl₄-induced liver cirrhosis rats, an oral glucose tolerance test (OGTT) was performed after an overnight fast. D-glucose (3.0 g/kg body weight) was administered by gavage and blood samples were obtained by tail bleeding at 0 (before glucose administration), 30, 60, and 120 min after the glucose load. Blood glucose levels (Haemoglucotest, 1-44 and Reflolux II reflectance meter, Boehringer-Mannheim, Mannheim, Germany) and serum insulin concentrations were measured with rat ELISA kit (Merocida AB, Uppsala, Sweden) (28).

Biochemical analysis. Alanine aminotransferase (ALT), aspartate aminotransferse (AST), albumin, total protein, total bilirubin, alkaline phosphatase (ALP), hyaluronic acid, and laminin were analyzed by Green Gross Reference Lab (Seoul, Korea).

Immunoprecipitation. Immunoprecipitation was performed as follows (29). After homogenizing liver tissue with 1 ml of lysis buffer (pH 7.5, 50 M Tris-HCl, 150 M NaCl, 1 M MgCl₂, 1 M CaCl₂, 1 M sodium orthovanadate, 100 M NaF, 1 M EGTA, 1% Triton X-100, 10% glycerol, leupeptin (5 μ g/ml) and 1 mmol/l phenylmethylsulfonyl fluoride) on ice, it was washed twice with ice-cold PBS. The homogenized liver tissues were then centrifuged at 15,000 g for 15 min at 4°C and supernatant was harvested. For immunoprecipitation, 20 μ l of anti-phosphotyrosine antibody agarose beads was incubated with supernatant containing 500 μ g of protein for 1 h at 4°C. Immunoprecipitates were washed three times with PBS containing 1% Nonidet P-40, three times with 100 M Tris-HCl pH 7.5 containing 100 M NaCl and 1 M EDTA.

PI 3-kinase assay. Crude PI 3-kinase was obtained by immunoprecipitation with antiphosphotyrosine antibody. Tissue lysates (500 μ g protein) were incubated with 20 μ l of antiphosphotyrosine antibody agarose beads. After washing, the



Figure 1. Effect of HMSCs on the body and liver weights in CCl_4 -induced liver cirrhosis rats. (A) Average weight change of rats induced with CCl_4 for 12 weeks. (B) Liver weights and relative liver weight in CCl_4 -induced liver cirrhosis. HMSCs and saline were injected after the CCl_4 induction for 8 weeks by tail vein. Values are expressed as the mean \pm SD (n=20). *p<0.05 vs. saline/olive, *p<0.05 vs. saline/CCl_4.

immunoprecipitates with PI 3-kinase activity were resuspended in 100 μ l of kinase assay buffer (20 M Tris-HCl, 75 M NaCl, 10 M MgCl₂, 200 μ g/ml phosphatidylinositol, 1 M EGTA, 20 μ M ATP, 10 μ Ci [γ -³²P]-ATP) and incubated for 30 min at room temperature with constant shaking. PI 3-kinase activity was measured by the phosphorylation of PI. The reaction was stopped by the addition of 100 μ l 1 M HCl and the reaction products were extracted with 200 μ l chloroform:methanol (1:1). The samples were centrifuged and the lower organic phase was harvested and applied to a silica gel thin layer chromatography (TLC) plate (Merck, Aichach, Germany) coated with 1% potassium oxalate. TLC plates were developed in chloroform : methanol : ammonium hydroxide : water (60:47:11.3:2), dried, and visualized by autoradiography.

Western blotting. Liver protein (40 μ g) was separated by SDS-PAGE gelelectrophoresis and transferred to nitrocellulose membrane (Schleicher & Schuell, Middlesex, UK). The membrane was blocked with 5% skim milk in 10 M Tris-HCl containing 150 M NaCl and 0.5% Tween-20 (TBS-T). After washing with TBS-T, the membrane was then incubated with primary antibodies (1:500) recognizing p-PKB, p-PKC- ζ , p-GSK-3, G-6-P, and PEPCK (Cell Signaling, Beverly, MA). After washing with TBS-T, horseradish peroxidase-HRPconjugated secondary antibody (New England Biolabs, Beverly, MA, 1:1000 in TBS-T) was applied to the membrane and the blot was developed using an enhanced chemiluminescence detection kit (Amersham, Piscataway, NJ).

Statistical analysis. Results were expressed as the mean \pm SD. Statistical analysis was performed using one-way ANOVA and *post hoc* test was performed for multiple comparisons. A p<0.05 or 0.01 was taken to indicate statistical significance. Statistical calculations were performed using SPSS software Windows (Version 10.0; SPSS, Chicago, IL).

Results

Body and liver weight. Rats given CCl_4 showed slower weight gain than rats with olive oil alone (Fig. 1). The average weight

of rats injected with CCl_4 for 8 weeks was 100-150 g lower than rats with olive oil alone. The mortality rates were about 10% in rats treated with CCl_4 after 8 weeks, while the olive oil-injected rats were all alive. Also, the final liver weight in the saline/ CCl_4 group was 56% of the saline/olive control. However, liver weights in the HMSCs/ CCl_4 group were 78% of the saline/olive control. This result showed that HMSC infusion prevented CCl_4 -induced loss of liver weight. A significant increase in relative liver weight (p<0.05) occurred after HMSC infusion in CCl_4 -induced liver cirrhosis. Relative liver weights in HMSCs/ CCl_4 group were 91% of the saline/ olive control.

Pathology. Liver cirrhosis in the rats was evaluated by two histological methods: H&E and MT staining. The results from two methods showed the same pattern. The histological analyses of the livers in saline/olive and HMSCs/olive groups showed normal architecture (Fig. 2). CCl_4 injection for 8 weeks induced extensive liver cirrhosis/fibrosis, as evidenced by both qualitative and quantitative histopathological examination. As shown in Fig. 2, liver morphology of CCl_4 -induced cirrhotic rats presented disruption of tissue architecture, extension of fibers, large fibrous septa formation, pseudo lobe separation and collagen accumulation. The extent of fibrosis was remarkably reduced after infusion of HMSCs. In both H&E and MT staining, the histological structure was obviously improved after HMSC infusion in CCl_4 -induced liver cirrhotic rat compared to the CCl_4 induction group.

Biochemical analysis. Serum markers for liver function such as ALT and AST were significantly decreased after HMSC infusion in CCl₄-induced liver cirrhosis rats (Table I, p<0.05). However, ALT and AST in saline/CCl₄ group continued to be elevated compared to saline/olive and HMSCs/olive groups. In addition, serum total protein and albumin concentrations were decreased in saline/CCl₄ group and stayed low, while serum total protein and albumin in HMSCs/CCl₄ group was significantly increased, compared to saline/CCl₄ group (p<0.05). ALP and total bilirubin levels were significantly decreased in HMSCs/CCl₄ group, as compared with saline/CCl₄ group



Figure 2. Histological analysis of liver sections. Liver sections were stained using H&E and MT. Original magnification x200.



Figure 3. Plasma glucose (A) and insulin levels (B) during oral glucose tolerance test after HMSC infusion in rat CCl_4 -induced liver cirrhosis. Plasma glucose levels were elevated during the oral glucose tolerance test, and an oral glucose load led to increases in insulin levels in control, cirrhosis, and HMSCs infusion groups. Values are expressed as the mean \pm SD *p<0.05 vs. saline/CCl₄.

(p<0.05). Hyaluronic acid and laminin levels, indicators of fibrosis/cirrhosis, were decreased in HMSCs/CCl₄ group and showed significant difference, compared to saline/CCl₄ group (p<0.05).

Insulin and glucose level after HMSC infusion in liver cirrhotic rats. To identify insulin and glucose intolerance both in CCl_4 -induced liver cirrhotic rats and after HMSC infusion in CCl_4 -induced liver cirrhotic rats, we measured insulin and

glucose levels on 0, 30, 60, 90, and 120 min after oral glucose load. As shown in Fig. 3, CCl₄-induced liver cirrhotic rats (saline/CCl₄) showed a tendency to elevate plasma insulin levels up to 60 min after an oral glucose load, while decreased slowly insulin levels from 90 to 120 min. However, insulin levels after HMSC infusion in CCl₄-induced liver cirrhotic rats (HMSCs/CCl₄) were decreased significantly from 60 to 120 min, compared to saline/CCl₄ (p<0.05). Similar to insulin levels, glucose levels in saline/CCl₄ were increased from 30 to 120 min, and showed remarkable increase at 60 min, compared to control (p<0.05). However, glucose levels of HMSCs/CCl₄ group showed significant decrease from 60 to 120 min, compared to saline/CCl₄ (p<0.05). These results indicate that HMSC infusion is effective on glucose intolerance and insulin resistance caused by liver cirrhosis.

Insulin signaling activity after HMSCs infusion in liver cirrhotic rats. To investigate the possible involvement of HMSCs on insulin-regulated signaling molecules in CCl₄-induced liver cirrhotic rats, we assessed activity of PI 3-kinase and expressions of PKB and PKC- using PI 3-kinase assay/ Western blotting, respectively. As shown in Fig. 4, phosphatidylinositol-3-phosphate (PI-3-P), which is formed by PI 3-kinase activity, was markedly decreased in saline/CCl₄, compared to saline/olive $(1.3\pm0.31, p<0.05)$. However, it was obviously increased in HMSCs/CCl₄, compared to saline/CCl₄ after HMSC infusion (3.2±0.23, p<0.05). The phosphorylation of PKB and PKC-2, downstream of PI 3-kinase, also were decreased in saline/CCl₄, whereas phosphorylation of these was significantly increased in HMSCs/CCl₄ (Fig 5, p<0.05). We also investigated whether HMSC infusion influenced the expression of GSK-3 in CCl₄-induced liver cirrhotic rats. As a result, the phosphorylation level of GSK-3 in HMSCs/CCl₄ was increased >30% after HMSC infusion, compare to saline/ CCl₄ (p<0.05).

Hepatic gluconeogenic enzymes after HMSC infusion in liver cirrhotic rats. Liver plays a prominent role in controlling blood glucose levels through hepatic glucose production. Thus, we investigated the expression of G-6-P and PEPCK,

	Groups							
Parameter	Saline/olive	HMSCs/olive	Saline/CCl ₄	HMSCs/CCl ₄				
ALT (Karmen/ml)	49.12±8.01	48.16±7.51	300.17±18.12ª	194.33±8.41 ^b				
AST (Karmen/ml)	44.17±9.23	47.28±6.35	350.21±36.39ª	260.14±18.16 ^b				
Albumin (g/dl)	3.42±0.07	3.52±0.09	2.24±0.12 ^a	3.03±0.14 ^b				
Total protein (g/dl)	7.13±0.16	6.91±0.13	4.48 ± 0.18^{a}	5.92±0.24 ^b				
ALP (U/l)	174.23±22.43	181.66±33.21	736.61±62.43ª	399.88±51.36 ^b				
Total bilirubin (mg/dl)	0.21±0.03	0.24±0.03	2.84±0.25 ^a	1.75 ± 0.14^{b}				
Hyaluronic acid (ng/ml)	46.25±2.11	44.31±2.27	568.42±25.11ª	267.91±28.22 ^b				
Laminin (µg/ml)	137.91±30.32	138.63 ± 20.58	780.01±39.11ª	359.24±50.77 ^b				

Table I.	Effects	of HMSC	ls on s	serum	parameters	in rat	CC	l₄-indu	ced l	iver	cirrhosis.
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All biomarkers in CCl_4 -induced group were improved after HMSCs infusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkakine phosphatase. Values are expressed as mean \pm SD. ^ap<0.05 vs. saline/olive; and ^bp<0.05 vs. saline/CCl₄, respectively.



Figure 4. Effect of HMSCs on phosphatidylinositol 3-kinase (PI 3-kinase) activity in rat with CCl₄-induced liver cirrhosis. PI 3-kinase activity was measured in anti-phosphotyrosine immunocomplex. PI-3-P, phosphatidylinositol-3-phosphate. Values are expressed as the mean \pm SD *p<0.05 vs. saline/CCl₄.

which are rapidly inhibited at the transcriptional level by insulin in CCl₄-induced liver cirrhotic rats (30). As expected, the expression of G-6-P and PEPCK in saline/CCl₄ was increased (Fig. 6). However, the expressions of G-6-P and PEPCK after HMSC infusion was remarkably decreased, compared to saline/CCl₄ (0.45 \pm 0.04 and 0.39 \pm 0.01 respectively, p<0.05). The results demonstrated that HMSCs decreased gluconeogenesis in CCl₄-induced liver cirrhosis.

Discussion

This is the first study to show that HMSCs can improve insulin resistance and glucose intolerence in CCl₄-induced liver cirrhotic rat model. Our results demonstrated that HMSCs inhibited hepatic gluconeogenesis by suppressing both G-6-P and PEPCK expressions and improve insulin resistance by increasing expressions of PI 3-kinase and PKB.

It is well established that cirrhotic subjects are glucose intolerant (31): 60-80% display impaired glucose tolerance,



Figure 5. Effect of HMSCs on the phosphorylation of protein kinase B (p-PKB), protein kinase C- ζ (p-PKC- ζ), and glycogen synthase kinase 3 (p-GSK-3) by Western blotting. Quantification of p-PKB, p-PKC- ζ , and p-GSK-3 expressions was performed by densitometry analysis. Data are the mean \pm SD of p-PKB/ β -actin, p-PKC- ζ/β -actin, and p-GSK-3/ β -actin at least three separate experiments. *p<0.05 vs. saline/CCl₄.



Figure 6. Effect of HMSCs on expressions of glucose-6-phosphatase (G-6-P) and phosphoenolpyruvate carboxykinase (PEPCK) by Western blotting. Quantification of G-6-P and PEPCK expression was performed by densitometry analysis. Data are the mean \pm SD of G-6-P/ β -actin PEPCK/ β -actin at least three separate experiments. *p<0.05 vs. saline/CCl₄.

and 10-30% are anticipated to develop diabetes (32). Moreover, cirrhotic patients have insulin resistance because a rise of plasma glucose entails an increase of insulin secretion (33,34). Insulin resistance has been defined as a subnormal biological response to a normal concentration of insulin (35). So to determine if cirrhotic animals were insulin resistant, we examined serum glucose and insulin concentration. High glucose and insulin levels were noted in CCl₄-induced liver cirrhotic rats. However, the levels of glucose and insulin were decreased in cirrhosis rats after HMSCs infusion. This is similar to results of Hess et al reporting that bone-marrow derived stem cell improved glucose tolerance by reducing glucose levels in steptozotocininduced hyperglycemic mice (36). Furthermore, to identify whether HMSCs improve the insulin resistance associated with liver cirrhosis, we assessed the expression of proximal part of the insulin signaling cascade. Glucose is mediated by IRS-1, PI-3 kinase, and other molecules. The IRS-1 protein, once phosphorylated at distinct tyrosine residues, acts as docking site for PI-3kinase. PKB and PKC-ζ is PI-3 kinase downstream molecules mediated insulin-stimulated glucose (37). In our study, it was observed that HMSCs increased the phosphorylation of PKB and PKC-ζ by activating PI 3-kinase. It is known that phosphorylated PKB induces phosphorylation and inactivation of GSK-3. Hence, phosphorylation and inactivation of GSK-3 results in activation of glycogen synthase and increased glycogen synthesis. Although we did not measure glycogen synthesis in the liver, our results of Western blotting showed that the phosphorylation of GSK-3 is significantly increased in CCl₄-induced cirrhotic rats after HMSCs infusion, compared with saline/CCl₄. Thus, these results revealed that HMSCs had a possibility to improve insulin resistance by phosphorylating and inactivating GSK-3 in CCl₄-induced cirrhotic rats.

In addition, we focused on pathways controlling hepatic glucose production. Increased gluconeogenesis is a main source of increased hepatic glucose production and the inability of insulin to regulate the rate-controlling gluconeogenic enzymes may contribute to this problem. Representative two insulin-responsive molecules for regulation of hepatic gluconeogenesis are G-6-P and PEPCK (9,38). G-6-P is a key enzyme in systemic glucose homeostasis, because it catalyzes the last biochemical reaction of glucose synthesis (39). In streptozotocin-induced

diabetes animal models, hepatic G-6-P enzyme activity, protein and mRNA expression are increased about 4-fold (40). Also, it has been reported that the over-expression of PEPCK, which is a rate-determining step in gluconeogenesis, increased hepatic glucose in diabetic animal model (41). In our present study, the expression of PEPCK and G-6-P were reduced up to approximately 40 and 50% after HMSCs infusion in CCl₄-induced liver cirrhosis group, respectively. In this regard, our results indicated that HMSCs were involved in gluconeogenesis by regulating expression of PEPCK and G-6-P in CCl₄-induced liver cirrhotic rats.

In conclusion, we demonstrated that HMSCs improve insulin resistance by increasing PI 3-kinase, PKB, PKC- ζ , and GSK-3 and alleviated gluconeogenesis by decreasing the expression of G-6-P and PEPCK in CCl₄-induced liver cirrhosis rats. Our study suggests that HMSCs might be a valuable therapeutic tool in the treatment of insulin resistance caused by liver cirrhosis.

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