Regenerative Therapy 26 (2024) 1048–1057

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Platelet-rich plasma-derived extracellular vesicles improve liver cirrhosis in mice

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ARTICLE INFO

Article history:
Received 14 September 2024
Received in revised form
18 October 2024
Accepted 23 October 2024

Keywords:
Platelet
Platelet-rich plasma-derived extracellular vesicles
Platelet-rich plasma
Liver cirrhosis

ABSTRACT

Introduction: Cirrhosis remains a significant clinical challenge due to its poor prognosis and limited treatment options, creating a high unmet medical need for the development of novel therapies. In this study, we analyzed the effects of a novel approach to treat cirrhosis using platelet-rich plasma-derived extracellular vesicles (PRPEV) in mice.

Methods: PRPEV were collected from platelet-rich plasma using ultrafiltration, and their proteomes were analyzed. The carbon tetrachloride (CCl_4)-induced cirrhosis model of mice was used to evaluate the effect of PRPEV administration and compared with the control group (n = 8). *In vitro* and *in vivo* mechanistic analyses of PRPEV administration were confirmed using real time-PCR and immunostaining.

Results: Gene ontology analysis based on the proteome revealed that PRPEV contain many factors associated with EV and immune responses. *In vitro*, PRPEV polarize macrophages into an anti-inflammatory phenotype. Following PRPEV administration, there was a decrease in serum alanine aminotransferase levels and reduction in liver fibrosis, while mRNA levels of regenerative factors were upregulated and *transforming growth factor β-1* was downregulated. Furthermore, the number of anti-inflammatory macrophages in the liver increased.

Conclusions: PRPEV may contribute to hepatocyte proliferation, anti-inflammation, and anti-fibrogenesis in the liver. This novel concept paves the way for cirrhosis treatment.

Abbreviations: Alb, Albumin; ALT, alanine aminotransferase; CCl_4, carbon tetrachloride; CCL2, chemokine (C–C motif) ligand 2; DAB, 3,3′-diaminobenzidine; DMEM, Dulbecco's Modified Eagle Medium; EV, extracellular vesicles; GO, Gene ontology; HSC, hepatic stellate cell; IL, interleukin; iNOS, induced nitric oxide synthase; LC, liver cirrhosis; PRP, platelet-rich plasma; PRPEV, platelet-rich plasma-derived extracellular vesicles; RT-PCR, real time-PCR; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2024.10.010

1. Introduction

Every year, around two million people worldwide die from liver diseases, including cirrhosis, accounting for 3.5 % of all annual deaths [1]. As cirrhosis progresses, liver failure, hepatocellular carcinoma, and gastrointestinal varices may develop, which can be fatal, requiring various treatments [2,3]. Currently, liver transplantation is the only curative treatment for cirrhosis; however, the number of patients with cirrhosis who can receive liver transplantation is extremely limited owing to the invasiveness of transplantation, donor shortage, lifelong use of immunosuppressive agents, and economic circumstances [4,5]. Therefore, a novel therapeutic approach for cirrhosis is necessary as an alternative to liver transplantation.

Platelets have been suggested to be a novel and promising avenue for the treatment of cirrhosis. Platelets contain many cytokines, chemokines, proteins, and growth factors. Furthermore, previous studies showed that platelets or platelet-rich plasma (PRP) play an important role in fibrolysis of the liver, and that cytokines, chemokines, proteins, and growth factors induced by platelets or PRP improves liver function in patients with cirrhosis [6–8]. Though platelets or PRP have several advantages in the treatment of cirrhosis; however, they have the disadvantage that their usage is mostly autologous administration due to their immunogenicity [9]. Extracellular vesicles (EV) of platelets may offer a potential solution to this problem. EV are membrane-bound vesicles released from the plasma membrane or endosomal compartments [10,11]. EV are small cellular particles, 30–400 nm in size, and their release from the membrane is highly conserved in biology [11]. Recent studies have shown that EV are important mediators of intercellular communication and encompass a variety of proteins, lipids, glycolipids, glycoproteins, DNA, mRNA, and microRNA [7,12]. Similar to other cells, platelets release a large number of EVs during activation, which can have potential clinical applications for transportation of RNA and proteins to cell receptors in other organs [10,12–14]. This mechanism would allow EVs to reach organs or tissues inaccessible to platelets, thereby contributing to long-distance intercellular communication [14]. Furthermore, EVs have the advantage of low immunogenicity and can be used for allogeneic administration [14–16].

We focused on platelet derived EVs, especially PRP derived EV (PRPEV), and explored their effect on cirrhosis. We also investigated the mechanisms of PRPEV, both *in vitro* and *in vivo*. Our findings will provide a scientific basis for further research on cirrhosis treatment.

2. Materials and methods

2.1. Mice

All animal experiments were conducted in compliance with the regulations of the ARRIVE guidelines and approved by the Institutional Animal Care and Committee at Niigata University (SA01135). C57BL/6 male mice were purchased from Charles River (Yokohama, Japan). The animals were housed in a specific pathogen-free environment and kept under standard conditions with a 12-h day/night cycle and access to food and water *ad libitum*.

2.2. Generation of a mouse model of carbon tetrachloride-induced liver injury

Cirrhosis was induced in 8-week-old male mice by intraperitoneal injection of 1.0 mL/kg carbon tetrachloride (CCl_4; Wako, Osaka, Japan) twice a week for 8 weeks. CCl_4 was dissolved in corn oil (Wako) at 1:10 ratio [17–22].

2.3. Preparation of PRP

Peripheral blood samples were collected from healthy volunteers between the ages of 20–40 (Supplemental Table 1). Technicians collected 11 mL of venous blood from the participants using a 23-gauge needle (Self-touch PSV sets, Osaka, Japan) in a tube containing 1 mL 3.8 % sodium citrate solution (Muto Pure Chemicals Co., LTD, Tokyo, Japan) as anticoagulant. The anticoagulated blood was carefully transferred into a new 15 mL tube containing 2.5 mL of lymphocyte separation solution (LSM; MP Biomedicals, Santa Ana, CA, USA), centrifuged at 800×*g* for 10 min at room temperature, and the supernatant was discarded. PRP was obtained by collecting 500 μL of the residual plasma on top of the buffy coat layer. Collection of blood samples from healthy volunteers was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of Niigata University (2024–0108).

2.4. Isolation and concentration and size distribution of PRPEV

To 10 mL of Advanced Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) with GlutaMAX TM (100x) (Thermo Fisher Scientific), 500 μL of the recovered PRP was added and allowed to stand at 37 °C for 48 h, following which the supernatant medium was collected. This was then transferred to a new 15 mL tube, centrifuged at 1200×*g* for 15 min at room temperature, and filtered through a 0.22 μm syringe filter (Sartorius Stedim Biotech, Goettingen, Germany) and concentrated to 1 mL with a 100 kDa cut-off ultrafiltration tube (Ultracel-100,000 NMWL, Merck Millipore, Ireland). In the process of PRPEV isolation, PRPEV collected from the five healthy volunteers were mixed together before using analysis. The concentration and size distribution of PRPEV were measured using a ViewSizer 3000 (HORIBA, Kyoto, Japan) nanoparticle size distribution and concentration analyzer.

2.5. Western blot

Total proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 4–20 % Novex Tris Glycine Mini Gels (Thermo Fisher Scientific) and transferred to Trans-Blot Turbo Transfer Pack (BIO RAD, Tokyo, Japan). Electrophoresis, blotting, and antibody treatment were performed using a Mini Gel Tank (Thermo Fishe Scientific), Pierce Power Blotter Stainer System (Thermo Fisher Scientific), and iBind Western Systems (Thermo Fisher Scientific) following the manufacturer's instructions. Primary antibodies (Exosome-anti CD9; 10626D; dilution: 1:50, and CD63; 10628D; dilution: 1:50 for western blotting) (Supplemental Table 2) were purchased from Thermo Fisher Scientific. Secondary antibodies (anti-mouse IgG horseradish peroxidase–linked whole antibody) were purchased from GE Healthcare (Chicago, IL, USA). All blots were obtained from the same experiment and processed in parallel.

2.6. Protein profiling of PRPEV

Standard data-independent acquisition (DIA) proteome analysis (Promega Co., Ltd, Tokyo, Japan) was performed using 800 μL of an isolated PRPEV fraction. The proteomic dataset was subjected to Gene Ontology (GO) analysis using the Database for Annotation, Visualization, and Integrated Discovery for Cellular Component and Biological Process Analyses [23].

2.7. PRPEV administration and analysis

Immediately after CCl4 administration at week 8, mice were injected with 300 μL of PRPEV in the tail vein four times per week. The control group received 300 μL of PBS in the same manner. CCl_4 administration was performed in the same manner, and mice were analyzed 48 h after the last dose of CCl_4 at week 9.

2.8. Reverse transcription real time-polymerase chain reaction (qRT-PCR)

Total RNA was reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen, Venlo, Netherlands). Gene expression analysis was performed using pre-validated QuantiTech primers (Supplemental Table 3) with QuantiTech SYBR reagent (Qiagen). Real time-PCR was conducted using a Step One and StepOnePlus Real Time- PCR System (Applied Biosystems, Foster City, CA, USA). Results were obtained from at least three separate samples. *Glyceraldehyde-3-phosphate dehydrogenase* was used as an internal control. The fold change in relative gene expression compared to the control was calculated by using the $2^{-\Delta\Delta CT}$ method.

2.9. Immunostaining

For immunostaining, 10 % formalin-fixed tissue was cut into 3-μm-thick sections. When necessary, heat-mediated antigen retrieval was performed using a 10 mM sodium citrate buffer (pH 9.0; Abcam, Cambridge, UK). For 3,3′-diaminobenzidine (DAB) staining, sections were blocked with 3 % hydrogen peroxide (Wako) for 10 min at room temperature and incubated with primary antibodies (Supplemental Table 4) overnight at 4 °C. Species-specific anti-IgG biotinylated antibodies were used for detection. The slides were stained using a Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA, USA) and DAB substrate (Muto Pure Chemicals, Tokyo, Japan). Nuclei were stained with hematoxylin (Vector Laboratories Inc.). Images were acquired using an HS All-in-One fluorescence microscope (BZ-X810) (Keyence, Osaka, Japan).

2.10. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 25 (International Business Machines Corporation, Tokyo, Japan) and Microsoft Excel (Microsoft, Washington, DC, USA). Data are presented as the mean ± standard deviation (SD), and data analyzed are normally distributed. The results were assessed using Student's *t*-test. Differences between groups were analyzed using one-way or two-way analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

Further description of the experimental procedures is provided in the supplemental information.

3. Results

3.1. PRPEVs contain many factors related to EV and immune response

The PRPEVs prepared from PRP by ultrafiltration using a previously reported protocol [24], showed highly enriched EV characteristics (Fig. 1a and b). To investigate the therapeutic effect of PRPEV on cirrhosis *in vivo*, we performed a comprehensive proteome analysis of the factors expressed in PRPEV (Supplemental Table 5). In the Cellular Component section of GO analysis based on the proteome, “Extracellular Exosome” was the highest, and many factors associated with EV placed at the top of the Cellular Component analysis (Fig. 1c). Furthermore, in the GO analysis of the Biological Process section based on the proteome, many factors related to the immune response were included at the top of the analysis (Fig. 1d). These findings suggest that PRPEV contains many factors related to EV, and that PRPEV might have a therapeutic effect on cirrhosis via an immune response.

3.2. PRPEVs have the potential to promote hepatocyte proliferation and polarize macrophages to anti-inflammatory M2 phenotype

As a preliminary step in examining the effects of PRPEV on cirrhosis *in vivo*, we performed *an in vitro* analysis of the effects of PRPEV on hepatocytes and macrophages, which are the main components of the liver. PRPEVs were added to cell-line-derived hepatocytes and primary culture-derived macrophages *in vitro* and the changes in mRNA levels 48 h after PRPEV addition were analyzed. Addition of PRPEV to hepatocytes upregulated the mRNA levels of *vascular endothelial growth factor* (*VEGF*) and *albumin* (*Alb*) (Fig. 2a). Furthermore, addition of PRPEV to macrophages showed a downregulation in the mRNA levels of the markers of inflammatory M1 phenotype (tumor necrosis factor [*TNF*]-α; induced nitric oxide synthase [*iNOS*]; and chemokine (C−C motif) ligand 2 [*CCl2*]) and showed an upregulation of the mRNA levels of the markers of M2 phenotype (*Fizz-1* and *Ym-1*) (Fig. 2b). These findings suggest that PRPEV may promote hepatocyte proliferation and induce macrophage polarization toward an anti-inflammatory M2 phenotype, suggesting that PRPEV may be effective against cirrhosis *in vivo*.

3.3. PRPEV administration decreased serum ALT levels and reduced liver fibrosis in cirrhotic model mice

Altogether, the results of the *in vitro* analysis suggest that PRPEV promotes hepatocyte proliferation and suppresses inflammation. To examine the therapeutic effect on cirrhosis, PRPEV were administered to the CCl_4-induced cirrhotic model mice via the tail vein ($n = 8$ per group). A simplified timeline from PRPEV administration to the analysis of the cirrhotic mouse model is shown in Fig. 3a. The administration dosage of PRPEV was determined by prioritizing feasibility; finally, PRPEV was administered four times a week at a dose of 300 μg. Evaluation of hepatobiliary enzymes one week after PRPEV administration showed that serum ALT levels were lower in the PRPEV administration group than in the control group (Fig. 3b). Furthermore, evaluation of the amount of liver fibrosis by Sirius red staining showed reduced liver fibrosis in the PRPEV administered group compared to that in the control group (Fig. 3c). These findings indicated that PRPEV may contribute to the suppression of inflammation and reduction of liver fibrosis in cirrhotic mouse models *in vivo*.

3.4. PRPEV administration upregulated mRNA levels of VEGF and Alb, and downregulated mRNA levels of transforming growth factor-β1 (TGF-β1) in the whole liver of cirrhotic model mice

In vivo examination showed that PRPEV decreased ALT levels and reduced liver fibrosis in a cirrhotic mouse model, and *in vitro* analysis showed that PRPEV might promote hepatocyte proliferation. Therefore, to elucidate the changes in the whole liver of the cirrhotic model mice after PRPEV administration, we performed a comprehensive real-time PCR analysis of hepatocyte proliferation (Fig. 4a), inflammation (Fig. 4b), and liver fibrosis (Fig. 4c). Consistent with the *in vitro* analysis, the PRPEV administration group showed an upregulation in the mRNA levels of *VEGF* and *Alb*. Furthermore, PRPEV administration group showed a downregulation in mRNA levels of *TGF-β1*. These findings suggest that

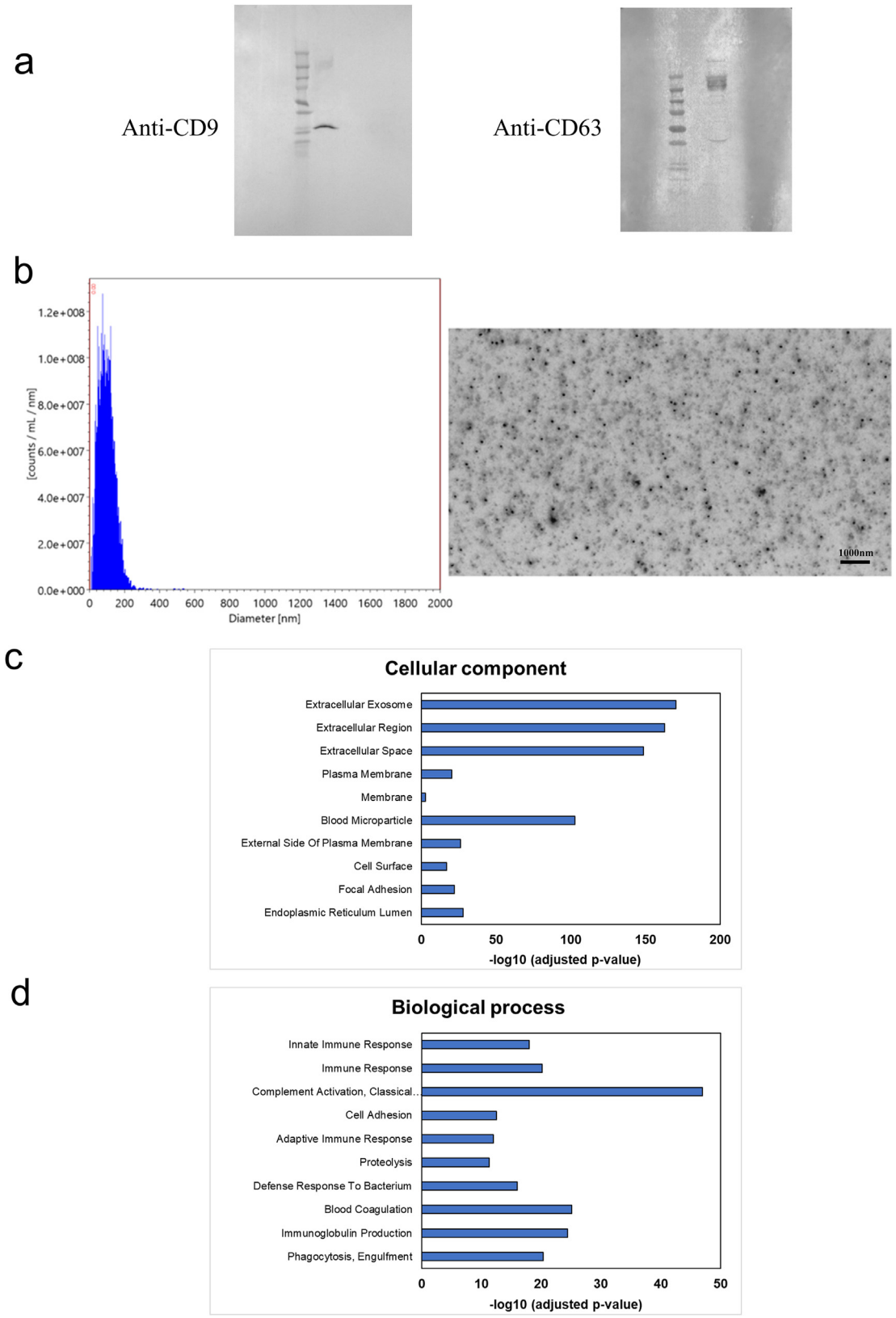

Fig. 1. Identification and characteristics of platelet-rich plasma-derived extracellular vesicles (PRPEV).
(a) Western blot analysis of CD 9 and CD 63 of PRPEV; n = 3 for each experiment. (b) Particle size distribution of PRPEV; n = 3 for each experiment. (c–d) Gene ontology analysis based on the proteome of PRPEV. The top 10 enriched terms in the Cellular Component and Biological Process are represented.

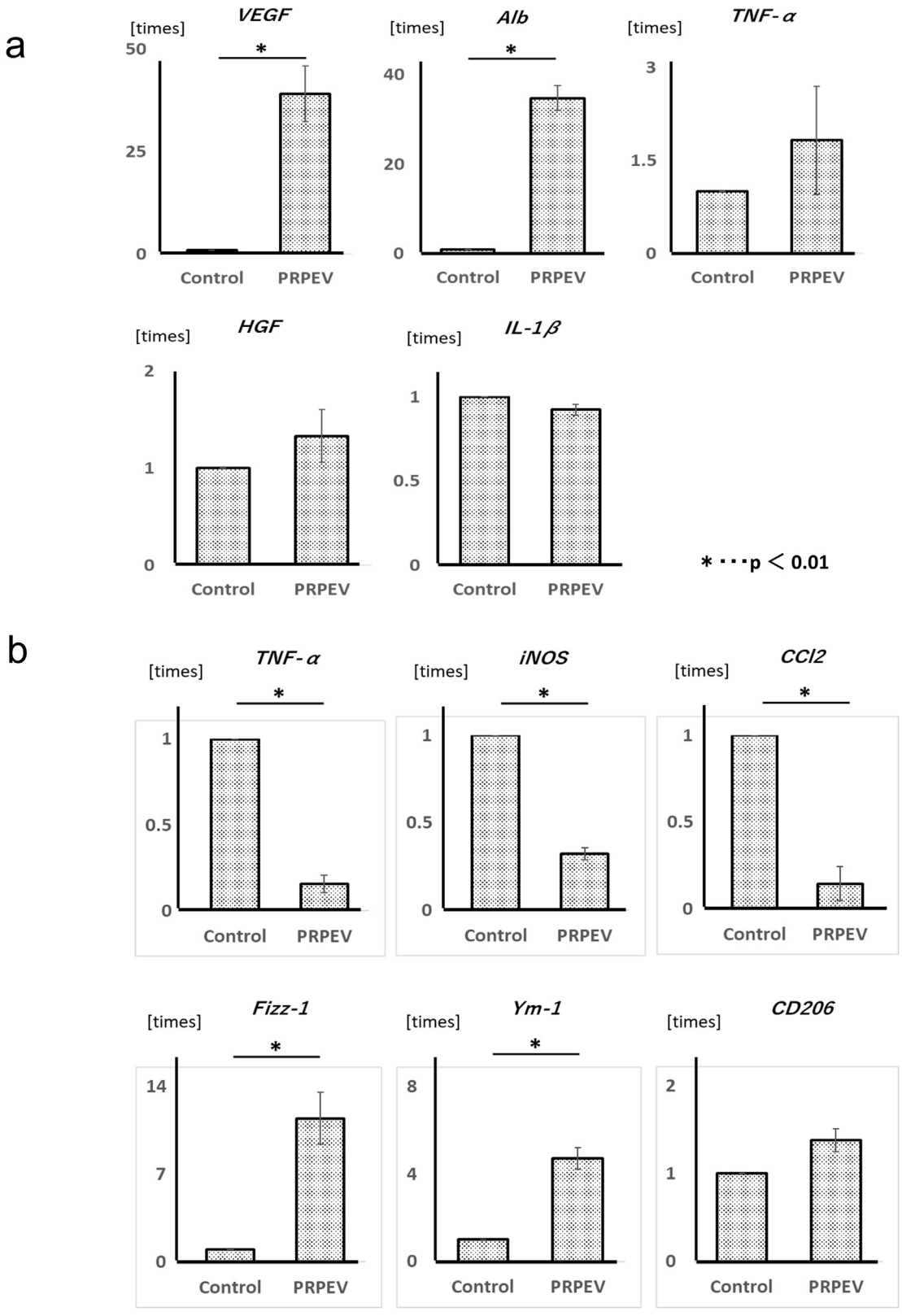

Fig. 2. *In vitro* analysis using hepatocytes and macrophages.
(a) Changes in the mRNA expression of vascular endothelial growth factor (VEGF), albumin (Alb), Tumor necrosis factor (TNF) -α, hepatocyte growth factor (HGF), and interleukin (IL) -1β in the hepatocytes (AML12) after addition of platelet-rich plasma-derived extracellular vesicles (PRPEV); n = 6 for each group. Data are presented as ratio to the standards as the mean ± SD, $p < 0.01$ (VEGF, PRPEV compared to Control), $p < 0.01$ (Alb, PRPEV compared to Control). (b) Changes in the mRNA expression of TNF-α, induced nitric oxide synthase (iNOS), chemokine (C–C motif) ligand 2 (CCl2), Fizz-1, Ym-1, and CD206 in the macrophages after addition of PRPEV; n = 6 for each group. Data are presented ratio to the standards as the mean ± SD, $p < 0.01$ (TNF-α, PRPEV compared to Control), $p < 0.01$ (iNOS, PRPEV compared to Control), $p < 0.01$ (CCl2, PRPEV compared to Control), $p < 0.01$ (Fizz-1, PRPEV compared to Control), $p < 0.01$ (Ym-1, PRPEV compared to Control).

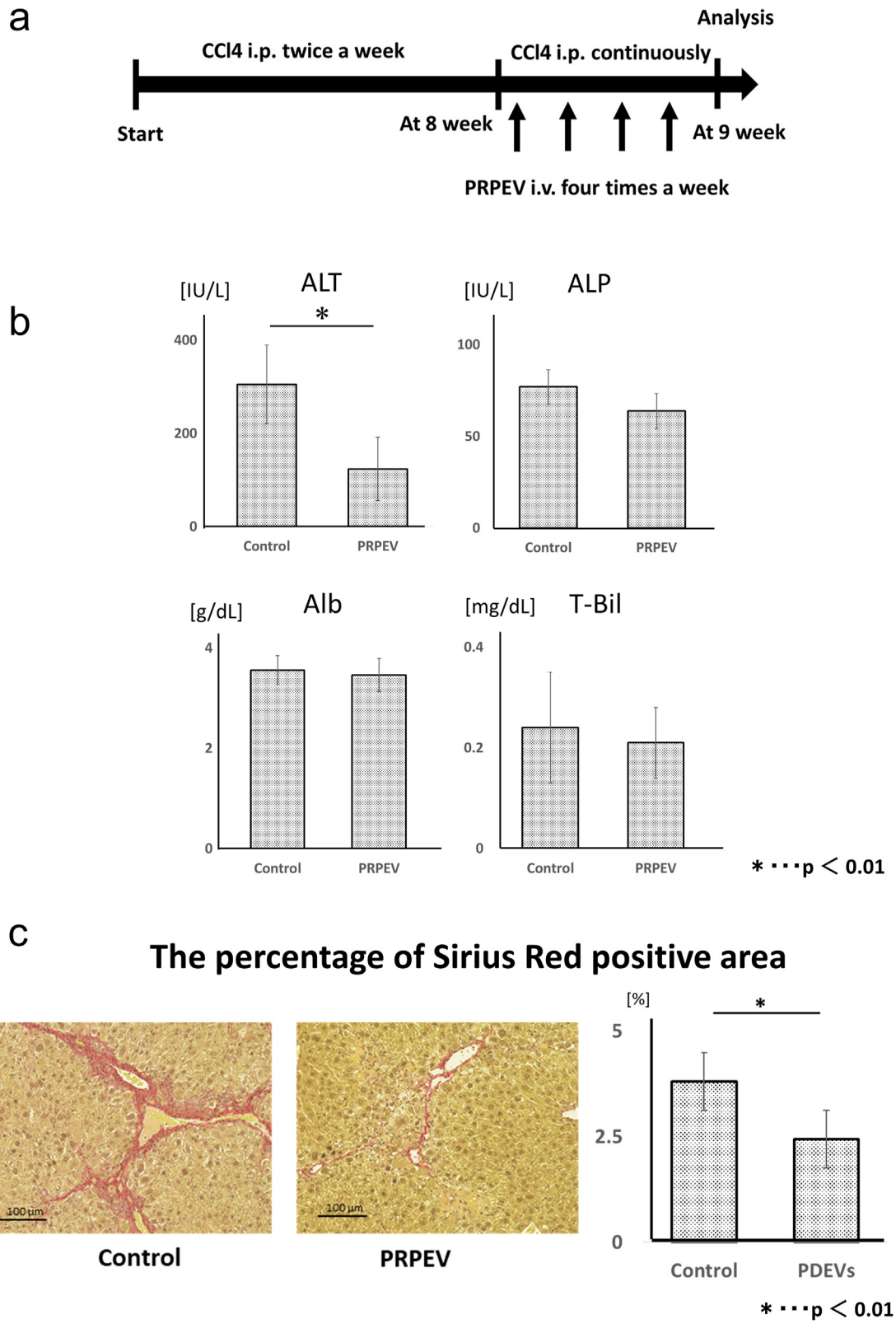

Fig. 3. Therapeutic effect of platelet-rich plasma-derived extracellular vesicles (PRPEV) on cirrhosis in mice.
(a) Schematic representation of the timeline from PRPEV administration to analysis of cirrhotic model mice. (b) Serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (Alb), and total bilirubin (T-Bil) after PRPEV administration. Data are presented as the mean ± SD, n = 8 in each group, $p < 0.01$ (ALT, PRPEV compared to Control). (c) Immunostaining with Sirius Red in the liver showing the degree of liver fibrosis in mice from Control and PRPEV group; n = 8 for each group. Data are presented as the mean ± SD, $p < 0.01$ (PRPEV compared to Control).

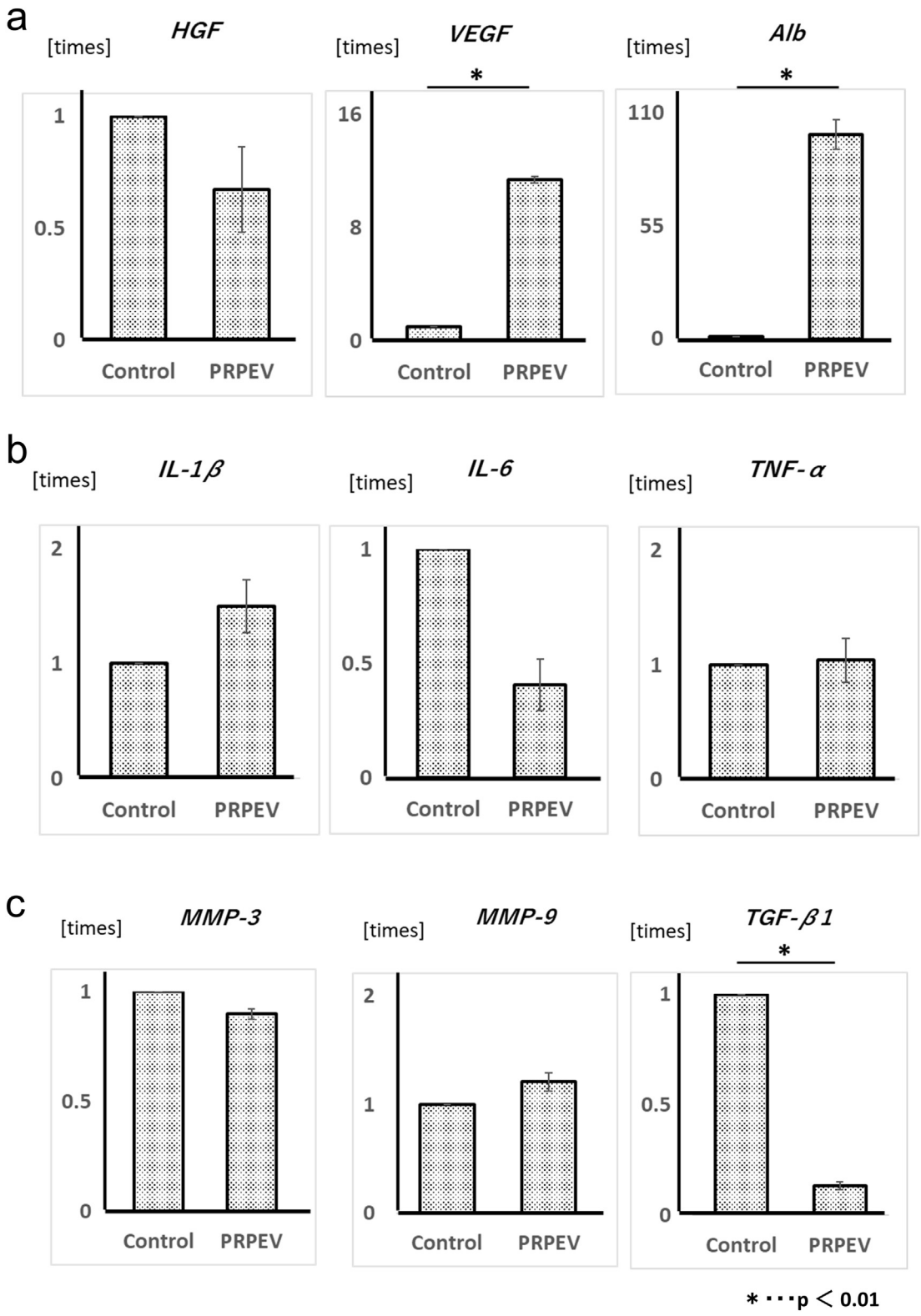

Fig. 4. Changes in the mRNA expression after platelet-rich plasma-derived extracellular vesicles (PRPEV) administration in the whole liver.
Data are presented ratio to the standards as the mean ± SD, n = 8 for each group. (a) Changes in the mRNA expression related to liver regeneration (hepatocyte growth factor [HGF], vascular endothelial growth factor [VEGF], and albumin [Alb]). $p < 0.01$ (VEGF, PRPEV compared to Control). (b) Changes in the mRNA expression related to inflammation (interleukin-1β [IL-1β], interleukin-6 [IL-6], and tumor necrosis factor-α [TNF-α]). (c) Changes in the mRNA expression related to liver fibrosis (matrix metalloplotainase-3 [MMP-3], matrix metalloplotainase-9 [MMP-9], and transforming growth factor-β1 [TGF-β1]). $p < 0.01$ (TGF-β1, PRPEV compared to Control).

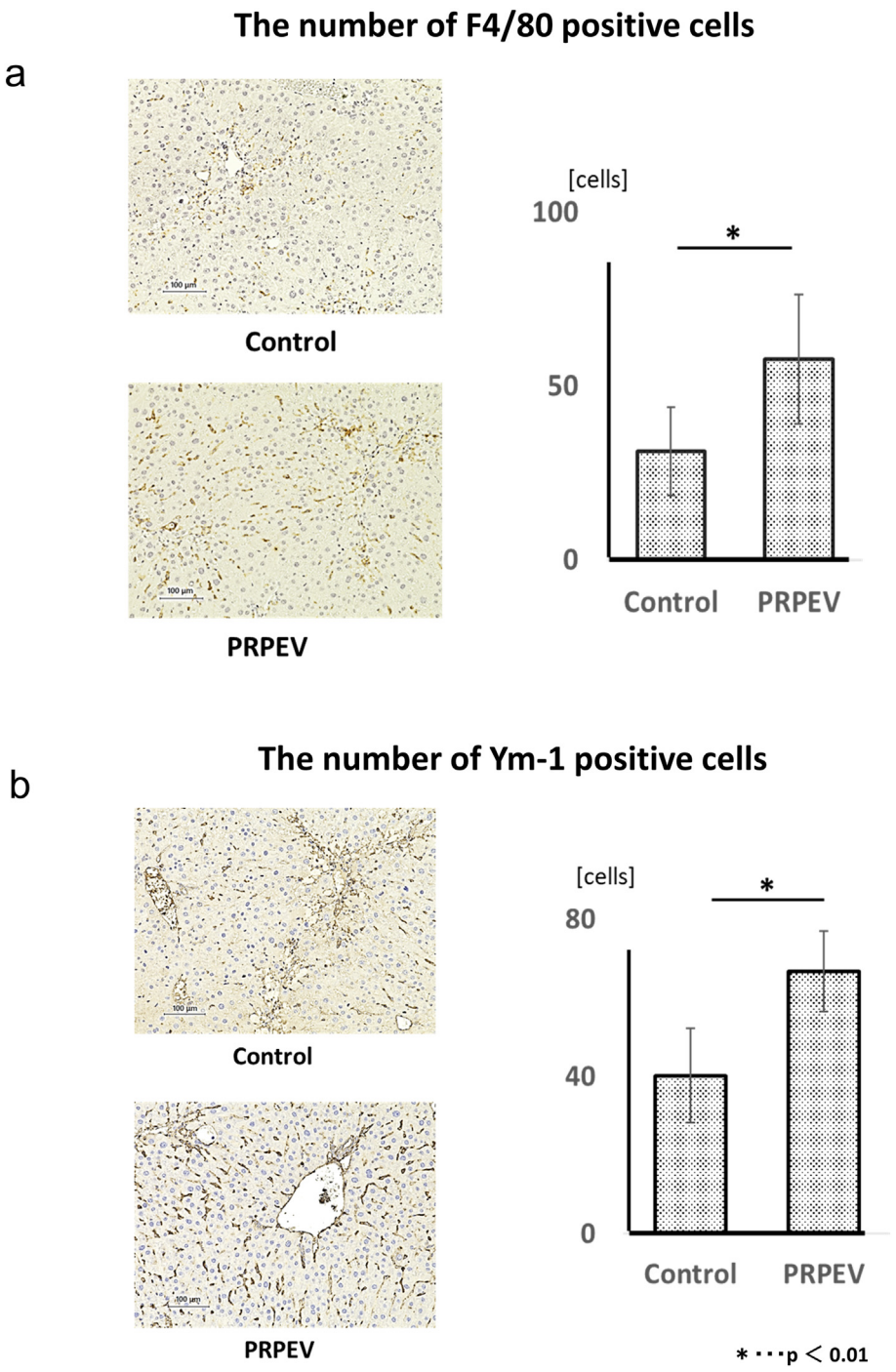

Fig. 5. Analysis of macrophages in the liver after platelet-rich plasma-derived extracellular vesicles (PRPEV) administration.
(a) Immunostaining for F4/80 in the liver; n = 8 for each group. The number of F4/80 positive cells in the liver was counted, and data are presented as the mean ± SD. $p < 0.01$ (PRPEV compared to Control). (b) Immunostaining for Ym-1 in the liver; n = 8 for each group. The number of Ym-1 positive cells in the liver was counted, and data are presented as the mean ± SD. $p < 0.01$ (PRPEV compared to Control).

PRPEV administration promotes hepatocyte proliferation and reduces liver fibrogenesis.

3.5. *PRPEV administration increased the number of anti-inflammatory M2 phenotype macrophages in the liver*

In vitro analysis showed that PRPEV may polarize macrophages to an anti-inflammatory M2 phenotype. To elucidate the changes in macrophages in the livers of cirrhotic model mice after PRPEV administration, we performed immunostaining for macrophage markers. One week after PRPEV administration, there was an increase in the number of F4/80 positive cells in the liver compared to that in the control group (Fig. 5a). Immunostaining for Ym-1, an M2 macrophage marker of anti-inflammation, showed an increase in the number of Ym-1 positive cells in the liver compared to that in the control group (Fig. 5b). These findings suggest that PRPEV increases the number of macrophages in the liver and further polarizes them toward an anti-

inflammatory M2 phenotype, which is consistent with the results of the *in vitro* analysis.

4. Discussion

Because of the large unmet medical needs for cirrhosis, basic research to identify novel therapeutic approaches for this condition is critical [25]. In this study, we examined PRPEV as a novel therapeutic agent for the treatment of cirrhosis. The PRPEV in this study were collected from the PRP supernatants by ultrafiltration, as previously reported [26,27]. First, we attempted to collect PRPEV by ultracentrifugation, which is the conventional method for EV collection; however, it was difficult to collect a sufficient amount of PRPEV for administration to cirrhotic model mice. Therefore, in this study, we explored the feasibility of PRPEV collection and used ultrafiltration-derived PRPEV. Based on the proteomic results, the ultrafiltration-derived PRPEV contained many factors associated with EV. Furthermore, based on the View-sizer results, the ultrafiltration-derived PRPEV were successfully collected with high enrichment. After confirming these findings, we proceeded with studying the investigations on the role of PRPEVs.

Before administering PRPEV to cirrhotic model mice, it was necessary to investigate the characteristics of PRPEV; therefore, we initially performed a proteome analysis of PRPEV. GO analysis based on the proteome of the PRPEV showed that "Extracellular Exosome" was at the top of the list of Cellular Component section. Furthermore, many EV-related proteins were detected in the list of Cellular Component section, supporting the notion that PRPEV have EV characteristics. In the Biological Process section, PRPEV contains many proteins related to "Immune Reaction", indicating that PRPEV affects immune cells, especially macrophages. Macrophages have been reported to change polarity under the influence of factors associated with immune response [28,29]; therefore, PRPEV may contribute to the polarization of anti-inflammatory M2 macrophages.

Macrophages are distinguished by their flexibility to adapt their functions and roles in response to their surroundings, as well as their various phenotypes. Among them, two phenotypes are representative: the classically activated M1 phenotype, which is an inflammatory macrophage with iNOS, TNF-α, and CCl2 as major markers; and the alternatively activated M2 phenotype, which is an anti-inflammatory macrophage with Fizz-1, Ym-1, and CD206 as major markers [29,30]. During liver injury, macrophages change their polarity to M1 macrophages to promote inflammation, while during the downregulation of inflammation, macrophages change their polarity to M2 type to contribute to tissue repair [29]. In this study, the addition of PRPEV to macrophages *in vitro* showed downregulation in mRNA expression of M1 inflammatory macrophages and upregulation in mRNA expression of M2 anti-inflammatory macrophages. Furthermore, immunostaining of the liver after PRPEV administration showed an increase in the number of F4/80 and Ym-1 positive cells. These results suggest that PRPEV might polarize macrophages to an anti-inflammatory M2 phenotype. Previous studies reported that macrophages polarize to the M2 phenotype by phagocytosing EV [31–33]. Furthermore, macrophages are abundant in the liver and contribute strongly to suppressing inflammation [30]; therefore, the decrease in serum ALT levels by PRPEV might be related to the anti-inflammatory function of macrophages.

In vivo CCl_4-induced cirrhosis was used as a mouse model. Continuous intraperitoneal administration of CCl_4 leads to cirrhosis [34]. Since this mouse model has been used in many studies related to cirrhosis, it was presumed to be appropriate for the validation of PRPEV in this study. Regarding the schedule of EV administration, we performed 300 μg PRPEV per dose four times weekly. EV may have dose-dependent effects, and unlike cell therapy, EV are less likely to cause embolic adverse events due to high-dose administration [25]; therefore, we planned an administration of a large dose of PRPEV as possible. There was no significant effect of once or twice weekly PRPEV administration (data not shown), though PRPEV administration more than five times weekly, was not feasible. Therefore, we performed four times weekly administration in this study.

PRPEV administration significantly decreased serum ALT levels *in vivo*. This suggests a macrophage-mediated effect, as described above. Although there was no significant change in the serum Alb levels after PRPEV administration, the mRNA expression of *Alb* and *VEGF* was significantly upregulated *in vitro* and *in vivo*, suggesting that PRPEV may contribute to the promotion of liver tissue repair [35]. Furthermore, in addition to a decrease in the serum ALT levels, PRPEV significantly reduced the extent of liver fibrosis. In general, fibrogenesis is caused by the activation of hepatic stellate cell (HSC) by stimulating factors such as TGF-β1 [36–38]. In this study, mRNA expression of *TGF-β1* was downregulated after PRPEV administration, suggesting that PRPEV might contribute to anti-fibrogenesis.

Although we examined and discussed the effect of PRPEV on cirrhosis, this study has a few limitations. First, the effects of PRPEV on liver cells, other than hepatocytes and macrophages, are unclear. From the results of the PRPEV proteome, PRPEV affected immune cells such as macrophages; however, we did not confirm its effect on other cells such as T-cells, B-cells, and dendritic cells. Furthermore, the downregulation in the mRNA levels of *TGF-β1* suggested that HSC were inactivated and led to the reduction of liver fibrosis; however, the detailed mechanism of HSC was not examined in this study. Further studies are needed to clarify the effects of PRPEV on cirrhosis. Another limitation concerns the adverse events associated with PRPEV administration. Though, there were no problems with the tolerance checks in mice in this study; however, the safety of EV in humans need to be examined. Thus, concerns remain regarding the use of PRPEV therapy in patients with cirrhosis.

In summary, this study showed that PRPEV may suppress inflammation in the liver through the effects of macrophages, promote hepatocyte proliferation, and contribute to anti-fibrogenesis (the mechanisms summarized in Graphical Abstract). Although several issues need to be resolved before the clinical application of PRPEV in patients with cirrhosis, we believe that this basic research on PRPEV will make a significant contribution to the development of novel treatments for cirrhosis.

Ethics statement

All animal experiments were approved by the Institutional Animal Care and Committee of Niigata University (SA01135). Collection of blood samples from healthy volunteers was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of Niigata University (2024–0108).

Funding information

This research was funded by a Grant-in-Aid for Scientific Research from the Japanese Society for the Promotion of Sciences (21K15993) awarded to Yusuke Watanabe and was funded by a Grant-in-Aid for Scientific Research from the Japanese Society for the Promotion of Sciences (22H03055) awarded to Shuji Terai.

Declaration of competing interest

None.

Acknowledgements

The authors thank Dr. Yoshitomi Kanemitsu of the Department of Omics and Systems Biology at Niigata University for assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2024.10.010.

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