ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2022. Vol. 38. N 4. P 231–241
doi: http://dx.doi.org/10.7124/bc.000A80

UDC 577.2:576.54

Study of the interaction between macrophages and human umbilical cord MSCs *in vivo* on the model of perotonitis in mice

P. O. Pikus[1,2], S. Y. Rymar[1,2], N. S. Shuvalova[2], V. A. Kordium[1,2]

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143

[2] State Institute of Genetic and Regenerative Medicine, NAMS of Ukraine
67, Vyshhorodska Str., Kyiv, Ukraine, 04114
polinaignatchenko7@gmail.com

Mesenchymal stem cells (MSCs) have unique properties that ensure the regeneration of damaged tissues, which allows using them in the cell therapy of various diseases. The realization of these properties is mainly related to the interaction of MSCs with macrophages. **Aim.** To study the interaction of the human umbilical cord MSCs with macrophages *in vivo* using a model of sterile inflammation of the peritoneal cavity in mice. **Methods.** Cytological methods for assessing acute inflammation of the peritoneal cavity of mice, isolation and cultivation of the human umbilical cord MSCs, study of the expression of the MSC surface markers by flow cytometry, determination of the phagocytic activity of mononuclear cells of peritoneal cavity, isolation of mononuclear RNA, RT-PCR, methods of statistical analysis. **Results.** The dynamics of the development of the MSCs' therapeutic effect after their transplantation into the peritoneal cavity of mice was studied; a method for assessing the therapeutic activity of various MSC preparations was developed; the fast polarization of macrophages to the M2 state after the injection of MSCs was shown, by studying the phagocytic activity of macrophages and expression of the *IL-10* gene. **Conclusions.** The most effective method to increase the therapeutic potential of MSCs is preconditioning with low doses of H_2O_2.

Keywords: MSCs, macrophages, peritonitis.

Introduction

Mesenchymal stem cells (MSCs) are the most promising candidates for use in cell therapy of various diseases. Many questions concerning the properties of the mechanism of MSCs' action remain open, despite a huge number of studies devoted to the investigation of the MSCs therapeutic properties and the conditions for their realization. To date, it is known that the therapeutic efficacy of MSCs is mainly due to their immunomodulatory properties,

© 2022 P. O. Pikus *et al.*; Published by the Institute of Molecular Biology and Genetics, NAS of Ukraine on behalf of Biopolymers and Cell. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited

which are realized as a result of the interaction of MSCs with cells of the immune system [1, 2]. The key participants in this interaction are monocytes and macrophages, which control inflammation in the body. The aim of our research was to study the interaction of the human umbilical cord MSCs with macrophages *in vivo* using a model of sterile inflammation of the abdominal cavity in mice.

Materials and Methods

The studies were carried out on male BALB/c mice aged 2–3 months with an average weight 25–30 g. During the experiment, all animals were kept under standard conditions, divided into control and experimental groups. All experiments were conducted in accordance with the accepted ethical standards for working with laboratory animals [3].

Induction of sterile abdominal inflammation in mice. Peritonitis in mice was induced by intraperitoneal injection of 1 ml of a 3 % solution of proteose peptone (France). Animals of the negative control group were injected with 1 ml of saline [4]. The number of macrophages and neutrophils in the peritoneal exudate as well as the total cell count were estimated to determine the development of inflammation. Exudate from the peritoneal cavity was obtained by washing the peritoneal cavity with 5 ml of cold α-MEM medium (HyClone, Thermo Scientific). The number of cells in the exudate was determined by direct counting in a Goryaev chamber using 0.4 % trypan blue solution to detect dead cells [5]. Macrophages and monocytes were identified after staining according to Romanovsky-Giemsa (counted in 20 light fields of the microscope). To evaluate the phagocytic activity, the peritoneal macrophages were incubated in Petri dishes on coverslips for adhesion, which allowed their separation from other cell types isolated from the peritoneal exudate. The phagocytic activity of macrophages was assessed by counting internalized and adherent *E. coli* in 100 phagocytic cells.

The phagocytic index was calculated using the following formula:

phagocytic index = number of ingested and adhered *E. coli* / number of macrophages × × 100 [6].

Statistical analysis. Statistical significances were determined using GraphPad Prism software, Version 8.0.1. The comparisons between multiple groups were evaluated via one-way analysis of variance (ANOVA) followed by Tukey's test. For all tests, $p < 0.05$ was considered significant.

Isolation and cultivation of human umbilical cord MSCs (hUC-MSCs). MSCs were isolated from a human umbilical cord after removal of the umbilical blood vessels by the explant method [7]. MSCs were cultivated during 24 h in α-MEM medium containing 10 % fetal bovine serum (HyClone, Thermo Scientific), 200 U/ml penicillin, and 200 μg/ml streptomycin and preconditioned MSCs with H_2O_2 (30 μM), melatonin (5μM), ascorbic acid (200 μM), γ-interferon (250U/ml), lipopolysaccharide (LPS) (1μg/ml). After the second passage, MSCs were evaluated for the surface marker expression and differentiation capacity [8]. The adipogenic, osteogenic, and chondrogenic differentiation potentials of MSCs were tested using the StemPro® Differentiation Kits (Gibco) (Figure 1B – D). [The] Surface markers of

MSCs were assessed by flow cytometry with FACS Aria (Becton Dickinson Lakes, NJ) using CD90 FITC, CD73 APC, CD105 PerCP — Cy5-5, CD45 FITC, CD34 APC. (Fig. 1E).

Isolation of RNA, analysis of gene expression in mice. The peritoneal exudate was centrifuged, and the cells were washed twice with phosphate-buffered saline**.** Total RNA was isolated using the NucleoSpin RNA Kit (Macherey-Nagel) according to the manufacturer's instructions. For cDNA synthesis, 200 ng of RNA were used as a template for

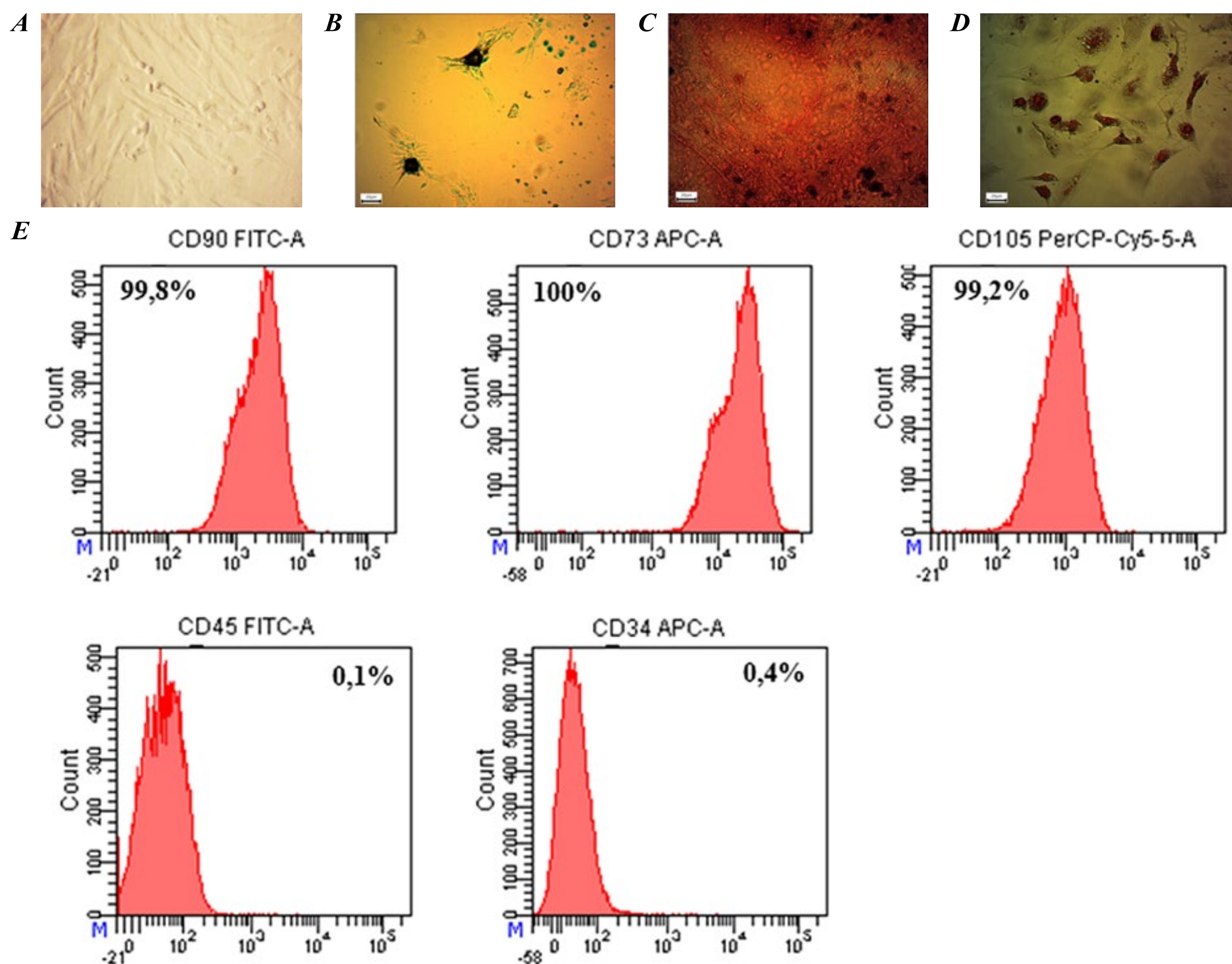

Fig. 1. The characteristics of hUC-MSCs: *A* — hUC-MSCs were isolated and cultured from the umbilical cord tissue. Morphology of hUC-MSCs shows successful proliferation. *B* — chondrogenic differentiation was detected by Alcian blue staining; *C* — osteogenic differentiation was detected by Alizarin red S staining; *D* — adipogenic differentiation was detected by Oil Red O staining; *E* — an expression of MSC surface markers from the human umbilical cord. Scale bar 20 μm.

reverse transcription (RT) with the RevertAid H Minus First Strand cDNA Kit (Thermo Scientific). The primer sequence used for RT-PCR was following:

for *IL-10* (forward) 5'-GCCGGGAAGA-CAATAACTGC-3',

(reverse) 5'-TCAGCTTCTCACCCAGG-GAA-3',

for *GAPDH* (forward) 5'-AAAAGGGTC-ATCATCTCCGC-3',

(reverse)5'-CCTGTTGCTGTAGCCG-TATT-3'.

The mRNA levels of the target genes were normalized against the mRNA level of *GAPDH*. ImageLab program was used for densitometry analysis of electrophoresis data.

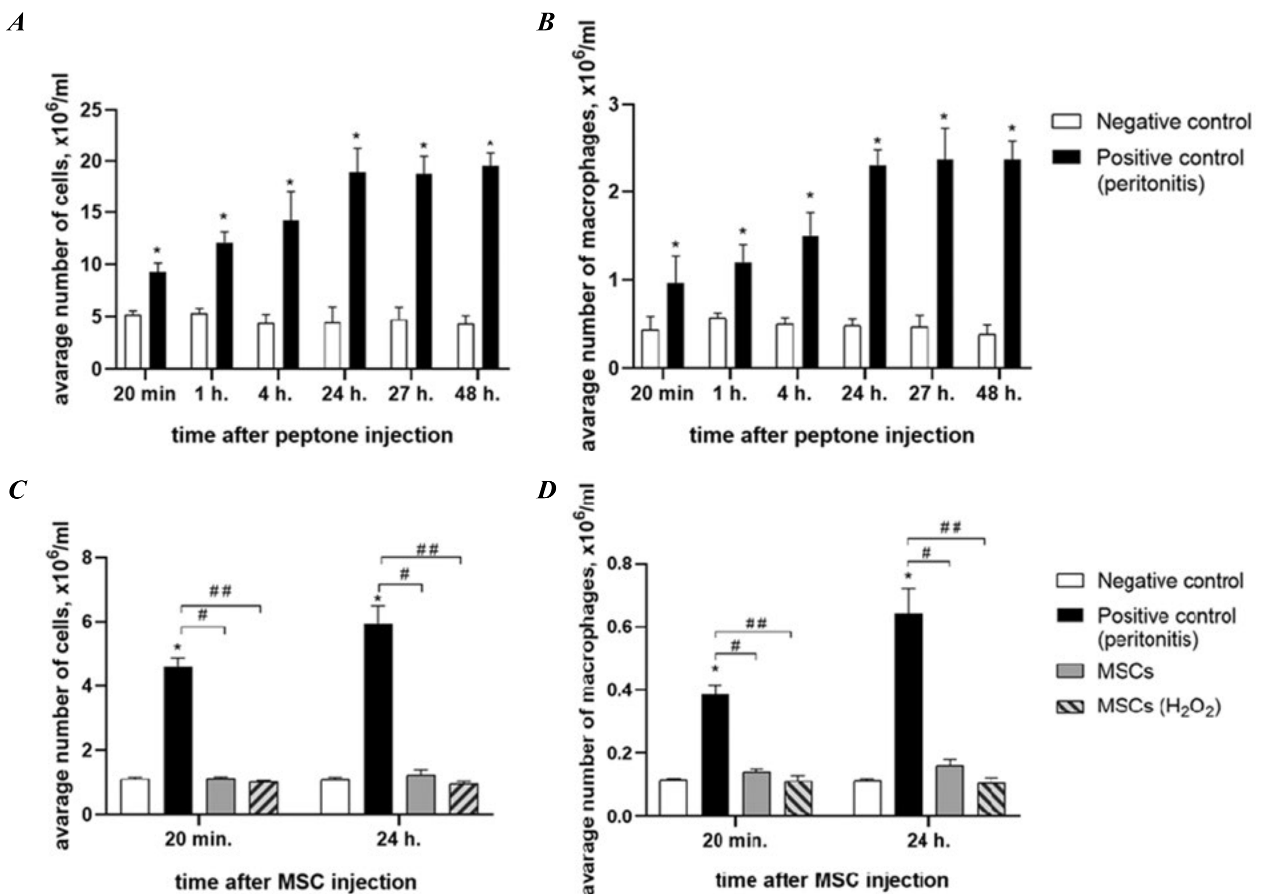

Fig. 2. *A – B* — The development of abdominal inflammation in mice following the injection of proteose peptone. The total number of cells (A) and the number of macrophages (B) in mouse exudate. *P< 0.5 – negative control vs. positive control. *C – D* — The effect of time of MSCs injection on inflammation. The total number of cells (C) and macrophages (D) from mice abdominal exudate after transplantation of native and preconditioned MSCs (20 min - inflammation prevention and 24 h - inflammation suppression). *P< 0.5 — negative control vs. positive control, #P< 0.5 — positive control vs. MSCs, ##P< 0.5 — positive control vs. MSCs (H_2O_2).

Results and Discussion

The administration of proteose peptone to mice caused the development of abdominal inflammation. The study of the development of peritoneal inflammation in mice after intraperitoneal injection of 1 ml of sterile 3 % proteose peptone showed that the total number of cells and the number of macrophages and monocytes in the exudate increased on average by 4.3 times and 4.7 times, correspondingly, 4 hours after the administration of peptone. An increase in the number of cells continues for 24 hours, after which the rate of the process slows down, the number of cells practically does not change after 48 hours (Fig. 2 A – B). All experiments regarding the effect of MSCs on the course of inflammation were performed during 24 hours following the proteose peptone administration.

Effects of MSCs dosage and administration time on inflammation of the abdominal cavity. In the first series of experiments, mice were intraperitoneally injected with MSCs at a dose of $50x10^3$ cells/mouse 20 min and 24 h after the administration of proteose peptone. Injection of MSCs 20 min after the administra-

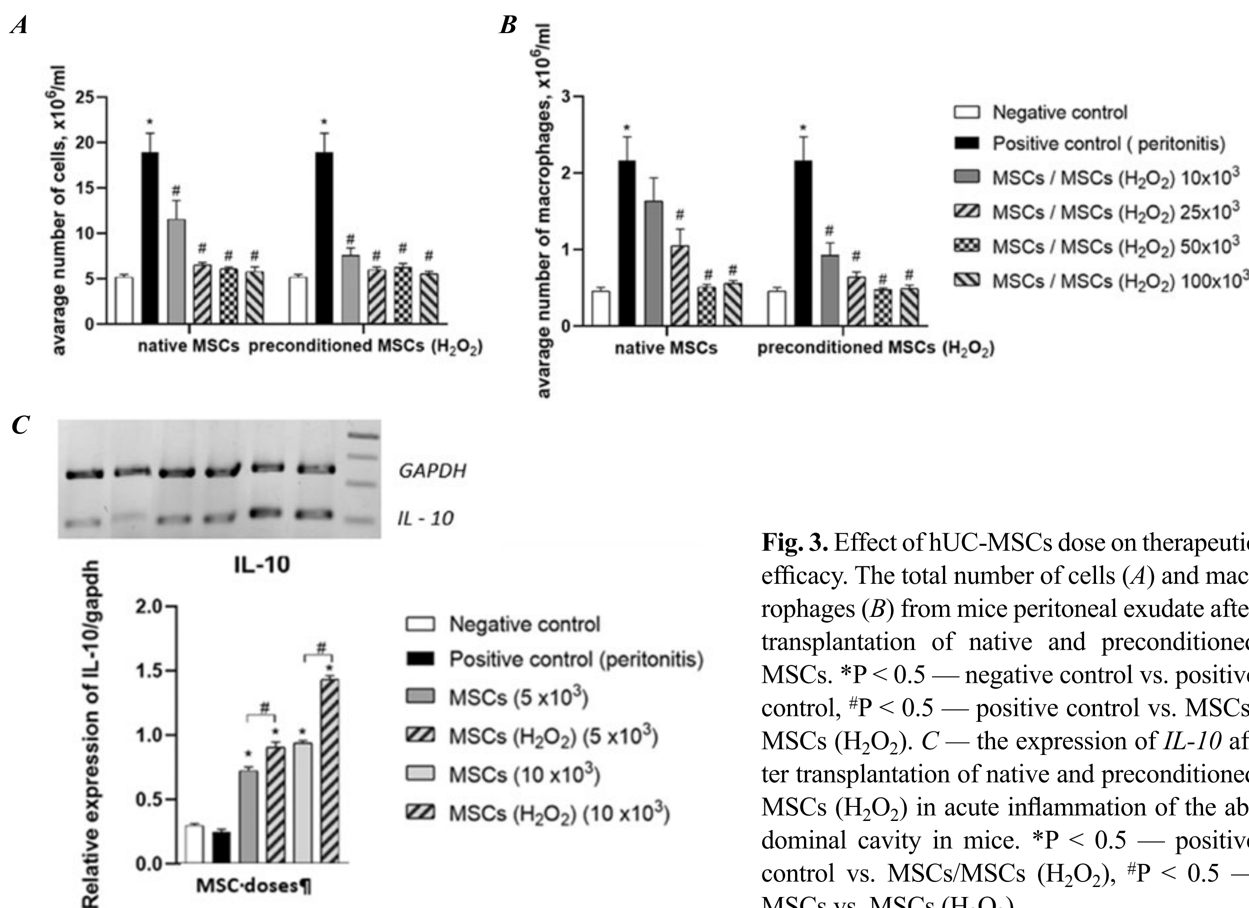

Fig. 3. Effect of hUC-MSCs dose on therapeutic efficacy. The total number of cells (*A*) and macrophages (*B*) from mice peritoneal exudate after transplantation of native and preconditioned MSCs. *P < 0.5 — negative control vs. positive control, #P < 0.5 — positive control vs. MSCs/MSCs (H_2O_2). *C* — the expression of *IL-10* after transplantation of native and preconditioned MSCs (H_2O_2) in acute inflammation of the abdominal cavity in mice. *P < 0.5 — positive control vs. MSCs/MSCs (H_2O_2), #P < 0.5 — MSCs vs. MSCs (H_2O_2).

tion of proteose peptone prevented the development of inflammation, and administration of MSCs 24 h later, when the inflammation was intense, stops inflammation (Fig. 2 C – D).

The amount of transplanted MSCs is a crucial factor in the therapeutic impact of MSCs. The study of the dependence of the total number of cells and the number of immune cells (macrophages, monocytes and neutrophils) in the exudate extracted from the peritoneal cavity on the dose of injected MSCs showed that the administration of doses greater than $50x10^3$ no longer affects the therapeutic effect. The signs of inflammation disappear when the therapeutic effect reaches its maximum (Fig. 3 A – B).

The dynamics of the development of the therapeutic effect after the introduction of MSCs. A low dose of MSCs ($5\ x10^3$ cells/mouse) administered intraperitoneally initiated the development of a therapeutic effect and reduced the number of macrophages in the exudate on average by 50 %. One of the goals was to find the conditions, at which this model is most sensitive to the quality of MSC preparation in order to compare the therapeutic potential of various MSC preparations. Development over time of the therapeutic effect of a low dose of the native MSCs and the MSCs preconditioned with H_2O_2 ($5\ x10^3$ cells/mouse) was studied. When MSCs are administered intraperitoneally the anti-inflammatory impact becomes apparent very fast. As soon as 15 min later the number of macrophages in the peritoneal cavity decreases by almost 1.8 times with the injection of the native MSCs and by 3 times with the injection of MSCs preconditioned with H_2O_2. Up to 24 h later, the number of macrophages in the peritoneal cavity decreases slightly, but only in the case of the injection of preconditioned MSCs, the signs of inflammation disappear completely. The maximum difference in the therapeutic effect[s] of the two preparations of MSCs is observed 15 min after the MSC[s] transplantation (Fig. 4). These results were used to compare the methods of MSCs preconditioning.

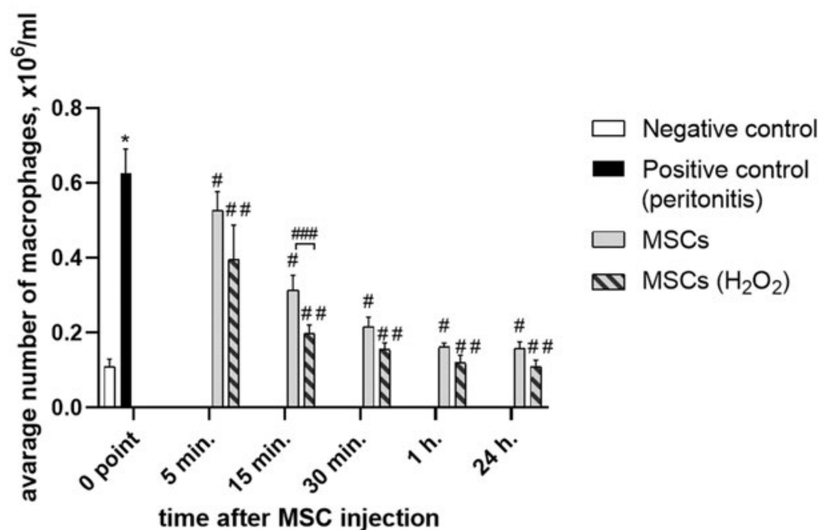

Fig. 4. Dynamics of the development of the therapeutic effect after the administration of native and preconditioned MSCs (H_2O_2). *P< 0.5 — negative control vs. positive control, $^{\#}$P< 0.5 — positive control vs. MSCs, $^{\#\#}$P< 0.5 — positive control vs. MSCs (H_2O_2), $^{\#\#\#}$P< 0.5 — MSCs vs. MSCs (H_2O_2).

Effects of MSCs intraperitoneal injection on abdominal cavity macrophages. Numerous studies, both *in vitro* and *in vivo*, have demonstrated that the interaction between MSCs and macrophages, or so-called "training of macrophages by MSCs" plays a crucial role in the immunomodulatory activity of MSCs [9, 10]. This interaction leads to polarization of macrophages, i.e. to the transition of classically activated pro-inflammatory M1 macrophages to the alternatively activated anti-inflammatory M2 macrophages [11, 12]. After the administration of proteose peptone, the number of pro-inflammatory M1 macrophages increases (Fig. 2 B), but their number quickly decreases after the transplantation of MSCs (Fig. 2 D), i.e. the signs of inflammation disappear quickly. This is probably connected to the phenotypic transition of pro-inflammatory M1 macrophages to the anti-inflammatory M2 phenotype. Since the most characteristic feature of M2 macrophages is a high level of the *IL-10* expression [13, 14], the study of the expression of *IL-10* by intraperitoneal macrophages was carried out. The results showed that injection of MSCs into the peritoneal cavity leads to an increase of the *IL-10* expression by macrophages, and the expression of *IL-10* after the injection of MSCs ($H_2O_{2)}$ is higher than after the injection of the native MSCs (Fig. 3 C).

The main property of macrophages is the ability to phagocytosis. The results of a study on the phagocytic activity of macrophages in the peritoneal cavity during sterile inflammation and after the transplantation of MSCs revealed that 15 min. after the transplantation of MSCs, the phagocytic index increased, and more intense growth could be observed with the injection of MSCs preconditioned with H_2O_2. After one hour, the phagocytic index starts to decline, and after 24 h, its value is similar to that observed in the control. The phagocytosis changes are accompanied by the

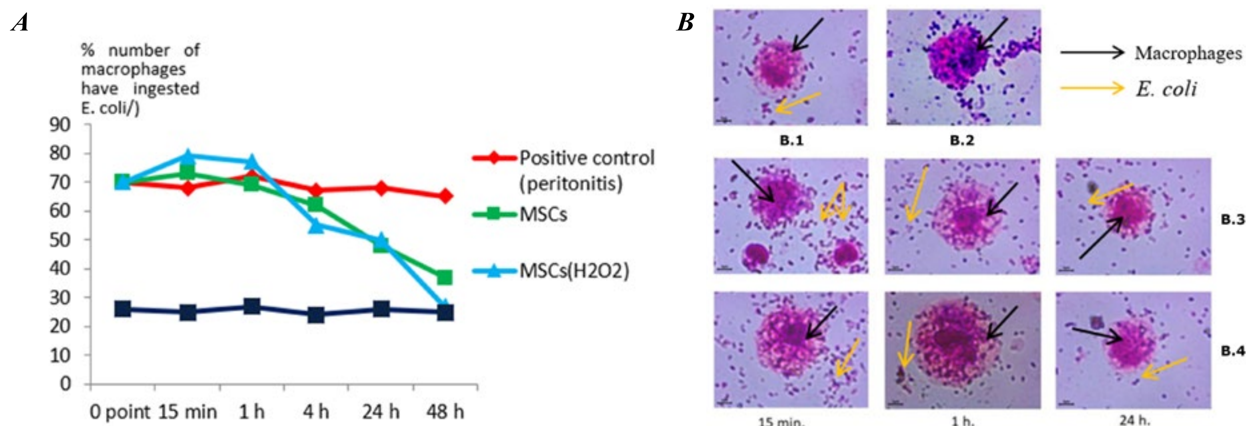

Fig. 5. Phagocytic activity of peritoneal macrophages during sterile inflammation and after MSCs injection. *A* — phagocytic activity of peritoneal macrophages from mouse exudate. *B1* — phagocytosis of *E. coli* by negative control macrophages, *B2* — phagocytosis of *E. coli* by macrophages of positive control (peritonitis), *B3* — phagocytosis of *E. coli* by macrophages after injection of native MSCs, *B4* — phagocytosis of *E. coli* by macrophages after injection of preconditioned MSCs (H_2O_2). Scale bar 5 μm.

changes in inflammatory signs (Fig. 5). Depending on the factors that induce polarization of macrophages, phagocytosis in some cases in M1 exceeds that observed in M2 [15], in others — phagocytosis is higher in M2 [16, 17]. *In vitro* studies show that the co-cultivation of MSCs and macrophages leads to the expression by macrophages of genes characteristic of the M2 phenotype and an increase of phagocytosis [18, 19]. This result was also observed in our *in vivo* study. The effects that we observe upon the transplantation of MSCs into the peritoneal cavity of mice allow us to consider the interaction of MSCs and pro-inflammatory macrophages in peritoneal cavity as an analogy for their co-cultivation. The change in the phagocytic activity of macrophages after the MSC transplantation into the peritoneal cavity coincides with an increase in the expression of *IL-10*, which indicates a polarization of pro-inflammatory macrophages into the M2 phenotype. Yet, in the body there is a rapid leaving of macrophages from the peritoneal cavity, which is associated with the resolution of inflammation, in contrast to the *in vitro* events. When the polarization of macrophages occurs in the body, more complex phenomena might be seen. It is assumed that in the inflammation resolution phase *in vivo*, macrophages can be activated neither classically nor alternatively but in hybridous way [20]. Our results demonstrate that the injection of MSCs, even in low doses ($5x10^3$ cells/mouse), leads to the polarization of macrophages into the anti-inflammatory M2 phenotype and to a rapid resolution of acute abdominal inflammation in mice.

Study of the therapeutic potential of MSCs preconditioned by various factors.

Numerous studies have demonstrated that MSCs are highly sensitive to the microenvironment. By modifying their secretome composition through preconditioning during *in vitro* cultivation, it is possible to increase the therapeutic potential of MSCs by enhancing survival after transplantation, promoting migratory abilities, and preventing apoptosis [21–23]. Preconditioning of MSCs by various physical, chemical and biological factors affects the therapeutic potential of MSCs in different ways. It was interesting to compare the effect of preconditioning by some factors on the anti-inflammatory efficacy of hUC-MSCs using one model — acute inflammation of the peritoneal cavity. The study of the dynamics of the development of the therapeutic effect of MSCs on inflammation of the peritoneal cavity in mice made it possible to define the dose of MSCs and the time of the effect development after the MSC transplantation, at which the system is most sensitive to the therapeutic efficacy of MSCs. The use of a high dose of MSCs, which leads to complete resolution of inflammation, does not allow us to see the difference in the anti-inflammatory activity of MSCs differently preconditioned. The use of a low dose of MSCs and observation of the effect 15 min after MSC administration did not lead to complete resolution of inflammation (Fig. 4) but made it possible to compare the therapeutic potential of MSC preparations after preconditioning with various factors. One of these factors was H_2O_2, which is known to enhance proliferation of MSCs, their migration, and survival, to increase the resistance to oxidative stress, and to enhance the therapeutic efficacy of MSCs at low concentrations H_2O_2 [24]. Additionally,

we used melatonin, which has antioxidant effects, enhancing the survival of MSCs after transplantation [25, 26], ascorbic acid, which can activate mitochondria, enhance MSC proliferation, suppress senescence, and improve therapeutic effects [27–29]. Another factor used to precondition MSCs is LPS, which induce[s] the transition of MSCs into a pro-inflammatory and immunosuppressive state by increasing the expression of pro-inflammatory cytokines [30, 31].

γ-Interferon is the substance that makes MSCs more effective at suppressing the immune system. It prolongs allograft life in graft against host disease and improves the migratory and reparative abilities of MSCs [32, 33]. The results of the studies on the therapeutic efficacy of MSCs after preconditioning demonstrated that preconditioning MSCs with H_2O_2, (30 µmol) results in the largest increase in therapeutic efficacy (Fig. 6). In an acute inflammation model, preconditioning MSCs with melatonin, ascorbic acid, and γ-interferon does not significantly improve the therapeutic efficacy of[preconditioned MSCs compared to the native MSCs. The therapeutic potential of MSCs is not altered by LPS preconditioning. Obviously, preconditioning with interferon-γ and LPS, which puts MSCs into a pro-inflammatory and immunosuppressive state, cannot be effective in an acute inflammation model.

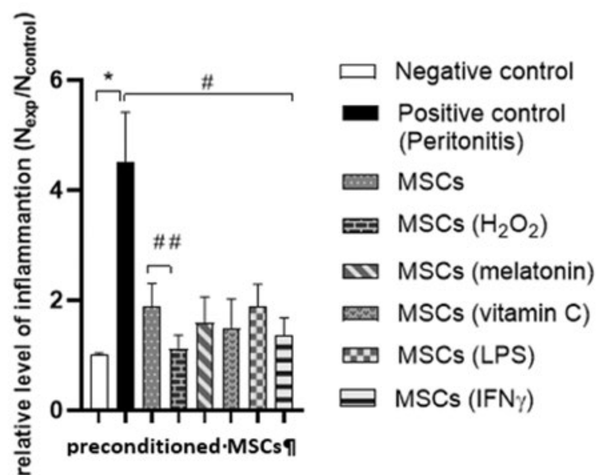

Fig. 6. Effect of MSCs preconditioning on their therapeutic potential. *P< 0.5 — negative control vs. positive control, #P< 0.5 — positive control vs. MSCs/preconditioning by various factors of MSCs, ##P< 0.5 — MSCs vs. MSCs (H_2O_2).

Conclusions

The study of the dynamics of the development of the therapeutic action of MSCs after their intraperitoneal administration showed that the anti-inflammatory effect develops very quickly. Even with a low dose of MSCs ($1.65x10^5$/kg), the number of macrophages in the peritoneal cavity declines significantly after 15 min, this is accompanied by an increase in the *IL-10* expression and enhanced phagocytosis. As a result the method has been developed that allows the evaluation of therapeutic efficacy of MSC preparations *in vivo*. This method is very important for the characterization of MSC preparations if their use in regenerative medicine is planned. The most effective method to increase the therapeutic potential of MSCs is preconditioning with low doses of H_2O_2.

REFERENCES

1. *Bernardo ME, Fibbe WE.* Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell.* 2013;**13**(4):392–402.
2. *Weiss ARR, Dahlke MH.* Immunomodulation by Mesenchymal Stem Cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol.* 2019;**10**:1191.

3. *Seshareddy K, Troyer D, Weiss ML.* Method to isolate mesenchymal-like cells from Wharton's Jelly of umbilical cord. *Methods Cell Biol.* 2008;**86**:101–19. doi: 10.1016/S0091-679X(08)00006-X.
4. *Caramanis C, Varonos DD.* The influence of acetylsalicylic acid, phenylbutazone, indomethacin, and flufenamic acid on the kinetics of leucocytes during acute inflammation. *Arch Toxicol Suppl.* 1980;**4**:485–91.
5. *Cain DW, O'Koren EG, Kan MJ, Womble M, Sempowski GD, Hopper K, Gunn MD, Kelsoe G.* Identification of a tissue-specific, C/EBPβ-dependent pathway of differentiation for murine peritoneal macrophages. *J Immunol.* 2013;**191**(9):4665–75.
6. *Chen HY, Weng IC, Li CS, Wan L, Liu FT.* Examination of galectins in phagocytosis. *Methods Mol Biol.* 2015;**1207**:201–13.
7. *Seshareddy K, Troyer D, Weiss ML.* Method to isolate mesenchymal-like cells from Wharton's Jelly of umbilical cord. *Methods Cell Biol.* 2008;**86**:101–19.
8. *Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E.* Minimal criteria for defining multipotent mesenchymal stromal cells. *The International Society for Cellular Therapy position statement.* Cytotherapy. 2006;**8**(4):315–7.
9. *Kim J, Hematti P.* Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. *Exp Hematol.* 2009;**37**(12):1445–53.
10. *Stevens HY, Bowles AC, Yeago C, Roy K.* Molecular crosstalk between macrophages and mesenchymal stromal Cells. *Front Cell Dev Biol.* 2020;**8**:600160.
11. *Vasandan AB, Jahnavi S, Shashank C, Prasad P, Kumar A, Prasanna SJ.* Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE2-dependent mechanism. *Sci Rep.* 2016;**6**:38308.
12. *Manole E, Niculite C, Lambrescu IM, Gaina G, Ioghen O, Ceafalan LC, Hinescu ME.* Macrophages and Stem *Cells*-Two to Tango for Tissue Repair? *Biomolecules*. 2021 May 6;**11**(5):697
13. *Lee KY.* M1 and M2 polarization of macrophages: a mini-review. *Med Biol Sci Eng.* 2019; **2**(1):1–5.
14. *Orecchioni M, Ghosheh Y, Pramod AB, Ley K.* Macrophage polarization: different gene signatures in M1(LPS+) vs. *classically and M2(LPS-) vs.* alternatively activated macrophages. Front Immunol. 2019;**10**:1084.
15. *Gratchev A, Kzhyshkowska J, Utikal J, Goerdt S.* Interleukin-4 and dexamethasone counterregulate extracellular matrix remodelling and phagocytosis in type-2 macrophages. *Scand J Immunol.* 2005; **61**(1):10–7.
16. *Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E.* Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. *Am J Respir Cell Mol Biol.* 2015;**53**(5):676–88.
17. *Lee DY, Lim JS, Cho KA.* Differential activation of macrophages based on their environment in advanced Age. *Chonnam Med J.* 2020;**56**(1):12–9.
18. *Ißleib C, Kurz S, Scholl S, Amberg B, Spohn J.* Plasticity of proinflammatory macrophages depends on their polarization stage during human MSC immunomodulation — an in vitro study using THP-1 and human primary macrophages. *Immuno.* 2021; **1** (4): 518–28.
19. *Hu C, Li L.* Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. *J Cell Mol Med.* 2018;**22**(3):1428–42.
20. *Bystrom J, Evans I, Newson J, Stables M, Toor I, van Rooijen N, Crawford M, Colville-Nash P, Farrow S, Gilroy DW.* Resolution-phase macrophages possess a unique inflammatory phenotype that is controlled by cAMP. *Blood.* 2008;**112**(10):4117–27.
21. *Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Gonçalves RM.* Mesenchymal stromal cell secretome: influencing therapeutic potential by cellular pre-conditioning. *Front Immunol.* 2018;**9**:2837.
22. *Ocansey DKW, Pei B, Yan Y, Qian H, Zhang X, Xu W, Mao F.* Improved therapeutics of modified mesenchymal stem cells: an update. *J Transl Med.* 2020;**18**(1):42.
23. *Li S, Deng Y, Feng J, Ye W.* Oxidative preconditioning promotes bone marrow mesenchymal stem cells migration and prevents apoptosis. *Cell Biol Int.* 2009;**33**(3):411–8.
24. *Guo L, Du J, Yuan DF, Zhang Y, Zhang S, Zhang HC, Mi JW, Ning YL, Chen MJ, Wen DL, Sun JH, Liu D,*

Zeng L, Zhang A, Jiang J, Huang H. Optimal H2O2 preconditioning to improve bone marrow mesenchymal stem cells' engraftment in wound healing. *Stem Cell Res Ther.* 2020;**11**(1):434.
25. *Tang Y, Cai B, Yuan F, He X, Lin X, Wang J, Wang Y, Yang GY.* Melatonin pretreatment improves the survival and function of transplanted mesenchymal stem cells after focal cerebral ischemia. *Cell Transplant.* 2014;**23**(10):1279–91.
26. *Yun SP, Han YS, Lee JH, Kim SM, Lee SH.* Melatonin rescues mesenchymal stem cells from senescence induced by the uremic toxin p-cresol via inhibiting mTOR-dependent autophagy. *Biomol Ther (Seoul).* 2018;**26**(4):389–98.
27. *Lee JH, Yoon YM, Han YS, Jung SK, Lee SH.* Melatonin protects mesenchymal stem cells from autophagy-mediated death under ischaemic ER-stress conditions by increasing prion protein expression. *Cell Prolif.* 2019;**52**(2):e12545.
28. *Fujisawa K, Hara K, Takami T, Okada S, Matsumoto T, Yamamoto N, Sakaida I.* Evaluation of the effects of ascorbic acid on metabolism of human mesenchymal stem cells. *Stem Cell Res Ther.* 2018; **9**(1):93.
29. *Theruvath AJ, Mahmoud EE, Wu W, Nejadnik H, Kiru L, Liang T, Felt S, Daldrup-Link HE.* Ascorbic Acid and Iron Supplement Treatment Improves Stem Cell-Mediated Cartilage Regeneration in a Minipig Model. *Am J Sports Med.* 2021;**49**(7):1861–70.
30. *Hou YS, Liu LY, Chai JK, Yu YH, Duan HJ, Hu Q, Yin HN, Wang YH, Zhuang SB, Fan J, Chu WL, Ma L.* Lipopolysaccharide pretreatment inhibits LPS-induced human umbilical cord mesenchymal stem cell apoptosis via upregulating the expression of cellular FLICE-inhibitory protein. *Mol Med Rep.* 2015;**12**(2):2521–8.
31. *Chu X, Xu B, Gao H, Li BY, Liu Y, Reiter JL, Wang Y.* Lipopolysaccharides improve mesenchymal stem cell-mediated cardioprotection by MyD88 and stat3 signaling in a mouse model of cardiac ischemia/reperfusion injury. *Stem Cells Dev.* 2019; **28**(9):620–31.
32. *Croitoru-Lamoury J, Lamoury FM, Caristo M, Suzuki K, Walker D, Takikawa O, Taylor R, Brew BJ.* Interferon-γ regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2,3 dioxygenase (IDO). *PLoS One.* 2011;**6**(2):e14698.
33. *Sivanathan KN, Gronthos S, Rojas-Canales D, Thierry B, Coates PT.* Interferon-gamma modification of mesenchymal stem cells: implications of autologous and allogeneic mesenchymal stem cell therapy in allotransplantation. *Stem Cell Rev Rep.* 2014; **10**(3):351–75.

Вивчення взаємодії між макрофагами та МСК пуповини людини *in vivo* на моделі перитоніту у мишей

П. О. Пікус, С. Ю. Римар, Н. О. Шувалова, В. А. Кордюм

Мезенхімальні стовбурові клітини мають унікальні властивості, які забезпечують відновлення пошкоджених тканин, що дозволяє використовувати їх у клітинній терапії різних захворювань. Реалізація цих властивостей в основному пов'язана із взаємодією МСК з макрофагами. **Мета.** Вивчення взаємодії МСК пуповини людини з макрофагами *in vivo* на моделі стерильного запалення черевної порожнини у мишей. **Методи.** Цитологічні методи оцінки гострого запалення черевної порожнини мишей; виділення та культивування МСК пуповини людини; дослідження експресії поверхневих маркерів МСК проточною цитометрією; визначення фагоцитарної активності мононуклеарів перитонеальної порожнини; виділення мононуклеарної РНК, ЗТ-ПЛР; методи статистичного аналізу. **Результати.** Досліджено динаміку розвитку терапевтичного ефекту МСК після їх трансплантації в черевну порожнину мишей; розроблено методику оцінки терапевтичної активності різних препаратів МСК; показано швидку поляризацію макрофагів до стану М2 після введення МСК шляхом вивчення фагоцитарної активності макрофагів та експресії гена *IL-10.* **Висновки.** Найбільш ефективним методом підвищення терапевтичного потенціалу МСК є прекондиціонування низькими дозами H_2O_2.

Ключові слова: МСК, макрофаги, перитоніт.

Received 24.09.2022