Expect the unexpected in the cell therapy of renal ischaemia

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Keywords: acute kidney injury; microenvironment; secretome; stem cells

Acute kidney injury (AKI) is a common complication in hospitalized patients associated with incremental increase in short- and long-term mortality [1]. There is a critical unmet medical need to explore new therapeutic approaches for AKI, and cell-based regenerative strategies represent a promising option. Similar to other competitive biomedical research disciplines, the field of experimental nephrology is experiencing a boom in regenerative cellular strategies [2]. One major focus is the induction of tissue regeneration by injection of the different populations of pluripotent stem cells (exogenous or replacement strategy). Accordingly, administration of bone marrow-derived haematopoietic, mesenchymal and unselected bone marrow-derived cells was extensively studied and beneficial outcomes were frequently reported [2]. The original simplistic concept envisaged successful homing of progenitor cells capable of transdifferentiation into healthy functional tubular epithelium. This initial excitement has been tampered with the findings facilitated by new cell-tracing techniques implicating rather inefficient engraftment and differentiation into functional tissue. According to current view, infused progenitors along with other resident tissue and local stem cells may respond to injury and contribute to the repair process by producing survival and tissue-protecting factors [3]. Promotion of the body’s own repair mechanisms (endogenous regeneration) together with utilization of it’s own resident or circulating stem cells is increasingly considered an alternative less invasive approach.

In their present study, Burger et al. employed CD133+ cells from human cord blood for the treatment of experimental AKI induced by 60 min warm ischaemia in mice. They relied on non-obese diabetic severe combined immunodeficient (NOD-SCID) mice deficient for B- and T-cells in order to avoid any immunological interference of the murine immune system with human progenitor cells. On the basis of the available literature reporting on the beneficial effects of CD133+ cells in cerebral [4] and myocardial ischaemia [5], the authors hypothesized similar effects in the renal setting. An experimental design in which CD133+ cells were systemically administered via the jugular vein immediately prior to reperfusion was chosen as feasible for clinical application. Cardiac surgery with cardiopulmonary bypass is the most common surgical procedure that results in haemodynamic instability and ischaemic AKI [6]. The possibility of anticipating AKI in such a clinical setting may allow timely cell therapy interventions. In spite of this attractive translational concept, the unexpected worst-case scenario occurred. Instead of preventing or ameliorating ischemic AKI, CD133+ cells enhanced the post-reperfusion inflammatory response, worsened tubular epithelial cell necrosis and apoptosis and led to consecutively severe renal function compromise. So why did cell therapy with putative CD133+ progenitors derived from human cord blood aggravate AKI despite a thoughtful experimental design?

In order to achieve therapeutic goals, treatment with CD133+ progenitor cells, as other cellular treatments, has to face several challenges encompassing the isolation procedure, homing to target tissue, cellular capability of secretion of protective mediators (paracrine effects), as well as interaction with the microenvironment (Figure 1). ‘Trouble-shooting’ usually begins with a standardized isolation process as a minimum quality requirement, the largest step to success and the central issue of the presented study. CD133 (prominin-1) was originally identified as a marker for primitive neural and haematopoietic (CD34+) stem cells. By definition, stem cells are capable of self-renewal and generating progenitor cells that continue to differentiate into lineage-committed mature cells. Progenitor cells, hence, are more lineage determined, and therefore carry a more limited differentiation potential and may proliferate for a finite number of divisions and lack the capacity to self-renew [7]. In this nomenclature, CD34 is a marker of premature, rather undifferentiated, barely lineage-committed stem and progenitor cells that is lost early during differentiation, whereas expression of, for example, CD34 characteristic for haematopoietic cells is maintained through later stages. One may consider this state of affairs confusing and controversial. At present, CD133 expression is demonstrated in undifferentiated epithelium, and different types of tumours and myogenic cells [8]. The CD133 molecule has several splice variants and undergoes post-translational modification dependent on the cellular maturation state among which glycosylation is of paramount biological importance. Obviously, CD133 antigen does not characterize one single,
Two additional broadly discussed and limiting issues in stem cell treatment are homing and interaction of applied cells with microenvironment via cell–cell and paracrine mechanisms. In the study by Burger et al., injected fluorescently labeled CD133+ cells were detected in blood 2 min after injection, which rapidly disappeared from the blood and were not detectable in the kidneys at any time point. Rapid disappearance and lack of homing to the injured tissue raises the possibility that CD133+ cells exacerbated AKI via negative paracrine actions through secretion of an ‘injurious secretome’. Interestingly, injected CD133+ cells were not harmful to sham-operated animals implicating a critical role of interaction of administered cells with the inflammatory microenvironment early after reperfusion. The biochemical milieu of ischemic and early reperfused tissue in a diabetic microenvironment is even more hostile and features acidosis, more pronounced inflammation and oxidative stress. The authors speculate that the oxidative burst during reperfusion may have negatively impacted on the applied cells by means of triggering production of injurious and not repair-inducing factors. Delayed administration of CD133+ as demonstrated in the study achieving protection from AKI [10] circumvented negative paracrine interactions with the microenvironment. Experienced clinicians are well aware that delayed therapies may also mean a missed window of therapeutic opportunity. Moreover, chronic disease conditions associated with increased oxidative stress as exemplified in patients with diabetes [17] may have limited functional repair capacity. For all these reasons, priming of stem and progenitor cells to enhance their therapeutic efficacy is a subject of intensive pre-clinical and clinical investigations [18]. The study by Burger et al. with all its shortcomings in mind forces us to pause, reflect and learn from negative results. From this perspective, the idea that a single shot of a single cell progenitor source may effectively achieve acute post-ischaemic renal repair is likely not going to be realized. As in many areas of complex disease interventions, combined, precisely timed, multi-step approaches that may incorporate various progenitor cells, their ‘secretomes’ and newly bioengineered tools may provide methods for effective therapy of AKI.

Conflict of interest statement. None declared.

(See related article by Burger et al. Human cord blood CD133+ cells exacerbate ischemic acute kidney injury in mice. Nephrol Dial Transplant 2012; 27: 3781–3789.)

References

5. Stamm C, Kleine HD, Choi YH et al. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for homogenous progenitor cell population. A glycosylated epitope of the CD133 molecule that is apparently characteristic of non-differentiated cells is recognized by the monoclonal antibody (MAb) AC133. Upon maturation, glycosylation of this epitope changes and the MAb AC133 cannot bind although CD133 is still expressed [9]. Studies reporting on beneficial effects of CD133+ cells in the treatment of cerebral and cardiac ischaemic insults relied on the AC133 antibody for isolation procedures [4, 5]. In contrast, Burger et al. used an antibody (MAb 293C3) directed against a different epitope (CD133/2) contained in the CD133 molecule and may have isolated more mature CD133+ cells. However, the receptor repertoire of the cells used in the present study may also differ from more mature CD34+ peripheral haematopoietic cells expressing CD133 of undetermined subtype which acted in a protective manner in a similar model of AKI induced by 25 min of warm kidney ischaemia in NOD-SCID mice when administered 24 h after reperfusion [10]. About one-third of the CD133+ cells (26%) used by Burger et al. expressed the kinase insert domain receptor/vascular endothelial growth factor receptor 2 (KDR/VEGFR2) compared with only few VEGFR2-positive cells in the study using CD34+ expressing CD133 cells which induced protection from AKI. VEGFR2 is found on endothelial progenitor cells [11] as well as on differentiated endothelial cells and macrophages. In addition, VEGFR2 is involved in the endothelium-pericyte crosstalk occurring during recovery from kidney injury. Blockade of VEGFR2 attenuated not only fibrosis and microvascular rarefaction but also inflammatory infiltration [12]. Conversely, activation of VEGFR2 may increase cellular permeability [13], which may act detrimental in the scenario of AKI via induction of inflammation. Subtle differences in isolation protocols may obviously have a profound impact on the biological properties of particular cell preparations and expected outcomes.

Timing of intervention and duration of injury could be important variables contributing to observed negative results. In contrast to the study by Burger et al., where cells were administered immediately prior to reperfusion, delayed administration (24 h upon induction of injury) of CD34+ peripheral haematopoietic cells expressing CD133 of undetermined subtype acted in a protective manner in a similar model of AKI [10]. Although elegant, the NOD-SCID mouse model with deficiency for B- and T-cells posed probably another challenge. SCID mice are less sensitive to ischaemic insult [14], yet diabetic background [15] and the lack of regulatory B- and T-cells could have disturbed this situation of ‘natural injury resistance’ [16].


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Received for publication: ..; Accepted in revised form: ..