

Scope for Renal Regeneration using Bone Marrow Derived Stem Cells – How Far Away From Bench to Bedside ?

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The combination of cell-based therapeutics with advances in stem cell research may have a role to play in chronic renal injury. Potential stem cells sources for therapeutic approach for end-stage renal failure are bone-marrow-derived stem cells (BMSCs), adult renal stem cells, embryonic stem cells and fetal renal stem cells. Studies of BMSCs contribution to renal parenchymal maintenance or repair have reported conflicting evidence. Some results appear to show that bone marrow derived cells contribute significantly to daily turnover of renal tissue and to a high degree during recovery from tissue damage. Others indicate that this is a rare event, seen only to a very limited extent following injury. This review article deals with aspects of potential clinical use of bone marrow-derived stem cells for renal regeneration in children.

Key Words: Bone Marrow; Stem Cells; Renal Regeneration

Introduction

Stem cell biology that is now nearly 3 decades old has been the subject of debate only since last decade with attempts to bring the laboratory results to clinical application and thus benefit human mankind [1, 2]. Chronic Renal Injury (CRI) is an irreversible deterioration of renal function that gradually progresses to end stage renal disease (ESRD) characterized by decline of renal function to a degree when life cannot be sustained without dialysis or transplantation. Dealing with CRI is a challenge even today with increasing number of renal transplants. The problem surfaces more so when one has to deal with children, as they would require the renal support system for about 4-6 decades, a period that may require 2-4 renal transplants to sustain for a life span of 60 years. Insufficiency of donor organs for cadaveric transplantation for children is a major hurdle. The rising concept of cell-based therapeutics has provided a framework around which new approaches are currently being generated including advances in stem cell research. This review article deals with the plausibility of stem cells approaches

for renal regeneration in children with the clinical use of bone marrow derived stem cells.

Embryologic Basis for Use of Stem Cells

When the ureteric bud (UB) and metanephrogenic mesenchyme (MM) meet, inductive signals are exchanged. The MM induces UB to grow, branch and arborize. UB in turn induces the MM to gain a stem-cell phenotype and multiply and then induces groups of stem cells to differentiate into nephrons. As the UB continues to grow and branch, its tips contact fresh stem cells and induce these into the nephrogenic pathway. There is a gradient of developmental age in a foetal kidney. The outermost cortex of stem cells are not yet committed to differentiation, the region just inside it has cells undergoing earliest phases of nephrogenic differentiation, and within that is the region that contains maturing nephrons and supporting stromal cells.

Embryological Basis of a Multicystic or Dysplastic Kidney

When the ureteric bud does not meet the nephrogenic blastema, normal nephrogenesis does not occur and

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leads to agenesis. If the bud originates from the mesonephric duct either caudal or cranial to the normal position, dysplasia may occur. At six weeks gestational period, the ureter goes through a period during which it has no lumen. As the metanephros is induced to develop functioning glomeruli and tubules, the ureter opens to become a conduit. Occasionally, this canalization fails to occur resulting in complete obstruction of the developing metanephros. Functioning nephrons fill with fluid and become cystic because there is no outlet for the fetal urine. This collection of cysts of varying sizes in the renal fossa is called a Multicystic kidney. Establishment of continuity with the normal ureter and drainage of the urine in hydronephrotic variety of multicystic kidney may allow the viable undifferentiated stem cells to undergo nephrogenic differentiation [3].

Need to Halt Progressive Renal Damage in Children

Chronic renal injury is defined as mild, moderate and severe, depending upon the glomerular filtration rate (GFR) of 50-80%; 25-50% or 10-25% of normal. Though mild and moderate CRI may be asymptomatic, severe CRI often presents as failure to thrive, acidosis, anemia, and renal osteodystrophy. Regardless of the etiology, once there is a critical loss of nephron mass, the renal failure is progressive. ESRD with GFR between 5-10% of normal presents with severe hyperkalemia, acidosis, fluid overload, pulmonary edema, and altered sensorium. The most common causes of CRI are depicted in Table 1.

Table 1: Causes of Chronic Renal Injury in children

Causes	Incidence
Obstructive Malformations	25%
Reflux Nephropathy	25%
Dysplastic Kidneys	20%
Focal Segmental Glomerulosclerosis	10%
Glomerulonephriti	10%
Polycystic Kidneys	2%
Others	8%

As a significant number of nephrons are damaged in CRI, the remaining nephrons compensate for the loss of renal function by undergoing progressive hyperperfusion and hyper filtration that in turn gradually causes increasing glomerular injury

and sclerosis. Thus stem cell based approaches that halt the ongoing renal damage would prove to be a boon in medical research if they can delay the need for a renal transplant [2].

Stem Cell Approaches for Renal Repair

Numerous studies have shown that renal cell repair and regeneration following acute renal injury follows a programme of de-differentiation, migration and proliferation, and restoration of differentiated function. The main purpose of introduction of supplementary cells to aid in the repair and regeneration of a damaged kidney is that they are rooted in the natural healing process [3]. Thus the aim is to accelerate the natural healing process through cellular supplementation.

Sources of Stem Cells

Four possible sources of stem cells for potential of therapeutic approach for the end-stage renal failure are [4]:

- (i) Bone-marrow-derived stem cells (BMSCs)
- (ii) Adult renal stem cells
- (iii) Embryonic stem cells
- (iv) Fetal renal stem cells

The plasticity in adult stem cell differentiation is a focus of debate.

However, the search for adult renal stem cells appears to be narrowing. Kim *et al.* demonstrated that the corticomedullary junction of the kidney contains renal-repairing, highly colony-forming multipotent stem cell-like tubular cells, thus forming the niche of adult renal stem cells [5]. These adult renal stem cells recently identified in the Bowman's capsule of adult human kidneys might prospectively be the ideal cell type for treatment of both acute and chronic renal injury because they display the potential to differentiate into multiple types of renal cells. The possible source for generation of renal cells is depicted in Figure 1A and B.

Possible Cell Source for Stem Cell Therapy in Renal Repair

- Adult renal stem cells (corticomedullary junction) -> Multiple renal cells
- Kidney mesenchymal stem cells -> neovascularization

- Embryonic stem cell -> Adopt renal progenitor fate in culture -> Patient at risk of ESRD
- Adult stem cell -> Adopt renal progenitor fate in culture -> Patient at risk of ESRD
- Bone marrow-derived stem cells-> Multiple renal cells

Chen *et al.* demonstrated that kidney mesenchymal stem cells are capable of differentiation toward endothelial and smooth muscle cell lineages *in vitro* and *in vivo*, support new blood vessel formation in favourable conditions and promote functional recovery of an ischemic kidney [6].

The renal regeneration group (RRG) of the University of Queensland hypothesized that it is possible to induce embryonic and/or adult stem cells to adopt a renal progenitor fate [7]. The isolation of renal progenitor cells can be achieved via sorting for specific cell surface markers. The renal stem cells administered to patients with or at risk of end stage renal disease (ESRD) can be recruited to, and functionally integrated into the damaged kidney. Glomerular and tubulo-interstitial damage in the ESRD kidney can be repaired by the administration of embryonic stem cell-derived or patient-derived renal progenitor cells. The use of embryonic tissue in research continues to provide valuable insights, subject of intense societal scrutiny and debate before it reaches the stage of clinical application. Embryonic stem cells, with their ability to generate nearly all cell types in the adult body and a possible source of

cells genetically identical to the donor, hold great promise but face ethical and political hurdles for human use.

However, bone marrow-derived stem cells (BMSCs) also represent an attractive and promising alternative, for renal repair, though it has been challenged by some authors.

Adult stem cells, when exposed to the right environments, are capable of contributing to several embryonic lineages, both *in vitro* and *in vivo*. BMSCs have been shown to give rise to small numbers of most renal cell types, including tubular cells, mesangial cells, podocytes, vascular cells and interstitial cells and improve renal function in many animal models of renal disease [8].

Bone Marrow Stem Cells Promising for Renal Regeneration

Paulsom *et al.* examined the kidney biopsies from male patients who had received transplants from female donors, as well as those from female mice that had received a male bone marrow transplants [9]. In both cases, Y-chromosome-containing (host or donor-derived, respectively) cells were identified within both tubules and glomeruli. Specific tubular epithelial markers used showed 3.8 to 7.9% of cortical tubular epithelial cells in the female recipients, 13 weeks following male bone marrow transplantation, contained a Y-chromosome.

Lin *et al.* isolated haematopoietic stem cells (HSC) from male mice and administered them to

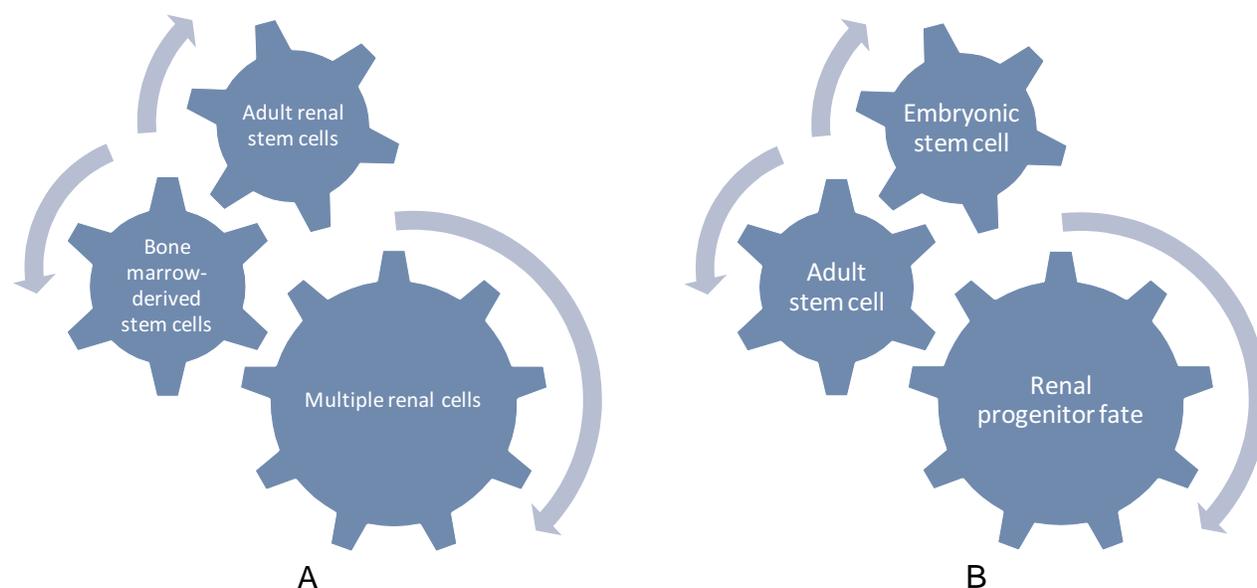


Fig. 1 A & B: Possible sources and mechanisms for generation of renal cells

lethally irradiated nontransgenic female mice following induction of ischemic injury by clamping of the renal artery for a 15-min period [10]. Striking contribution of donor-derived cells to renal proximal tubules, 4 weeks after transplantation were seen in the recovering kidney with no contribution in animals where transplantation was carried out without concomitant induction of ischemic injury. Regenerating kidneys were found to contain $8.3 \pm 3.2\%$ of Y-chromosome positive cells, spread throughout approximately 80% of proximal tubules that contained some cells co-expressing α -galactosidase and proximal tubule-specific markers.

Another study also reported that stem cell transplantation ameliorated the effects of ischemic injury, as blood urea nitrogen levels in the group that received transplantation were indistinguishable from unirradiated controls and lower than those of animals that did not receive stem cells over a 7-day period following injury [11]. These studies conclude that BMSCs make a significant contribution to the regeneration of renal tubules following acute injury and may even be involved in daily turnover of parenchymal cells.

Recently, Qian *et al.* isolated rat and human bone marrow mesenchymal stem cells (MSCs). After being co-cultured with injured kidney tissues in transwell dishes *in vitro*, the rat MSCs became rounded renal tubular epithelial-like cells, and highly expressed renal markers such as cytokeratin 18 (CK18) and aquaporin-1 (AQP1) [12].

Human MSCs were infused into rats with ARF, and techniques of microscopy, histology, PCR, RT-PCR and fluorescence *in situ* hybridization were used to characterize the MSCs after transplantation. It was found that there were more exogenous human MSCs localized to injured kidney tissues and the kidney recovery rate in the transplanted MSC group was higher than in the control group. Genes associated with human renal tubular epithelial cells such as AQP1 and parathyroid hormone receptor 1 were detected. Thus it was suggested that the injured kidney tissue induced rat and human MSCs to differentiate into renal tubular epithelial-like cells *in vitro* and *in vivo*, and exogenous human MSCs could home specifically to injured regions and efficiently cure rat ARF.

MR imaging has been used to visualise

intravascularly administered magnetically labelled mesenchymal stem cells *in vivo*, that promoted recovery of rat acute renal failure [13].

It has been reported that haematopoietic lineage marrow cells, but not cloned cultured MSCs, can play a role not only in normal wear-and-tear turnover of renal tubular cells, but also in repair after tubular injury [14].

Lin *et al.* showed the differentiation of BM-derived haematopoietic stem cells to renal tubular epithelial cells (RTECs) following renal injury, suggesting the possibility that the boosting of haematopoietic stem cells to peripheral circulation by G-CSF may accelerate tubular regeneration [10]. However, this prospect is still controversial in light of data from other authors that showed that haematopoietic stem cells did not differentiate to RTECs during repair of the post-ischemic kidneys [15, 16]. However, they did show differentiation of haematopoietic stem cells to vascular endothelial cells in post ischemic kidneys, and suggested the role of haematopoietic stem cells in vasculogenesis [15, 16].

Choi *et al.* evaluated the hypothesis that the treatment with mesenchymal stem cells MSCs could improve renal function and attenuate injury in chronic renal failure (CRF) [17]. They did modified 5/6 nephrectomy in Sprague-Dawley female rats (8 weeks old). The rats in the MSC group received an injection of MSCs (1×10^6 cells) via tail vein 1 day after nephrectomy. No significant differences in blood urea nitrogen and creatinine concentration were observed between MSC group and untreated CRF group. However, the weight gain in the MSC group was greater than those in the CRF group after 4 months. Proteinuria in the MSC group was less than that in the CRF group over time. Y chromosome was detected in the kidney of MSC group. Although no significant difference was observed between two groups, the histologic analysis suggested that MSCs have positive effect against glomerulosclerosis.

Renoprotective Effect of Bone Marrow-Derived Stem Cells (BMSCs)

The mechanisms proposed for the renoprotective effect of Bone marrow-derived stem cells (BMSCs) include:

1. *Transdifferentiation* : The process of transdifferentiation avoids complications from

immunogenicity of introduced cells by obtaining the more easily accessible stem cells of another tissue type from the patient undergoing treatment, expanding them *in vitro*, and reintroducing them as a therapeutic agent.

2. Paracrine factors
3. Immunomodulatory effects

Current opinion attributes this renoprotective effect mainly to paracrine factors supporting regeneration by local renal cells and to immunomodulatory effects, rather than to transdifferentiation of bone marrow cells into renal cells.

Controversial Reports – Debatable Issue

Contrary to the above, there are also several studies that have produced evidence that the contribution of bone marrow cells towards renal regeneration is a rare event. Szczyпка *et al.* (2005) transplanted whole bone marrow from male mice into lethally irradiated female recipients and examined the kidneys of otherwise healthy animals in addition to subjects with folic acid (FA)-induced renal injury [18]. Detection and characterization of donor-derived cells was by galactosidase activity, Y-chromosome detection by fluorescent *in situ* hybridization (FISH), and IHC for Lotus tetragonolobus lectin (proximal tubule-specific) and the pan-hematopoietic marker CD34. They concluded that though it is possible for bone marrow-derived cells to adopt proximal tubule cell fate, this is an extremely rare event, even in response to FA-induced injury.

Gupta *et al.* (2002) examined kidney biopsies from male patients who had received transplants from female donors and found only rare (affecting <1% of tubules) instances of Y-chromosome-positive cells within the tubular epithelia in patients recovering from acute tubular necrosis and none at all in cases without resolving acute tubular necrosis [19].

Wagers *et al.* (2002) repopulated lethally irradiated hosts with single, GFP-marked, haematopoietic stem cells and looked for GFP-positive, potentially transdifferentiated cells based on any or all of the following properties: morphology, tissue-specific marker expression, and lack of expression of the hematopoietic marker CD45 [20]. Of all the tissues examined (brain, liver, kidney, gut, skeletal muscle, cardiac muscle, and lung), cells

meeting criteria for being considered as transdifferentiated were observed only in brain and liver, probably due to cell fusion events.

Various studies have shown that bone marrow stem cells can rescue mice from acute renal tubular damage under a conditioning advantage (irradiation or cisplatin treatment) favouring donor cell engraftment and regeneration. However, it has been demonstrated that exogenous BMCs do not rescue non-irradiated mice from acute renal tubular damage caused by HgCl₂, despite establishment of chimerism and cell proliferation in bone marrow and spleen [21].

Reasons for Disparity in Results from Different Studies

1. Differing detection methods of donor-derived cells,
2. Differing methods and/or degrees of rigor in excluding donor-derived hematopoietic cells from analysis,
3. Different starting populations of donor cells
4. Different injury models.

Problems in Clinical Application of Bone Marrow Cells for Renal Regeneration

1. Stem cell therapy may be effective only in the initial few months of life when the kidney still has potential to develop. The increase of glomerular filtration is very fast in the first three months of life, and gets slower till the adult level is reached at the end of the second year. The level of glomerular filtration in a newborn is about 30% of its value in an adult. The adult values of the renal blood flow are reached by the end of the first year of life. The neonatal kidney has reduced urine concentration capacity which reaches the concentration capacity of the adult level at the age of 18 months [22].
2. The bone marrow in patients with chronic renal injury is usually hypoplastic. The tap is dry. One may have to resort to Erythropoietin injections to stimulate the bone marrow to form red blood cells. However, some studies in the past have demonstrated a significant abundance and an increased recombinant human erythropoietin sensitivity of Bone marrow and circulating erythroid progenitors in anaemic children with

end-stage renal disease ESRD with no evidence of the presence of uremic inhibitors to erythropoiesis [23].

3. The dose of the bone marrow cells may have to be repeated several times.

Future Perspectives

The potential impact of advances in stem cell technology on the entire prospective cell based therapeutic approaches for the treatment of renal failure is enormous. It is through various scientific methodologies that the promise of stem cells may be completely explored and brought to clinical use for

the benefit of developmental malformations in children that are an avenue for maximum potential use of stem cells [24,25].

Conclusions

Studies of BMSCs contribution to renal parenchymal maintenance or repair have reported conflicting evidence. Some results appear to show that bone marrow-derived cells contribute significantly to daily turnover of renal tissue and to a high degree during recovery from tissue damage. Others indicate that this is a rare event, seen only to a very limited extent following injury.

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