Define Mesenchymal Stem Cell from Its Fate: Biodisposition of Human Mesenchymal Stem Cells in Normal and Concanavalin A–Induced Liver Injury Mice

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ABSTRACT

The pharmaceutical industry and clinical trials have been revolutionized by mesenchymal stem cell–based therapies. However, the pharmacokinetics of transplanted cells has been little characterized in their target tissues under healthy or disease condition. A quantitative polymerase chain reaction analytical method with matrix effect was developed to track the biodistribution of human mesenchymal stem cells in normal mice and those with Concanavalin A (Con A)–induced liver injury. Mesenchymal stem/stromal cell (MSC) disposition in blood and different organs were compared, and relevant pharmacokinetic parameters were calculated. Human MSCs (hMSCs) and mouse MSCs (mMSCs) displayed a very similar pharmacokinetic profile in all tested doses: about 95% of the detected cells accumulated in the lung and 3% in the liver, and almost negligible cells were detected in other tissues. A significant double peak of hMSC concentration emerged in the lung within 1–2 hours after intravenous injection, as with mMSCs. Prazosin, a vasodilator, could eliminate the second peak in the concentration–time curve (AUC) by 10% in the first 2 hours. The injury caused by Con A was significantly reduced by hMSCs, and the Cmax and AUC0–8 (AUC from time 0 to 8 hours) of cells in the injured liver decreased by 54 and 50%, respectively. The Cmax and AUC would be improved with the alleviation of congestion through the administration of heparin. The study provides a novel insight into the pharmacokinetics of exogenous MSCs in normal and Con A–induced liver injury mice, which provides a framework for optimizing cell transplantation.

SIGNIFICANCE STATEMENT

Mesenchymal stem/stromal cells (MSCs) are known for their potential as regenerative therapies in treating several diseases, but an insufficient understanding of the pharmacokinetics of MSCs restricts their future application. The current study was the first to elucidate the pharmacokinetics and possible factors, including dosage, species, and derived sources, in a systematic way. The study further revealed that Concanavalin A–induced liver injury significantly prevented cells from entering the injury site, which could be reversed by the diminished congestion achieved by heparin.

Introduction

Since chimeric antigen receptor T-cell therapies were approved by the US Food and Drug Administration, cell therapies have attracted increasing attention for their excellent therapeutic potency. As a multipotent progenitor cell product, mesenchymal stem/stromal cells (MSCs) are well known for their potential in regenerative medicine and treatment of diseases such as hepatitis and liver failure (Ringlen et al., 2006; Zhang et al., 2014; Lee et al., 2015; Shi et al., 2017), Crohn’s disease (Lopez-Santalla et al., 2020), and myocardial infarction (Golpanian et al., 2015). After hundreds of clinical trials conducted in different countries, the positive phase III results of Remestemcel-L (Mesoblast Ltd) for graft-versus-host disease (Galipeau and Sensebe, 2018) suggested that MSCs could be the next promising cell product to be approved. Although intensive studies have focused on the therapeutic benefits of MSCs, insufficient information about the pharmacokinetic process blurs their future application. It is an essential but difficult task to estimate the contribution of MSC

ABBREVIATIONS: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the concentration–time curve; Con A, Concanavalin A; Cy5, cyanine 5; hMSC, human mesenchymal stem cell; IVIS, in vivo imaging system; mMSC, mouse mesenchymal stem cell; MSC, mesenchymal stem/stromal cell; NHS, N-hydroxysuccinimide; P/S, penicillin and streptomycin; PCR, polymerase chain reaction; PK, pharmacokinetic; qPCR, quantitative polymerase chain reaction.
exposure to treatment efficacy (Brooks et al., 2018). However, there have been conflicting results regarding tissue distribution and residence time in vivo. For example, Lee et al. (2009) found that the venous-administered MSCs disappeared within 5 minutes, about 83% of cells accumulated in the lungs, and less than 0.05% of cells remained in mice after 48 hours. Nyibizi et al. (2004) reported that GFP-labeled MSCs could survive in mice lung up to 150 days. The route of administration also complicated the residence time of MSCs, and it was reported that MSCs could live more than 5 months when they were injected into muscles (Gao et al., 2001; Braid et al., 2018). Besides, it is still questionable whether MSC studies should be conducted on immunodeficiency animal models. Since the survival time of MSCs may triple in such animals (Zangi et al., 2009), it seems less plausible to translate preclinical results from these animals into real clinical performance in the future.

Collectively, there are several fundamental questions about MSCs in vivo distribution that have not been systematically answered: As an “immune privileged” cell product (Zhang et al., 2015), is there a significant difference in MSCs’ pharmacokinetic behaviors among species? What is the relationship between dose and exposure in its target organs? Could pathologic condition affect MSCs’ distribution and clearance?

In this study, MSCs were labeled with hydrophilic dye and detected by an in vivo imaging system (IVIS). The conventional quantitative polymerase chain reaction (qPCR) method was optimized by considering matrix effects to estimate human MSC disposition in mice organs quantitatively. The pharmacokinetics of human MSCs (hMSCs) and mouse MSCs (mMSCs) were investigated simultaneously to clarify the impact of species differences. A double peak of hMSC concentration curve in the lung was observed when the dosage exceeded 2 × 10^7 cells per kilogram, and the vasodilator prazosin eliminated the double peak and increased hMSC accumulation in the lung. Furthermore, we identified distinct pharmacokinetic (PK) properties in the Concanavalin A (Con A)–induced acute hepatitis model with a significant decrease in C_max and area under the concentration-time curve (AUC) in the liver; the potential reason might be liver congestion, which could be ameliorated by a coadministration of heparin.

**Materials and Methods**

**Materials.** Concanavalin A and pentobarbital sodium were purchased from Sigma (St Louis, MO). The cell culture medium (α-minimal essential medium), Dulbecco’s modified Eagle’s medium containing low glucose (1 g/L), penicillin and streptomycin (PS; 100 μg/mL), FBS, and 0.25% trypsin/EDTA were obtained from Thermo Fisher Scientific (Madison, WI). Glutamine (100 μg/mL), and cyanine 5 (Cy5)–N-hydroxysuccinimide (NHS) were purchased from Meilunbio (Dalian, China). Glycine was obtained from Sino Pharm Chemical Reagent Co., Ltd. (Shanghai, China). Prazosin was purchased from Meryer Chemical Technology Co., Ltd. (Shanghai, China), and heparin was from J&K Chemical Ltd (Shanghai, China).

**Animal Study.** Adult male C57/BL6 mice (6–8 weeks) were purchased from the Shanghai Laboratory Animal Co. (Shanghai, China). All animals were maintained under the specific-pathogenfree (SPF) condition with a constant temperature (23 ± 1.5 °C) and humidity (70 ± 20%) on a 12-hour light/dark cycle. All animal experiments and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Material Medica, Chinese Academy of Sciences (permit number: 2019-02-PGY-29).

**In Vivo Transplantation and Imaging of MSCs.** Mice were given 200 μl hMSCs or mMSCs (4 × 10^3, 1 × 10^4, 2 × 10^4 cells/kg) through the tail vein. At the indicated time points (15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours), mice were anesthetized with pentobarbital (60 mg/kg) and sacrificed; anti-coagulated blood and selected organs were collected. Organs were washed by PBS, and fluorescence intensities were measured by an IVIS (Caliper Life Sciences, Hopkinton, MA) with filter set as follows: Excitation/Emission 640/680 nm. For prazosin experiments, mice were intraperitoneally injected with prazosin (5 mg/kg) 30 minutes before MSCs. For heparin assay, mice were injected intravenously with 800 U/kg heparin 1 hour before giving MSCs.

**Establishment of Murine Con A–Induced Hepatitis Model.** Male C57/BL6 mice were randomly divided into groups (n = 3 for each group): the normal control, PBS, Con A group (10 mg/kg) and Con A + hMSC (2 × 10^7 cells/kg) group.

To examine the therapeutic effect, all mice were anesthetized with pentobarbital (60 mg/kg) and sacrificed, and blood and livers were harvested 24 hours after the Con A challenge. Blood samples were collected and centrifuged (3,000 rpm, 4 °C) for 15 minutes to obtain serum and stored at −80 °C for further analysis. Liver specimens were dissected and placed in 10% formaldehyde for subsequent histologic analysis, and the rest was stored at −80 °C.

**H&E Staining.** Liver tissues were randomly selected for pathology analysis. H&E staining was performed on 5-μm–thick liver sections of paraffin-embedded formaldehyde-fixed tissues to evaluate for pathologic changes.

**Human MSC Culture.** Human MSCs were kindly provided by Hai Li and Huiming Xu (Renji Hospital, affiliated to Shanghai Jiao Tong University School of Medicine). Human MSCs (female) were prepared and identified as described in previous study (Zhong et al., 2020). The umbilical cord was obtained with informed consent. The collection and use of the umbilical cord were approved by the Institutional Ethical Review Committee of Shanghai Children’s Medical Center, Shanghai Jiao Tong University School of Medicine. Umbilical cord tissues were cut and digested, then attached to culture plates individually, followed by the addition of z-minimal essential medium containing 10% FBS and 1% PS, and then incubated at 37 °C with 5% CO_2. Approximately 12 days later, the cells were cultured on new plates for further expansion and the next experiments.

**Mice Adipose–Derived MSC Isolation and Culture.** Mice adipose–derived MSCs were harvested from male C57/BL6 mice (25 g) according to a previously published protocol as previously reported (Estes et al., 2010). Primary mMSCs were cultured with Dulbecco’s modified Eagle’s medium supplemented with 10% FBS plus 1% glutamine and 1% PS in 5% CO_2 at 37 °C.

When the cell density reached 80–90% confluence, the cells were washed twice with PBS and harvested with 0.25% trypsin/EDTA. The detached cells were centrifuged at 1000 rpm for 3 minutes. The resultant pellet was obtained by centrifugating (1000 rpm, 3 minutes) and resuspended in a fresh medium for further use.

**Cell Labeling with Cy5-NHS.** After collection, cells were washed by PBS (pH 7.4). Then the pellet was incubated with Cy5-NHS (10 μM, pH 8.0) for 30 minutes. Glycine (100 mM) was added to terminate the reaction, and then cells were washed with PBS (pH 7.4) three times.

**Blood Biochemical Analyses.** The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum were measured by biochemical assay kits (Jiancheng Bioengineering, Nanjing, China), according to the manufacturer’s instructions.

**Cell Viability Assay.** MSCs were seeded into 96-well plates (3 × 10^3 cells/well) for 24 hours prior to the experiment. After that, 10 μl CCK-8 (Yeussen Biotest, Shanghai, China) was added to each well and incubated for 2 hours at 37 °C in a dark place, and then the absorbance at 450 nm wavelength was measured by a microplate reader (BioTek, Winooski, VT).

**ELISAs.** The concentration of serum cytokines, including interferon-γ, tumor necrosis factor-α, and interleukin-6, were detected by...
 genomic DNA extraction kit (Magen, China) was used to extract gDNA from tissue based on the manufacturer’s protocol. Briefly, about 10 mg tissues were weighted and then added to tubes with 220 μl lysis buffer and 10 μl proteinase K (20 mg/ml). The tissue suspension was digested in a water bath at 55°C overnight. Ten microliters RNase was then added, followed by further incubation at 70°C for 10 minutes. The gDNA was purified and eluted following the manufacturer’s protocol.

Human-Specific Alu qPCR Primers. Quantitative polymerase chain reaction (PCR) primers were designed targeting the human-specific unique sequence of Alu (Thi Tran and Kitami, 2019) and β-actin. The primer set for human-specific Alu repeat was as follows: forward, 5’-CTTGCAAGGCGGAGATT-3’; reverse, 5’-GAGACGGGAGTCTCGGCTCTGTGC-3’. The primer set for both human and mouse actin was as follows: forward, 5’-TACGAAATGCCGCTGGTACAT-3’; reverse, 5’-ATCATTGTGGCAACGAGCG-3’.

qPCR Amplification. Quantitative real-time PCR assays were performed by Applied Biosystems ABI 7500 System (Bio-Rad Laboratories, Hercules, CA), and the reaction mix was loaded into 96-well PCR plates (Invitrogen, Waltham, MA). The qPCR assay was performed in a volume of 20 μl that contained 10 μl qPCR SYBR Green Mix (Yeasen Biotech, China), 1 μl forward and reverse primers, and 200 ng template gDNA diluted in water. The SYBR Green PCR reaction conditions were as follows: hold stage was 95°C for 10 minutes, cycling was 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds.

Cell Number–Cycle Threshold Standard Line Establishment. Cells (10^6, 2.5 × 10^6, 6.25 × 10^6, 3.125 × 10^6, 7.8 × 10^5, 2 × 10^5, 500, 250, 125, 60) were mixed with 10 mg mice organ tissues to extract DNA. The standard curve of cycle threshold values versus cell number (logarithmic form) was obtained through qPCR amplification. The number of hMSCs per unit weight in test tissues was calculated according to the standard curve.

For detecting the cells in blood, gradient-diluted Cy5-labeled MSCs (10^5, 5 × 10^5, 2.5 × 10^5, 1.25 × 10^5, 6.25 × 10^4, 3.13 × 10^4, 1.56 × 10^4, 7.8 × 10^3, 390, and 0) were mixed with 200 μl anticoagulant blood measured at wavelength of 640 nm excitation/680 nm emission, and the standard curve of fluorescent intensity and cell numbers were obtained. The calibration curve was constructed by linear regression of the fluorescent intensity and cell numbers of standard samples. The number of hMSCs per milliliter in test tissues was calculated according to the standard curve.

Statistical Analysis. GraphPad Prism 8.0 software was used for statistical analysis, and all data were expressed as means ± S.D. Two-tailed, unpaired t test and one-way ANOVA followed by Tukey’s multiple-comparison tests were used to analyze comparisons of two or more than two groups, respectively. The effects of drug treatments over time were analyzed using two-way ANOVA followed by Sidak’s multiple-comparison tests. A value of P < 0.05 was considered to be statistically significant.

PK parameters including Cmax, AUC, elimination half-life, and mean residence time were calculated by noncompartmental analysis with WinNonlin software (version 6.2, Pharsight, Cary, NC).

Results

Biodisposition of hMSCs in Mice After Intravenous Administration. An imaging method for labeling cells with Cy5-NHS (Supplemental Fig. 1; Supplemental Fig. 2) and an optimized qPCR method (Supplemental Fig. 3) were employed to detect hMSC distribution in mice. Briefly, Cy5-NHS–labeled hMSCs were injected intravenously at various doses (4 × 10^6, 1 × 10^7, and 2 × 10^7 cells/kg). The animals were anesthetized and then sacrificed at different time points, and organs were collected. The fluorescence intensities of the liver and lung were measured by IVIS.

The concentration curves of hMSCs in the lung and liver are displayed in Fig. 1, and related PK parameters are listed in Table 1. The parameters were calculated according to qPCR results. The half-life in the liver and lung was about 20 hours and 6 hours, respectively (Table 1). Nearly 95% of the detected hMSCs were distributed in the lung, and almost no cells were detectable in other organs (including heart, spleen, and kidney). The fluorescence results were also detected (Supplemental Table 1; Supplemental Fig. 4), and the results were very close to qPCR.

The AUC of hMSCs in the liver increased with dose; however, its AUC in the lung disproportionately rose at a higher dose, which was attributed to the double peak that appeared in the lung at approximately 1 hour after administration (Fig. 1). Human MSC concentrations in blood were calculated; relevant parameters indicated that the half-life was about 7 hours, and 80% of detected hMSCs in blood were cleared within 8 hours (Supplemental Fig. 4).

Comparison of hMSC and mMSC Elimination Process After Intravenous Injection. More importantly, we asked whether the derived source made a difference in the distribution of transplanted cells. To maximize the species differences, MSCs isolated from mouse adipose tissue were used as an allogeneic control and investigated by the IVIS method. The dose of 2 × 10^7 cells per kilogram was selected for

Fig. 1. Pharmacokinetics of hMSCs in mice lung and liver by qPCR. The lung (A) and liver (B) were collected, and human Alu repeat was analyzed by qPCR assay after the mice were intravenously administered hMSCs at doses of 4 × 10^6, 1 × 10^7, and 2 × 10^7 cells per kilogram. The data were expressed as means ± S.D. (n = 3), and the experiments were repeated two times.
subsequent comparison. Interestingly, the elimination processes of mMSCs and hMSCs were very similar in the liver and lung (Fig. 2).

**Vasodilator Prazosin Hydrochloride Eliminated hMSC “Double Peak” in the Lung.** Since the double peak in the PK curve was observed for the first time, we hypothesized that the possible reason might be the recaptured hMSCs in the pulmonary capillaries. To investigate the impact of microvessel sizes on hMSCs disposition, prazosin, an alpha-adrenergic antagonist, was used to dilate blood vessels. Human MSCs were intravenously administered 30 minutes later after intravenous injection of prazosin (5 mg/kg). It was found that prazosin could increase the accumulation of hMSCs in the lungs (Fig. 3A); more MSCs entered the lungs within 1 hour. hMSC AUC and Cmax both increased by about 10% (Table 2). Although the cells accumulated in the lung increased after prazosin administration, there were no obvious alterations in the lung clearance process or distribution in the liver (Fig. 3B).

**Distribution of hMSCs Under Con A-Induced Liver Injury.** Next, we examined the link between hMSC exposure in the liver and its efficacy in the mice hepatitis model. At first, we examined hMSCs’ capacity to attenuate inflammation and injury in Con A–induced hepatitis. hMSCs were administered through the tail vein 4 hours after administration of Concanavalin A (10 mg/kg). Next, the fluorescence signals of various organs were measured (Fig. 4, A and B; Supplemental Fig. 6). As we expected, the accumulation of hMSCs in the liver was significantly augmented by heparin (Fig. 5A): the Cmax in the liver increased by about 30% at 4 hours, and Cmax returned to the same as the hMSC group after 24 hours (Fig. 5B). The increased hMSC amount in liver was connected with the improvement of liver damage (Fig. 5E). So was the decrease of liver ALT and AST levels and the proinflammation factors (tumor necrosis factor-α, interferon-γ, and interleukin-6) (Fig. 5, C and D; Supplemental Fig. 7). Taken together, these results indicated that congestion in the liver could be the potential risk hindering the hepatic distribution of therapeutic MSCs in the Con A–induced model.

**Discussion**

There are numerous reports about the biologic characteristics of stem cells and their therapeutic effects, whereas few studies have been conducted about their disposition and fate in target organs. The purpose of this study was to characterize hMSC distribution in mice with optimized qPCR/IVIS methods.

### Table 1: Pharmacokinetic parameters in mice lung and liver after multidose hMSC administration

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Lung</th>
<th>Liver</th>
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<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>AUCmax (h x cells x 10^7/kg)</td>
<td>17.9 ± 7.34</td>
<td>121 ± 10.2</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.25 ± 0</td>
<td>0.25 ± 0</td>
</tr>
<tr>
<td>Cmax (cells x 10^7/kg)</td>
<td>48.2 ± 4.28</td>
<td>155 ± 35</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>6.71 ± 0.06</td>
<td>7.12 ± 0.54</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>4.68 ± 1.05</td>
<td>5.71 ± 1.21</td>
</tr>
</tbody>
</table>

AUCmax, AUC from zero to observed; MRT, mean residence time; t1/2, elimination half-life, Tmax, peak time

Fig. 2. Pharmacokinetics of Cy5-labeled mMSCs and hMSCs in C57 mice by IVIS imaging assay. The fluorescence intensity of MSCs in mice lung (A) and liver (B) were measured by IVIS after giving hMSCs or mMSCs to C57 mice at a dose of 2 x 10^7 cells per kilogram. The data were analyzed by Graphpad Prism software and expressed as means ± S.D. (n = 3), and the experiments were repeated two times.
and clarify the contribution of liver hepatitis to hMSCs kinetics in the liver.

To record therapeutic cell residence time in vivo, multiple strategies have been adapted, including fluorescence (Braid et al., 2018), transgenic (Prigent et al., 2015; Braid et al., 2018), isotopic (Ghazavi et al., 2017), magnetic iron (Liu and Ho, 2017), and flow cytometry (Stuckey et al., 2006; Elman et al., 2014; Nguyen et al., 2014), whereas membrane dyes (PKH26, DiR(DiIC18(7); 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine iodide)) (Li et al., 2013; Vaegler et al., 2018) and nuclear dyes (Hoechst 33342, DAPI (4',6-diamidino-2-phenylindole)) (Creane et al., 2017) are still the most conventional labeling methods. However, these lipophilic dyes can bind noncovalently to the lipid regions of target cell membranes (Jensen, 2012) and accumulate in the liver or spleen due to their leakage from the damaged cells. On the contrary, the hydrophilic dye Cy5-NHS can covalently bind to living cells, and it did not show nonspecific binding in the liver after the elimination of hMSCs in vivo (Supplemental Fig. 1C).

To have a more precise understanding of the amounts of transplanted cells in organs, an improved qPCR method with previous studies (McBride et al., 2003; Creane et al., 2017) that simply used mice DNA mixed with human DNA to establish a standard curve (Yokoo et al., 2006; Shim et al., 2017) that simply used mice DNA mixed with human DNA to establish a standard curve (Yokoo et al., 2006; Shim et al., 2017), our optimized method simulates the complexity of real detection environments since the matrix effect was included. In this study, to obtain hMSC standard curves with a matrix effect, different amounts of cells were mixed with 10 mg mice tissue homogenates before DNA extraction. The results indicated that approximately 500 hMSCs could be detected from 10 mg tissue homogenates, which is equivalent to about 200 hMSCs in 200,000 mice cells (Supplemental Fig. 3). Compared with previous studies (McBride et al., 2003; Creane et al., 2017) that simply used mice DNA mixed with human DNA to establish a standard curve (Yokoo et al., 2006; Shim et al., 2017), our optimized method simulates the complexity of real detection environments since the matrix effect was included.

**Human MSC concentrations in different organs were measured at three doses. Importantly, it was found that 90% of the hMSCs were stuck in the lungs, whereas 2–3% dwelled in other organs (<0.5%). However, 95% of MSCs will be eliminated from the body within 24 hours. Interestingly, a double-peak phenomenon was noticed in the time-concentration curve in the lung after intravenous administration (Fig. 3), which was never reported before according to our knowledge. It was reported that MSCs could be captured by the pulmonary capillaries because their size (20–30 µm) is large than the capillaries (10–15 µm) (Fischer et al., 2009). Some hMSCs might be stuck in the capillaries during their first circulation, and the remaining free cells may be trapped later during the following circulations, forming the second peak in the lung. To test this hypothesis, prazosin was used to dilate blood vessels and accelerate the migration of MSCs to deeper capillary positions. The double peak disappeared, and thus both the Cmax and AUC in the lung increased significantly. Prazosin also promotes stuck cells’ migration to other target tissue like the liver, as we expected. Our results were in opposition to those of Gao et al. (2001), who claimed that vasodilator drugs could reduce cell entrapment in the lung. However, they used 111In-oxine–labeled MSCs to trace radioactivity in the lung 48 hours after hMSC administration. According to our experience, tracing radiolabeled MSCs is not a good method to detect MSCs quantitatively because of the short half-life and cytotoxicity of radioactive material.**

**One of the main concerns in current MSC preclinical studies is the interspecies variety. Here, mMSC and hMSC kinetics in mice were both investigated. To maximize the variances, MSCs isolated from different tissues were used: mice adipose–derived MSCs (mMSCs) were compared with...**

**TABLE 2. Pharmacokinetic parameters of hMSC in liver and lung after prazosin administration**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Liver</th>
<th>Lung</th>
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<tbody>
<tr>
<td></td>
<td>hMSC</td>
<td>Prazosin + hMSC</td>
</tr>
<tr>
<td>AUClastb,2 (h × FU × 10⁶)</td>
<td>193.4 ± 14.4</td>
<td>228.4 ± 16.3</td>
</tr>
<tr>
<td>AUClasta,2 (h × FU × 10⁶)</td>
<td>419.9 ± 34.7</td>
<td>660.3 ± 67.6</td>
</tr>
<tr>
<td>Tₘax (h)</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Cₘax (FU × 10⁶)</td>
<td>113.4 ± 21.5</td>
<td>152.3 ± 21.0</td>
</tr>
</tbody>
</table>

FU, fluorescent units; Tₘax, peak time. *P < 0.05; **P < 0.01 compared with the hMSC group.
human umbilical cord–derived MSCs (hMSC). Interestingly, the in vivo PK pattern of two kinds of MSCs was quite close (Fig. 2), which implied that the species of cells would not greatly influence the fate of MSCs in vivo. As immunodeficient animal models may not reflect the clinical situation correctly, it is feasible to conduct hMSC PK studies in wild-type animals directly.

For conventional chemical compounds, it is well accepted that their PK profile may be altered significantly due to disease. Here, Con A–induced immune liver injury was employed to investigate the PK profile of MSCs under disease. The liver damage was alleviated after hMSC administration (Fig. 4), which was consistent with previous reports (Akla et al., 2012; Tamura et al., 2016). It was reported that more MSCs tend to migrate to the injury sites to alleviate inflammation (Gao et al., 2016). However, in this study, the AUC0–4 (AUC from time 0 to 4 hours) of hMSCs in the Con A–induced injury liver reduced 50% compared with the control group. The phenomenon that fewer hMSCs could access the liver under liver injury within the first 4 hours could not be explained by a tendency to gather in the injury location. In fact, the homing effect did not happen until approximately 4–24 hours after hMSC administration: the concentration of hMSCs in the liver in the diseased mice was 40% more than in the normal group.

The discrepancy of hMSC pharmacokinetics and pharmacodynamics suggested that disease might interfere with the distribution of MSCs. Much congestion occurred in the liver after Con A administration (Ye et al., 2018), which was also observed in our results (Fig. 5), and the congestion might prevent MSCs from entering the liver. When the congestion was alleviated by heparin, the Cmax in the liver increased by about 30%, resulting in further lower liver inflammation (Fig. 5). Our results also explained why a simple increasing dosage may not necessarily bring about

![Fig. 4.](https://example.com/f4.png) The exposure and efficacy of hMSCs in mice with Con A–induced liver injury. Fluorescence intensities of hMSC in the mice lung (A) and liver (B) were analyzed by IVIS. ALT (C) and AST (D) concentrations in mice blood were measured to indicate liver functions after hMSC administration. *P < 0.05, ***P < 0.005 compared with the hMSC group; †P < 0.05; ##P < 0.01 compared with the Con A group. Data were expressed as means ± S.D.; statistical difference was determined by two-way ANOVA followed by Sidak’s multiple-comparison tests (n = 3), and the experiments were repeated two times.

### TABLE 3
Pharmacokinetic parameters of hMSC in liver and lung between Con A injury model and vehicle group

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Liver</th>
<th>Con A + hMSC</th>
<th>Lung</th>
<th>Con A + hMSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–4 (h × FU × 10^6)</td>
<td>174.6 ± 15.83</td>
<td>90.23 ± 12.02**</td>
<td>1841.00 ± 153.30</td>
<td>1276.00 ± 279.40*</td>
</tr>
<tr>
<td>AUC4–24 (h × FU × 10^6)</td>
<td>462.2 ± 42</td>
<td>554.80 ± 104.30</td>
<td>7825.00 ± 575.50</td>
<td>7915.00 ± 1651.00</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C_{max} (FU × 10^6)</td>
<td>59.53 ± 8.26</td>
<td>33.33 ± 8.32*</td>
<td>643.30 ± 74.50</td>
<td>450.00 ± 141.70</td>
</tr>
</tbody>
</table>

FU, fluorescent units; AUC0–4, AUC from time 0 to 4 hours; AUC4–24, AUC from time 4 to 24 hours; T_{max}, peak time. *P < 0.05; **P < 0.01 compared with the hMSC group.
enhanced therapeutical outcomes in some cases (Marino et al., 2008; Wu et al., 2008): the distribution of MSCs in the target site might be restricted by a disease condition like congestion. Pharmacokinetic research of MSCs under disease might help to find adverse factors on MSC distribution and potential ways to improve efficacy.

Fig. 5. Pretreated heparin increased the exposure and efficacy of hMSCs in Con A–induced liver injury mice. The hMSCs fluorescence intensities in the mice lung (A) and liver (B) were analyzed by IVIS. ALT (C) and AST (D) concentrations in mice serum were measured to indicate liver functions after hMSC administration. (E) Classic images of control, Con A, Con A + hMSC, and Con A + hMSC + heparin groups were examined by H&E staining. Magnification ×20. Scale bar = 100 μm. Black arrow indicates inflammation infiltration; red rectangle indicates hemorrhage; blue circle indicates degeneration and necrosis. *P < 0.05; **P < 0.01; ***P < 0.001 compared with the control group; *P < 0.05; **P < 0.01; ***P < 0.001 compared with the Con A group; #P < 0.05; ##P < 0.01; ###P < 0.001 compared with the hMSC + Con A group. Data were expressed as means ± S.D.; statistical difference was determined by two-way ANOVA followed by Sidak’s multiple-comparison tests (n = 3), and the experiments were repeated three times.
However, there are also some limitations to this study. Firstly, in our experiment, we only performed the multiple dose of hMSC in male mice. In previous studies, the hMSC distribution showed no significant changes between male and female mice (Creane et al., 2017), but related multidose pharmacokinetics studies in female are also needed to analyze the impact of gender on the elimination pattern. Secondly, because the study capacity of IVIS is limited and we have to measure each tissue’s fluorescence in time, we could not handle too many animals at each time point. However, every study in our manuscript was conducted at least twice, some in triplicate. So the result in this study is enough to support our conclusion.

It is interesting to note that mMSCs underwent a similar eliminating process as hMSCs in the mice. One possible reason is that our mMSCs had been cultured and expanded in vitro. It was reported the usage of certain growth supplements and/or cytokines would increase MSC immunologic profiles (immunogenic major histocompatibility complex molecules, class I and II human leukocyte antigens), which may accelerate the elimination of mMSCs in mice (Huang et al., 2010; Salvadori et al., 2019; Wang et al., 2019) and make the PK profiles of hMSCs and mMSCs close to each other. Since the in vitro culture is inevitable during MSC manufacture, it is reasonable to speculate that the difference of PK profile raised by allogeneic and xenogeneic MSC transplantation may not be as significant as expected before. And it is feasible to use hMSCs’ in vitro PK profile to predict their clinical cell fate. However, other possible explanations could not be completely excluded and deserve further exploration. Another issue that should be addressed is that multiple dose of MSCs have been adopted in clinical trials (Zhang et al., 2012; Suk et al., 2016; Kabat et al., 2020), which was not investigated in this paper. Therefore, investigating the elimination pattern of MSC after multiple doses will benefit the field in future as well.

Conclusion

In our present study, optimized qPCR and IVIS methods were applied to track hMSC elimination in mice in more accurately. It was found that the intravenously injected cells mainly gathered in the lungs and that more than 95% of cells were quickly cleared within 24 hours, which indicated that MSCs need to be modified to increase their survival in vivo. The mechanisms of exogenous cell clearance were similar, so it is feasible to refer to in vivo PK results in mice to design clinical studies.

Finally, the congestion in the liver induced by Con A greatly prevented MSCs from entering the liver, leading to the discrepancy of pharmacokinetics and pharmacodynamics, which showed the that damage induced by the disease may prevent cells from reaching the target site rather than exerting its homing effects. The decreased cell distribution in the liver may be reversed after the alleviation of congestion in the liver and remarkably improve therapeutic effects. We believed that profound knowledge of the pharmacokinetics of MSCs in disease is fundamental for the optimization of future transplantation and self-application.

Ethics Statement. This study was conducted and followed the recommendations of the Institutional Animal Care and Use Committee, Shanghai Institute of Materia Medica (SIMM). The protocol was approved by the Institutional Animal Care and Use Committee, SIMM. The collection and use of the umbilical cord were approved by the Institutional Ethical Review Committee of Shanghai Children’s Medical Center, Shanghai Jiao Tong University School of Medicine.

Authorship Contributions

Participated in research design: Han, Chenhui Ma, Peng, Z. Wu, Xu, Huang, Li, Pan. Conducted experiments: Han, Chenhui Ma, J. Wu, Zhang, Chen Ma. Performed data analysis: Han, Chenhui Ma. Contributed new reagents or analytic tools: Xu, Q. Jiang, Huang, Li. Wrote or contributed to the writing of the manuscript: Han, Chenhui Ma, Pan.

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