Amelioration of Acute Renal Failure by Stem Cell Therapy—Paracrine Secretion Versus Transdifferentiation into Resident Cells

Administered Mesenchymal Stem Cells Protect against Ischemic Acute Renal Failure through Differentiation-Independent Mechanisms. *Am J Physiol Renal Physiol* E-pub February 15, 2005

Although the kidney has been suspected, though not definitively proven, to contain organ-specific pluripotent stem cells, their role in regeneration after renal injury is uncertain (1–3). Recently, however, several investigators found evidence for a renoprotective role of non–organ-specific stem cells in acute renal failure. Arriero et al. (4) reported dramatic protection of the kidney against ischemia/reperfusion injury after injection of *in vitro* expanded skeletal muscle-derived stem cells, differentiated along the endothelial lineage (but not after injection of nondifferentiated stem cells). The renal function after ischemia was improved and engraftment of the transplanted cells into the renal microvasculature was documented. Morigi et al. (5) studied the cisplatin model of acute renal failure: Injection of mesenchymal stem cells (MSC) of bone marrow origin, but not injection of hematopoietic cells, protected syngeneic female mice against severe tubular injury and renal functional impairment. Furthermore, engraftment of MSC into the vigorously proliferating tubular epithelial cell layer was documented by demonstration of Y-chromosome–containing cells. In the same model, Iwasaki et al. (6) showed that pretreatment with G-CSF and M-CSF with the rationale to mobilize bone marrow–derived stem cells prevented renal tubular injury and accelerated recovery of renal function. Furthermore, cells expressing markers of bone marrow–derived cells were documented in the tubular epithelial cell layer. Finally, in acute renal failure after ischemia/reperfusion injury, Lin et al. (7) showed that hematopoietic stem cells contributed to the regeneration of renal tubular epithelial cells. In the kidneys of nontransgenic female recipients that had been subjected to unilateral ischemia, the authors showed that still after 4 wk β-galactosidase–expressing Y chromosome–positive cells from transgenic male donors were detected. The authors suggested that their findings are accounted for by "transdifferentiation," *i.e.*, phenotypic conversion of pluripotent somatic stem cells of one tissue type to another tissue type as had previously been postulated by other authors (8,9). The authors could not exclude, however, cell hybridization, *i.e.*, the possibility that bone marrow–derived cells adopted the phenotype of other cell lineages by fusion (10,11).

The recent paper of Tögel et al. now introduces a possibility which is alternative or complementary to the above interpretations. Some background information may be helpful at this point. Stem cell therapy has gained much attention in cardiology after controlled trials suggested its efficacy (12,13), but the mechanism involved has not been clearly defined (14). Apart from differentiation of stem cells into cardiomyocytes (15), somewhat dubious because of the above caveat (10,11) and some new evidence (16) against this possibility, alternative possibilities have been suggested such as angiogenesis (*i.e.*, capillary formation) (17,18) and paracrine effects by stem cell secretions. The latter mechanism was suggested after bone marrow–derived mononuclear cells were shown to express vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and angiopoietins (19–21). An impressive example of paracrine effects has also been provided by the documentation of an immunomodulatory effect, in this case of allogeneic MSC in cocultures with immune cells: T(H)1 cells decreased IFN-γ and T(H)2 cells increased IL-4 secretion (22). There are even unpublished observations that graft-versus-host reactions are mitigated by such stem cells.

In this study by Tögel et al., MSC were isolated from rat femurs. They were well characterized by isolation as adherent cells and their specificity was validated by their ability to differentiate into osteocytes and adipocytes. The MSC were fluorescence-labeled by carboxy-fluorescein diacetate (CFDA). Two different rat strains were used. The renal arteries were clamped for 40 min and thereafter 10⁶ MSC (or equal numbers of fibroblasts) were injected into the carotid...
artery. Administration of the cells immediately after ischemia/reperfusion or after 24 h caused faster recovery of serum creatinine compared with injection of vehicle or syngeneic fibroblasts. Using different techniques including genetic markers (Y-chromosome), the fluorescence-labeled MSC could be transiently detected in control and postischemic kidneys, surprisingly mostly in the glomerular capillaries although some were attached to peritubular capillaries. After 24 and 72 h, however, MSC were no longer demonstrable in the kidneys using different techniques for detection. Renal injury scores by histology as well as apoptosis scores were lower and the mitogenic index was higher in the MSC-treated animals. Measurement of gene expression in the kidney provided support for the hypothesis of paracrine actions. By real time PCR after injection of MSC, but not of fibroblast control injection, the expression of proinflammatory cytokines (IL-1β, TNF-α, INFγ, iNOS) was lower and the expression of the anti-inflammatory cytokine IL-10 was higher, while no difference was found with respect to some molecules known to play a role in the recovery from acute renal failure (e.g., VEGF-A, -B, -C, -D; EGF; IGF-1; bone morphogenetic protein-7 [BMP7]).

The authors concluded that in this model the beneficial effect of MSC did not result from transdifferentiation of stem cells into renal parenchymal cells, but was primarily the result of paracrine effects causing downregulation of proinflammatory and upregulation of anti-inflammatory cytokines. The study was well controlled and a laudable effort was made to strictly characterize the injected stem cells. The conclusion would also be in line with recent findings with stem cell therapy in other organs (21,22).

To explain the differences with previous renal studies on this topic, one has to point to several important differences with respect to species, type of stem cells, time course of renal injury, etc. Nevertheless, this fascinating paper illustrates that matters are much more complex than we thought only a few years ago. Most likely stem cell therapy will not be a panacea and before drawing definite conclusions with respect to its therapeutic potential, safety and feasibility issues must be resolved. Currently, to quote G.B. Shaw, “We have the privilege to be confused on a much higher level.”

References
Posttransplant Skin Carcinoma—Fighting Immunosuppression by Local Immunostimulation


T. Stasko, M.D. Brown, J.A. Carucci, S. Euvrard, T.M. Johnson, R.D. Sengelmann, E. Stockfleth, W.D. Tope; International Transplant-Skin Cancer Collaborative; European Skin Care in Organ Transplant Patients Network

Malignancy after renal transplantation has become an ever more important clinical issue after longer graft survival rates have been achieved and increasingly older patients are accepted for transplantation (1). The magnitude of the issue had been underestimated in the past because in clinical practice other issues such as rejection and infection had dominated. When adult patients survived long enough, however, it was noted that 10 yr after transplantation the risk of malignancy was 13.8-fold higher than in the background population (2) and 10 times higher than in hemodialyzed patients (3). It is also of note that an increased frequency is not found for all types of cancer, but preferentially for squamous cell carcinoma of the skin, lip, cervix, vulva (4) and for posttransplant lymphopro-
liferative disorders (1). In most countries, squamous-cell carcinoma, but also basal-cell carcinoma, account for >90% of all skin cancers in transplanted patients (5–7), with the notable exception of Japan (8). The frequency of squamous skin cancers is particularly remarkable in young adults having received their graft during childhood (9). The highest frequency of squamous skin cancer has been reported from Australia where a mostly fair-skinned population is exposed to intense ultraviolet sunlight: The cumulative incidence, calculated by life-table analysis, increased progressively from 7% after 1 yr to 45% after 11 yr and to 70% after 20 yr (7,10,11). The deleterious effect of ultraviolet light is apparently related to DNA mutations leading to the formation of thymidine dimers and inactivation of the tumor suppressor gene p53 (1). Papillomaviruses apparently play an ancillary role in skin cancer formation (12).

Not only is the risk of onset of squamous cell carcinoma increased, but the risk of a second skin cancer is also impressive: 25% of patients will have a second lesion after 13 mo, and 50% will have a second lesion after 3.5 yr (13). This is presumably so because squamous cell carcinoma arises on the “soil” of an actinic keratosis as a precancerous epidermal lesion in sun-exposed areas of the skin (14). Other precursor lesions comprise multiple warts, keratoacanthomas (difficult to distinguish from squamous cell carcinoma even by histology) and occasionally Bowen’s disease, an intraepidermal carcinoma (4).

In renal graft recipients, not only is the incidence of squamous cell carcinoma of the skin higher and does one see more frequently local recurrence as well as de novo second tumors; the skin tumors also grow more rapidly than in non-immunosuppressed individuals and they metastasize more frequently (15,16). The incidence of skin cancer is dependent on the degree of immunosuppression (10,11). An important role of immunosuppression is also suggested by the greater frequency in graft recipients on triple (prednisolone, azathioprine, cyclosporine) as compared with double agent (prednisolone, azathioprine) immunosuppressive therapy (6). Furthermore, a 5-yr randomized controlled trial documented a lower incidence of skin cancer on low-dose than on standard-dose cyclosporine (17). In line with this notion, decreased skin cancer was noted after cessation of immunosuppressants (18). Finally, skin cancers are more frequent in the more heavily immunosuppressed heart transplant recipients (6,19) and less frequent in the less heavily immunosuppressed liver transplant recipients (20). More detailed data will come forward from the International Transplant Skin Cancer Collaborative (ITSCC) and the Skin Care in Organ Transplant Patients (SCOP) Collaborative Group (11). The novel immunosuppressant rapamycine inhibits tumor growth in vitro and in experimental models (21)—whether it will ultimately reduce the tumor burden in transplanted patients remains to be seen, although preliminary short-term observations look encouraging (22).

The management of skin carcinoma has become an important clinical issue and was the subject of a recent review and of a consensus conference of the ITSCC and the European Skin Care in Organ Transplant Patients Network (4,23). We refer the reader to these publications and touch here only on some points that are of specific interest to the nephrologist. The mainstay is histologically-controlled surgical excision with potential adjuvant radiotherapy and chemotherapy (bleomycin, fluorouracil, cisplatin) for metastasizing tumors. There is a role for isoretinoin (24), but the often recommended use of IFNα risks graft loss from rejection. In view of the high risks of recurrence and the appearance of new tumors on the soil of predisposing skin lesions, the main challenge is prevention. This necessitates patient education (sun protection), follow-up, and dermatological monitoring (1,25).

What else can one do?

One intervention, although risky, is tapering (18) or even stopping immunosuppressive medication (16). There is controlled evidence documenting benefit from topical and systemic administration of retinoids in graft recipients (26–31), although the latter possibly not without risk because of induction of IFN-γ production (32) and the resulting potential risk of acute rejection (26,32). A rebound is also often seen when the treatment is terminated. Schaier et al. found that retinoids are highly renoprotective in experimental renal disease (33), including allografts (34). There may therefore be benefits beyond the skin, although this has not been proven in humans.
A new aspect has been the recent introduction of topical treatment with immune response modifiers, e.g., imiquimod and resiquimod (4,35). They have been used for superficial basal-cell carcinoma (36) and actinic keratosis in nontransplanted patients (37), and more recently in graft recipients (32,38,39). The action of imiquimod, approved in 1997 by the US Food and Drug Administration for external genital and perianal warts, is based on a novel principle, *i.e.*, stimulation of both the innate and adaptive arms of the immune system resulting in (locally) enhanced immune function. This action is mediated through the pathogen recognition or Toll-like receptor–7. Interaction with this receptor stimulates both the release of cytokines such as IFNα, interleukins (IL-1, IL-6, and IL-12), as well as TNFα (40); it promotes intracellular and extracellular killing by nonphagocytic and phagocytic cells (41), and activates the transcription factors AP-1 and NFκB (42,43). In parallel the adaptive immune response is upregulated, resulting in stimulation of the TH1 and inhibition of the TH2 pathways. Langerhans-type antigen-presenting cells are activated, migrate to lymph nodes, and present antigen to T cells (44).

What is the clinical evidence for its efficacy? In a randomized, double-blind, vehicle-controlled study, the efficacy and safety of locally applied 5% imiquimod cream was examined in 36 elderly patients with actinic keratosis. The lesions were totally cleared in 84% of the patients and partially cleared in a further 8%, leaving no scarring or discoloration (45), and the success was maintained long-term (46). A heavy inflammatory reaction of the skin predicted success, suggesting that it is part of the substance’s mechanism of action.

This strategy has by now been successfully applied in renal transplant recipients with premalignant skin lesions, particularly actinic keratosis and viral warts. Some observations suggest that part of the imiquimod action is related to TH1 cellular-mediated elimination of human papillomavirus by this local immune response modifier (12).

If these preliminary results are born out by more complete long-term observations, this strategy may offer the possibility to outsmart the deleterious effects of systemic immunosuppression by stimulating the immune response locally in the skin.

References
11. Ulrich C, Schmook T, Sachse MM, Sterry W, Stockfleth E: Comparative epidemiology and


**Treating Microalbuminuria—Cosmetic Exercises with Urine Chemistry or Effective Reduction of Clinical Events?**

**Effects of Fosinopril and Pravastatin on Cardiovascular Events in Subjects with Microalbuminuria.** *Circulation* 110: 2809–2816, 2004


Recently there has been intense interest in the issue of microalbuminuria as reflected by a number of reviews and guidelines. Microalbuminuria was first described by diabetologists (1,2) as a predictor of cardiovascular and renal risk in diabetic patients (3–5). Today microalbuminuria has become a well-established risk factor in non-diabetic subjects as well. Several observational studies established that in non-diabetic subjects microalbuminuria and even high normal albuminuria increase the risk of cardiovascular events and cardiovascular death (6–9). In some studies the risk increased parallel to increasing albumin excretion rates.

In diabetic subjects it has been found that even albuminuria in the upper normal range caused
a striking increase of cardiovascular risk (by a factor of 9.8) and renal risk (by a factor of 12.4) (10). Similarly, in nondiabetic individuals with normoalbuminuria as well, the results of the Heart Outcomes Prevention Evaluation (HOPE) Study (11) and the Losartan Intervention For Endpoint Reduction (LIFE) Study (12) show a progressive increase of cardiovascular end points when albuminuria is higher than the approximate population median. High normal albumin excretion rates in nondiabetic subjects are equally associated with a higher risk to develop frank microalbuminuria (13), just as they are in diabetic patients (10).

In diabetic (14) as well as in nondiabetic patients with overt proteinuria it has been shown that treatment with blockers of the renin-angiotensin system diminishes the cardiovascular risk and that this goes in parallel with the reduction of proteinuria.

What had not been shown so far was whether interventions reduce cardiovascular endpoints in individuals who have nothing but microalbuminuria as a risk factor. Clarification of this point would be of considerable interest to justify screening for albuminuria in nondiabetic patients.

Asselbergs et al. selected an angiotensin-converting enzyme (ACE) inhibitor and a statin for intervention in such subjects, the latter particularly because, in the past, reduction of albuminuria during administration of statins had been seen in some (15,16) but not all studies. It is not easy to find a suitable cohort of patients for such a trial, but the PREVEND Study provided a unique opportunity for this endeavor. In the Dutch city of Groningen, 40,856 subjects (47.8% of the inhabitants) had sent in their morning urine. A subgroup of 1439 individuals with persistent urinary albumin concentration >10 mg/L in the morning urine, BP <160/100 mmHg, and total cholesterol <8 mmol/L was randomized to receive either the ACE inhibitor fosinopril (20 mg/d) or matching placebo on the one hand, or the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor pravastatin (40 mg/d) or matching placebo on the other hand. The mean duration of follow-up was 46 mo. This population was relatively healthy: Mean age 51 yr, mean systolic BP 130/76 mmHg, mean cholesterol 5.8 mmol/L, and only 3.4% had a history of a cardiovascular event. The median urinary albumin excretion was no more than 22.8 mg/24 h (range 15.8 to 41.3). The primary endpoint was the combined incidence of cardiovascular death or hospitalization for one (or several) of the following: Nonfatal myocardial infarction or myocardial ischemia, heart failure, peripheral vascular disease, cerebrovascular accident.

The results are truly remarkable for a middle-aged population with albumin excretion in the upper normal or low microalbuminuric range, with a low prevalence of diabetes, relatively normal BP and cholesterol, as well as with few individuals having a history of cardiovascular events—and despite a sobering compliance (about 65% in this asymptomatic population).

Fosinopril caused an extremely modest reduction of BP while pravastatin lowered LDL-cholesterol substantially from 4.0 to 3.8 mmol/L. As anticipated, fosinopril lowered urinary albumin from 23.5 to 17.6 mg/24 h while pravastatin, in contrast to previous reports (15,16), failed to do so.

What about cardiovascular endpoints? Subjects receiving fosinopril had a 40% lower incidence of the primary cardiovascular endpoint compared with the placebo arm, i.e., 3.9% versus 6.5%, but P < 0.098. The reduction of cerebrovascular events was striking (0.2 versus 2.3% (P < 0.05). Subjects with urinary albumin in the highest quintile (>50 mg/d) had a significantly higher risk for developing a cardiovascular event on placebo than on fosinopril. The risk in this subgroup was reduced by fosinopril from 13% to 5.2%, reducing the relative risk by 60%. In contrast, pravastatin did not lower the incidence of the primary endpoint (−13%; P = 0.649).

The data of this interventional trial are in line with observational data in the LIFE Study suggesting that reduction of albumin excretion is associated with a lower risk for the primary cardiovascular endpoint (17).

What can we learn from this trial, which assessed a relatively small cohort with very low cardiovascular risk and urinary albumin values considerably lower than in previous studies?

The HOPE Trial (18) and the Europa (EUROpean trial On reduction of cardiac events with Perindopril in stable coronary Artery disease) trial (19) had shown that ACE inhibitors reduce
cardiovascular endpoints in populations at high cardiovascular risk. Despite the biostatistical borderline significance, the results of the PREVEND IT study suggest that screening for urinary albumin concentrations in the high normal or microalbuminuric range will identify individuals at high cardiovascular risk who might benefit from treatment with an ACE inhibitor (but somewhat surprisingly not with a statin).

Why is microalbuminuria such a powerful predictor? Although microalbuminuria is an independent predictor of risk, it is also associated with an array of other cardiovascular risk indicators, the metabolic syndrome (20), insulin resistance (21) and a high risk of de novo diabetes (22) which is reduced, however, by renin-angiotensin system blockade (23), high body mass index (24), evidence of oxidative stress (25), smoking (26), hypertension (27), and even developmental abnormalities of the kidney (“nephron underdosing”) (28).

What is currently not well understood is the link between albuminuria and the cardiovascular risk, and this continues to be a fascinating challenge to nephrology. The time-honored Steno hypothesis ascribed the cardiovascular risk in microalbuminuric patients to microinflammation and endothelial dysfunction (29). There is indeed substantial evidence for this hypothesis in microalbuminuric patients (30). Recent findings of cross-talk between podocytes and endothelial cells in the glomerulus may provide a potential link between endothelial cell dysfunction and deranged glomerular permselectivity (31–33).

Open questions remain. Apart from the obvious need to see the results confirmed in a larger prospective study, the following issues immediately come to one’s mind. Which is the best method for detecting urinary albumin in the kidney (34)? Which are the most appropriate cut-offs? In this age where economic aspects are of ever more importance in medicine we also need information on whether screening for albuminuria is cost-effective. We should also know the frequency of side effects in the long run and compliance problems. Even in the short run compliance was remarkably low in the disciplined but asymptomatic Dutch.

Nevertheless, the study raises the hope that blockade of the renin-angiotensin system provides benefits beyond BP lowering (35), even in low-risk individuals presenting with nothing but urinary albumin values in the high normal or microalbuminuric range. The public health importance of this issue is obvious.

References


