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In vivo tracking of ^{111}In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis

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Abstract

Background: Several animal and few human studies suggest the beneficial role of bone marrow mesenchymal stem cells (MSCs) in liver cirrhosis. However, little is known about the fate of MSCs after infusion in cirrhotic patients. We evaluated stem cell biodistribution after peripheral infusion of MSCs in four cirrhotic patients.

Methods: After three passages of MSCs, the patients received a total of 250–400×10^6 cells, of which only 50% of the cells were labeled. Specific activities of 0.21–0.67 MBq/10^6 cells were maintained for the injected labeled MSCs. Planar whole-body acquisitions (anterior/posterior projections) were acquired immediately following infusion as well as at 2 h, 4 h, 6 h, 24 h, 48 h, 7th and 10th days after cell infusion.

Results: After intravenous infusion, the radioactivity was first observed to accumulate in the lungs. During the following hours to days, the radioactivity gradually increased in the liver and spleen, with spleen uptake exceeding that in the liver in all patients. Region-of-interest analysis showed that the percentage of cells homing to the liver (following decay and background corrections and geometric mean calculation) increased from 0.0%-2.8% at immediately post-infusion images to 13.0–17.4% in 10th-day post-infusion. Similarly, the residual activities in the spleen increased from 2.0%-10.2% at immediately post-infusion images to 30.1%-42.2% in 10th-day post-infusion. During the same period, the residual activities in the lungs decreased from 27.0–33.5% to 2.0–5.4%.

Conclusion: The infusion of MSCs labeled with ^{111}In-oxine through a peripheral vein is safe in cirrhosis. Cell labeling with ^{111}In-oxine is a suitable method for tracking MSC distribution after infusion.

Keywords: ^{111}In-oxine; Mesenchymal stem cell; Cirrhosis

1. Introduction

Cirrhosis and chronic liver failure are among the most frequent causes of morbidity and mortality throughout the world, with the majority of preventable cases attributed to excessive alcohol consumption, viral hepatitis or nonalcoholic fatty liver disease [1]. Orthotopic liver transplantation is the standard treatment modality in patients with decompensated cirrhosis [2]. However, it has several limitations such as shortage of organ donors, high cost, and several complications [2]. Despite progress in medical and surgical treatment, alternative strategies are needed to improve the outcome of patients with cirrhosis.

Stem cell therapy may be a potential alternative to liver transplantation. Bone marrow is a reservoir of various stem cells, including hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Several studies have suggested MSC transplantation can reduce liver fibrosis in animal models of cirrhosis [3–6].

Infusion of the cells directly into the vessels feeding the liver (e.g., portal vein or hepatic artery) may improve delivery and subsequent engraftment of the cells into the liver. This method requires an invasive procedure for angiography, and may lead to serious adverse effects, such

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as hemorrhage or contrast nephropathy [2]. In a Phase 1 trial, we have shown MSC transplantation through a peripheral vein is safe and feasible in human cirrhosis [7]. However, there is a concern that after peripheral vein infusion of MSCs, many of the cells may entrap in the lung during their first pass through this organ. On the other hand, it has been shown that some MSCs infused through a peripheral vein can escape the lung, engraft into the liver and reverse CCl4-induced acute liver injury [8]. Thus, there is a need to track MSCs after peripheral vein infusion in cirrhosis, and to provide information about the biodistribution of the cells delivered by such a route, in order to confirm adequate homing of the injected cells to the target organ.

Among the different methods examined [9–16], magnetic resonance imaging (MRI) and radioactive labeling with radiopharmaceuticals are the most widely used methods for tracking infused stem cells [17]. However, some authors claim that MRI tracking of stem cells suffers from low sensitivity [18,19]. An important limitation of MRI is that it may not distinguish iron-labeled cells from free iron particles. Hence, we chose nuclear medicine techniques to evaluate biodistribution of stem cells infused via the intravenous route. Three well-established cell labeling radioagents are ^{18}FDG, ^{99m}Tc-HMPAO and ^{111}In-oxine, with short and longer half-lives, respectively. The time period during which radiolabeled cell distribution can be observed is limited by the decay of their label [20]. Since we were interested in cell homing over several days, we selected ^{111}In as the radionuclide (with a half-life of 67 h). ^{111}In-oxine is an Auger electron emitter which internalizes nonspecifically into both normal and malignant cells [21]. Based on the study of Andersson et al. [22], the decline of intracellular ^{111}In concentration is most prominent during the first 6 h post-labeling and appears to remain stable thereafter.

We conducted a pilot study on human to evaluate stem cell biodistribution after peripheral infusion of MSCs in patients with liver cirrhosis by labeling a fraction of the injected cells using ^{111}In-oxine and imaging the distribution pattern over a 10-day period.

2. Materials and methods

2.1. Patients

Four patients with decompensated liver cirrhosis were included in the study. Informed consent was assigned by the patients and documented in the medical record. The project was approved by the ethics committee and the research council of Digestive Disease Research Center, Tehran University of Medical Sciences [ClinicalTrials.gov ID: NCT00476060].

2.2. Preparation of MSCs

A total of 200 ml bone marrow was aspirated from four different sites of the iliac crest in the right and left side (50 ml at each site) of each patient. The procedure of stem cell isolation was performed in a clean room (FS 209 E & ISO 14 644). The harvested bone marrow samples were placed in sterile tubes and were diluted 1:2 with 2 mM EDTA/PBS. The mononuclear fraction was isolated by density gradient centrifugation at 435×*g* for 30 min at room temperature using Ficoll–Hypaque solution (Inno-TRAin, Germany) and seeded at a density of 1×10^6cells/cm^2 into T75 cell culture flasks (Nunc, Austria). The cells were plated in Dulbecco's modified Eagle's medium-low glucose (DMEM-LG, GIBCO, UK), supplemented with 10% fetal calf serum (Sigma, Germany) and 1% penicillin-streptomycin (Gibco, UK), and cultured at 37°C in a 5% CO_2 atmosphere. After 3 days, nonadherent cells were removed and the adherent cells were cultured for another 7 days with media changes every 3 days. Cells were grown to confluence, then harvested by incubation with 0.25% trypsin/1mM EDTA (GIBCO, UK), centrifuged at 1200 rpm for 5 min, and subcultured at a 1:3 split ratio in new culture flasks. After reaching confluence for the second time (after 8 days), the harvested cells were defined as passage 1, and the replated cells were cultured and serially subcultured until passage 3 [23].

At the end of the last passage, they were washed with tyrode salt (Sigma, Germany) and incubated with M199 medium (Sigma, Germany) for an hour. Cells were detached with trypsinization and washed with normal saline supplemented with 1% human serum albumin (Blood Research & Fractionation Co, Iran) and heparin three times, and resuspended at 1–1.5×10^6/ml density in M199. This washing process eliminates trace amounts of fetal bovine serum as well. Also, bacteriological tests were performed on the samples for every passage and at the time of injection. Then, the cells were labeled with ^{111}In-oxine before intravenous infusion.

Table 1
Age and sex of the patients, the estimated number of infused MSCs, specific activity and also the labeling efficiency of the samples

Patient	Age	Sex	Estimated number of infused MSCs	Specific activity of the injected labeled cells	Labeling efficiency
1	62	F	270×10^6	0.67 MBq/10^6 cells	36.2%
2	17	M	290×10^6	0.71 MBq/10^6 cells	38.8%
3	29	M	250×10^6	0.41 MBq/10^6 cells	52.0%
4	62	M	400×10^6	0.21 MBq/10^6 cells	53.0%

2.3. ^{111}Indium-oxine labeling of MSCs

In each patient, the solution containing the MSCs was divided into two equal portions: The first portion was used for radiolabeling (hence, only 50% of cells were labeled with ^{111}In-oxine [24]) and the second portion infused to the patients without radiolabeling. For radiolabeling, the first portion was initially washed with phosphate-buffered saline, mixed and suspended with ^{111}In-oxine at the concentration of approximately 1.85 MBq/10^6 cells, and then incubated for 30 min at the room temperature.

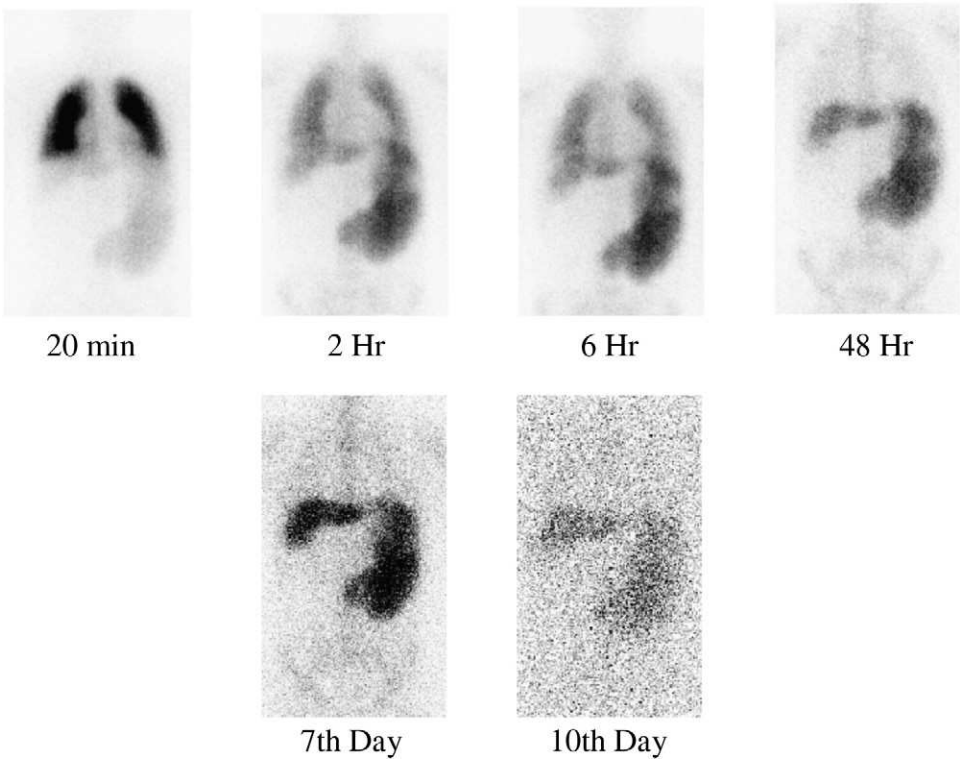

Fig. 1. Distribution of radiotracer in the lungs, liver and spleen. Following intravenous infusion, the labeled MSCs first accumulated in the lungs, and gradually shifted to the liver and spleen during the following hours to days.

Cells were centrifuged at 320 g for 7 min, and the supernatants were removed. After repeating the above cell washing, the ^{111}In-oxine radiolabeling efficiency and specific activity of the MSCs (radiotracer activity per 10^6 cells) were measured (following background and physical decay corrections). In each patient, the labeled and unlabeled portions of MSCs were infused separately from two different cubital veins, simultaneously. Immediately before infusion, cell labeling efficiency and specific radioactivity were determined. Cell viability of all samples was >99% by trypan blue exclusion.

2.4. Imaging procedure

Planar whole-body acquisitions (anterior and posterior projections) were acquired immediately following infusion as well as at 2 h, 4 h, 6 h, 24 h, 48 h, 7 days and 10 days after stem cell infusion (Dual head gamma camera, ADAC, USA). Scanning speed was set at 12 cm/min in all acquisition procedures. The SPECT images were obtained at 24th-hour post-infusion. The photopeaks set at 171/245 Kev for ^{111}In.

2.5. Data analysis

In all patients, ^{111}In biodistribution was quantified by drawing regions of interest (ROIs) around the lungs, liver and spleen on the anterior and posterior views of the planar whole-body images at different time points after infusion. The percent of organ activity was calculated by geometric mean [(anterior counts×posterior counts) $^{1/2}$] calculation.

2.6. Follow-up visits and outcome measures

The following tests were performed just before infusion, and also in the second and fourth week post-infusion: complete blood cell counts, PT and international normalized ratio (INR), serum albumin, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT),

Table 2
Residual activity in the liver and spleen at different time points

	2 h	4 h	6 h	24 h	48 h	7th day	10th day
Patient 1							
Lung	33.5%	31.1%	19.2%	14.7%	12.0%	4.0%	2.0%
Liver	2.8%	4.1%	4.1%	4.3%	4.7%	12.0%	13.5%
Spleen	2.0%	3.1%	3.3%	4.0%	5.4%	21.3%	30.1%
Patient 2							
Lung	27.0%	26.0%	21.1%	13.0%	12.2%	8.7%	5.4%
Liver	0.0%	3.6%	3.6%	3.9%	4.3%	11.0%	13.0%
Spleen	2.5%	4.2%	5.1%	7.0%	9.3%	14.0%	42.0%
Patient 3							
Lung	29.8%	24.3%	20.1%	13.6%	12.1%	6.8%	2.9%
Liver	2.1%	5.1%	5.4%	7.7%	8.2%	9.1%	15.6%
Spleen	10.2%	12.7%	15.0%	25.1%	25.7%	37.6%	40.2%
Patient 4							
Lung	30.1%	26.9%	20.8%	12.6%	12.0%	5.7%	3.0%
Liver	7.9%	9.3%	10.1%	11.9%	11.9%	15.1%	17.4%
Spleen	7.3%	8.8%	8.8%	9.1%	14.9%	32.2%	33.0%

Table 3
Clinical and laboratory data of the patients during the follow-up visits

	Patient 1 (cryptogenic cirrhosis)													
	Edema	PT (s)	INR	Serum albumin (g/dl)	Cr (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	AST (IU/ml)	ALT (IU/ml)	AFP (mcg/L)	Hb	WBC	Plt	MELD score
Baseline	2+	14.1	1.2	3.5	2.13	1.82	0.53	27	13	3.05	10.5	5400	135000	18
Two weeks post-infusion	2+	13.7	1.2	3.2	2.05	2.03	0.59	40	24	2.83	11.7	5910	71000	18
Four weeks post-infusion	1+	13.7	1.2	3.5	1.83	1.52	0.52	41	27	2.47	12.2	7940	82000	16
	Patient 2 (cryptogenic cirrhosis)													
	Edema	PT (s)	INR	Serum albumin (g/dl)	Cr (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	AST (IU/ml)	ALT (IU/ml)	AFP (mcg/L)	Hb	WBC	Plt	MELD score
Baseline	2+	18	1.7	2.5	0.8	2.7	0.45	73	67	1.89	10.5	3050	31000	16
Two weeks post-infusion	2+	17.6	1.9	2.1	0.57	2.3	0.63	79	53	1.46	9.8	2200	42000	17
Four weeks post-infusion	2+	16.7	1.8	2.1	0.58	2.41	0.63	72	45	1.48	9.9	2290	38000	16
	Patient 3 (cryptogenic cirrhosis)													
	Edema	PT (s)	INR	Serum albumin (g/dl)	Cr (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	AST (IU/ml)	ALT (IU/ml)	AFP (mcg/L)	Hb	WBC	Plt	MELD score
Baseline	1+	15	1.52	3.7	1.2	2.0	–	39	16	4.5	12.7	6300	100000	15
Two weeks post-infusion	1+	16.8	1.6	3.8	1.31	1.56	0.49	38.0	27.0	4.26	14.7	4520	92000	16
Four weeks post-infusion	1+	15.5	1.4	3.5	1.28	2.01	0.57	40.0	28.0	4.20	14.5	4640	84000	15
	Patient 4 (hemochromatosis)													
	Edema	PT (s)	INR	Serum albumin (g/dl)	Cr (mg/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	AST (IU/ml)	ALT (IU/ml)	AFP (mcg/L)	Hb	WBC	Plt	MELD score
Baseline	1+	16.5	1.6	3.0	0.7	3.09	1.8	230	150	1.69	11.0	2700	55000	16
Two weeks post-infusion	1+	16.6	1.6	2.5	0.75	4.07	2.04	265	165	1.69	10.9	2360	50000	14
Four weeks post-infusion	1+	>36.7	>6.9	2.7	0.72	5.63	2.78	292	176	1.84	11.5	3190	55000	35

PT, prothrombin time; INR, international normalized ratio; Cr, serum creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; Hb, hemoglobin; WBC, white blood cells; Plt, platelet; MELD, model for end stage liver disease.

serum alkaline phosphatase, serum total and direct bilirubin, alfa-fetoprotein. In addition, 10 ml of the patients' serum samples were collected and stored frozen at −70°C at each follow-up visits.

Interval histories were taken and physical examinations performed at each follow-up visits. Peripheral edema was graded as follows: 0: no edema; trace: indention caused by pressure over the dorsum of the foot; 1+: indention at shin; 2+: indention at knee; 3+: indention above knee; 4+: generalized edema (indention over hip and low back).

2.7. Evaluation of safety and feasibility

Adverse effects were evaluated at each follow-up visits according to the above-mentioned schedule. Clinical, laboratory, and safety-related data were prospectively collected. Procedural complications were defined as any hemodynamic instability during the cell infusion. Major side effects were defined as development of any of the following complications during the follow up: acute renal failure, worsening hepatic decompensation that requires urgent liver transplantation, progressive elevation in serum AFP.

3. Results

Table 1 summarizes the patients' age, sex, estimated number of infused MSCs (of which only 50% of cells were labeled), specific activity of injected labeled cells, and also the labeling efficiency.

3.1. Whole-body imaging

After intravenous infusion, the radioactivity was first observed to accumulate in lungs, and gradually shifted to liver and spleen (Fig. 1). Based on the initial views (those projections which were obtained immediately after infusion), liver and spleen uptake could be distinguished from the background activity just in the fourth patient who suffered from a milder form of the disease (as confirmed by his lower MELD score, as compared to the other three patients). In the other patients, the initial hepatic and splenic accumulation of radioactivity was visualized only after 2 h (except the third patient who showed delineation of the splenic activity in the immediately post-infusion images — due to his huge splenomegaly) (Fig. 1).

During the following hours to days, the radioactivity gradually increased in liver and spleen, with spleen uptake exceeding that in liver in all patients (Fig. 1). ROI analysis showed that the percentage of cells shifted toward the liver (following decay and background corrections and geometric mean calculation) increased from 0.0%-2.8% in the immediate post-infusion images to 13.0%-17.4% in 10th-day post-infusion. Similarly, the residual activities in the spleen increased from 2.0%-10.2% at immediately post-infusion images to 30.1%-42.2% in 10th-day post-infusion (Table 2). During the same period, the residual activities in the lungs decreased from 27.0–33.5% to 2.0–5.4%.

On SPECT images after the 24th hour, the activity distribution was homogenous throughout the liver in all the patients, but the splenic activities were nonhomogeneous with foci of severely decreased or absent radiotracer uptake, even evident in planar imaging (Fig. 1).

No unexpected adverse events or complications were observed during stem cell infusion and within 1-month follow-up period. Clinical and laboratory data of the patients at the follow-up visits are summarized in Table 3.

4. Discussion

Our study is the first report of MSCs tracking after transplantation in human cirrhosis. This study showed that after peripheral vein infusion of MSCs, most of the cells were initially entrapped within the lung capillaries during their first pass, but over the next 48 h, a significant proportion of the cells escaped from the lung capillary system and migrated to the liver and spleen (Table 2). Furthermore, we showed that the cells can be visualized in vivo up to at least 10 days after initial infusion, and it is possible to quantify and follow them using radiolabeling with ^{111}In-oxine. In this study, infusion of the MSCs labeled with ^{111}In-oxine was safe in liver cirrhosis patients within 1 month of follow-up.

MRI tracking of the cells labeled with iron particles is another acceptable method for tracking the cells in regenerative medicine. However, some authors believe that MRI tracking of stem cells suffers from disadvantages such as the limited number of probes available [18]. Schoepf et al. [25] asserted that with clinical 1.5 T MR equipment and clinically applicable contrast agents, it was not feasible to trace the in vivo distribution of intravenously injected hematopoietic cells to more than one final target organ or to depict the migration of the transplanted cells to several subsequent target organs over time. Based on the study of Kraitchman et al. [19], one of the main disadvantages of MRI tracking is the lower sensitivity of the method, compared with radionuclide techniques. In fact, initial cell-labeling techniques were hampered by limited concentration of internalized contrast agent, which resulted in a limited sensitivity of MRI to detect the labeled cells. To compensate for this limited sensitivity, experimental cell-tracking studies were performed using MR imagers with very high magnetic field strengths of up to 14 T [15].

On the other hand, several studies already reported that indium- or technetium-labeled stem cell imaging can be used to monitor the fate of transplanted stem cells [26,27]. Radionuclide techniques may provide a noninvasive and semiquantitative method to sequentially assess the in vivo distribution and homing of MSCs to the intended site [28]. In our experience, ^{111}In (which has long half-life of 67 h) provides the opportunity to look at cell homing over several days. In the current study, the activity continues to accumulate in the liver and spleen during the entire 10 days after infusion.

Kang et al. [29] evaluated the homing and distribution of peripheral HSCs in patients who were afflicted by myocardial infarction after injection using ^{18}F-FDG-labeled stem cell PET [29]. They found that after intravenous injection, the total amount of stem cells in the liver and spleen were 21.3% and 42.1% of the injected dose, respectively, at 4 h post-infusion [29]. Caveliers et al. [30] evaluated stem cell homing after intracoronary injection of peripheral blood stem cells in patients with chronic ischemic cardiomyopathy. In their study, the ROI analysis in patients showed that the respective estimated liver and spleen uptake was approximately 25% and 3.5% after 12 h.

In our study, the absorbed activity increased gradually during the study period; however, the total activity in the liver was less than that reported by Caveliers et al. [30] and Kang et al. The differences as compared to our findings (with decreased hepatic and increased splenic homing) may be explained by the fact that our patients suffered from advanced liver cirrhosis. Splenomegaly which is present in cirrhosis, and the hepatofugal flow of the portal vein observed in portal hypertension, can be responsible for decreased hepatic homing found in this study [31,32].

Our study has some limitations. It is possible that MSCs labeled with ^{111}In-oxine are phagocytosed by the reticulo-endothelial cells when they are trapped initially in the lung capillary. Isotopic tracking cannot exclude the possibility that ^{111}In-oxine signals were detected from free radioactive indium released from labeled cells (although based on the previous reports the extent of such phenomenon is very limited [22]) or the retoculoendeothelial cells which could theoretically phagocytize nonviable MSCs (due to time-dependent cytotoxic effect of the ^{111}In-oxine, after infusion, a limited portion of MSCs would lose viability [23]). In fact, no one can be sure that the observed radioactivity in vivo reveals the presence of viable MSCs in the organs having radioactivity. However, it should be emphasized that this limitation is not limited to scintigraphic tracking methods: MR tracking of stem or other cells has a critical flaw that the ferromagnetic particles do not represent viable cells in vivo. We simply do not know whether MR signals mean magnetic particles in the “live” cells or “already dead” cells or even particles in the engulfed/digested cells.

The beneficial effects of bone marrow stem cells in liver fibrosis have been demonstrated in several animal studies [3–6]. Few human studies have assessed the therapeutic

efficacy of stem cells in the treatment of patients with liver cirrhosis [2,7,33–35]. In these limited studies, the cells were either administered intravenously [7] or injected into the portal vein [33,34] or hepatic artery [2,33,34]. None of these studies addressed the fate of these cells in vivo.

In our study (Table 3), no significant improvement in liver function was noted after 1 month period of follow-up. Our main explanation is the fact that may be longer follow-ups are needed to explore the therapeutic and beneficial effects of infused MSCs on liver cirrhosis, as theoretically they have to generate functionally active hepatocytes (a process which is time dependent). Lack of improvement may also be explained by the fact that homing of the infused cells to the target organ (liver) has been occurred in just a limited number of MSCs. This explanation is boosted by our study findings, which show that up to the 10th post-infusion day, less than 14% of the activity was located in the patients' livers and more than 30–40% of the activity was trapped in the enlarged spleens. As another explanation it should be kept in mind that may be some degrees of the toxic effects of the In-oxine on MSCs would decrease the therapeutic effects of the therapeutic measure of MSC infusion. Although we have previously shown that a limited proportion of MSCs lose their viability upon binding to In-oxine [23], and in our study just half of the infused cells were labeled with radiotracer and also as low as possible radiotracer was applied for radiolabeling of stem cells (the dose with the lowest cytotoxicity, as determined by our previous in vitro experiment [23]), we can not exclude the negative impact of In-oxine on the function and viability of MSCs, however. Furthermore, the efficacy of this potential new treatment strategy needs to be evaluated in a larger group of patients.

In conclusion, we found in vivo tracking of MSC with ^{111}In-oxine is safe and feasible in cirrhosis. Cell labeling with ^{111}In-oxine is a suitable method for tracking of cell distribution up to 10 days after infusion.

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