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Therapeutic potential of conditioned medium derived from bone marrow mesenchymal stromal cells cocultured with hepatocytes in alleviation of CCl_4-induced liver damage in mice

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Summary

Mesenchymal stromal cell-derived conditioned medium therapy is a rapidly developing field of research that seems to have a significant impact on the treatment of various diseases. In the present study, the therapeutic effect of conditioned medium derived from bone marrow mesenchymal stromal cells (BMSCs) co-cultured with hepatocytes (H/BMSCs CM) was investigated for alleviation of CCl_4-induced liver damage in mice.

Materials and methods

Hepatocytes were co-cultured with BMSCs for 20 h. H/BMSCs CM were then collected. Mice were intraperitoneally injected with CCl_4 twice a week for 7 weeks, followed by injection of H/BMSCs CM. Four weeks after the CM treatment, serum levels of ALT, AST, alkaline phosphatase (ALP), and albumin were determined, liver histopathology was examined, and ELISA assay was performed to measure the serum levels of IL-4 and IFN-γ.

Results

The CM-treated group demonstrated marked decrease in serum levels of ALT and AST compared to RPMI group, without significant differences in the levels of alkaline phosphatase and albumin. Histopathological study demonstrated noticeable improvement in CM treatment group. CM treatment caused an increase in IL-4 production compared to RPMI group, and did not affect the release of IFN-γ.

Our results have shown that H/BMSCs CM treatment attenuates CCl_4-induced injury. Further studies should be carried out to elucidate the mechanism of action in order to achieve intended therapeutic effect which can be translated and optimized.

Keywords

Mesenchymal stromal cells, bone marrow, hepatocytes, co-culture, conditioned medium, liver damage.

Introduction

Following their discovery over 50 years ago, mesenchymal stromal cells (MSCs) have become one of the most studied cellular therapeutic products [1]. A large number of research have shown the beneficial effects of MSCs-based therapies to treat different diseases [2]. Several mechanisms have been proposed to explain the therapeutic effect of mesenchymal stromal cells in repairing damaged tissues.

Yet, many reports do not support the MSCs differentiation to replace injured tissue [3]. Various studies on growth factors derived from mesenchymal stromal cells have shown that the secreted factor alone without the mesenchymal stromal cell itself may cause tissue repair under various conditions including tissue/organ damage [4]. The medium in which mesenchymal stromal cells are cultured and secrete different growth factors and cytokines is called conditioned medium (CM). CM is a promising alternative that can overcome the poor engraftment of the transplanted mesenchymal stromal cells and potential risk of cancer development [5]. It may present a better option in the field of the future regenerative medicine [6]. As compared to cell-based therapies, MSC-CM (non-cell-based) therapies are generally preferred because they are less likely to trigger immune response, thus suggesting their safer usage. They are also more amenable to reformulation to support different routes of administration [7]. In addition, preparation of CM is more economical since it can be produced at large scale from available MSC populations under current good manufacturing practice (cGMP) conditions. Moreover, CM can be stored for a relatively long period without any toxic cryoprotectants, such as dimethyl sulfoxide (DMSO) [6]. Thus, it may be manufactured, freeze-dried, packaged, and transported more easily than mesenchymal stromal cells [5]. However, CM should be administered more frequently because the half-life of cytokines and growth factors are mostly shorter [8]. Therefore, recent studies have used the secreted factors rather than direct application of MSCs [9].

The present study aimed to investigate therapeutic effect of conditioned medium derived from bone marrow mesenchymal stromal cells co-cultured with hepatocytes in alleviation of CCl_4-induced liver damage in mice.

Materials and methods

Animals

Healthy male Balb/c mice (6-8 weeks) were housed in plastic cages, in the room with controlled temperature (24±2°C) and light regimen (12:12 h light/dark cycle), provided with food and water *ad libitum*. All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals and the ethical standards of our institution.

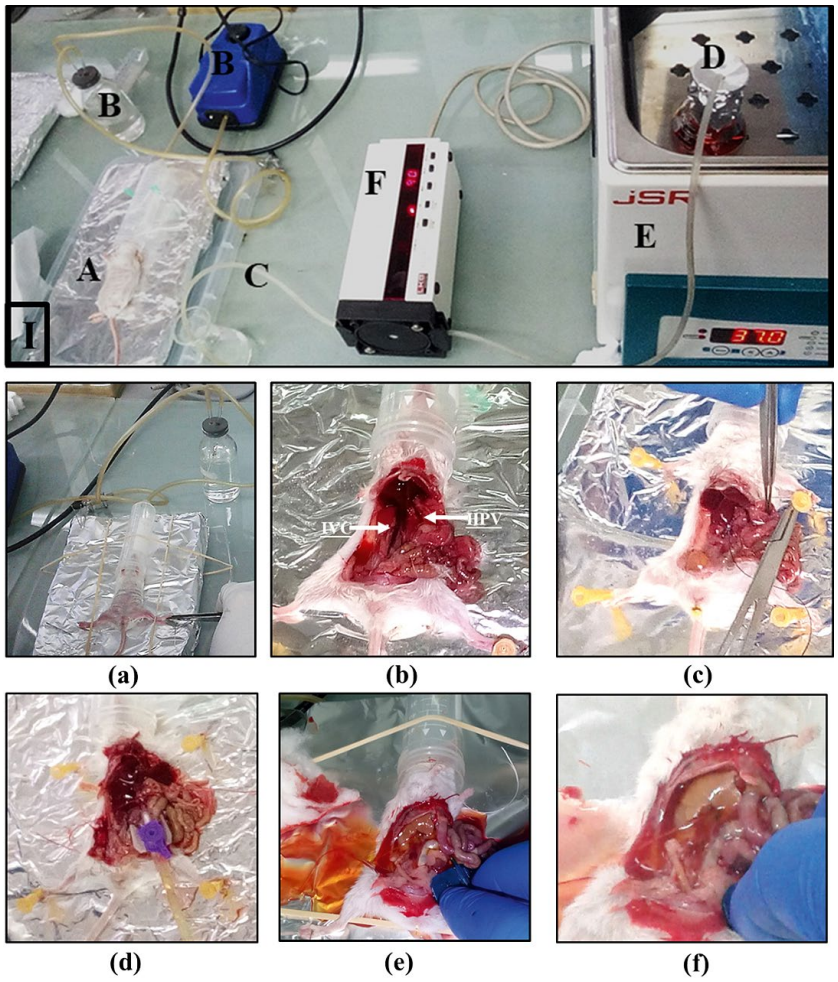

Figure 1. Mouse hepatocytes isolation (perfusion method). (I): Pictorial representation of perfusion system setup (A: surgical table, B: anesthesia machine, C: perfusion tube, D: perfusion solution, E: water bath for warming perfusion media, F: peristaltic pump), (a): Gas anesthesia with Isoflurane. (b): The abdomen was opened, exposing the hepatic portal vein (HPV) and the inferior vena cava (IVC). (c): Silk ties were used to secure the catheter. (d): IV catheter inserted into the portal vein, and connected to the perfusion tube (e): The vena cava was cut for blood/fluid drainage. (f): The liver should blanch while the blood is flushed out.

Hepatocyte isolation

Hepatocytes were isolated using a modified two-step ethylenediaminetetraacetic acid (EDTA)/collagenase protocol, according to [10] with slight modifications. The mouse was deeply anesthetized with Isoflurane (Fig. 1). After sterilizing the skin, the abdomen was opened *via* an upper abdominal transverse incision, and the intestines were pushed to the left of the animal's torso, to expose the hepatic portal vein (HPV) and the inferior *vena cava* (IVC). A sterile cannula was inserted into the portal vein for the antegrade perfusion. The cannula was secured using 6-0 silk ties and then was connected to the perfusate tube, avoiding introduction of air. The flow rate (5 mL/min), was controlled by a peristaltic pump. The liver was first perfused with calcium and magnesium-free Hank's balanced salt solution (Sigma-Aldrich, USA), containing 0.5 mM EDTA at 5 mL/min for 7 min. Once the solution begins to perfuse the liver, the *vena cava* was cut for blood/fluid drainage. The liver was then perfused with calcium and magnesium-free Hank's balanced salt solution, containing 1 mg/mL collagenase (Sigma-Aldrich, USA), and 5 mmol/L $CaCl_2$ at 5 mL/min for 7 min.

All the perfusion solutions were pre-warmed and maintained at 37°C. The perfusion was considered successful when the whole organ was completely blanched and the endpoint when the tissue was visibly digested and the capsule started to separate from the organ surface. The liver was removed and placed in a petri dish with RPMI (37°C), and the Glisson's capsule enclosing the liver was carefully separated using a pair of forceps, in order to disperse the hepatocytes. The resulting cell suspension was passed through a 65-µm nylon mesh to remove cell clumps. The suspension was then centrifuged at 1000 rpm for 2-3 min. The supernatant was discarded, and the remaining cell suspension was washed three times with RPMI. The hepatocyte pellet was gently resuspended in RPMI, and the viability was determined by trypan blue exclusion method.

Co-culture of mouse BMSCs and hepatocytes

BMSCs were isolated from the tibias and femurs of intact BALB/c mice using flushing method. For co-culture of BMSCs and hepatocytes, 1.5×10^6 BMSCs (at passage 0 (P0)) were cultured in serum-free RPMI medium in order to exclude possible effects on liver regeneration caused by fetal bovine serum (FBS), and incubated at 37°C with 5% humidified CO_2 for 4 h. Once BMSCs were attached to the flask, the culture medium was discarded and 1.5×10^6 of freshly isolated hepatocytes were added to the culture.

Following ca. 20 hours of hepatocyte-BMSCs co-culture, the conditioned medium was collected and centrifuged at 1000 rpm for 10 min, then filtered (0.22 µm pore size) and kept at -80°C until use.

Experimental Protocol

The model liver injury was induced by CCl_4. The mice were injected intraperitoneally with CCl_4 (1 mL/kg body weight) dissolved in olive oil, twice a week for 7 weeks. Then, the mice were injected into the tail vein with 0.2 ml of conditioned medium (CM) from the co-culture of hepatocytes and BMSC (CM group), or with (0.2 ml) of serum-free RPMI medium as vehicle control (RPMI group). Four weeks after CM treatment, all animals were sacrificed, and their livers and venous blood were collected.

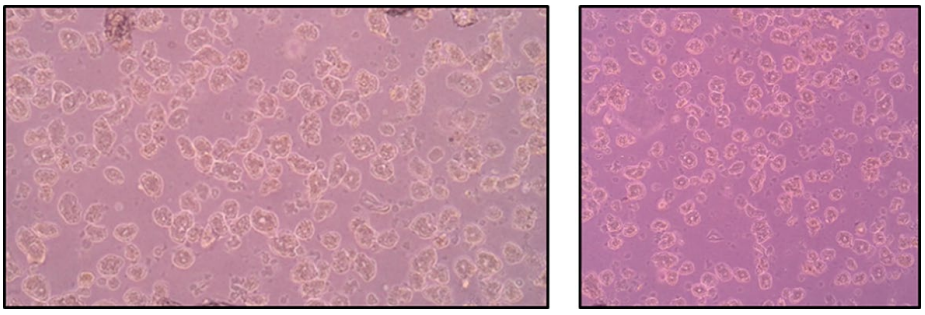

Figure 2. Light-microscopic pictures of cultured mouse hepatocytes. Hepatocytes show a cubic morphology and a clear and distinct nucleus. Some bi-nucleated cells were found

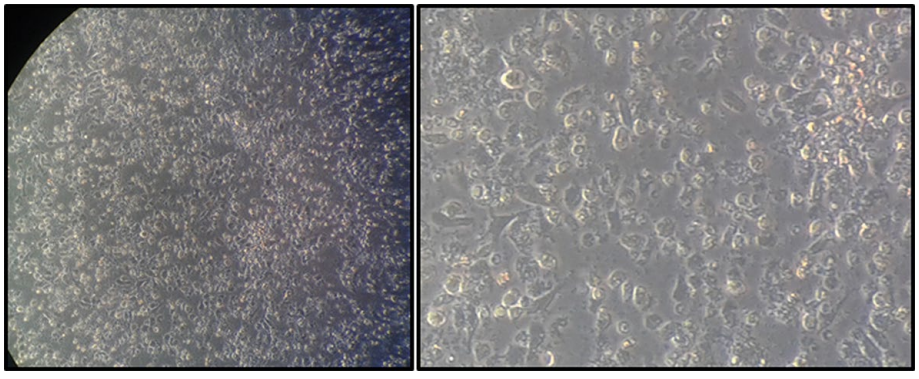

Figure 3. Light-microscopic pictures of mouse of BMSC/hepatocytes co-culture

The body mass and liver weight in the mice were determined at the end of the experiment. The liver-to-body weight ratio was calculated as follows: Liver-to-body ratio = (liver weight (g)/(mouse body weight (g)-liver weight (g) *100.

Biochemical indices, cytokines and histopathology

At the time of sacrificing, venous blood samples were collected and centrifuged at 3000 rpm for 5 minutes to separate blood serum. The levels of albumin, alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in the serum were detected. Serum interleukin-4 (IL-4) and IFN-γ levels were detected by ELISA kits (Sun Red, China) according to the manufacturer's instructions.

Liver tissue samples were collected and fixed in 10% formalin. The specimens were then processed to form paraffin sections and stained with hematoxylin and eosin (H&E). The sections were examined for histopathological changes under light microscope.

Statistical analysis

All values were presented as mean ± SD. The statistical difference was analyzed using t-test for independent samples (SPSS 17 software). $P<0.05$ was considered statistically significant.

Results

Isolation and culture of hepatocytes

The yield of viable hepatocytes was (83.46×10^6), and their viability was 78%. Isolated hepatocytes were examined under an inverted microscope (Fig. 2). Freshly isolated hepatocytes were round-shaped and suffered from stress, presumably, due to the isolation process. With time, the cells gradually recovered from the stress. 24 hours later, the hepatocytes showed a cubic morphology that resembles the organization of liver tissue, with clear and distinct nuclei (some of them are bi-nucleated).

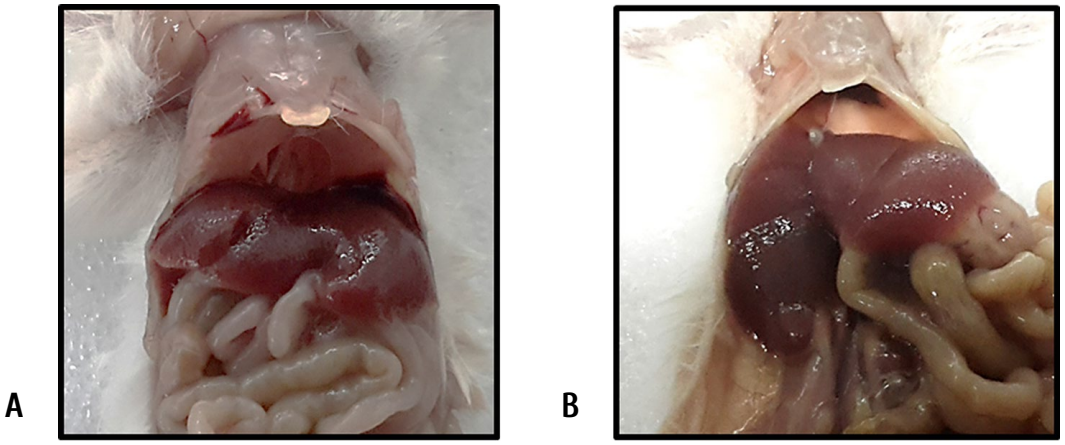

Figure 4. Morphologic examination of the livers. (A) In the RPMI group, the liver had irregular surface with necrotic spots and showed pale brown color. (B) In the mice receiving CM-treatment, the liver surface was slightly coarse, less swollen, more reddish, and lustrous than those of the RPMI group

Table 1. Body weights, liver weights and liver-to-body weight ratios of the studied groups

	Liver Weight (g)	Body Weight (g)	Body Weight – Liver Weight	Liver to body Ratio
RPMI group	1.49±0.08[a]	22.8±0.9[a]	21.3±0.9[a]	7.02±0.6[a]
CM group	1.37±0.05[b]	21.3±1.22[b]	20±1.2[b]	6.8±0.3[a]

All values are represented as mean ± SD. Mean values with different letters over the same column are significantly different at ($P<0.05$).

Table 2. Serum levels of ALT, AST, ALP, and albumin in the studied groups

	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dl)
RPMI group	55.12±12.41[a]	111.2±12.2[a]	151.2±8[a]	2.47±0.1[a]
CM group	47.46±7.3[b]	87.3±2.2[b]	153.5±10[a]	2.26±0.3[a]

All values are represented as mean ± SD. Mean values with different letters over the same column are significantly different at ($P<0.05$).

Distribution of murine BMSCs and hepatocytes in co-culture

The hepatocytes in co-culture appeared in small cell clusters, with the presence of BMSCs intermingled throughout the hepatocyte culture (Fig. 3). Most BMSCs attached to the culture flask surface, and hepatocytes attached to the BMSCs layer.

Effects of CM treatment on liver and body weight

The results of this study showed non-significant differences in the liver weight to body weight ratio in CM treated group compared to the RPMI group (Table 1).

Biochemical serum indexes

CM treated group showed significantly decreased serum levels of ALT and AST compared to RPMI group. The levels of albumin and ALP were not significantly different (Table 2).

Histopathology

The liver in the RPMI-treated group exposed to CCl_4 demonstrated irregular surface upon morphologic examination. They had rough surface with necrotic spots. The livers were slightly swollen, and showed decreased glossiness, with a pale brown color. In the mice receiving CM treatment, the surface of the liver was slightly rough, less swollen, more reddish, and lustrous than in those from the RPMI group. Necrotic spots on the surface were noticeably reduced (Fig. 4).

The results of histological analysis are shown in Fig. 5. In the CCl_4-exposed RPMI group, the livers showed loss of normal architecture. The cord-like arrangement of hepatocytes was disrupted. The main histopathologic findings were marked hepatocellular necrosis, hepatocytes were swollen with vacuolated cytoplasm. In some specimens we observed severe infiltration with inflammatory cells, vascular dilatation and congestion. Dilated blood sinusoids were also observed in some parts.

These histopathological changes were remarkably alleviated in histological sections of CM-treated group. The sections showed nearly normal organization of hepatic lobules. The cord-like arrangement of hepatocytes was restored, as well as minimal disruption of hepatic cellular structure, well-preserved cytoplasm, reduced inflammation with low presence of inflammatory cells.

Detection of cytokines

Serum IL-4 and IFN-γ levels were detected by ELISA. IL-4 level considerably increased in CM treated group compared with the RPMI group. CM treatment did not affect the level of IFN-γ compared to RPMI group (Fig. 6).

Discussion

Bone marrow mesenchymal stromal cells MSC(M) were isolated, cultured, and characterized in our previous study [11].

In the current study, the conditioned medium was prepared by co-culture of hepatocytes with BMSCs at passage 0 (P0 BMSCs), in order to avoid the decline in cell functions with further passaging [12].

Various methods have been employed in attempt to isolate hepatocytes. These include mechanical, chemical, and enzymatic methods [13]. In this study, two-step EDTA/collagenase protocol was used to isolate hepatocytes. Collagenase perfusion of the liver to obtain hepatocytes has been performed since the early 1950s and has been permanently improved [14]. In this study, the liver was perfused *via* the portal vein instead of *vena cava* due to its ease of access within abdomen and that this vein feeds directly into the liver [14]. The whole isolation procedure, from the animal anesthesia to cell seeding, was completed as quickly as possible (in 40-50 min) to obtain sufficient number of viable cells, and we obtained a high cellular yield with good viability.

At the first step, removal of calcium ions by EDTA from epithelial cells is essential to disrupt the Ca^{2+}dependent E-cadherin molecules between adjacent hepatocytes which results in rapid destruction of intercellular junctions resulting in loss of cell-to-cell contacts, and thus improving quantity and quality of the dispersed hepatocytes. At the second step, we introduced collagenase into the liver lobes thus causing disruption of the supporting extracellular matrix. Ca^{2+} is added to the perfusion medium with collagenase, since the presence of Ca^{2+} is required for enzymatic activity of collagenase during the perfusion [15]. Low-speed centrifugation was used to isolate hepatocytes since the viable hepatocytes have higher density and can be easily purified from the non-parenchymal cells and dead hepatocytes [14].

Liver diseases affect approximately 17.5% of the population. According to World Health Organization statistics, more than a hundred million people worldwide are suffering from liver disease [16]. The limited half-life of transplanted cells, potential tumorigenic risk, and other risks of MSCs have led to the further development of acellular therapies [17]. Therapy with conditioned medium derived from mesenchymal stromal cells is a rapidly advancing field that may exert substantial impact on the treatment of different diseases/conditions [5]. Various mesenchymal stromal cell-derived conditioned media were produced by different approaches and processing, and tested in various diseases, mostly showing good results [4]. Hepatocytes and mesenchymal stromal cells (MSCs) are attractive sources of cell-based therapies for the liver diseases. The co-transplantation of hepatocytes and MSCs may improve therapeutic efficiency for the treatment of liver injury. In this study, we have tested the synergistic effect of the factors or secretome produced by hepatocytes and BMSCs in enhancing the regenerative capacity of liver.

To induce liver injury in mice, CCl_4 was used. Carbon tetrachloride is one of the most commonly used hepatotoxins as an experimental model of liver disease [18]. CCl_4 is metabolized in the liver *via* mitochondrial cytochrome P450 (CYP450), and the resulting free radicals damage the lipid membranes of hepatocytes by lipid peroxidation, thus leading to the release of cellular contents into the extracellular matrix (ECM), which generates a myriad of inflammatory signals in the liver. High level of inflammation leads to apoptosis and further liver damage [19].

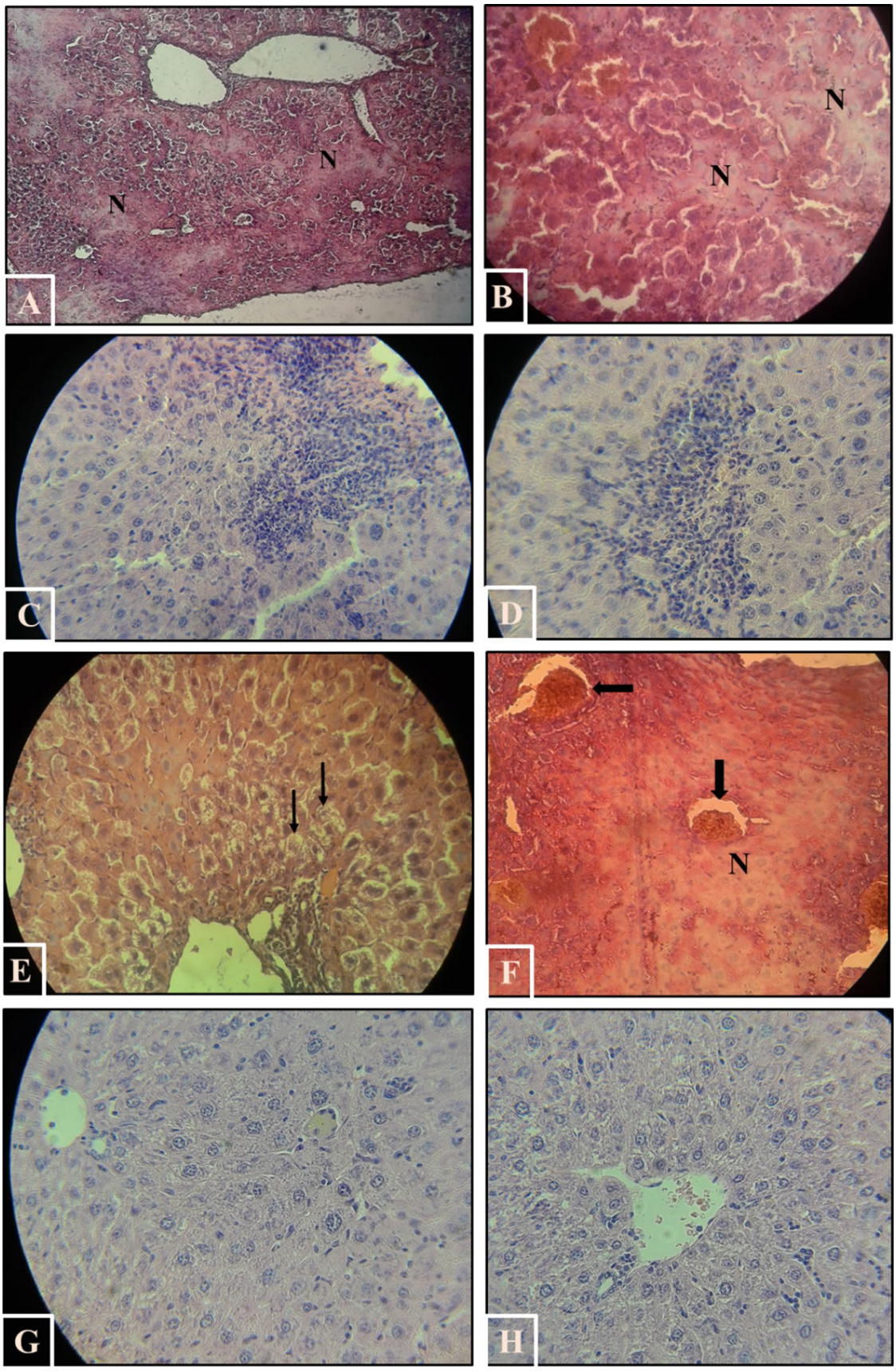

Figure 5. H&E-stained sections in the livers of the experimental groups. (A, F). RPMI group. A-B. Marked affection with disorganization of hepatic architecture, hepatocytes necrosis (N) can be seen. C, D. severe infiltration of inflammatory cells. E. hepatocytes with vacuolated cytoplasm (arrows). F. Vascular congestion (arrows). (G, H). CM treated group. Nearly normal organization of hepatic lobules. Hepatocytes appeared nearly similar to that of the normal mouse.

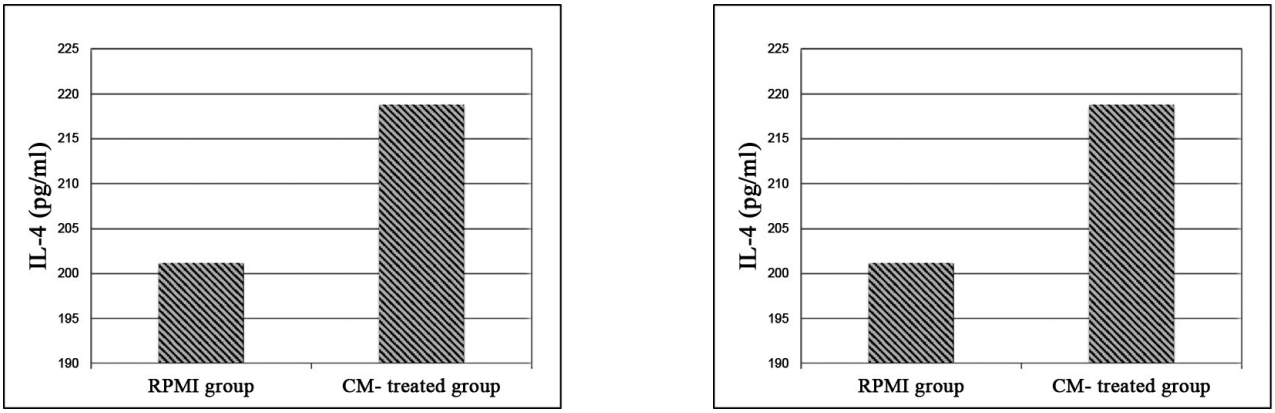

Figure 6. Serum levels of IL-4 (pg/ml), and IFN-γ (pg/ml) cytokines. Serum were collected one month after CM treatment, and cytokines were measured with ELISA assay

Our results showed a marked decrease in the levels of ALT and AST enzymes in the CM-treated group compared to the RPMI group (controls). Serum ALT and AST are usually elevated in liver damage [20]. ALT and AST are enzymes basically located within hepatocytes. Hence, when liver cells are damaged or die, the transaminases are released into bloodstream, where they can be measured. Therefore, their levels reflect the degree of liver injury [18]. However, the decrease in ALT and AST levels after CM treatment suggests that H/BMSCs CM may promote regenerative capacity of the liver and improve injured liver tissue. On the other hand, no improvement was observed in hepatocyte functions in terms of albumin secretion.

The therapeutic effect of CM in the liver damage caused by CCl_4 was evident by comparing the histological sections of the liver in the studied groups. Histological study results were in consistent with biochemical results (alleviation of Alt and AST levels). The liver in the RPMI group demonstrated irregular morphology at morphologic examination. Microscopical examination also showed several histopathological changes induced by CCl_4. These results supported that 7 weeks of CCl_4 administration clearly altered the liver structure and health, causing liver damage and changes to macroscopic pattern of the mouse liver. A considerable improvement in external morphology and histopathological changes and in the hepatic lobular architecture was observed after CM treatment of mice.

Serum IFN-γ and IL-4 levels were detected by ELISA. The serum IL-4 level considerably increased after CM treatment. This result proves the therapeutic effect of H/BMSCs CM. IL-4 is one of the widely acknowledged immune regulatory cytokines able to suppress inflammation [21]. This cytokine commonly represents Th2 responses [22]. However, the results indicated that CM treatment did not affect the release of IFN-γ.

In summary, H/BMSCs CM treatment was effective in alleviation of CCl_4-induced liver damage in mice. This treatment may present a novel adjunctive therapy in drug-induced liver toxicity. CM is mediating this effect, probably, through maintaining the liver homeostasis which primarily includes induction of hepatocyte regeneration [7].

It is shown that MSC-conditioned media (MSC-CM) contain a variety of cytokines, chemokines, and growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), angiopoietin-1, erythropoietin, and stromal derived factor-1 (SDF-1), which have multiple positive impacts on tissue regeneration [3]. The liver trophic factors secreted by MSCs, in particular, hepatocyte growth factor (HGF) could be the crucial player in liver regeneration [9].

Hepatocytes also secrete various cytokines that modulate both hepatocyte metabolism and liver inflammation [23]. Previous study identified over 200 proteins in the conditioned medium of rat hepatocyte including plasma, extracellular, and intracellular proteins [24]. Another report identified 691 secreted proteins in the conditioned medium of primary human hepatocytes, such as alpha-1-antitrypsin, alpha-1-antichymotrypsin, alpha-1-acid glycoprotein 1, and albumin. Different factors in CM derived from hypatocytes cocultured with BMSCs act together to promote regeneration [25]. Previous study showed that the culture of human hepatocytes (HCs) in medium previously conditioned by cocultured HCs and MSCs demonstrated improved function, whereas no effect was observed when HCs cultured in medium conditioned by MSCs alone, which indicates that the presence of HCs is needed to stimulate MSCs to produce the relevant factors/cytokines in the culture medium [26].

Further studies are required to evaluate efficacy and safety of CM therapy, and to determine optimal cell source, culture conditions, duration of medium conditioning, the optimal injection route and dosage which can profoundly affect clinical outcome, and to elucidate the exact mechanism of action. Hence, the intended therapeutic effect may be translated and optimized, in order to develop standardized methods for production of various conditioned media and validation of their usage in various diseases.

Conclusion

Our results indicate that H/BMSCs CM is effective in stimulating liver regeneration after CCl_4-induced injury. Despite great number of promising results with mesenchymal stromal cell-conditioned media, and the need for efficient

treatment of the patients suffering from liver diseases, the use of CM in human clinical trials needs more studies to analyze and characterize the cytokines and growth factors secreted in CM in order to understand the effects of these compositions on different body tissues, and to make sure that this CM does not have the potential to cause severe adverse effects in humans. We also need to try CM of different mesenchymal stromal cells to achieve maximal healing effect in different diseases. We hope that this research will stimulate and encourage such efforts towards harnessing the therapeutic potential of the CM.

Conflict of interest

None declared.

Acknowledgments

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References

1. Wright A, Arthaud-day ML, Weiss ML. Therapeutic Use of Mesenchymal Stromal Cells: The Need for Inclusive Characterization Guidelines to Accommodate All Tissue Sources and Species, Front. Cell Dev. Biol, 9, 1-18 (2021).

2. Hmadcha A, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V. Therapeutic Potential of Mesenchymal Stem Cells for Cancer Therapy, Front. Bioeng. Biotechnol, 8(43), 1-13 (2020).

3. Saheli M, Bayat M, Ganji R, Hendudari F, Kheirjou R, Pakzad M, Najar B, Piryaei A. Human mesenchymal stem cells-conditioned medium improves diabetic wound healing mainly through modulating fibroblast behaviors. Arch Dermatol Res. 2020; 312(5):325-336. doi: 10.1007/s00403-019-02016-6

4. Pawitan JA. Prospect of stem cell conditioned medium in regenerative medicine. Biomed Res Int. 2014;2014:965849. doi: 10.1155/2014/965849

5. Mehling BM, Manvelyan M, Benesh G, Wu DC. Characterization of human umbilical cord mesenchymal stem cells-derived conditioned medium. J. Stem Cell Res. Ther. 2016; 1(6): 218-220. doi: 10.15406/jsrt.2016.01.00038

6. Pokrovskaya LA, Zubareva EV, Nadezhdin SV, Lysenko AS, Litovkina TI. Biological activity of mesenchymal stem cells secretome as a basis for cell-free therapeutic approach. Res Results Pharmacol. 2020; 6, 57-68. doi: 10.3897/rrpharmacology.6.49413

7. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther. 2014; 5(3):76. doi: 10.1186/scrt465. PMID: 24915963

8. Aal SA, Abdelrahman SA, Raafat N. Comparative therapeutic effects of mesenchymal stem cells *versus* their conditioned media in alleviation of CCL_4-induced liver fibrosis in rats: Histological and biochemical study. JMH. 2019; 3(1):1-20. doi: 10.21608/jmh.2019.10310.1051

9. Azhdari Tafti Z, Mahmoodi M, Hajizadeh MR, Ezzatizadeh V, Baharvand H, Vosough M, Piryaei A. Conditioned media derived from human adipose tissue mesenchymal stromal cells improves primary hepatocyte maintenance. Cell J. 2018; 20(3):377-387. doi: 10.22074/cellj.2018.5288

10. Weglarz TC, Degen JL, Sandgren EP. Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. Am J Pathol. 2000; 157(6):1963-1974. doi: 10.1016/S0002-9440(10)64835-3

11. Joujeh D, Ghrewaty A, Soukkarieh C, Almarrawi A and Darwicha J. An optimized protocol for mouse bone marrow mesenchymal stromal cells isolation and culture. Cell Ther Transplant,10(3-4): 61-70 (2021).

12. Jiang T, Xu G, Wang Q, Yang L, Zheng L, Zhao J, Zhang X. *In vitro* expansion impaired the stemness of early passage mesenchymal stem cells for treatment of cartilage defects. Cell Death Dis. 2017; 8:2851. doi: 10.1038/cddis.2017.215

13. Lee D, Lee K. Hepatocyte isolation, culture, and its clinical applications. Hanyang Med Rev 2014; 34(4):165-172. doi: 10.7599/hmr.2014.34.4.165

14. Cabral F, Miller CM, Kudrna KM, Hass BE, Daubendiek JG, Kellar BM, Harris EN. Purification of hepatocytes and sinusoidal endothelial cells from mouse liver perfusion. J Vis Exp. 2018 Feb 12;(132):56993. doi: 10.3791/56993

15. Li WC, Ralphs KL, Tosh D. Isolation and culture of adult mouse hepatocytes. Methods Mol Biol. 2010; 633:185-196. doi: 10.1007/978-1-59745-019-5_13

16. Sarvandi SS, Joghataei MT, Parivar K, Khosravi M, Sarveazad A, Sanadgol N. *In vitro* differentiation of rat mesenchymal stem cells to hepatocyte lineage. Iran J Basic Med Sci. 18(1), 89-97. PMID: 25810881

17. Driscoll J, Patel T. The mesenchymal stem cell secretome as an acellular regenerative therapy for liver disease. J Gastroenterol. 2019; 54(9):763-773. doi: 10.1007/s00535-019-01599-1

18. Moharib M, Hammam O, Salman F, El-naggar M, Sherif S. Transplantation of Modified and Fresh Hepatocyte Reduces Hepatotoxicity Induced by Carbon Tetrachloride. Life Sci J. 2014; 11(8): 641-652.

19. Mishra AP, Siva AB, Gurunathan C, Komala Y, Lakshmi BJ. Impaired liver regeneration and lipid homeostasis in CCl_4 treated WDR13 deficient mice. Lab Anim Res. 2020;36(1):41. doi: 10.1186/s42826-020-00076-8

20. Gaim K, Gebru G, Abba S. The effect of arsenic on liver tissue of experimental animals (fishes and mice) – A review article. Int J Sci Res Publ. 2015; 5(1), 1-9.

21. Ma K, Gong T, Ao Q, Yan Y, Song B, Huang H, et al. Intracerebral transplantation of adipose-derived mesenchymal stem cells alternatively activates microglia and ameliorates neuropathological deficits in Alzheimer' s disease mice. Cell Transplant. 2013; 22(1):113-126. doi: 10.3727/096368913X672181

22. Njoku DB. Suppressive and pro-inflammatory roles for IL-4 in the pathogenesis of experimental drug-induced liver injury: a review. Expert Opin Drug Metab Toxicol. 2010; 6(5):519-531. doi: 10.1517/17425251003601979

23. Sheng L, Jiang B, Rui L. Intracellular lipid content is a key intrinsic determinant for hepatocyte viability and metabolic and inflammatory states in mice. Am J Physiol Endocrinol Metab. 2013; 305(9):E1115-23. doi: 10.1152/ajpendo.00401.2013

24. Farkas D, Bhat VB, Mandapati S, Wishnok JS, Tannenbaum SR. Characterization of the secreted proteome of rat hepatocytes cultured in collagen sandwiches. Chem Res Toxicol. 2005; 18(7):1132-1139. doi: 10.1021/tx0500225

25. Franko A, Hartwig S, Kotzka J, Ruoß M, Nüssler AK, Königsrainer A, et al. Identification of the secreted proteins originated from primary human hepatocytes and HepG2 cells. Nutrients. 2019; 11(8):1795. doi: 10.3390/nu11081795

26. Fitzpatrick E, Wu Y, Dhadda P, Hughes RD, Mitry RR, Qin H, et al. Coculture with mesenchymal stem cells results in improved viability and function of human hepatocytes. Cell Transplant. 2015; 24(1):73-83. doi: 10.3727/096368913X674080

Терапевтический потенциал среды, кондиционированной стволовыми клетками костного мозга для ослабления повреждения печени у мышей, вызванного CCl_4

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Резюме

Терапия с применением сред, кондиционированных стволовыми клетками, является быстро развивающейся областью науки, которая может внести существенный вклад в лечения различных болезней. В настоящей работе изучался терапевтический эффект кондиционированной среды из культуры стволовых клеток костного мозга (СККМ), которые кокультивировали с гепатоцитами (КС ККМ) с целью ослабления CCl_4-индуцированного повреждения печени у мышей.

Материалы и методы

Гепатоциты кокультивировали с СККМ в течение 20 час., затем собирали кондиционированную среду от них. Мышам экспериментальной группы вводили внутрибрюшинно CCl_4 дважды в неделю в течение 7 недель, после чего вводили кондиционированную среду от кокультур СККМ и гепатоцитов. Через 4 недели определяли уровни АЛТ, АСТ, ALP и альбумина в сыворотке, оценивали гистопатологию печени, и определяли уровни IL-4 и IFN-γ в сыворотке посредством ИФА.

Результаты

Группа, леченная введением кондиционированной среды СККМ/гепатоцитов, имела значительно сниженные уровни АЛТ и АСТ по сравнению с мышами, которым вводили среду RPMI без существенных различий в уровнях ALP и альбумина. Гистологическое исследование показало выраженное ослабление патологической картины печени в группе, леченной кондиционированной средой от кокультур СККМ/гепатоцитов. Введение конционированной СККМ среды вызывало повышение продукции IL-4 и не нарушало выхода IFN-γ.

Выводы

Наши результаты показали, что введение сред из кокультур СККМ/гепатоцитов сопровождается ослаблением повреждений печени, вызванных CCl_4. Необходимы дальнейшие исследования, чтобы выяснить механизм действия и достичь желаемого терапевтического эффекта, который может быть репродуцирован и оптимизирован.

Ключевые слова

Стволовые клетки, костный мозг, гепатоциты, кокультура, кондиционированная среда, печеночная патология.