Survival and distribution of injected haematopoietic stem cells in acute kidney injury

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Keywords: acute kidney injury, haematopoietic stem cells, ischaemia/reperfusion, kidney disease, nephron repair

ABSTRACT

Background. Endogenous bone marrow-derived cells are known to incorporate into renal epithelium at a low rate. Haematopoietic stem cells (HSCs) rather than mesenchymal stem cells (MSC) are responsible for this phenomenon. MSCs have the potential to ameliorate kidney function after acute kidney injury (AKI) without directly repopulating the tubules. However, little is known about the short-term effect of HSCs.

Methods. In this article, we analysed the survival rate and organ distribution of isolated rat HSCs injected into the renal artery after ischaemic renal injury, using quantitative real-time PCR, as well as their impact on renal function and histomorphology.

Results. Intra-arterially injected Lin−CD90+ HSCs were detected in the kidney at significant amounts only within the first 24 h after injection and were virtually absent by Day 2. Compared with control animals, no differences were seen after HSC administration with respect to renal function and histomorphology.

Conclusions. Injected HSCs do not appear to significantly contribute to tubular repair or ameliorate renal damage in ischaemic AKI although they may show considerable engraftment in various organs. These data further challenge the concept that injection of HSCs may be used as a therapeutic approach in treating AKI.

INTRODUCTION

Acute kidney injury (AKI) is found in 5% of all hospitalized patients and in up to 50% of patients with sepsis [1, 2]. With a mortality of up to 60% in patients on the intensive care unit, AKI thus provides one of the big challenges in modern acute care nephrology. Furthermore, it is becoming increasingly evident that not only renal failure necessitating replacement therapy is associated with poor outcome but that even a small rise in serum creatinine confers a marked mid- or long-term risk of death or for the development of end-stage renal disease [3]. To date, no effective therapies are at hand to prevent or...
treat AKI specifically. In the last 10 years, the mounting knowledge of the plasticity of adult stem cells raised the hope for a new and potent therapeutic approach in this respect. Early investigations using chimeric mice have demonstrated that bone marrow-derived stem cells (BMSCs) home to the injured kidney and become integrated into the tubular epithelium, the predominant site of injury in AKI [4]. Subsequently, several attempts have been undertaken to exploit this capacity of integration and trans-differentiation by injecting BMSC at the onset of AKI [5–9]. The majority of these studies, for several reasons, focused on the use of bone marrow-derived multi-potent stromal cells, also referred to as mesenchymal stem cells (MSCs). Although a beneficial impact of MSCs on kidney function in various AKI animal models has been clearly shown by various groups, including this group, this is apparently not caused by direct tubular incorporation but rather paracrine or endocrine effects [10–12]. Much less is known about the effect of haematopoietic stem cells (HSCs), the other major fraction of the BMSC, in AKI. Given the many advantages of MSCs on one side, like relatively simple harvesting from various tissues (e.g. adipose tissue) and immunomodulatory properties impeding immune response to name a few, and some major disadvantages of HSCs on the other side, like putative kidney-threatening effects (demonstrated at least in the setting of HSC transplantation for cancer), therapeutic application of HSCs certainly poses a big challenge to clinical use. However, since early in ontogenetic development haematopoiesis arises in the aorta-gonad-mesonephros region, which is also the origin of the later kidney [13], it is the HSCs that were originally considered especially useful for kidney repair. In addition, HSCs are known to promote vascular regeneration in various organs which might aid in the repair of the damaged peritubular microvasculature that is found in AKI [14]. Here, we investigated the incorporation, organ distribution and functional impact of syngeneic HSCs delivered directly to the kidney via injection into the renal artery in a rat model of ischaemic AKI.

SUBJECTS AND METHODS

HSC harvest and purification

Bone marrow cells were obtained by flushing the femurs and tibiae of male Lewis rats (Charles River, Sulzfeld, Germany) with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and heparin. Mononuclear cells were isolated after centrifugation on Ficoll-Hypaque and washed twice with phosphate-buffered saline (PBS). The remaining red blood cells were depleted using erythrolysin. HSCs were enriched using the magnetic-activated cell sorting system (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) and validated RNA quality through electrophoresis measurement on the Nanodrop 1000 (Peqlab, Erlangen, Germany) including an on-column DNAse digest. We determined RNA concentration through photometric analysis using a FACSCanto flow cytometer (Becton Dickinson) according to previously described protocols [15]. In brief, 5 × 10^5 cells were resuspended in 100-μL FACS buffer containing anti-CD45R-PE, anti-CD3-PE, anti-CD11b/c-PE and anti-CD90-PerCP (BD Biosciences, as above). Unspecific binding was determined by labelling cells with IgG-PE, IgG-PerCP or IgG control antibody (BD Biosciences). After washing, cells were fixed with 1% paraformaldehyde prior to analysis. FACS analysis results were confirmed by using a second monoclonal anti-CD90 antibody (anti-CD90.1, Miltenyi Biotec, Bergisch Gladbach, Germany).

To rule out the possibility of a contamination with MSCs the enriched Lin^−^CD90^+^ cells were tested by flow cytometry using a FACSCanto flow cytometer. Bone marrow-derived stem cells (BMSCs) were harvested by flushing the femurs and tibiae of male Lewis rats (Charles River, Sulzfeld, Germany) with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and heparin. Mononuclear cells were isolated after centrifugation on Ficoll-Hypaque and washed twice with phosphate-buffered saline (PBS). The remaining red blood cells were depleted using erythrolysin. HSCs were enriched using the magnetic-activated cell sorting system (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) and validated RNA quality through electrophoresis measurement on the Nanodrop 1000 (Peqlab, Erlangen, Germany) including an on-column DNAse digest. We determined RNA concentration through photometric analysis using a FACSCanto flow cytometer (Becton Dickinson) according to previously described protocols [15]. In brief, 5 × 10^5 cells were resuspended in 100-μL FACS buffer containing anti-CD45R-PE, anti-CD3-PE, anti-CD11b/c-PE and anti-CD90-PerCP (BD Biosciences, as above). Unspecific binding was determined by labelling cells with IgG-PE, IgG-PerCP or IgG control antibody (BD Biosciences). After washing, cells were fixed with 1% paraformaldehyde prior to analysis. FACS analysis results were confirmed by using a second monoclonal anti-CD90 antibody (anti-CD90.1, Miltenyi Biotec, Bergisch Gladbach, Germany).

Rat haematopoiesis PCR array

Whole bone marrow cells (control) and Lin^−^CD90^+^ cells were obtained from five rats and analysed individually. RNA was prepared using the Direct-zol RNA Miniprep Kit (Zymo Research, Irvine, CA) including an on-column DNase digest. We determined RNA concentration through photometric measurement on the Nanodrop 1000 (Peqlab, Erlangen, Germany) and validated RNA quality through electrophoresis using a Tapestation (Agilent, Santa Clara, CA). Equal amounts of RNA per sample (160 ng) were reverse transcribed using the RT2 First Strand Kit (Qiagen, Hilden, Germany). The resulting cDNA was used for qPCR analysis on the 384-well RT2 Profiler PCR Array Rat Hematopoietic Stem Cells (PARN-054ZE, Qiagen, SABiosciences) that profiles the expression of 84 genes related to the development of blood cell lineages from HSCs through progenitor stem cells. Quantitative PCR was
performed using the 7900 HT qPCR cycler (Applied Biosy-
systems, Foster City, CA), and the resulting data were exported
through SDS v2.4 (Applied Biosystems) selecting a manual
threshold of 0.2 and automatic baseline settings. All exper-
iments were conducted using biological triplicates. ACTB was
used as an endogenous control for normalization.

**Myeloid colony-forming unit assay**

Bone marrow cells were extracted from five rats and pro-
cessed individually. Unprocessed bone marrow cells (control)
or purified Lin^− CD90^+ HSCs were used with the MethoCult
GF R3774 assay (StemCell Technologies, Grenoble, France)
according to the manufacturer’s protocol. In brief, a 10 × stock
solution of the indicated cells was prepared in Iscove’s
MDM with 2% FBS. This cell suspension (0.3 mL) was added
to 3 mL of the Methocult mixture. Then, 1.1 mL of this mix
with a final cell count of 2.5 × 10^5 was seeded in each 35 mm
dish. Every step was run as technical duplicate. The dishes
were incubated in a cell culture incubator (37°C, 5% CO₂,
>95% humidity). Colonies of the MethoCult GF R3774 assay
were counted after 8 days of incubation using a stereo
microscope.

**Labelling of the cells for histological cell tracking**

Lin^− CD90^+ cells were stained with 10-μM PKH26GL red
fluorescent cell linker kit (Sigma-Aldrich, Taukirchen,
Germany) according to the manufacturer’s protocols. In brief,
cells were suspended at a density of 5 × 10^6 cells/mL in
Diluent C and mixed with PKH26 to obtain a final dye con-
centration of 10 μM. After incubating for 5 min at room tem-
perature, FCS was added to terminate the reaction. Cells were
washed three times and diluted in PBS to give a final cell
density of 10^6 cells/250 μL. Cell viability was determined by
trypan blue dye exclusion and found to be ∼90%. More than
95% of the PKH26-labelled cells exhibited fluorescence. For all
experiments, enriched LIN^− CD90^+ HSCs were used within 30
min after purification and staining procedures, i.e. ∼4 h after
sacrificing of donor animals.

**Experimental animals and protocols**

Protocols and procedures for animal experiments were ap-
proved by the regional government’s Animal Care and Use
Committee (Bezirksregierung Köln, No. 50.203.2-K 7, 22/04),
and conformed with the ‘Guide for the Care and Use of Lab-
oratory Animals’ (NIH publication No. 85–23, National
Academy Press, Washington DC, revised 1996). For all exper-
iments, female inbred Lewis rats weighing 150–200 g were
used (Charles River, Sulzfeld, Germany). Ischaemia/reperfusion
injury as a well-established AKI model was induced by the
removal of the right kidney and the clamping of the left renal
artery for 70 min under ketamine/xylazine anaesthesia. The
abdominal aorta was prepared and small visceral arteries
leaving the aorta in the proximity of the left renal artery were
occluded using an electrocautery device (ERBE Elektromedi-
zin GmbH, Tübingen, Germany). After restoration of renal
blood flow and a reperfusion period of 15 min, 250-μL PBS
containing 10^6 male HSCs or PBS alone was administered via
a 27-G needle inserted into the distal aorta after clamping the
aorta above and below the left renal artery. The injection site
was closed using fibrin glue, and the clamps were removed to
restore aortic and renal blood flow. For quantitative assess-
ment of HSCs, rats (n = 7/group) were sacrificed after 2 h, 24
h, 2 days and 7 days after I/R. Histomorphologic analysis was
conducted after 24 h and after 2 days (n = 7/group), samples
for tracking of PKH26-labelled cells were collected at 2 h, 24 h,
2 days and 7 days (n = 7/group). Blood samples were collected
directly prior to the surgical procedure (baseline), after 2 h, 24
h, 2 days, 3 days, 5 days and 7 days after I/R (n = 7).

**DNA preparation and quantitative real-time PCR**

Rats were sacrificed and the kidneys, lung, spleen, liver,
heart and brain were removed, minced, snap frozen and stored
at −80°C. Kidneys were processed as whole organs (455–550
mg) to account for a possible inhomogeneous cell distribution,
while all other organs were processed as pieces of 450–550 mg
each (spleen ∼200 mg). Lysis was carried out in ATL lysis
buffer (1 mL/100 mg tissue) and 100-μL Proteinase K
(Qiagen). DNA was isolated using the DNeasy tissue kit
(Qiagen) and 5 μL of the final volume (100 μL) was added to
the PCR reaction. Quantitative assessment of HSC survival
was achieved using a method we recently established [10].
Briefly, real-time PCR amplifying the SRY gene located on the
Y chromosome of the male donor HSCs was performed on the
iQ iCycler platform (Biorad, Munich, Germany) using the
Qiagen Hotstar Taq-polymerase. The primer sequences
(MWG, Ebersberg, Germany), probes (MGB probes, Applied
Biosystems) and corresponding annealing temperatures are
listed in Table 1. All samples were run as triplicates. External
standards were generated by injecting a dilution series of 10^6
male HSCs into 500-mg specimens of each organ of female
rats in vitro (spleen 200 mg). The total DNA content of the
samples slightly differed from the values derived from the
external standard samples due to varying sample weight or ef-
ciency of the DNA isolation process. To correct for this error,
we included amplification of the autosomal encoded gene
osteopontin (OPN) as a marker for total DNA content. For
this purpose, a dilution series of a stock consisting of a
mixture of 10^6 male MSC and 500-mg female organ tissue was
generated. Amplification of both OPN and SRY genes was per-
formed by real-time PCR. The CT ratios (OPN and SRY,
respectively) of each sample of this dilution series and the
stock itself were calculated and plotted against each other
showing linear dependency. In the experimental setting, the
CT ratio of the OPN amplification of each specimen and of
the external standard was entered in the function derived by
linear regression analysis to adjust the CT values of the SRY
amplification, thereby enabling us to correct for, albeit small,
differences in sensitivity.

The numbers given in Figures 2a and 4 were derived by
calculating the average of the assessed absolute male cell
numbers of all examined animals at each time point and pre-
sented as percentage of the amount of injected cells.

As shown recently, this protocol accurately and reliably
detects male cells at quantities as low as 10^5 cells in each whole
organ [10].
Histology

Histologic assessment of tubular necrosis was determined semiquantitatively using the method reported by Chatterjee et al. [16]. Briefly, a grid (25 squares) was used to determine the number of line intersects involving tubular profiles in random cortical fields (5-μm slices, haematoxylin and eosin staining). One hundred intersections were examined for each kidney, and a score from 0 to 3 was given for each tubular profile involving an intersection: 0 = normal histology; 1 = tubular cell swelling, brush border loss, nuclear condensation, with up to one-third of the tubular profile showing nuclear loss; 2 = same as for score 1, but greater than one-third and less than two-thirds of the tubular profile show nuclear loss; and 3 = greater than two-thirds of the tubular profile showing nuclear loss. The total score for each kidney was calculated by the addition of all 100 scores with a maximum score of 300.

Tracking of PKH26-positive cells

Frozen kidney samples were cut into 5-μm slices, air dried, fixed in 2% paraformaldehyde for 15 min and stored until analysis at 4°C. Specific fluorescence of PKH26 was analysed using an Axiovert 200-M microscope equipped with a charge-coupled device camera (Carl Zeiss). For better visualization of the glomerular structure, we used a polyclonal guinea pig anti-nephrin antibody (Fitzgerald, North Acton, MA) and a secondary goat Alexa 488-coupled anti-guinea pig antibody (Invitrogen, Carlsbad, CA). Nuclear staining was performed with DAPI.

Serum creatinine and urea measurements

Blood samples were obtained by puncturing the tail vein, centrifuged at 5000 × g for 10 min at 4°C, and the supernatant was stored at −80°C. Serum creatinine and urea levels were determined using an auto-analyser (Roche 917 Analyser, Roche Diagnostics) and expressed as milligrams per 100 mL.
**Statistical analysis**

All data are presented as mean ± SEM. The entire data collection except for the PCR array analysis was done with Excel (Microsoft, Redmond), statistical analysis was performed with SPSS 12.0.1 (Chicago) using student’s two-tailed t-test for unpaired values. The exported data of the rat haematopoiesis PCR array was analysed employing the RT2 Profiler PCR Array Data Analysis web tool (Qiagen, SABiosciences), which calculates statistical significance by a two-tailed student’s t-test. P < 0.05 was considered statistically significant. Gene lists for the Venn Diagram were transformed to mouse Entrez Gene IDs using DAVID [17, 18] and the NCBI HomoloGene batch function. Intersections of the lists were created using L2N [19]. Significance was calculated employing Fisher’s exact test comparing the overlap of both lists and chi-square test to examine expected and actual frequencies.

**RESULTS**

**Lin<sup>−</sup>CD90<sup>+</sup> HSC Enrichment**

Flow cytometry of bone marrow cells depleted of red blood cells demonstrated the Lin<sup>−</sup>CD90<sup>+</sup> cell population to be 2–3% of the whole cell population, which is consistent with earlier findings [15] (Supplementary material Figure 1). After purification by magnetic-activated sorting, 98% of the cells were Lin<sup>−</sup>CD90<sup>+</sup> and no Lin<sup>+</sup>CD90<sup>−</sup> cells were detected (Supplementary material Figure 2). Adherent cells were found at a density of <5 cells/cm² confirming that contaminating mesenchymal stem cells were not present in this cell population in a significant number (not shown).

**Characterization of Lin<sup>−</sup>CD90<sup>+</sup> HSCs**

Rat HSCs are not nearly as well characterized as mouse or human, and apart from CD90 [20], no well-described HSC surface markers are reported. However, there is a significant overlap of CD90<sup>+</sup> and lineage-commited (Lin<sup>+</sup>) cells, as seen in FACS analysis of whole bone marrow (data not shown) and, consistently, McCarthy et al. described a rat HSC population as CD90<sup>−</sup>CD45RC<sup>−</sup> [21]. In analogy to common mouse-HSC enrichment protocols, we, therefore, used Lin<sup>−</sup>CD90<sup>+</sup> cells in our experiments. To prove the stem cell properties of enriched Lin<sup>−</sup>CD90<sup>+</sup> cells, we investigated differential expression patterns of conserved HSC-related genes using quantitative PCR array technique as well as myeloid colony-forming unit (CFU-GM) assays.

Of 84 investigated transcripts related to the development of blood cell lineages, we identified 17 genes that were significantly up-regulated by at least 3-fold in our Lin<sup>−</sup>CD90<sup>+</sup> cell population (Figure 1a, complete list of all investigated
transcripts in Supplementary material Table 1). Fifteen of these genes are well described in literature as being related to HSCs and/or haematopoietic progenitor cells [22–29]. Among these are the established HSC surface markers CD34 and Kit as well as the key haematopoietic transcription factors Gata1, Gata2, Tal1, Lmo2 and Runx1. In addition, bioinformatic comparison of our data with two large-scale transcriptome analyses [23, 30], together identifying 3350 HSC-specific genes revealed a highly significant overlap (Supplementary material Figure 3, Supplementary material Table 2).

CFU-GM assays clearly showed an enhanced differential potential of Lin−CD90+ cells when compared with whole bone marrow cells, the numbers of GM colonies being 66.7 ± 6.5/2.5 × 10^3 cells and 9.5 ± 0.5/2.5 × 10^3 cells (P < 0.0009), respectively (Figure 1b).

**HSCs survival in the damaged kidney**

To assess the potential of HSCs in renal repair after the induction of ischaemic AKI, a well-established ischaemia/reperfusion model was induced by removal of the right kidney and clamping the left renal artery for 70 min in anaesthetized rats. After restoration of renal blood flow in the remaining kidney and a reperfusion period of 15 min, 250-μL PBS containing 10^6 male HSCs or PBS was administered into the renal artery. Quantitative assessment of cell numbers was achieved using a recently described real-time PCR-based method that yields highly accurate and reproducible results [10]. In the control animals receiving only PBS, no signal was obtained by real-time PCR (therefore not shown in figures). We found a significant entrapment of HSCs in the injured kidney 2 h after injection; 15.01 ± 4.24% of the injected 10^6 cells were detected at that early time point. Cell counts dropped rapidly to 0.29 ± 0.07% at Day 2 but increased by Day 7 to 0.67 ± 0.22% (Figure 2a).

**HSCs localize to glomeruli and disappear within 24 h**

Kidneys removed 2 h after application of PKH26-labelled HSCs showed localization of fluorescent cells in almost all

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**FIGURE 4:** Detection of intra-arterially injected male HSCs in the lung, liver, spleen, heart and brain of female rats at different time points (Day 0 denominates the first assessment time point 2 h post-injection). Numbers given as percentage of the injected amount of 10^6 HSCs.
glomeruli but not in renal vessels or the tubulointerstitial area. No fluorescent cells were detected at 24 h, Day 2 or Day 7 (Figure 2b).

**Impact of injected HSCs on kidney function after renal injury**

Serum creatinine and serum urea levels peaked at Day 1 after induction of AKI followed by a steady decline over the next few days. There were no statistically significant differences between the group that had received HSCs and the control group, neither with respect to peak values nor in the time kinetics (Figure 3a), indicating that HSC injection does not show a considerable benefit in this model. This finding was consistent with the results of the histologic total severity score, which did not show any difference of histomorphologic features characteristic for AKI at Days 1 and 2 (Figure 3b).

**Distribution of HSCs in organs other than the kidney**

We next studied the fate of the injected cells in the whole organism. Interestingly, the majority of HSCs could be detected in the microcirculation of the lung with 55.39 ± 6.88% of the total amount of cells residing there 2 h after injection. As in the kidney, most of the HSCs disappeared by Day 2 and reappeared at Day 7. In contrast, cell counts were low at 2 h post-injection in spleen (0.14 ± 0.04%), heart (0.51 ± 0.04%) and brain (1.06 ± 0.17%) but increased over the course of the following days to 2.62 ± 1.25%, 1.15 ± 0.45% and 5.23 ± 2.8% at Day 7, respectively. The liver harboured 14.28 ± 2.59% of injected cells 2 h after injection and 5.13 ± 1.47%, 10.27 ± 2.64% and 5.44 ± 1.79% at Days 1, 2 and 7, respectively (Figure 4).

**DISCUSSION**

In the lack of effective therapies, stem cell technology has recently emerged as a new and promising treatment option for AKI. With their plasticity and capability to trans-differentiate into cells of various tissue types, stem cells derived from the bone marrow have been extensively studied with respect to their potential to enhance recovery of the injured tubular epithelium. The impact of endogenous BMSCs was investigated by several studies using chimeric mice that had undergone bone marrow transplantation (BMT). Seven days after induction of AKI, donor-derived cells accounted for up to 10% of the repopulated tubular epithelium [5, 31]. For some time MSCs have been considered the major fraction of BMSCs responsible for this phenomenon and, indeed, therapeutic injection of MSCs after induction of I/R leads to a significant attenuation of AKI, both, in functional and histomorphologic terms [6, 9]. However, subsequently, it has been demonstrated that MSCs do not engraft directly into the epithelial, and it is now accepted that paracrine or endocrine actions convey the reported beneficial effects. In turn, this makes HSCs the prime BMSC fraction responsible for the cell replacement effect seen with whole BMT. However, the role of HSCs in the setting of renal damage is still debated. Early HSC mobilization experiments using G-CSF or M-CSF yielded conflicting results showing either beneficial or detrimental effects on kidney repair, depending on the AKI model used [11, 32, 33]. Similarly, the impact of injected exogenous HSCs in AKI has not yet been deciphered consistently. Initial studies using a model of cisplatin-induced AKI did not show significant beneficial effects [6]. In contrast, in a murine BMT model, Lin et al. [34] analysed the contribution of HSCs in tubular regeneration after ischaemia/reperfusion injury (I/R). Isolated HSCs of Rosa29 mice were injected into irradiated recipients that had been subjected to I/R. Using several detection methods, donor HSCs were identified in the S3 segment of the proximal tubule. However, this was 4 weeks after I/R and the short-term effect of HSC transplantation in terms of structural and, more importantly, functional restoration of the kidney was not examined in this investigation. Recently, Fang et al. [35] robustly repopulated bone marrow after lethal irradiation by transplanting plastic non-adherent enhanced green fluorescent-positive marrow cells as a source of haematopoietic lineage-committed bone marrow cells (HLMCs) and plastic-adherent MSCs in mice. After induction of AKI by administration of HgCl2, only HLMCs but not MSCs were found to be incorporated into the tubular epithelium. Together, these latter studies suggest that intrinsic HSCs may indeed play an important role in tubular turnover as well as in tubular regeneration after AKI. However, cell replacement by HSCs presumably works at a considerably low rate, and this seems to be even more so if exogenous HSCs are administered directly after AKI, i.e. without establishing a robust BMT model prior to the renal insult. There is also some evidence that HSC administration is only effective under a conditioning advantage (e.g. irradiation) [36]. The question as to whether renal damage can be ameliorated in the short run by acute injection of exogenous HSCs early after the onset of ischaemic AKI and, in the absence of such a survival advantage (resembling the situation encountered in the clinical setting), has not been addressed appropriately so far. More precisely, the fate of acutely injected HSCs under these circumstances in terms of renal engraftment, survival and organ distribution remains elusive.

In order to close this gap, we administered syngeneic bone marrow-derived Lin-CD90+ cells, after thoroughly determining their HSC properties, via the left renal artery directly after inducing ischaemic AKI in the rat without prior bone marrow ablation. HSC injection did not attenuate the extent of renal failure as mirrored by peak serum creatinine levels nor did it accelerate functional recovery. Moreover, histomorphologic features of AKI were also not influenced by HSC application. Accurate quantitative assessment of cell distribution revealed that the HSCs were entrapped in relevant numbers within the injured kidney only at very early time points. These cells were predominantly located in the first capillary bed they were to encounter after injection, i.e. the glomerular tuft, and were no longer detectable by microscopy at later time points. Although the sensitivity of histologic retrieval of labelled cells might be diminished over time by loss of the fluorescent dye PKH26 and, hence, incorporated HSCs might have escaped detection
by microscopy, this suggests a passive behaviour of the injected HSCs that are washed out of the kidney within hours. We were not able to detect HSCs in either the tubular epithelium or the peritubular vasculature, consistent with the notion that genuine cell replacement by HSCs, if at all, occurs at a low rate. Given this spatial and temporal cell distribution, it seems unlikely that injected HSCs can substantially contribute to renal repair in the early phase after injury when most of the functional recovery takes place in the rat I/R model. However, low but readily detectable cell numbers were found at Day 7 arguing for a sustained engraftment in tubular epithelium at a slow rate in the kidney, again consistent with earlier findings. Thus, with respect to an effective treatment, one would clearly have to augment the HSCs capacity for renal engraftment and thereby speed up the process of tubular repopulation. To this end, Li et al. recently showed that haematopoietic stem and progenitor cells can be converted into renal-like cells ex vivo by sequentially treating them with a combination of protein factors and that these cells ameliorate AKI in a mouse model of I/R [37]. Unfortunately, again the effect was not attributable to direct tubular cell replacement.

Nonetheless, AKI in humans hardly resembles the rodent I/R model with its rapid recovery but rather is a protracted disease and its resolution often is a matter of weeks or even months. Cell replacement even at low rates in this setting might still be advantageous for the clinical outcome. In this study we did not look at cell counts in the kidney beyond Day 7 but rather at a sustained engraftment at a slow rate in the kidney, again consistent with earlier findings. Thus, with respect to an effective treatment, one would clearly have to augment the HSCs capacity for renal engraftment and thereby speed up the process of tubular repopulation. To this end, Li et al. recently showed that haematopoietic stem and progenitor cells can be converted into renal-like cells ex vivo by sequentially treating them with a combination of protein factors and that these cells ameliorate AKI in a mouse model of I/R [37]. Unfortunately, again the effect was not attributable to direct tubular cell replacement.

Stem cell therapy, by its nature, always carries the risk of yet unknown side effects. We therefore not only examined the HSC counts in the kidney, but also in other organs. Not surprisingly, the vast majority of administered cells were found in the lung, i.e. the next capillary bed the cells entered after exiting the kidney. Likewise, we had anticipated a certain cell entrapment in the liver and the spleen considering their being part of the extramedullary haematopoietic system. In contrast, the readily detectable and, over the first 7 days, steadily growing cell counts in the heart and the brain were completely unexpected. The absolute HSC counts in the latter organs at Day 7 even outnumbered the detected amount of cells in the kidney. Thus, in this model, clearly there is no preferential renal tropism of the injected HSCs, which might further hamper their use as a therapeutic tool.

In conclusion, the administration of isolated HSCs early after renal I/R does not ameliorate AKI nor do the cells contribute to cell replacement in the short term. Whether there are beneficial effects as a therapeutic tool in the long run is unknown but must be doubted in the face of considerable HSC engraftment in various other organs.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

ACKNOWLEDGMENTS

The authors thank Ruth Herzog for excellent technical assistance.

FUNDING

This work was supported by the Koeln Fortune Program of the University of Cologne (to V.B.).

CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this paper have not been published previously in whole or part.

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Received for publication: 8.5.2012; Accepted in revised form: 27.9.2012