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INTRAPORTAL INDUCTION OF MESENCHYMAL STEM CELLS FOR IMMUNOSUPPRESSION INDUCTION IN LIVER TRANSPLANTATION

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Background. Despite the effectiveness of modern immunosuppressive therapy protocols, acute rejection remains a significant challenge in liver transplantation (LT), occurring in up to 40% of cases. One promising strategy to improve graft tolerance and reduce rejection rates is the use of mesenchymal stem cells (MSCs). Administering MSCs directly into the regional circulation of the transplanted liver offers the potential to enhance the effects of standard immunosuppressive therapy by exerting a localized immunosuppressive effect at the graft site. **Objective:** to evaluate the clinical efficacy of intraportal administration of MSCs during the induction phase of immunosuppressive therapy in patients undergoing LT. **Materials and methods.** A randomized prospective study was conducted involving two groups of LT recipients. In the experimental group (n = 14), patients received an intraportal infusion of MSCs during transplantation at a dose of 20×10^6 cells. The control group (n = 14) underwent standard transplant reperfusion without MSC administration. The study assessed the safety of the MSC infusion procedure, graft function, incidence and severity of acute rejection, renal function, and tacrolimus levels. Additional assessments included histological and immunohistochemical analyses, as well as fluorescence *in situ* hybridization (FISH). **Results.** No complications associated with MSC administration were observed. The MSC group demonstrated faster restoration of graft function, with significantly lower levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by postoperative day 4 ($p < 0.05$), and normalization of AST achieved by day 10. The incidence of acute rejection was lower in the MSC group (21%) compared to the control group (28%), with only mild to moderate rejection observed in the MSC group. Additionally, expression of matrix metalloproteinase-10 (MMP10) was significantly reduced in the MSC group ($p = 0.01$). Tacrolimus levels were lower in the MSC group, yet adequate immunosuppression was maintained. This correlated with faster renal function recovery, with serum creatinine levels on day 4 significantly lower in the MSC group compared to controls (80 *vs* 101 μmol/L, $p < 0.05$). FISH analysis confirmed the presence of MSCs within the liver graft tissue on postoperative day 7. **Conclusion.** Intraportal administration of MSCs during LT is a safe approach that enhances faster graft function recovery, reduces the severity of acute rejection, and mitigates tacrolimus-associated nephrotoxicity. These findings support the potential of MSC therapy as a valuable adjunct to standard immunosuppressive regimens in LT.

Keywords: liver transplantation, mesenchymal stem cells, intraportal infusion, acute kidney injury, graft rejection.

INTRODUCTION

Liver transplantation (LT) remains one of the most effective treatment options for patients with diffuse and focal liver lesions at end stages of the disease. Current data indicate that five-year survival after LT from brain-dead donors reaches approximately 75%, while ten-year survival approaches 70% [1, 2]. A critical determinant of long-term transplant success is the use of immunosuppressive therapy (IST), which helps prevent graft rejection, ensuring long-term patient survival.

Despite advances in modern immunosuppressive protocols, the incidence of acute rejection during the early postoperative period remains quite high, with rates reported as high as 40% [3, 4]. One promising avenue is the application of cellular biotechnology, particularly the use of mesenchymal stem cells (MSCs). Owing to their potent immunomodulatory properties, MSCs are increasingly regarded as a potential adjunct or alternative to conventional IST, offering fewer complications and side effects [5–7].

Of particular interest is the use of local MSC therapy, achieved by introducing the cells directly into the

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regional blood flow of the liver graft. This approach enables the creation of a high concentration of the cellular product within the target organ, thereby enhancing therapeutic effectiveness. This targeted approach can significantly enhance the effectiveness of standard immunosuppression protocols by modulating immune response mechanisms directly within the transplanted liver [8, 9].

In this regard, the present study aimed to evaluate the clinical efficacy of intraportal administration of MSCs during the induction phase of IST in liver transplantation.

MATERIALS AND METHODS

Study design

To assess the efficacy of intraportal MSC administration, we conducted an interventional, randomized, prospective, comparative study involving two groups (n = 28). The main group included 14 patients who received intraportal MSC infusion during transplantation, while the comparison group comprised 14 recipients who underwent standard donor liver reperfusion without MSCs.

Inclusion criteria included patients with liver cirrhosis listed for transplantation; age ≥18 years; liver graft from a deceased donor; and transplantation performed using the classic technique (resection of the retrohepatic segment of the portal vein). Exclusion criteria included: age <18 years; split or living-related LT; non-standard portal reconstruction (e.g., reno-portal, cava-portal, or porto-caval shunt anastomosis); retransplantation; primary graft non-function; or severe graft dysfunction requiring retransplantation.

Endpoints of the study: 1) primary endpoints – frequency of complications associated with intraportal application of MSCs; frequency of histologically confirmed graft rejection; intensity of immune-inflammatory reactions based on immunohistochemical expression of matrix metalloproteinase-10 (MMP10) and caspase-3 (Casp3); 2) secondary endpoints – dynamics of liver function recovery, dynamics of kidney function recovery, tacrolimus levels, frequency of postoperative complications, duration of treatment.

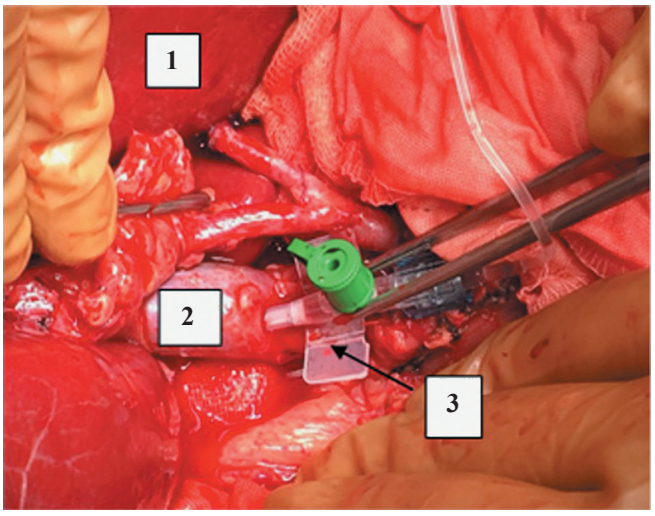

Fig. 1. Intraoperative intraportal infusion of mesenchymal stem cells. 1 – Liver graft; 2 – Portal vein; 3 – Venous catheter

Characteristics of the cell product

To meet the research objectives, biomedical cell product (BMCP) "Human Mesenchymal Cells TU BY 100660677.001" (registration certificate No. IM-7.101480, registration number Mn-7.117650-1402, dated May 29, 2014) was used. The BMCP was produced from allogeneic MSCs isolated from the adipose tissue of brain-dead donors and complied with the minimum criteria for MSCs established by the International Society for Cellular Therapy (ISCT, 2006) [10].

Intraportal administration of MSCs

The developed method for intraportal administration of MSCs involved the following steps:

1. A 16G venous catheter was introduced into the portal vein of the graft and connected to a syringe containing the cell product. The infusion volume was 20 million MSCs suspended in 20 ml of 0.9% NaCl solution.
2. MSCs were administered after portal blood flow was initiated, at an infusion rate of 2 ml/min (Fig. 1).
3. Upon completion of the infusion, the catheter was carefully removed from the portal vein, and the puncture site was sutured.

Histological and immunohistochemical examination of transplant

Puncture biopsy of the liver graft was performed on postoperative day 7 (POD 7), as well as when clinically indicated in cases of graft dysfunction. The presence or absence of rejection was assessed according to the Banff classification. The Rejection Activity Index (RAI) was applied to quantitatively determine the severity of acute cellular rejection. Humoral rejection was identified by immunohistochemistry (IHC) through detection of the complement fragment C4d, which is associated with antibody-mediated tissue injury [11–13].

To further evaluate the intensity of the alloimmune response, IHC analysis was also performed to assess tissue expression of matrix metalloproteinase-10 (MMP-10) and caspase-3 (Casp3) [14, 15].

Molecular cytogenetic studies

Molecular cytogenetic analysis was conducted using fluorescence *in situ* hybridization (FISH) to verify the presence of MSCs in the transplanted liver. This was achieved by detecting alpha satellite sequences in the Xp11.1–Xq11.1 region and satellite DNA III in the Yq12 region. To ensure accurate identification of MSCs, a pre-

requisite was adherence to the principle of gender mismatch between the donor, recipient, and administered MSCs – that is, the donor and recipient had to differ in sex from the MSC product being administered [16].

Statistical analysis

All statistical analyses were performed using Statistica 8.0 software. The Shapiro–Wilk test was applied to assess the normality of data distribution. Data with non-normal distributions were expressed as median (25th–75th percentiles). For comparisons of quantitative variables between groups, the Mann–Whitney U test (MW) was employed. Categorical variables were analyzed using Fisher's exact test (F).

RESULTS

The study and control groups were comparable in terms of clinical and demographic features (Table 1). The mean age of patients in the MSC group was 46 years (39–52), while in the control group it was 47 years (40–55) (MW, $p > 0.05$). Gender distribution was also similar: in the MSC group, there were 7 men (50%) and 7 women (50%), whereas the control group included 8 men (57%) and 6 women (43%) (F, $p > 0.05$).

In the MSC group, the indications for liver transplantation were: hepatitis B (HBV) cirrhosis – 1 patient (7%); HBV + HDV cirrhosis – 2 patients (14%); HCV cirrhosis – 2 patients (14%); HCV cirrhosis with hepatocellular carcinoma (HCC) – 3 patients (21%); cryptogenic cirrhosis – 3 patients (21%); cryptogenic cirrhosis with giant cell transformation – 1 patient (7%); PSC with cholangiocellular carcinoma – 1 patient (7%); cirrhosis secondary to autoimmune hepatitis (AIH) – 1 patient (7%).

In the control group, the indications were distributed as follows: HBV cirrhosis – 1 patient (7%); HBV + HDV cirrhosis – 1 patient (7%); HCV cirrhosis – 3 patients (21%); HCV cirrhosis with HCC – 1 patient (7%); cirrhosis due to non-alcoholic steatohepatitis – 1 patient (7%); cryptogenic cirrhosis – 2 patients (14%); cirrhosis from Wilson–Konovalov disease – 2 patients (14%); cirrhosis due to PSC – 1 patient (7%); primary biliary cirrhosis – 1 patient (7%); cirrhosis secondary to AIH – 1 patient (7%) (F, $p > 0.05$).

In the MSC group, immunosuppression induction (IS) was achieved with glucocorticosteroids (GCS) in 10 patients (71%), while 4 patients (29%) received a combination of GCS and interleukin-2 receptor antagonists (IL2RA, Basiliximab). In the control group, 9 patients (64%) received GCS, and 5 patients (36%) received GCS + IL2RA (F, $p > 0.05$) [17].

Maintenance IS consisted of standard triple therapy with calcineurin inhibitors (tacrolimus), mycophenolate mofetil (MMF), and GCS (methylprednisolone). Tacrolimus was initiated on POD 1 at 0.1 mg/kg/day, but its administration was delayed in cases of acute kidney injury until renal function normalized or showed stable improvement. In patients with stable graft function under acute kidney injury (AKI), tacrolimus trough levels were maintained at a lower threshold (<5 ng/ml) [17].

Management of rejection episodes followed established protocols: in acute cellular rejection (ACR), patients received pulse methylprednisolone therapy; in antibody-mediated rejection (AMR), plasmapheresis and intravenous immunoglobulin were administered. For cases of immunological graft dysfunction, everolimus (a macrolide immunosuppressant) was added as a fourth agent, and the MMF dosage was escalated to 2000 mg/day [17].

The study showed that intraportal administration of MSCs was safe and did not result in local complications related to catheter placement, such as thrombosis, bleeding, vascular rupture, or injury to the posterior wall of the vena cava. Likewise, no systemic complications were observed, including hypotension, cardiac arrhythmia, hyperthermia, or allergic reactions.

Importantly, MSC infusion did not cause local hemodynamic disturbances within the graft. Portal vein blood

Table 1

Clinical parameters of patients

Parameter	MSC	Control	MW, p
Recipients			
MELD score, points	18 (10; 23)	19 (14; 24)	>0.05
Na, mmol/L	137 (134; 140)	137 (136; 138)	
Bilirubin, μmol/L	67 (18; 126)	59 (25; 118)	
INR	1.45 (1.19; 1.81)	1.4 (1.19; 1.77)	
Urea, mmol/L	4.6 (3.9; 6.7)	4.9 (4.45; 9.25)	
Creatinine, μmol/L	61 (51; 95)	65 (65; 101)	
GFR, mL/min	56 (43; 75.5)	53 (28; 70)	
Donor factors			
Donor age, years	49 (40; 54)	48 (41; 60)	>0.05
Days in the ICU	4 (3; 5)	4 (3; 5)	
Hb, g/L	125 (102; 141)	130 (104; 150)	
AST, U/L	49 (38; 68)	62 (46; 76)	
ALT, U/L	33 (20; 91)	40 (24; 81)	
Na, mmol/L	148 (142; 158)	151 (147; 162)	
INR	1.27 (1.03; 1.4)	1.2 (0.94; 1.32)	
Operation:			
Blood loss, mL	1500 (900; 2000)	1300 (1000; 2000)	>0.05
Total ischemia time, min	515 (480; 570)	555 (460; 600)	
Warm ischemia time, min	45 (35; 50)	46 (40; 52)	
Anhepatic phase duration, min	50 (38; 60)	55 (46; 60)	

flow velocity after reperfusion and MSC administration in the main group was 33 (27; 41) cm/s, compared with 36 (29; 42) cm/s in the control group (MW, $p > 0.05$).

Histological examination of intraoperative liver graft biopsies in both groups, obtained after reperfusion, confirmed the absence of microcirculatory thrombosis (Fig. 2).

On the first postoperative day, patients in both groups exhibited biochemical signs of graft dysfunction, primarily attributable to preservation and ischemia–reperfusion injury (Fig. 3).

Subsequently, progressive improvement in graft function was observed in all patients; however, recovery was notably faster in those who received local therapy with MSCs (Fig. 3).

By POD 4, serum transaminase levels were significantly lower in the MSC group compared to controls. Specifically, the AST level was 125 (85; 321) U/L in the MSC group versus 190 (140; 352) U/L in the control group (MW, $p = 0.02$). Similarly, ALT levels were 279 (125; 456) U/L and 358 (211; 606) U/L, respectively (MW, $p = 0.04$) (Fig. 4).

In the main group, normalization of AST levels was achieved by POD 10, with a median value of 34 (19; 51) U/L. In contrast, patients in the control group had AST levels that remained above the normal range at POD 10, reaching 53 (29; 92) U/L (MW, $p = 0.04$) (Fig. 5).

No significant differences between the groups were observed in the recovery dynamics of bilirubin, alkaline phosphatase, gamma-glutamyl transferase, or international normalized ratio (MW, $p > 0.05$).

Histological examination of liver graft biopsies revealed acute cellular rejection (ACR) in 3 patients (21%) of the main group. Of these, 2 cases were mild (RAI score 4) and 1 was moderate (RAI score 6). In the control group, rejection was confirmed in 4 patients (28%):

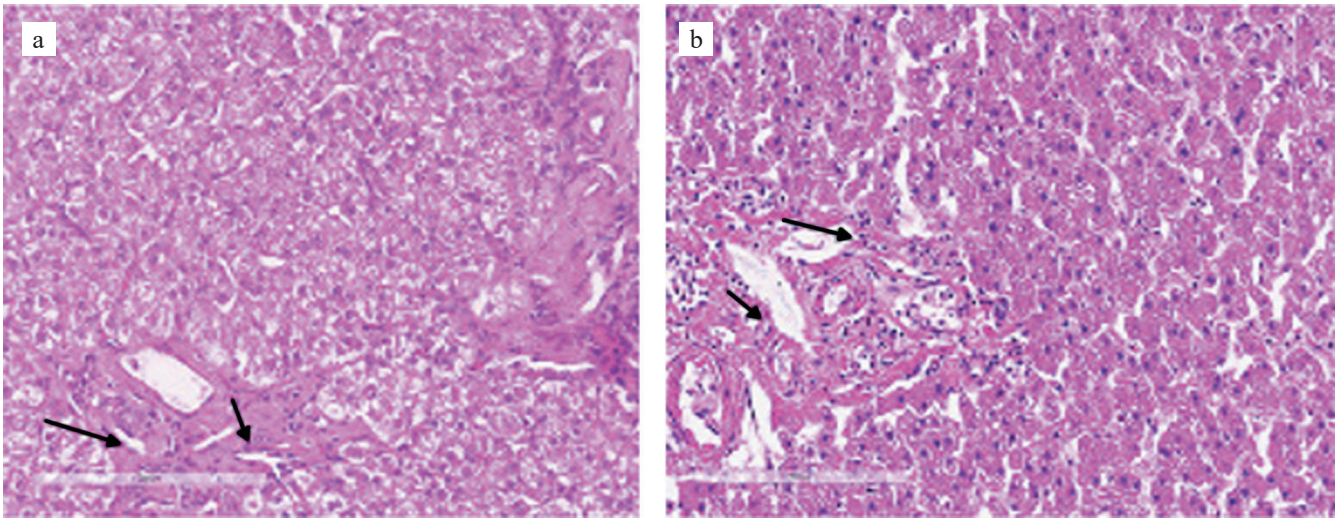

Fig. 2. Intraoperative liver transplant biopsies (hematoxylin and eosin staining, 200× magnification). Arrows indicate portal capillaries with open lumens. a – Biopsy after reperfusion and intraportal MSC administration (main group); b – Biopsy after reperfusion without MSCs (control group)

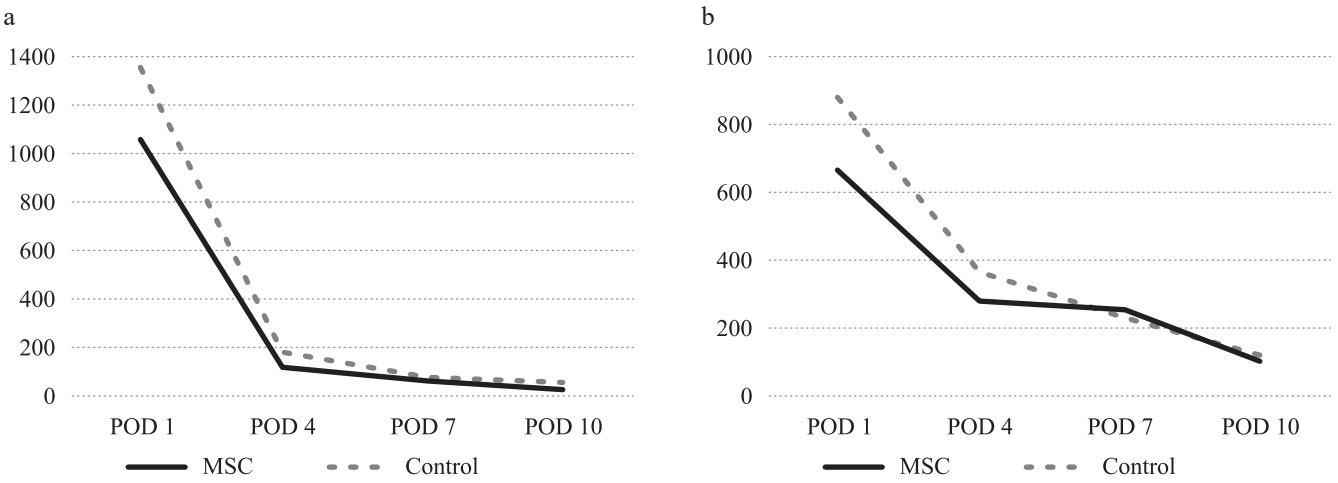

Fig. 3. Dynamics of AST and ALT levels in the study groups. a – AST levels (U/L); b – ALT levels (U/L)

3 with ACR and 1 with antibody-mediated rejection (AMR). The severity of ACR was higher in the control group, with 1 moderate case (RAI score 7) and 2 severe cases (RAI score 8) (F, $p > 0.05$) (Table 2).

Immunohistochemical analysis of liver biopsies obtained on POD 7 included assessment of MMP-10 and caspase-3 expression to quantify the severity of immunological graft injury (Table 2).

In the MSC group, MMP-10 expression in hepatocytes was significantly lower, with a median value of 5% (3; 25), compared with 20% (10; 30) in the control group (MW, $p = 0.01$) (Figs. 6, 7).

Fig. 7 demonstrates more intense MMP-10 expression in the control group (Fig. 7, b) compared with the MSC group (Fig. 7, a).

Immunohistochemical assessment of caspase-3 expression revealed no statistically significant differences between groups, with values of 70 (60; 85)% in the MSC group and 75 (70; 90)% in the control group (MW, $p > 0.05$).

Table 2

Comparative histological characteristics of liver transplant biopsies

	MSC (n = 14)	Control (n = 14)
Rejection	3 (21%)	4 (28%)
ACR	3	3
Mild (RAI 4–5) Moderate (RAI 6–7) Severe (RAI 8–9)	2 1 –	– 1 2
AMR	–	1
MMP10, %	5 (3; 25)*	20 (10; 30)
Caspase-3, %	70 (60; 85)	75 (70; 90)

Note: * indicates a statistically significant difference compared to the control group ($p < 0.05$).

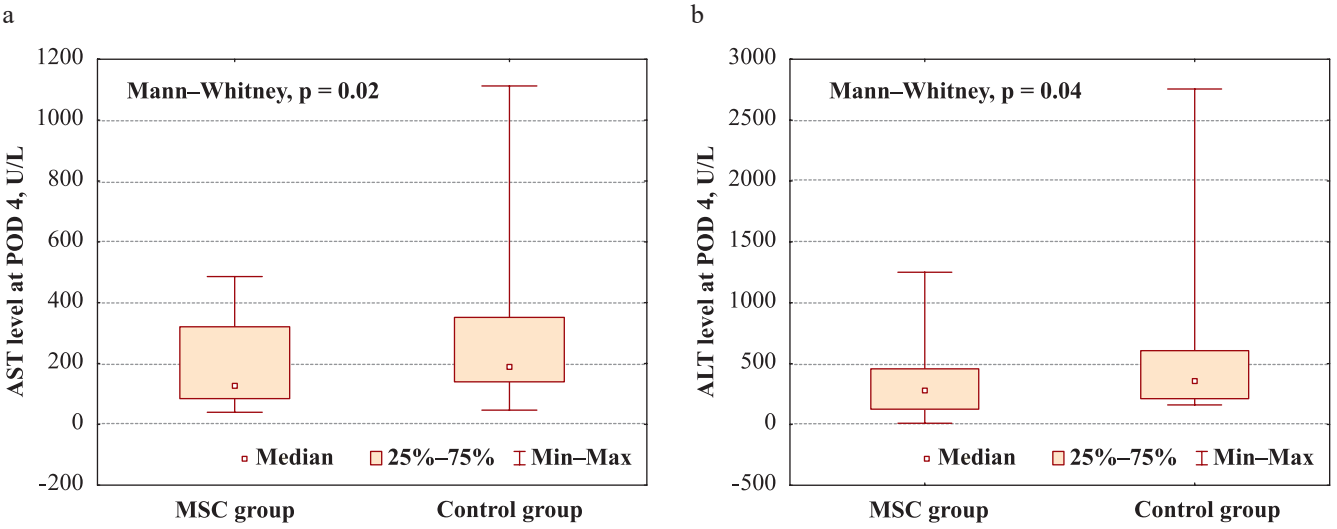

Fig. 4. Mean AST and ALT levels in the study groups on postoperative day (POD) 4. a – AST level; b – ALT level

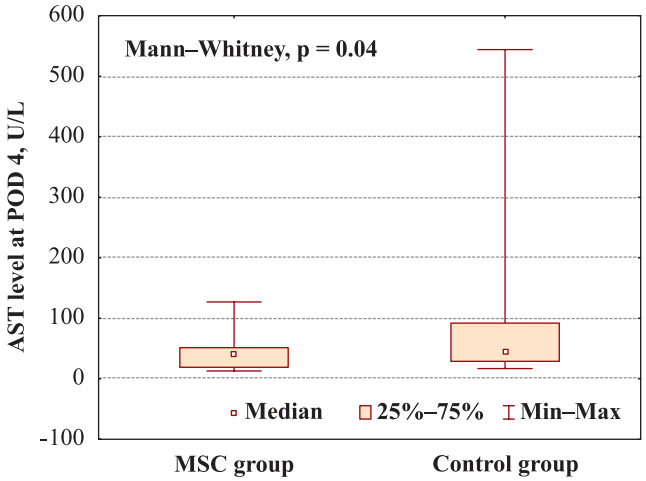

Fig. 5. Mean AST levels in the study groups on postoperative day (POD) 10

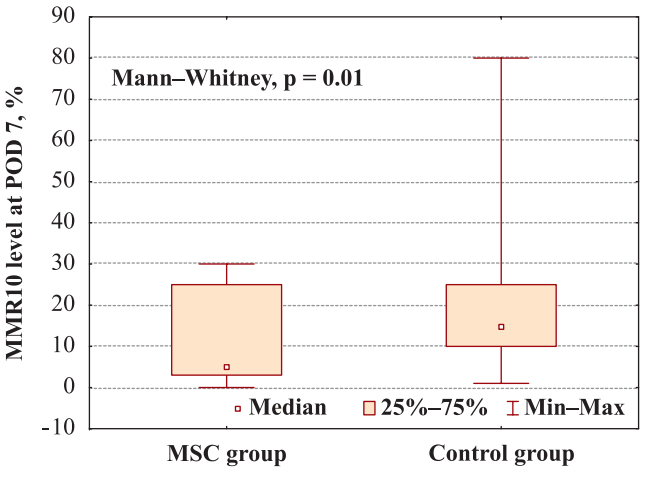

Fig. 6. Mean MMP10 expression levels in liver transplant biopsies at postoperative (POD) day 7

FISH analysis performed on POD 7 confirmed the presence of MSCs, identifiable by a karyotype distinct from that of both the donor and the recipient (Fig. 8).

Determination of tacrolimus levels showed consistently lower concentrations of the immunosuppressant in the MSC group throughout the early postoperative period (Table 3).

On POD 14, this difference reached statistical significance: tacrolimus levels were 5.2 (2.6; 6.7) ng/ml in the MSC group versus 6.7 (4.3; 9.5) ng/ml in the control group (MW, $p = 0.04$).

The dynamics of renal function are summarized in Table 4. During the first two postoperative days, patients in both groups demonstrated elevated urea and creatinine levels, along with reduced glomerular filtration rate, reflecting the development of perioperative renal injury.

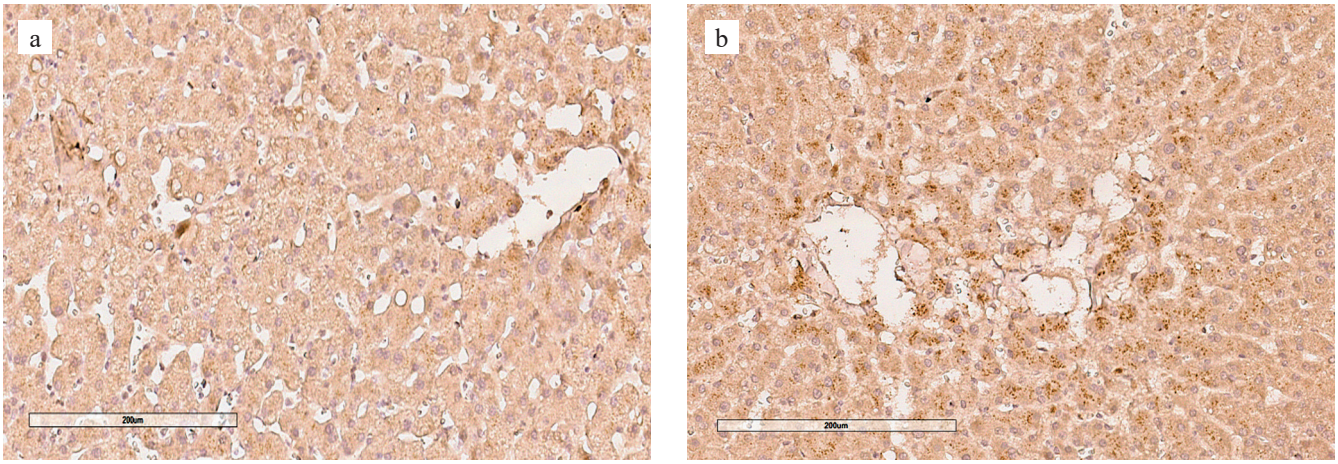

Fig. 7. Immunohistochemical (IHC) staining of MMP-10 expression in liver transplant biopsies (200× magnification): a – MSC group; b – control group

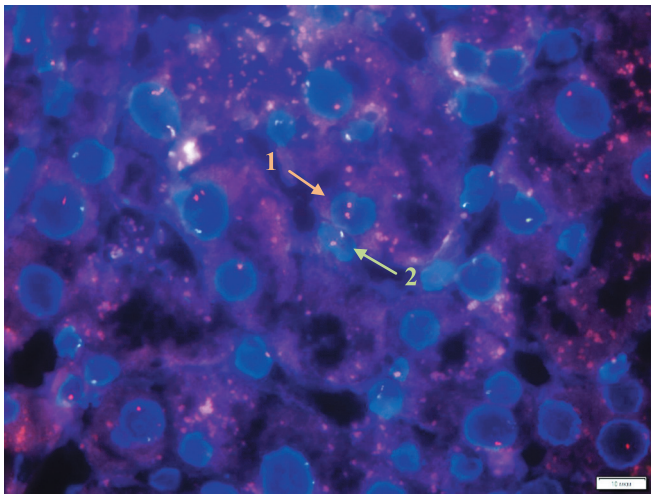

Fig. 8. FISH analysis of liver transplant biopsy: (1) – cell with two copies of alpha satellite sequences in the centromeric region of chromosome X (Xp11.1–Xq11.1); (2) – remaining cell population containing both an alpha satellite sequence in the centromeric region of chromosome X (Xp11.1–Xq11.1) and satellite DNA III in the Yq12 region of chromosome Y

According to the KDIGO (2012) international guidelines [18], AKI is defined by one of the following criteria: (1) an increase in serum creatinine by more than 1.5 times baseline, (2) an absolute increase in creatinine of 26.5 μmol/L within 48 hours, or (3) a reduction in urine output to 0.5 mL/kg/h for at least 6 hours.

Analysis of renal function showed that the ratio of POD 1 creatinine to baseline was 1.51 (1.13; 2.19) in the MSC group and 1.58 (1.32; 1.88) in the control group (MW, $p > 0.05$). On day 2, these ratios were 1.63 (1.28; 2.49) and 1.64 (1.26; 2.43), respectively (MW, $p > 0.05$). The absolute increase in creatinine from baseline to day 1 was 45 (8; 90) μmol/L in the MSC group and 41 (21; 53) μmol/L in the control group (MW, $p > 0.05$), while from baseline to day 2 the increase was 39 (13; 125) and 50 (24; 84) μmol/L, respectively (MW, $p > 0.05$). These findings indicate the presence of postoperative renal injury in both groups (Table 5).

Table 3

Comparative characteristics of tacrolimus levels between the groups

Days	Group	POD				
		2	4	7	10	14
Tac, ng/mL	MSC	0 (0; 0.6)	0.8 (0; 2)	3.2 (0.8; 4.9)	4.9 (3; 8.2)	5.2* (2.6; 6.7)
	Control	1 (0; 2.5)	2 (0.9; 3.4)	4.1 (2.1; 6.1)	5.7 (3.3; 7.1)	6.7 (4.3; 9.5)

Note: * indicates a statistically significant difference compared to the control group ($p < 0.05$).

The absence of statistically significant differences in creatinine ratios and absolute changes on POD 1 and 2 compared with baseline confirmed the homogeneity of the groups with respect to renal function and indicated that MSCs did not influence early development of AKI.

Because of AKI, initiation of tacrolimus therapy was delayed in both groups. The time to treatment initiation was comparable: 3 (2; 4) days in the MSC group and 2 (1; 4) days in the control group (MW, p = 0.15). Clinically, this finding indicates that the timing of tacrolimus administration did not affect the postoperative course or clinical outcomes in either group.

However, patients in the MSC group had lower tacrolimus levels and faster recovery of renal function. By POD 4, urea levels were 10.8 (8; 17.2) mmol/L in the MSC group versus 14 (7.4; 18) mmol/L in the control group (MW, p = 0.03), and creatinine levels were 80 (62; 123) μmol/L versus 101 (70; 132) μmol/L, respectively (MW, p = 0.04). Based on these observations, a correlation analysis was performed to assess the relationship between renal function and tacrolimus level.

On POD 4, a direct correlation was found between tacrolimus and creatinine levels: higher tacrolimus concentrations were associated with elevated creatinine levels (Sp, p = 0.008) (Fig. 9).

Table 4

Comparative characteristics of laboratory indicators of kidney function.

Days	Group	POD						
		0	1	2	4	7	10	14
Urine, mmol/L	MSC	4.6 (4.1; 6.6)	10.75 (9; 13)	16.15 (12.2; 20.4)	10.8* (8; 17.2)	8.25 (5.1; 12)	7.05 (5.5; 10.1)	7.85 (5.2; 11)
	Control	4.9 (3.4; 7.6)	8 (6.5; 13)	14.7 (9.2; 20.8)	14 (7.4; 18)	6.1 (4.4; 8.4)	7.2 (5.8; 10.2)	7.6 (5.1; 12.2)
Creatinine, μmol/L	MSC	61 (52; 91)	110 (79; 154)	128 (70; 186)	80* (62; 123)	78.4 (57; 99)	78 (61; 108)	86 (65; 94)
	Control	65 (57; 84)	112 (81; 137)	118 (84; 166)	101 (70; 132)	84 (63; 108)	78 (66; 102)	82 (65; 107)
GFR, mL/min	MSC	56 (34; 70)	37 (20; 61)	31 (19; 47)	33 (14; 44)	45.5 (25; 57)	42.5 (32; 59)	38 (28.3; 57)
	Control	53 (45; 64)	32.25 (24; 45)	25 (16.2; 38)	31 (18; 50)	33 (23; 56)	41 (29; 54)	38 (24.7; 59)

Note: * indicates a statistically significant difference compared to the control group (p < 0.05).

Table 5

Characteristics of groups according to development of AKI in the early postoperative period

Creatinine, μmol/L	MSC (n = 14)	Control (n = 14)	MW, p
POD 1 / POD 0	1.51 (1.13; 2.19)	1.58 (1.32; 1.88)	>0.05
POD 2 / POD 0	1.63 (1.28; 2.49)	1.64 (1.26; 2.43)	>0.05
Δ POD 1 – POD 0	45 (8; 90)	41 (21; 53)	>0.05
Δ POD 2 – POD 0	39 (13; 125)	50 (24; 84)	>0.05

Table 6

Early postoperative complications following liver transplantation

Complication	MSC (n = 14)		Control (n = 14)	
Vascular	1	7%	0	0%
– arterial (hepatic artery stenosis)	1	7%	0	0%
Biliary	2	14%	1	7%
– bile leakage	1	7%	0	0%
– anastomotic stricture	1	7%	1	7%
SSI (surgical site infection)	1	7%	2	14%
– superficial	0	0%	1	7%
– deep	1	7%	1	7%
Intra-abdominal hemorrhage	1	7%	1	7%

The incidence of early postoperative complications was comparable between the groups and did not differ significantly (F, p > 0.05) (Table 6).

The median length of stay in the intensive care unit was 3 (2; 4) days in the MSC group and 3 (2; 5) days in the control group (MW, p > 0.05). The total duration of inpatient treatment after transplantation was slightly shorter in the MSC group – 17 (14; 20) days compared with 19 (15; 24) days in the control group – although this difference was not statistically significant (MW, p > 0.05).

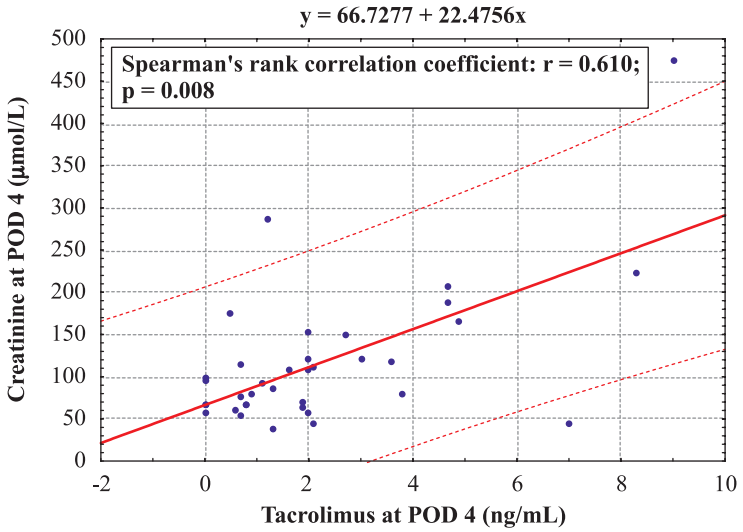

Fig. 9. Correlation between serum creatinine levels and tacrolimus concentration on postoperative day (POD) 4

DISCUSSION

The results of this study demonstrated both the safety and potential efficacy of intraportal administration of MSCs in liver transplantation. The absence of local or systemic complications related to MSC infusion, together with preserved portal hemodynamics and the lack of microthrombosis in biopsy samples, confirmed the safety of the developed technique.

Analysis of graft function revealed faster recovery of liver function in the MSC group, reflected by significantly lower transaminase levels on POD 4 and earlier normalization of AST by POD 10.

Another positive finding was a trend toward reduced frequency and severity of acute rejection in the MSC group. Although the overall incidence of immunological graft dysfunction did not differ significantly between groups (21% in the MSC group *vs* 28% in controls), only mild to moderate cellular rejection occurred in the MSC group, whereas cases of severe cellular and antibody-mediated rejection were observed in the control group. This was supported by significantly lower MMP10 expression in MSC-group biopsies, suggesting less severe immunological injury to the graft.

The beneficial effects observed following intraportal administration of MSCs may be due to several mechanisms of action. First, MSCs secrete a range of anti-inflammatory mediators, including IL-10 and TGF-β, which suppress T-lymphocyte activation and proliferation. Second, they modulate the function of antigen-presenting cells and reduce the production of proinflammatory cytokines. Third, MSCs promote the expansion of regulatory T cells, which play a central role in maintaining immunological tolerance to the graft [5–7].

The detection of administered MSCs within the graft on POD 7, as confirmed by FISH analysis, demonstrates their ability to persist in the target organ, which may underlie the observed immunomodulatory effects.

Although MSCs have a low immunogenic profile, characterized by weak HLA class I expression and absence of HLA class II antigen expression, the possibility of an immune response from the recipient cannot be ruled out, particularly in the context of repeated cell administrations or insufficient immunosuppression [19]. In our study, no clinically significant immune reactions against MSCs or cases of MSC-associated acute rejection were observed. This outcome was likely facilitated by the standard immunosuppressive regimen used after liver transplantation, the single local administration of MSCs into the graft, and the intrinsic immunosuppressive effect of MSCs themselves on the immune system.

The potential to reduce tacrolimus levels in the MSC group while maintaining adequate immunosuppression is a particularly important finding. Given the observed correlation between tacrolimus and creatinine levels, lowering calcineurin inhibitor exposure may help mitigate nephrotoxicity and accelerate renal recovery, as evidenced in the MSC group by POD 4.

The absence of differences in the frequency of other postoperative complications, together with the trend toward shorter hospitalization in the MSC group, further supports the safety and potential clinical value of the proposed technique.

Our findings are in line with previous reports on the use of cell therapy in solid organ transplantation. Sun et al. (2018) demonstrated both safety and efficacy of local MSC administration via the renal artery in kidney transplantation [8].

Popp et al. (2011) showed the safety of MSC infusion into the portal vein of a liver transplant [9]. Their study highlighted the potential of MSCs to partially replace calcineurin inhibitors and confirmed the effectiveness of combining MSC therapy with mycophenolates, thereby supporting our proposed immunosuppression strategy aimed at minimizing calcineurin inhibitor exposure.

Taken together, our findings reinforce the potential of MSCs as an adjunct to standard immunosuppressive therapy and align with existing evidence on the safety and efficacy of cell therapy in solid organ transplantation. These results underscore the promise of MSCs in preventing rejection and enhancing liver graft function.

CONCLUSION

This study led to the development and successful implementation of a safe technique for intraportal administration of mesenchymal stem cells during liver transplantation. The approach demonstrated several important clinical advantages:

1. Safety – no local or systemic complications were observed during MSC administration.
2. Efficacy in graft function recovery – faster normalization of liver function parameters was achieved in the MSC group.
3. Immunomodulatory effects – a trend toward reduced severity of rejection episodes and lower expression of immune injury markers was observed.
4. Reduction of nephrotoxicity risk – the possibility of lowering tacrolimus levels without compromising immunosuppressive efficacy was demonstrated, thereby reducing the nephrotoxic burden of calcineurin inhibitors.

Overall, these findings highlight the promise of intraportal MSC infusion as an adjunct to standard immunosuppressive therapy in liver transplantation.

The authors declare no conflict of interest.

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