Mesenchymal stem cells and chronic renal artery stenosis

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Abstract
Renal artery stenosis is the main cause of renovascular hypertension and results in ischemic nephropathy characterized by inflammation, oxidative stress, microvascular loss and fibrosis with consequent functional failure. Considering the limited number of strategies that effectively control renovascular hypertension and restore renal function, we propose that cell therapy may be a promising option based on the regenerative and immunosuppressive properties of stem cells. This review addresses the effects of mesenchymal stem cells (MSC) in an experimental animal model of renovascular hypertension known as 2 kidney-1 clip (2K-1C). Significant benefits of MSC treatment have been observed on blood pressure and renal structure of the stenotic kidney. The mechanisms involved are discussed.

Introduction
Atherosclerotic renovascular disease is the main cause of secondary hypertension and prolonged renal ischemia may result in irreversible damage leading to end-stage kidney disease. Renovascular hypertension is a renin-angiotensin-aldosterone-system (RAAS)-dependent hypertension. The reduction in the renal perfusion pressure stimulates renin secretion and thus, RAAS activation. Angiotensin II (Ang II) has a pivotal role in the genesis of the renovascular hypertension, however, after the onset phase, hypertension maintenance has a strong neurogenic component, clearly demonstrated in the two-kidney one-clip (2K-1C) rat model of renovascular hypertension (7, 26). Renal ischemia may increase the renal sympathetic nerve activity (rSNA) contributing to the maintenance of hypertension by modulating renin secretion and renal tubular sodium reabsorption (23). Moreover, the persistent renal hypoperfusion results in ischemic nephropathy. In addition to the oxidative stress caused
by ischemia, hypoxia per se is able to initiate immune responses and macrophage accumulation within the kidney (10), and the sustained decline in the renal blood flow results in microvascular loss that aggravates the hypoxic injury spreading the inflammation resulting in fibrosis and renal failure that, in turn, perpetuates the hypertension (5). Thus, renal artery stenosis is associated with three major clinical syndromes: ischemic nephropathy, hypertension and its consequences on the cardiovascular system.

Chronic renal ischemia: cell therapy

Considering the complex and multifactorial pathogenic mechanisms, the control of renovascular hypertension is a challenge. Many antihypertensive drugs, including the renin angiotensin system (RAS) blockers, are not always effective, and 60% of patients are or become refractory to the treatment (28). Moreover, recent clinical trials have failed to demonstrate greater benefit from angioplasty in terms of blood pressure in long-term follow up (18). Even when blood pressure returns to normal levels after revascularization, the impact of prolonged renal ischemia remains poorly understood. In addition, the contralateral kidney is subjected to hypertension and may develop hypertensive nephropathy, contributing to the progression of end-stage kidney disease.

Chronic kidney disease (CKD) is characterized by progressive fibrosis of the renal parenchyma, which is irreversible. Over the last decade stem cells have emerged as a new regenerative therapy for many diseases, including CKD (17). Mesenchymal stem cells (MSC) are interesting candidates for cellular therapies due to their regenerative and immunosuppressive capacities. MSC are undifferentiated adult cells that can be isolated from a variety of tissues but primarily bone marrow stroma. These multipotent stem cells can differentiate to cells of the mesenchymal lineage such as
osteocytes, adipocytes, and chondrocytes, and potentially other cell types. Directed
differentiation can be achieved by culturing MSC in defined conditions (14, 27). The
mononuclear cell fraction contains a complex assortment of different stem/progenitor
cells that are known to migrate to damaged tissues, including ischemic areas, to secrete
soluble factors such as growth factors and cytokines that can influence the function of
other cells including local cells, macrophages and T cells (1). The expected results are
cell proliferation, angiogenesis, reducing apoptosis, oxidative stress (11) and
inflammation by mediating immunomodulation and thus contributing to tissue repair
(6).

**Effects of Mesenchymal Stem Cell treatment**

The beneficial effects of MSC therapy have been evaluated in renovascular
hypertension through unilateral renal artery clipping in rats (2K-1C model). In this
model, the partial occlusion of the renal artery is expected to reduce renal plasma flow
by 50%. Oliveira-Sales and coworkers demonstrated that MSC, administered through
tail vein at the 3rd and 5th weeks after left renal artery clipping did not normalize systolic
blood pressure (SBP) but prevented further increase in SBP when compared with
untreated 2K-1C animals (24).

The benefit of this type of cell therapy was also obtained in other models of
hypertension, including a model of pulmonary hypertension (PH) (14) and a model of
congestive heart failure in spontaneously hypertensive rats (SHR) (2). In the PH model,
MSCs were intravenously implanted and significantly reduced the progression of PH
resulting in lower levels of mean right ventricular pressure and mean pulmonary arterial
pressure improving the cardiac function (19). Braga et al, 2008 (2), also reported
beneficial effects after systemic administration of MSCs in SHR with congestive heart
failure induced by myocardial infarction after coronary artery ligation. These Authors showed a reduction of myocardial remodeling associated with improvement in echocardiographic parameters after four weeks of treatment with MSC. Although in many protocols cells are administered directly into the damaged tissue, in the Oliveira-Sales study, cells were systemically injected in order to evaluate whether these cells would reach other potentially damaged organs and tissues including the central nervous system (CNS), a site potentially important in this model of renovascular hypertension (20). Interestingly, MSC were predominantly found in the kidney cortex and medulla, but also in the medulla oblongata in the CNS. The rise in the sympathetic nerve activity is crucial to maintain renovascular hypertension (23) and recent evidence indicates that an increase in the activity of neurons of the paraventricular nucleus and rostral ventrolateral medulla, triggered by Ang II (7) and oxidative stress (25), are the major mechanisms involved in the maintenance of sympathoexcitation of the cardiovascular system in renovascular hypertension. Thus, the presence of MSC in the CNS may indicate that, even a functional disturbance may be enough to attract MSC. In fact, we showed previously that MSC treatment reduced the RSNA in 2K-1C rats leading to reduction in SBP and indirectly reduced the impact of hypertension that the contralateral kidney was exposed (24).

Increased levels of Ang II are imperative to initiate the elevation in SBP after renal artery stenosis, however, the role of Ang II in the renovascular hypertension goes beyond the systemic hypertension. It has been recognized that the tissue activation of RAS results in inflammation, since Ang II is able to stimulate the proinflammatory transcription factor NF-κB (31), that in turn upregulates the transcription of many inflammatory chemokines (29). Persistent inflammation results in fibrosis. Ang II also induces fibrosis via transforming growth factor-β (TGF-β). (3, 21). The renal
inflammatory and fibrogenic mechanisms mediated by Ang II appears to be relevant in the renovascular hypertension since it was observed that 6 weeks after renal artery occlusion, the expression of the molecules involved in the Ang II synthesis including angiotensinogen, renin and angiotensin II converting enzyme, were upregulated within the stenotic kidney (19). Also, there was an elevation in the expression of the AT$_1$ receptor together with a reduction in the AT$_2$ receptor, the latter is known to display opposite effects to those triggered by AT$_1$ receptors and may act as an endogenous AT$_1$ antagonist (22). Thus, the downregulation of the AT$_2$ receptor indicates that the responses mediated by AT$_1$ receptor may be enhanced in the stenotic kidney. These results suggest that, independently of circulating RAS, intrarenal RAS activation can induce renal damage in the stenotic kidney by triggering inflammatory and fibrosis cascades. We observed that the upregulation of the RAS components in the clipped kidney was blunted by cell therapy. Also MSC induced a decrease in the expression levels of AT$_1$ receptor together with an increase in AT$_2$ receptor levels (24), contributing to reduce the intrarenal RAS overactivity in the stenotic kidney. In parallel with the suppression of intrarenal RAS, MSC treatment was also efficient to reduce circulating RAAS since a decrease in the plasma renin activity and Ang II levels were observed in the MSC treated 2K-1C rats (unpublished data). Several studies showed that the functional regeneration of ischemic tissue by improved neovascularization and subsequent tissue repair is critically dependent on the mobilization and integration of endothelial progenitor cells (EPCs) into the ischemic tissue (8). However, Ang II decreases EPCs numbers and function in vitro and in vivo resulting in profoundly diminished endothelial regeneration (9). Ang II accelerated the onset of senescence in an Ang II– infusion rat model and the inhibition of EPCs senescence by an AT$_1$R blocker may improve the functional activity of EPCs for potential cell therapy (16).
Indeed, in a cell culture study, Ang II increased the rate of senescence of EPCs as well, and that this appears to be a consequence of its ability to stimulate gp91phox expression and thus superoxide formation (13). Taken together with our results, it is possible to speculate that the suppression of RAS activity by the MSCs has also an additional benefit on the neovascularization induced by cell therapy.

As pointed out, inflammation is a pivotal process resulting in fibrosis and, we found that the pro-inflammatory cytokines IL-1β and TNFα were upregulated in the stenotic kidney of the 2K-1C animals. The inflammatory cascade triggered by IL-1β and TNFα was attenuated by MSC treatment with a decrease in the expression levels of both IL-1β and TNFα and by an increase in the anti-inflammatory cytokine IL-10 (24). This response was likely mediated by the immunomodulatory activity of the MSC, since MSC have been described to inhibit the proliferation and function of a broad range of immune cells, including T cells, B cells, natural killer cells and dendritic cells. Several soluble factors have been shown to play a major role in the immunosuppressive effects of MSC, including hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), TGF-β1, indoleamine 2,3-dioxygenase (IDO), nitric oxide and IL-10 (30). Moreover, it is important to consider that the beneficial effect of MSCs was probably also mediated by a suppression of the intrarenal RAS (21)

The ischemic nephropathy caused by chronic renal artery stenosis is characterized by marked functional and structural deterioration of the stenotic kidney (12). The structural alterations comprise a process known as microvascular rarefaction which is a hallmark change that characterizes the stenotic kidney (4). We observed that chronic renal artery stenosis induced by 2K-1C model, had a profound impact in the integrity of capillary vessels with a dramatic microvascular rarefaction and MSC treatment significantly restored the microvascular tree (24). The neovascularization
induced by MSC is dependent on its angiogenesis capacity, by mechanisms that are not completely understood. Evidence suggests that the improvement of the renal architecture and the neovascularization is not dependent on the transdifferentiation capacity of the MSC but it has been attributed to its immunomodulation capacity with suppression of the pro-inflammatory cytokines promoting cell survival and reducing apoptosis (32).

The beneficial effects of MSC in the stenotic kidney, by suppressing intrarenal RAS, reducing inflammation and inducing neovascularization were reflected by less fibrotic areas and by a functional improvement of the ischemic kidney, including increase in the renal plasma flow and glomerular filtration rate (unpublished data). The mechanisms involved in the renal dysfunction induced by 2K-1C model and the potential benefits of MSC treatment are summarized in the Figure 1.

The impact of renal artery stenosis on the ischemic kidney have been subject of extensive studies, however, much less attention has been given to the contralateral kidney, but it is important to consider that the contralateral kidney is exposed to severely elevated blood pressure and it is target of many factors released by the ischemic kidney. In fact, we observed that, in spite of more preserved architecture, the contralateral kidney exhibited features of hypertensive nephropathy, including the presence of atrophic glomeruli and increased collagen deposition, both manifestations are indicative of cortical and medullary fibrosis. These structural changes resulted in a functional impairment of the contralateral hypertensive kidney. Interestingly, MSC treatment reduced collagen deposition but had only discrete effects in the contralateral kidney function including glomerular filtration rate and renal plasma flow (unpublished data).
Stem cell therapy emerges as a potential strategy to treat chronic renal artery stenosis and its complication on cardiovascular and renal systems. The paracrine immunomodulation capacity of MSC appears to be a relevant mechanism to improve renal function, but additional studies are necessary to delineate the mechanistic effects of MSC to reconstruct the renal parenchyma and to interfere with CNS activity. Moreover, for a clinical application, it is pivotal to evaluate the long-term benefits of this kind of cell therapy when the artery occlusion is or is not resolved. So, clinical investigations of cell-based therapy in human subjects are needed.

Figure 1: Multiple mechanisms responsible for renal dysfunction in chronic renal artery stenosis induced by renal artery clipping model and the effects of MSC treatment.

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CHRONIC RENAL ARTERY STENOSIS (2K-1C)

Renin-Angiotensin System
Renal sympathetic nerve activity
Hypertension

Local mechanisms:
- Microvascular loss
- Renal tissue inflammation
- Oxidative stress
Renal fibrosis and dysfunction

MSCs THERAPY (IV)

Intrarenal RAS activity in stenotic kidney
Sympathoexcitation
Prevent the progressive increase of arterial blood pressure
Vascular rarefaction in the stenotic kidney
 IL-1 and TNF-alfa
 IL-10
Renal fibrosis and proteinuria