

REVIEW

Open Access



Transition of clinical and basic studies on liver cirrhosis treatment using cells to seek the best treatment

Shuji Terai^{*} , Atsunori Tsuchiya, Yusuke Watanabe and Suguru Takeuchi

Abstract

The liver is a highly regenerative organ; however, its regeneration potential is reduced by chronic inflammation with fibrosis accumulation, leading to cirrhosis. With an aim to tackle liver cirrhosis, a life-threatening disease, trials of autologous bone marrow cell infusion (ABMi) therapy started in 2003. Clinical studies revealed that ABMi attenuated liver fibrosis and improved liver function in some patients; however, this therapy has some limitations such as the need of general anesthesia. Following ABMi therapy, studies have focused on specific cells such as mesenchymal stromal cells (MSCs) from a variety of tissues such as bone marrow, adipose tissue, and umbilical cord tissues. Particularly, studies have focused on gaining mechanistic insights into MSC distribution and effects on immune cells, especially macrophages. Several basic studies have reported the use of MSCs for liver cirrhosis models, while a number of clinical studies have used autologous and allogeneic MSCs; however, there are only a few reports on the obvious substantial effect of MSCs in clinical studies. Since then, studies have analyzed and identified the important signals or components in MSCs that regulate immune cells, such as macrophages, under cirrhotic conditions and have revealed that MSC-derived exosomes are key regulators. Researchers are still seeking the best approach and filling the gap between basic and clinical studies to treat liver cirrhosis. This paper highlights the timeline of basic and clinical studies analyzing ABMi and MSC therapies for cirrhosis and the scope for future studies and therapy.

Keywords: Liver, Cirrhosis, Fibrosis, Mesenchymal stromal cell, Macrophage, Exosome

Introduction

The liver is a highly regenerative organ, but long-term chronic inflammation followed by accumulation of fibrosis leads to cirrhosis. Generally, in compensated cirrhosis, the regenerative ability of the liver is retained when the damage caused by factors, such as hepatitis viruses and alcohol, is resolved; however, in decompensated cirrhosis, the regenerative ability weakens. Even if the causative factors are withdrawn, liver fibrosis sometimes worsens owing to infection or bleeding. This status is considered as a “point of no return” (Fig. 1); thus, early resolution of the causes of liver cirrhosis and

regression of liver fibrosis are important to induce the ability of endogenous liver regeneration. To date, liver transplantation is considered the most effective therapy for cirrhosis; no other therapy has been approved for regressing liver fibrosis and for inducing endogenous liver regeneration [1–7].

Basic and clinical studies to develop a new treatment for cirrhosis are being performed vigorously. However, there are still gaps between basic and clinical study data in terms of therapeutic effects [8–10]. Here, the basic studies related to cirrhosis treatment using bone marrow cells, mesenchymal stromal cells (MSCs), and exosomes derived from MSCs are introduced [3, 11–18] and previous and ongoing clinical studies on cirrhosis [13, 19–21] are discussed with the prospect of developing effective therapies.

* Correspondence: terais@med.niigata-u.ac.jp

Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan



© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

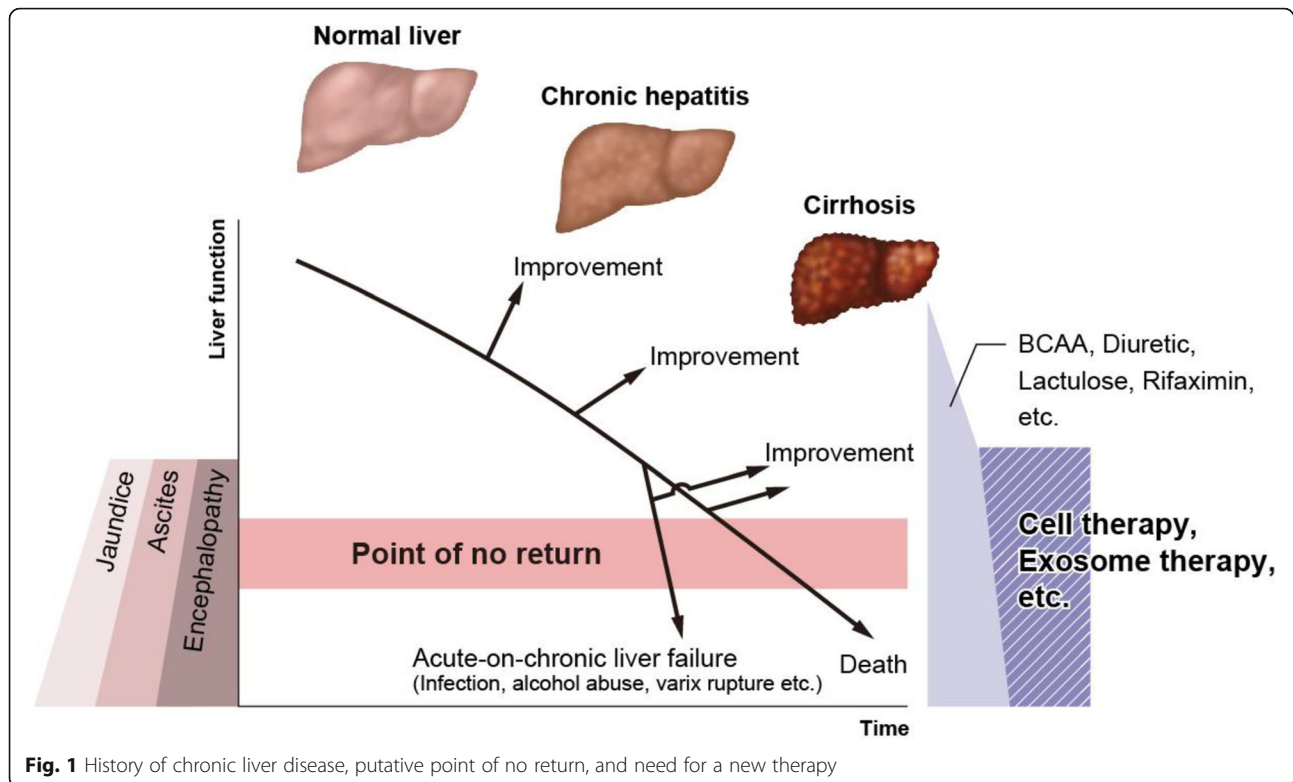


Fig. 1 History of chronic liver disease, putative point of no return, and need for a new therapy

Findings from basic studies

Bone marrow cells

Approximately 20 years ago, an *in vivo* mouse model was developed to monitor the effects of administration of GFP-positive bone marrow cells in carbon tetrachloride (CCl_4)-induced cirrhotic liver [11]. The intravenously administered cells migrated into the cirrhotic liver and attenuated liver fibrosis. This phenomenon is important because heterogeneous bone marrow cells include cells that can be used for regressing liver fibrosis. Furthermore, the repopulation of bone marrow-derived GFP-positive round-shaped hematopoietic-like cells expressing matrix metalloproteinase was identified in the damaged area of the liver [12]. However, the specific phenotype of these working cells attenuating liver fibrosis could not be identified.

Mesenchymal stromal cells and macrophages

As a next step, bone marrow cells crucial for attenuating liver fibrosis have been analyzed. The majority of the bone marrow cells are hematopoietic cells, whereas MSCs constitute a minor proportion. MSCs have garnered the attention of researchers worldwide as one of the candidate cells for the treatment of cirrhosis. The therapeutic effects of MSCs determined using animal models of cirrhosis have been reported [4]; however, gaining mechanisms of this therapy is difficult, requiring multi-directional analysis [22]. Some studies have

reported that hepatocyte damage can be reduced by reducing inflammation or oxidative stress [23]. A few others have reported the effects of stellate cells [24]. There are several reports on the relationship between MSCs and macrophages [1, 5]. The relationship between bone marrow-derived MSCs and macrophages has been extensively evaluated. Some humoral factors from MSCs have been found to alter the polarity of macrophages into an anti-inflammatory phenotype. The therapeutic effects of bone marrow-derived MSCs and macrophages and the combination of MSCs and macrophages (total cell numbers were the same in each group) were evaluated. Unexpectedly, the combined therapy exerted the highest therapeutic effect on fibrosis. The distribution of MSCs and macrophages has also been analyzed in mice [16]. MSCs and macrophages were derived from Ds-Red transgenic and GFP transgenic mice, respectively. The GFP-positive bone marrow cell-derived macrophages and Ds-Red-positive MSCs were then injected into a mouse with CCl_4 -induced cirrhosis, and the distribution of these cells was analyzed in the liver, lung, and spleen by two-photon excitation microscopy. A small percentage of MSCs repopulated in the cirrhotic liver soon after injection, whereas most of the MSCs were trapped in the lung and disappeared after 7 days. On the contrary, many macrophages migrated into the damaged area of cirrhotic livers after injection for a week; some of them were phagocytizing hepatocyte debris. When hepatocyte

debris was added to the macrophages, the macrophages started to produce oncostatin M and vascular endothelial growth factor, suggesting phagocytosis induces the regeneration of the liver [16]. From a series of results combined with previous observations, it is proposed that MSCs trapped in the lung function as “conducting cells,” attenuate liver damage and inflammation, and provide signals to “working cells,” namely, macrophages, to attenuate liver fibrosis (Fig. 2).

Exosomes derived from mesenchymal stromal cells

Recently, in the analysis to identify key factors, exosomes secreted by MSCs have garnered attention [25]. Exosomes are extracellular vesicles of approximately 100 nm, acting as cargo for various proteins, miRNAs, and lipids for cell–cell communication [26]. Exosomes from MSCs are thought to be key factors involved in communications between MSCs and immune cells, especially macrophages. Exosomes after intravenous administration are mainly distributed in the liver and lung within 24 h and rapidly reduced thereafter [27]. Reportedly, macrophages are the main effector of exosomes [28]. On the basis of these findings, it may be ideal to use exosomes from MSCs for treating liver cirrhosis. Another attraction about exosomes is that they can be

modified by pre-conditioning or gene editing of exosome-producing cells [29]. As MSCs are known to exert anti-inflammatory effects when inflammation in the host is high [30, 31], the use of MSCs pre-conditioned with cytokines or chemokines before harvesting the exosomes is an attractive strategy. For example, interferon- γ -preconditioned exosomes (γ -exosomes) could change the polarity of macrophages into an anti-inflammatory phenotype more effectively than exosomes without stimulation. γ -Exosomes also increase the migration and phagocytic abilities of macrophages. The characteristics of γ -exosomes were further evaluated in vitro using stellate cells; γ -exosomes did not inhibit the activation of stellate cells. The therapeutic effects of γ -exosomes were evaluated in the CCl₄-induced cirrhosis model and their anti-inflammatory and anti-fibrotic effects were found to be superior to those of the MSCs and conventional MSC-derived exosomes. The effects of γ -exosomes in the CCl₄-induced cirrhosis mouse were further evaluated by single-cell RNA-seq and two-photon excitation microscopy. γ -Exosomes were found to increase the frequency of anti-inflammatory macrophages in the liver and turn circular, increasing the number of macrophages that could contact the hepatocyte debris and migrate into the damaged area of the liver in a fixed time. The proteins

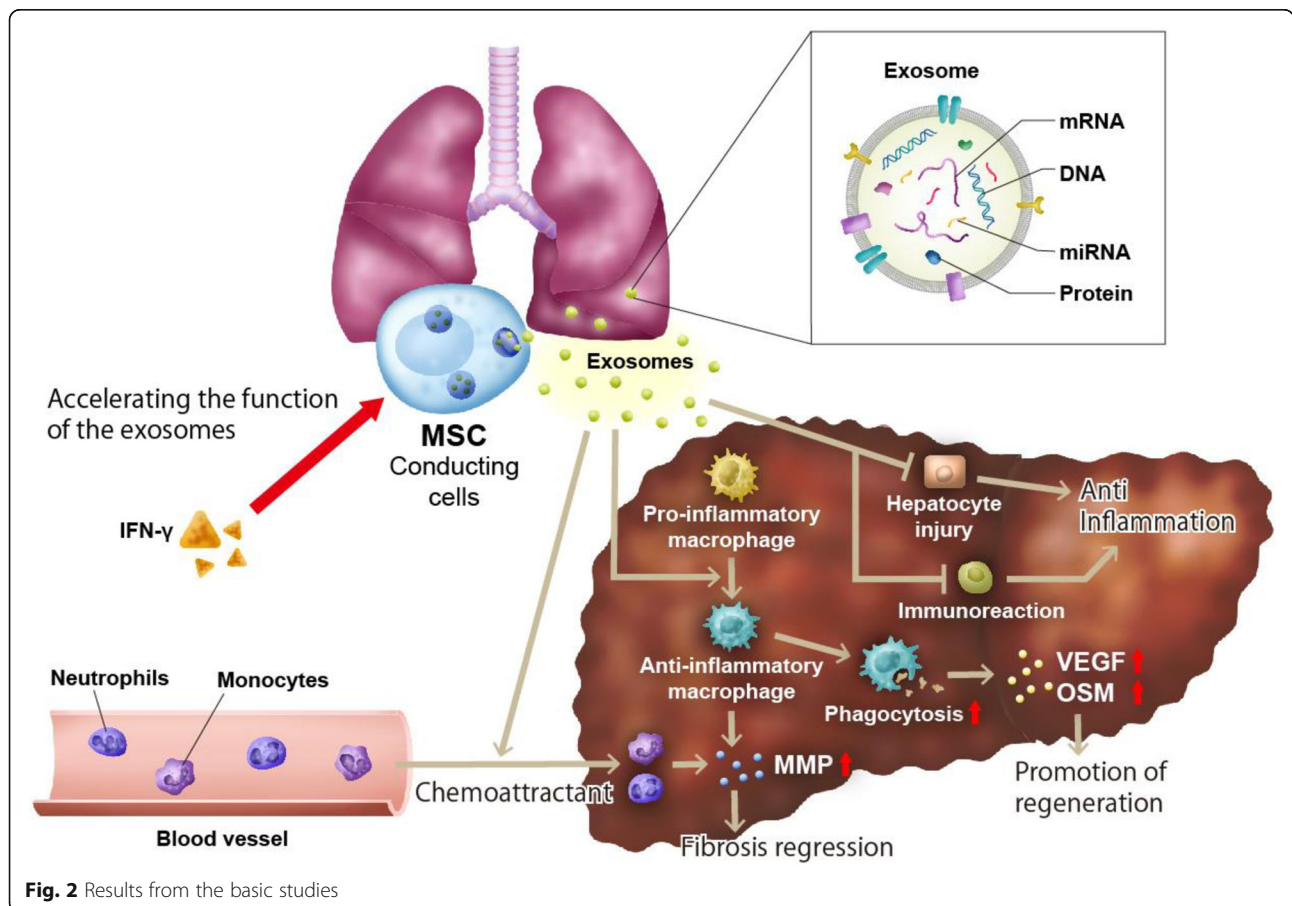


Fig. 2 Results from the basic studies

and miRNAs were also compared between γ -exosomes and conventional exosomes, and it was found that they had changed after IFN- γ stimulation. Among these proteins, annexin-A1, lactotransferrin, and aminopeptidase N have been thought to play an important role in the polarization of macrophages [18]. From these findings, it could be concluded that MSCs can function as “conducting cells” through exosomes (especially γ -exosomes) by inducing the anti-inflammatory “working cells” (macrophages) and exert higher therapeutic effects in patients with cirrhosis (Fig. 2). Exosomes can be used as a stable drug delivery system.

Clinical studies

Autologous bone marrow cell infusion therapy

In parallel with the basic studies, a clinical study of cell therapy for decompensated cirrhosis was performed to overcome the “point of no return” [1]. First, autologous bone marrow cell infusion (ABMi) therapy was developed for patients with decompensated cirrhosis. For ABMi therapy, 400 ml of autologous bone marrow cells was harvested from the ilium; mononuclear cells were obtained and then infused from the peripheral vein. Owing to the need for general anesthesia, the criteria of ABMi therapy for patients with cirrhosis were as follows: serum total bilirubin under 3.0 mg/dl, PLT over 50,000/ml, and no obvious cardiovascular disease. The first human clinical study of ABMi therapy for patients with decompensated cirrhosis started in November 2003 and the treatment efficacy was reported in 2006 [19]. This study was expanded as a multi-center project for cirrhosis owing to the varying etiologies, including hepatitis B virus infections [20] and alcoholic related liver diseases [21]. Owing to the difficulties in evaluating the regression of fibrosis, at that time, some of the responders of ABMi therapy showed morphological improvement in the irregularity of cirrhosis, as observed in the computed tomography images, suggesting that ABMi therapy attenuates fibrosis [19]. In addition to improving the morphology, ABMi therapy decreased the serum granulocyte-colony stimulating factor and interleukin-1 β levels and increased the number of proliferating cell nuclear antigen-positive proliferative hepatocytes [13] and cytokeratin 7-positive “liver progenitor cells” [20]. From these observations, it can be concluded that ABMi therapy attenuates liver fibrosis via sequential activation of liver regeneration.

MSC therapy

While there were several responders to ABMi therapy, this therapy is relatively invasive and has some difficulties in spreading. Therefore, ABMi therapy has been shifted to autologous MSC therapy. Bone marrow cells, adipose tissue, and umbilical cord tissues are major sources of MSCs [22]. Some studies have reported the

efficacy of autologous MSC therapy [8, 9]; however, the therapeutic effects appear to be restricted compared with those expected from the basic studies. It is well known that MSCs depict low immunogenicity; thus, allogeneic MSC use for therapy without immunosuppressive agents is increasing in a variety of fields. Although adipose tissue is easily accessible during plastic surgery, harvesting allogeneic bone marrow cells is difficult in Japan owing to regulations. Therefore, in the first Japanese trial using allogeneic MSC therapy, adipose tissue was selected as the source for allogeneic MSCs. Phase I/II clinical study was started in 2017 using allogeneic adipose tissue-derived MSC therapy for decompensated cirrhosis with Rohto Pharmaceutical Co., Ltd. The study aimed to evaluate the safety and effectiveness of this therapy is ongoing. Compared to the favorable results from the basic studies, strong effects have not been observed in clinical studies. Researchers and clinicians are now seeking the optimal timing, disease stage, and cell types or culture conditions of cells to treat liver fibrosis effectively.

Conclusions

Herein, the advances in basic studies using bone marrow cells, MSCs, and MSC-derived exosomes and clinical studies using autologous bone marrow cells and MSC therapy conducted in the past 20 years were discussed. Considering the basic studies, attenuating liver fibrosis and activating endogenous regenerative ability seem to induce anti-inflammatory “tissue repair” macrophages. The Forbes group of Edinburgh University reported phase I autologous macrophage therapy and its safety [32]. In addition to macrophages, the regulation of a variety of immune cells, including regulatory T cells, NK cells, and hepatic stellate cells, would be important to induce the activation of this endogenous regenerative ability [33]. Recent technological advances, such as two-photon excitation microscopy and single-cell RNA-Seq, have helped to demonstrate this phenomenon [18]. In addition, in the last two decades, researchers have focused on developing an evaluation tool for fibrosis. While invasive liver biopsy was the only tool for the accurate assessment of fibrosis, several non-invasive tools, such as serum markers, Mac-2 binding protein glycosylated isomer [34], autotaxin [35], and Pro-C3 [36], FibroScan [37], and elastography [38] using ultrasound sonography and magnetic resonance imaging, have been developed. It is important to determine markers or methods that are most efficient for the evaluation of fibrosis in clinical studies. These results can be used for the development of more effective therapy, induction of anti-inflammatory “tissue repair” macrophages, and evaluation of fibrosis and regeneration using the latest methods. At this point, exosomes are attractive candidates for next-generation cirrhosis therapy; however, promising alternative treatments can emerge (Fig. 3) [18, 39].

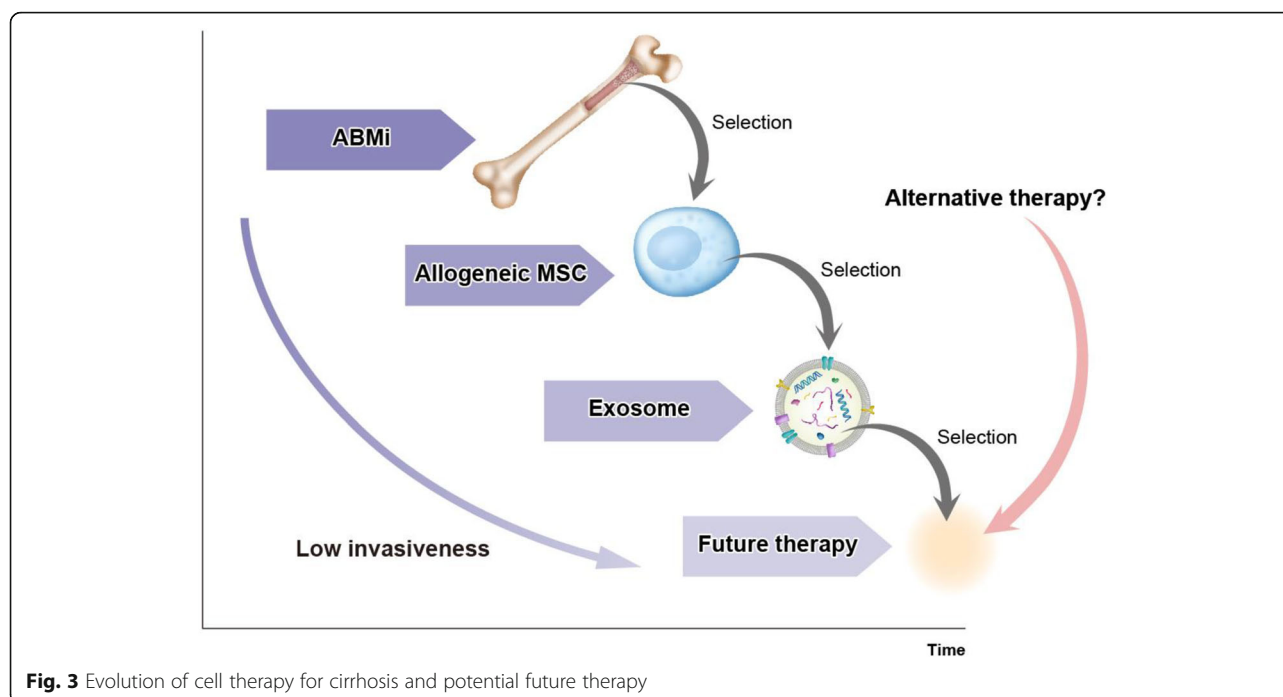


Fig. 3 Evolution of cell therapy for cirrhosis and potential future therapy

Continuous clinical and basic studies may be necessary for future therapy. A recent study has demonstrated that exosomes are an attractive alternative therapy for cirrhosis [18]. MSC-derived exosomes can be obtained from a variety of tissues, such as bone marrow cells, adipose tissues, umbilical cord tissues, and dental pulp. Theoretically, exosomes can be obtained from MSCs derived from induced pluripotent stem cells or embryonic stem cells. In addition, the contents of exosomes can be manipulated by preconditioning or gene editing, suggesting that more appropriate exosomes can be produced. However, exosome therapy is limited by the abundance of exosomes from cells and confirmation of uniformity of exosomes. However, new technologies and associated regulations to overcome these problems are expected. There are currently no Food and Drug Administration-approved exosome products, and the International Society for Cellular and Gene Therapies and the International Society for Extracellular Vesicles recognize the potential of extracellular vesicles (including exosomes) from MSCs and possibly other cell sources as treatments for several diseases [40]. Now, the Japanese Society for Regenerative Medicine has also formulated a working group to develop exosomes for the therapy. Other therapeutic cell-free strategies using peptides or oral drugs are also in progress [7]. In this milieu, cirrhosis treatment with cell and cell-free strategies has started to move to the next stages, allowing us to seek the best therapy.

Abbreviations

MSC: Mesenchymal stromal cell; CCl₄: Carbon tetrachloride; ABMi: Autologous bone marrow cell infusion

Authors' contributions

ST and AT: conception and design. YW and ST: collection data. ST and AT: manuscript writing. The authors read and approved the final manuscript.

Funding

This study was supported by the Research Program on Hepatitis from the Japan Agency for Medical Research and Development (AMED) (20fk0210070h0001) and by a Grant-in-Aid for Scientific Research (B) (19H03636) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. This study was partially supported by ROHTO Pharmaceutical Co., Ltd.

Availability of data and materials

All data needed to evaluate the conclusions in the paper are provided in the paper. Additional data related to this study may be requested from the authors.

Declarations

Ethics approval and consent to participate

N/A.

Consent for publication

N/A.

Competing interests

ST received research fund from ROHTO Pharmaceutical Co., Ltd. ROHTO Pharmaceutical Co., Ltd. supports a clinical trial of MSCs.

Received: 8 July 2021 Accepted: 6 September 2021

Published online: 16 September 2021

References

1. Terai S, Tsuchiya A. Status of and candidates for cell therapy in liver cirrhosis: overcoming the "point of no return" in advanced liver cirrhosis. *J Gastroenterol.* 2017;52(2):129–40. <https://doi.org/10.1007/s00535-016-1258-1>.
2. Terai S, Tanimoto H, Maeda M, Zaitu J, Hisanaga T, Iwamoto T, et al. Timeline for development of autologous bone marrow infusion (ABMi)

- therapy and perspective for future stem cell therapy. *J Gastroenterol.* 2012; 47(5):491–7. <https://doi.org/10.1007/s00535-012-0580-5>.
3. Tanimoto H, Terai S, Taro T, Murata Y, Fujisawa K, Yamamoto N, et al. Improvement of liver fibrosis by infusion of cultured cells derived from human bone marrow. *Cell Tissue Res.* 2013;354(3):717–28. <https://doi.org/10.1007/s00441-013-1727-2>.
 4. Tsuchiya A, Kojima Y, Ikarashi S, Seino S, Watanabe Y, Kawata Y, et al. Clinical trials using mesenchymal stem cells in liver diseases and inflammatory bowel diseases. *Inflamm Regen.* 2017;37(1):16. <https://doi.org/10.1186/s41232-017-0045-6>.
 5. Tsuchiya A, Takeuchi S, Watanabe T, Yoshida T, Nojiri S, Ogawa M, et al. Mesenchymal stem cell therapies for liver cirrhosis: MSCs as “conducting cells” for improvement of liver fibrosis and regeneration. *Inflamm Regen.* 2019;39(1):18. <https://doi.org/10.1186/s41232-019-0107-z>.
 6. Watanabe Y, Tsuchiya A, Terai S. The development of mesenchymal stem cell therapy in the present, and the perspective of cell-free therapy in the future. *Clin Mol Hepatol.* 2021;27(1):70–80. <https://doi.org/10.3350/cmh.2020.0194>.
 7. Iwasawa T, Nojiri S, Tsuchiya A, Takeuchi S, Watanabe T, Ogawa M, et al. Combination therapy of Juzentaihoto and mesenchymal stem cells attenuates liver damage and regresses fibrosis in mice. *Regen Ther.* 2021;18: 231–41. <https://doi.org/10.1016/j.reth.2021.07.002>.
 8. Mohamadnejad M, Alimoghaddam K, Bagheri M, Ashrafi M, Abdollahzadeh L, Akhlaghpour S, et al. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. *Liver Int.* 2013;33(10):1490–6. <https://doi.org/10.1111/liv.12228>.
 9. Suk KT, Yoon JH, Kim MY, Kim CW, Kim JK, Park H, et al. Transplantation with autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: Phase 2 trial. *Hepatology.* 2016;64(6):2185–97. <https://doi.org/10.1002/hep.28693>.
 10. Huang KC, Chuang MH, Lin ZS, Lin YC, Chen CH, Chang CL, et al. Transplantation with GXHPC1 for liver cirrhosis: phase 1 trial. *Cell Transplant.* 2019;28(1_suppl):1005–115. <https://doi.org/10.1177/0963689719884885>.
 11. Terai S, Sakaida I, Yamamoto N, Omori K, Watanabe T, Ohata S, et al. An in vivo model for monitoring trans-differentiation of bone marrow cells into functional hepatocytes. *J Biochem.* 2003;134(4):551–8. <https://doi.org/10.1093/jb/mvg173>.
 12. Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H, et al. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. *Hepatology.* 2004;40(6):1304–11. <https://doi.org/10.1002/hep.20452>.
 13. Mizunaga Y, Terai S, Yamamoto N, Uchida K, Yamasaki T, Nishina H, et al. Granulocyte colony-stimulating factor and interleukin-1beta are important cytokines in repair of the cirrhotic liver after bone marrow cell infusion: comparison of humans and model mice. *Cell Transpl.* 2012;21(11):2363–75. <https://doi.org/10.3727/096368912X638856>.
 14. Iwamoto T, Terai S, Hisanaga T, Takami T, Yamamoto N, Watanabe S, et al. Bone-marrow-derived cells cultured in serum-free medium reduce liver fibrosis and improve liver function in carbon-tetrachloride-treated cirrhotic mice. *Cell Tissue Res.* 2013;351(3):487–95. <https://doi.org/10.1007/s00441-012-1528-z>.
 15. Kojima Y, Tsuchiya A, Ogawa M, Nojiri S, Takeuchi S, Watanabe T, et al. Mesenchymal stem cells cultured under hypoxic conditions had a greater therapeutic effect on mice with liver cirrhosis compared to those cultured under normal oxygen conditions. *Regen Ther.* 2019;11:269–81. <https://doi.org/10.1016/j.reth.2019.08.005>.
 16. Watanabe Y, Tsuchiya A, Seino S, Kawata Y, Kojima Y, Ikarashi S, et al. Mesenchymal stem cells and induced bone marrow-derived macrophages synergistically improve liver fibrosis in mice. *Stem Cells Transl Med.* 2019; 8(3):271–84. <https://doi.org/10.1002/sctm.18-0105>.
 17. Watanabe T, Tsuchiya A, Takeuchi S, Nojiri S, Yoshida T, Ogawa M, et al. Development of a non-alcoholic steatohepatitis model with rapid accumulation of fibrosis, and its treatment using mesenchymal stem cells and their small extracellular vesicles. *Regen Ther.* 2020;14:252–61. <https://doi.org/10.1016/j.reth.2020.03.012>.
 18. Takeuchi S, Tsuchiya A, Iwasawa T, Nojiri S, Watanabe T, Ogawa M, et al. Small extracellular vesicles derived from interferon-gamma pre-conditioned mesenchymal stromal cells effectively treat liver fibrosis. *NPJ Regen Med.* 2021;6(1):19. <https://doi.org/10.1038/s41536-021-00132-4>.
 19. Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, et al. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. *Stem Cells.* 2006;24(10):2292–8. <https://doi.org/10.1634/stemcells.2005-0542>.
 20. Kim JK, Park YN, Kim JS, Park MS, Paik YH, Seok JY, et al. Autologous bone marrow infusion activates the progenitor cell compartment in patients with advanced liver cirrhosis. *Cell Transplant.* 2010;19(10):1237–46. <https://doi.org/10.3727/096368910X506863>.
 21. Saito T, Okumoto K, Haga H, Nishise Y, Ishii R, Sato C, et al. Potential therapeutic application of intravenous autologous bone marrow infusion in patients with alcoholic liver cirrhosis. *Stem Cells Dev.* 2011;20(9):1503–10. <https://doi.org/10.1089/scd.2011.0074>.
 22. Wang LT, Liu KJ, Sytwu HK, Yen ML, Yen BL. Advances in mesenchymal stem cell therapy for immune and inflammatory diseases: use of cell-free products and human pluripotent stem cell-derived mesenchymal stem cells. *Stem Cells Transl Med.* 2021;10(9):1288–303. <https://doi.org/10.1002/sctm.21-0021>.
 23. Miyaji T, Takami T, Fujisawa K, Matsumoto T, Yamamoto N, Sakaida I. Bone marrow-derived humoral factors suppress oxidative phosphorylation, upregulate TSG-6, and improve therapeutic effects on liver injury of mesenchymal stem cells. *J Clin Biochem Nutr.* 2020;66(3):213–23. <https://doi.org/10.3164/jcbn.19-125>.
 24. Najimi M, Berardis S, El-Kehdy H, et al. Human liver mesenchymal stem/progenitor cells inhibit hepatic stellate cell activation: in vitro and in vivo evaluation. *Stem Cell Res Ther.* 2017;8(1):131. <https://doi.org/10.1186/s13287-017-0575-5>.
 25. Witwer KW, Van Balkom BWM, Bruno S, et al. Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. *J Extracell Vesicles.* 2019;8(1):1609206. <https://doi.org/10.1080/20013078.2019.1609206>.
 26. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science.* 2020;367(6478):eaau6977. <https://doi.org/10.1126/science.aau6977>.
 27. Kang M, Jordan V, Blenkiron C, Chamley LW. Biodistribution of extracellular vesicles following administration into animals: a systematic review. *J Extracell Vesicles.* 2021;10(8):e12085. <https://doi.org/10.1002/jev.2.12085>.
 28. Imai T, Takahashi Y, Nishikawa M, Kato K, Morishita M, Yamashita T, et al. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. *J Extracell Vesicles.* 2015;4(1):26238. <https://doi.org/10.3402/jev.v4.26238>.
 29. Xu M, Yang Q, Sun X, Wang Y. Recent advancements in the loading and modification of therapeutic exosomes. *Front Bioeng Biotechnol.* 2020;8: 586130. <https://doi.org/10.3389/fbioe.2020.586130>.
 30. Kawata Y, Tsuchiya A, Seino S, Watanabe Y, Kojima Y, Ikarashi S, et al. Early injection of human adipose tissue-derived mesenchymal stem cell after inflammation ameliorates dextran sulfate sodium-induced colitis in mice through the induction of M2 macrophages and regulatory T cells. *Cell Tissue Res.* 2019;376(2):257–71. <https://doi.org/10.1007/s00441-018-02981-w>.
 31. Ikarashi S, Tsuchiya A, Kawata Y, Kojima Y, Watanabe T, Takeuchi S, et al. Effects of human adipose tissue-derived and umbilical cord tissue-derived mesenchymal stem cells in a dextran sulfate sodium-induced mouse model. *Biores Open Access.* 2019;8(1):185–99. <https://doi.org/10.1089/biores.2019.0022>.
 32. Moroni F, Dwyer BJ, Graham C, Pass C, Bailey L, Ritchie L, et al. Safety profile of autologous macrophage therapy for liver cirrhosis. *Nat Med.* 2019;25(10): 1560–5. <https://doi.org/10.1038/s41591-019-0599-8>.
 33. Campana L, Esser H, Huch M, Forbes S. Liver regeneration and inflammation: from fundamental science to clinical applications. *Nat Rev Mol Cell Biol.* 2021;22(9):608–24. <https://doi.org/10.1038/s41580-021-00373-7>.
 34. Ishii N, Harimoto N, Araki K, Muranushi R, Hoshino K, Hagiwara K, et al. Preoperative Mac-2 binding protein glycosylation isomer level predicts postoperative ascites in patients with hepatic resection for hepatocellular carcinoma. *Hepatol Res.* 2019;49(12):1398–405. <https://doi.org/10.1111/hepr.13412>.
 35. Ogawa M, Tsuchiya A, Watanabe T, Tetsu T, Kimura N, Matsuda M, et al. Screening and follow-up of chronic liver diseases with understanding their etiology in clinics and hospitals. *JGH Open.* 2020;4(5):827–37. <https://doi.org/10.1002/jgh3.12406>.
 36. Daniels SJ, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A, et al. ADAPT: an algorithm incorporating PRO-C3 accurately identifies patients with NAFLD and advanced fibrosis. *Hepatology.* 2019;69(3):1075–86. <https://doi.org/10.1002/hep.30163>.
 37. Nouredin N, Alkhoury N, Brown KA, Nouredin M. Driving nonalcoholic steatohepatitis forward using the fibroScan aspartate aminotransferase score, but obey the traffic lights. *Hepatology.* 2020;72(6):2228–30. <https://doi.org/10.1002/hep.31498>.

38. Ajmera VH, Liu A, Singh S, Yachoa G, Ramey M, Bhargava M, et al. Clinical utility of an increase in magnetic resonance elastography in predicting fibrosis progression in nonalcoholic fatty liver disease. *Hepatology*. 2020; 71(3):849–60. <https://doi.org/10.1002/hep.30974>.
39. Tsuchiya A, Takeuchi S, Iwasawa T, Kumagai M, Sato T, Motegi S, et al. Therapeutic potential of mesenchymal stem cells and their exosomes in severe novel coronavirus disease 2019 (COVID-19) cases. *Inflamm Regen*. 2020;40(1):14. <https://doi.org/10.1186/s41232-020-00121-y>.
40. Borger V, Weiss DJ, Anderson JD, et al. International Society for Extracellular Vesicles and International Society for Cell and Gene Therapy statement on extracellular vesicles from mesenchymal stromal cells and other cells: considerations for potential therapeutic agents to suppress coronavirus disease-19. *Cytotherapy*. 2020;22(9):482–5. <https://doi.org/10.1016/j.jcyt.2020.05.002>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

