Experimental Intravenous Cell Therapy of Acute and Chronic Renal Failure

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Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 1, pp. 52-57, January, 2007
Original article submitted November 11, 2006

The therapeutic effect of intravenous injection of human fetal bone marrow mesenchymal stem cells or summary culture of kidney cells were studied on models of chronic or acute renal failure in outbred albino rats. Both cell types promoted improvement and normalization of the renal function in rats with stable chronic renal insufficiency (2 weeks after kidney cell injection, 1 month after bone marrow mesenchymal stem cell injection). Renal function remained normal or subnormal during the delayed period (3-3.5 months after injection). In rats with latent stage of chronic renal insufficiency, exacerbation was induced by additional 40-min ischemia. All rats receiving intravenous injection of saline died. Improvement of the functional parameters started 2 weeks after injection of kidney cells or bone marrow mesenchymal stem cells, and normalization was observed after 1.1-5 months. During the delayed period (after 3-4 months), functional parameters retained at normal or subnormal levels. In experimental series III, all rats with acute renal failure intravenously injected with saline (control) died from uremia on days 2-4. After injection of kidney cells 50% rats survived and renal function in these animals returned to normal after 2 weeks. After injection of bone marrow mesenchymal stem cells 83% rats survived, functional parameters returned to normal after 3 weeks.

Key Words: *fetal stem and progenitor cells; cell therapy; chronic renal insufficiency; acute renal ischemia*

The potentialities of cell therapy for improving the functions of damaged organs, including the kidneys, are extensively studied in recent years. The therapeutic effect of stem and progenitor cells is due to their high regeneration potential, capacity to differentiate into various types of mature cells, and secretion of growth factors and cytokines by them.

We showed that injection of human fetal bone marrow mesenchymal stem cells (BM-MSC) or summary culture of human fetal kidney cells into the renal parenchyma of rats with chronic renal insufficiency (CRI) led to gradual normalization of functional parameters of this organ after 11-12 days [1]. Intraparenchymatous injection of BM-MSC into the kidney of rats with postischemic acute renal failure (ARF; 90 min thermal ischemia of an only kidney) improved rat survival to 80%, all functional parameters returned to normal after 3 weeks; after injection of summary kidney cell culture animal survival was 17%.

In this study we evaluated the efficiency of intravenous therapy with kidney cell culture and bone marrow MSC for CRI and ARF.

The possibility of stem cell release from systemic circulation and their fixation in the renal tissue was shown in studies demonstrating the presence

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0007-4888/07/1431-0160

of labeled endogenous and exogenous (injected) bone marrow cells in various structures of the damaged kidney (renal tubular epithelium, glomerular capillaries, mesangium) [6-9,11]. The effects of these cells are believed to be responsible for regeneration of damaged renal structure.

MATERIALS AND METHODS

Experiments were carried out on 37 outbred albino rats (200-280 g).

In experimental series I and II, chronic renal insufficiency was induced in 20 rats by unilateral nephrectomy and coagulation of $^1/_2$-$^2/_3$ of the parenchyma under ether narcosis. The severity of functional disorders was evaluated after 1-1.5 months. The rats were placed for 24 h into metabolic cages for urine collection and at the end of this period the blood was collected from the caudal vein. The concentrations of urea, creatinine, sodium, and potassium were measured in urine and blood samples and creatinine clearance and sodium reabsorption were calculated with consideration for 24-h diuresis. The animals with 50% and more reduced creatinine clearance <50% (which corresponded to compensated CRI stage according to N. A. Lopatkin classification [2]) formed group 1 (n=8), while rats with less pronounced decrease in this parameter corresponding to latent CRI stage comprised group 2 (n=12). In group 1 animals, laparotomy was carried out under ether narcosis and 1.5×10^6 cultured human fetal BM-MSC suspended in 1 ml saline (4 rats) or 1.5×10^6 cultured human fetal (10-13 weeks) kidney cells in the same volume of saline (4 rats) were injected into the vena cava inferior. In group 2 rats, exacerbation of CRI was induced by 40-min clamping of the renal vascular pedicle; 3-5 min after resumption of circulation 1.5×10^6 BM-MSC or kidney cells were injected into the vena cava inferior, similarly as in series I (4 rats per subgroup). Four control rats received 1 ml saline into the vena cava inferior instead of cell culture.

In series III, postischemic ARF was induced by unilateral nephrectomy and 90-min clamping of the vascular pedicle in the remaining kidney. Five minutes after resumption of circulation in the kidney 1.5×10^6 human fetal BM-MSC (6 rats), kidney cells (6 rats), or 1 ml saline (5 rats) were injected into the vena cava inferior.

The efficiency of cell therapy for CRI and ARF was evaluated by animal survival and by the dynamics of biochemical parameters characterizing renal function for 2-2.5 months.

The method for isolation of human fetal stem cells was described in detail [1]. Nonviable human fetuses of different gestation terms obtained during medical abortion served as the donor material. Aborted material was obtained from medical institutions licensed by the Ministry of Health of the Russian Federation and working within the framework of legislation of the Russian Federation (Order No. 302 of 28.12.1993 of the Ministry of Health and Supplement No. 3 of 05.04.1994). Summary kidney cell suspension was prepared by enzyme treatment of fetal kidney tissue. Viable cells were counted using trypan blue and propidium iodide. Samples with viability ≥90% were used. The cells were cultured in DMEM and F-12 (1:1) supplemented with 10% FCS and 0.02% gentamicin in 25-cm^2 culture flasks (Cornig) at 37°C in an incubator with humid atmosphere and 5% CO_2 until confluence. BM-MSC were isolated from human fetal tubular bone marrow. The cells were washed from the bone marrow with DMEM containing 2 mM EDTA as the anticoagulant, centrifuged for 30 min at 2000g in Ficoll-urografin density gradient (1.077 g/ml), and mononuclear cell fraction was collected at the interphase. The resultant cells were resuspended in complete nutrient medium (DMEM and F-12 (1:1) with 10% FCS and 0.02% gentamicin) and transferred into culture flasks (25 cm^2, Cornig). After BM-MSC adhesion to plastic, nonadherent cells were removed, while adherent cells were cultured until confluence.

For injection to animals, adherent cells were dissociated with 0.25% Trypsin-EDTA, centrifuged for 5 min in DMEM at 2000g, and the precipitate was resuspended in DMEM. The resultant suspension (cell viability ≥98%) was recentrifuged for 5 min at 2000g and diluted with saline to a concentration needed for injection to animals.

The results were statistically processed by nonparametric tests for small samples using Statistica 6.0 software.

RESULTS

The most pronounced changes in all parameters of renal function (before cell therapy) were observed in experimental series I (Table 1). They corresponded to compensated CRI stage according to N. A. Lopatkin classification. In series II, all parameters were also significantly worse than in intact animals and the status of these animals was evaluated as latent CRI stage.

Injection of cultured BM-MSC and kidney cells led to gradual improvement of all functional parameters. This recovery was more rapid in experiments with kidney cells (Fig. 1). In these experiments all functional parameters normalized as soon

TABLE 1. Initial Renal Function in Rats with CRI in Experimental Series I and II

Parameter	Intact rats	Series I	Series II
Blood creatinine, μmol/liter	46±2	85±3***	75±4**
Blood urea, mol/liter	4.4±0.1	6.7±0.4**	5.8±0.5*
Creatinine clearance, ml/min/kg	3.32±0.12	1.74±0.05***	2.33±0.07**
Sodium reabsorption, %	99.64±0.05	99.09±0.80*	99.24±0.10*

Note. *p<0.05, **p<0.01, ***p<0.001 compared to intact rats.

as after 2 weeks and remained normal for more than 2 months. Later, an increase in blood creatinine concentration and reduction of creatinine clearance and sodium reabsorption were observed, but these values remained at subnormal levels. Recovery of renal function was slower after BM-MSC injection: more than 1 month was needed for attaining normal or subnormal values. However, later all parameters, except sodium reabsorption, remained normal during the entire period of observation. Sodium reabsorption remained at a subnormal level.

In experimental series II (rats with less pronounced renal dysfunction corresponding to latent CRI stage), exacerbation of renal insufficiency was induced by additional 40-min ischemia simulating potential exacerbation of CRI after surgery on the kidney with clamping of renal vessels, *e.g.* nephrolithotomy. Stem cell suspension was injected several minutes after resumption of circulation in the kidney.

In control series all animals injected with saline died from uremia within 5 days, while in experimental series I and II survival rate was 100%.

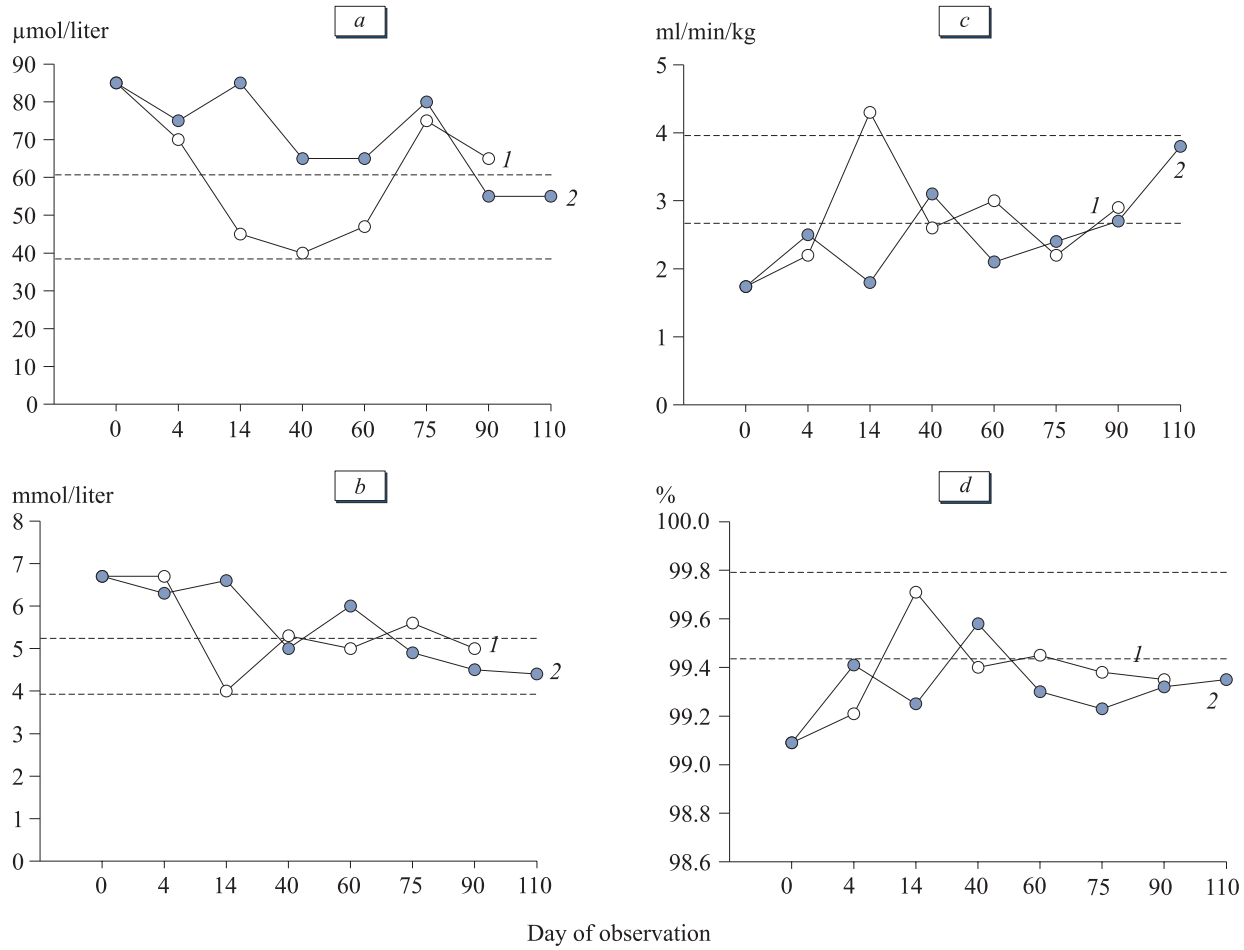

Fig. 1. Dynamics of blood creatinine (*a*) and urea (*b*) concentrations, creatinine clearance (*c*), and tubular sodium reabsorption (*d*) in rats with compensated CRI (series I) after injection of human fetal kidney cells (*1*) or BM-MSC (*2*). Here and in Figs. 2, 3: area between horizontal lines shows the range of values in intact rats.

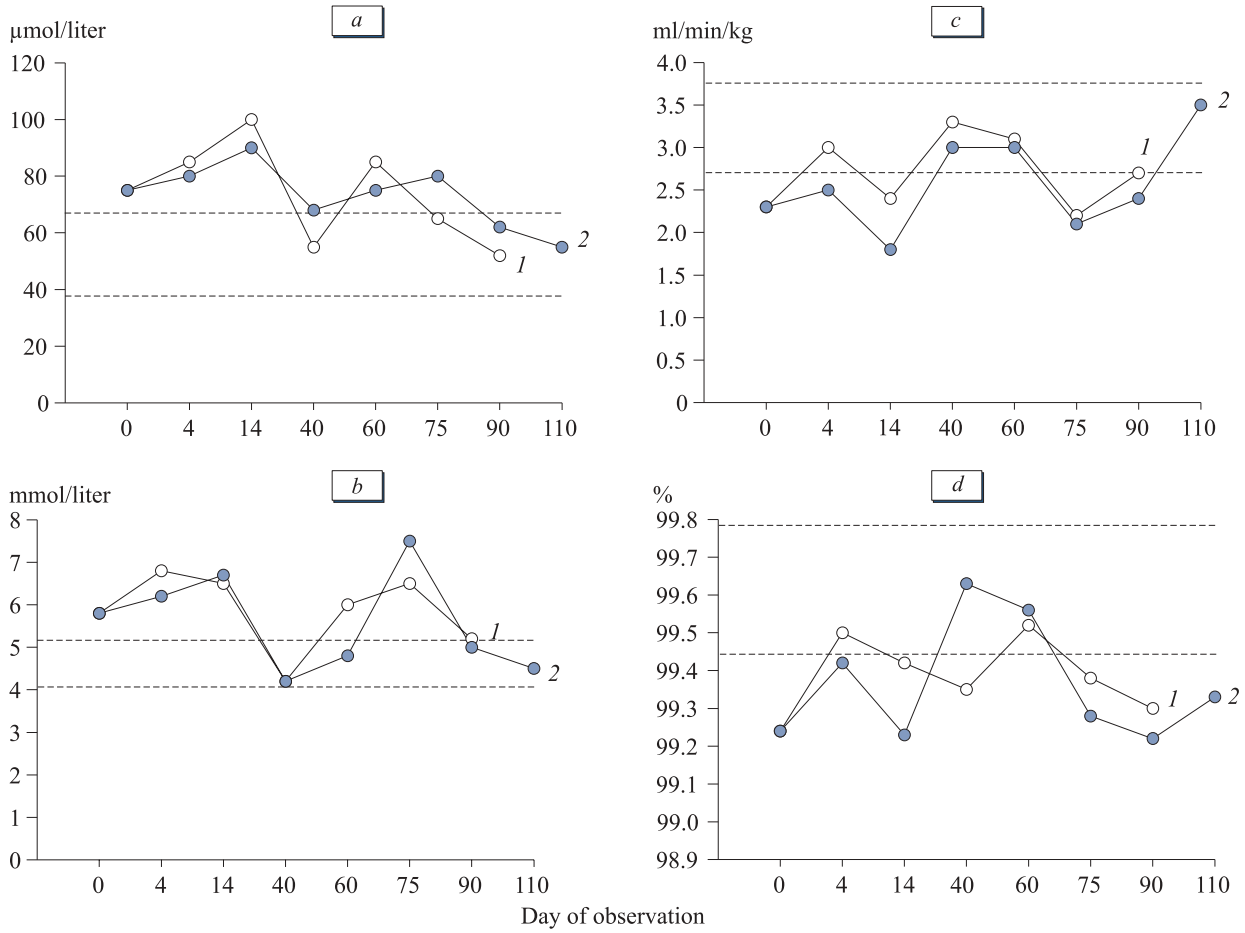

Fig. 2. Dynamics of blood creatinine (*a*) and urea (*b*) concentrations, creatinine clearance (*c*), and sodium reabsorption (*d*) in rats with CRI exacerbation (series II) after intravenous injection of human fetal kidney cells (*1*) or BM-MSC (*2*).

Evaluation of the dynamics of renal functional parameters after intravenous injection of stem cells showed a favorable effect of both variants of cell therapy. The main functional parameters gradually recovered after a 2-week latent period, during which elevation of blood creatinine and urea concentrations was observed (Fig. 2). The dynamics of the parameters (except tubular sodium reabsorption) was about the same in experiments with kidney cells and BM-MSC: normalization after 1-1.5 months and temporary elevation of blood creatinine and urea in parallel with reduction of creatinine clearance after 2-2.5 months. At later terms (after 3-4 months), renal function improved again and functional parameters returned to normal (Fig. 2, *a-c*). Sodium reabsorption returned to normal 4 days after injection of kidney cells, but later a trend to gradual deterioration of this characteristic was observed, and during the delayed period it remained at a lower boundary of normal or was subnormal (Fig. 2, *d*). In experiments with BM-MSC, sodium reabsorption in the renal tubules was reduced during 2 weeks, while later this parameter returned to normal and remained at this level for 2-2.5 months. A stable reduction of this parameter was observed at later terms.

Comparison of the results in series I and II showed that the use of kidney cells more effectively accelerated the therapeutic effect in experiments with stable CRI (series I) than in CRI exacerbation induced by additional ischemic exposure (series II). The dynamics of functional parameters after injection of BM-MSC was similar in series I and II. The therapeutic effect manifested only after 2 weeks.

A transitory deterioration of functional parameters was observed after 2-2.5 months; its cause is not yet clear.

It is noteworthy that the filtration function of the renal glomeruli was restored more fully than the reabsorption function of the renal tubules after cell therapy with both kidney cells and BM-MSC. Sodium reabsorption after normalization decreased again to subnormal values during the delayed period.

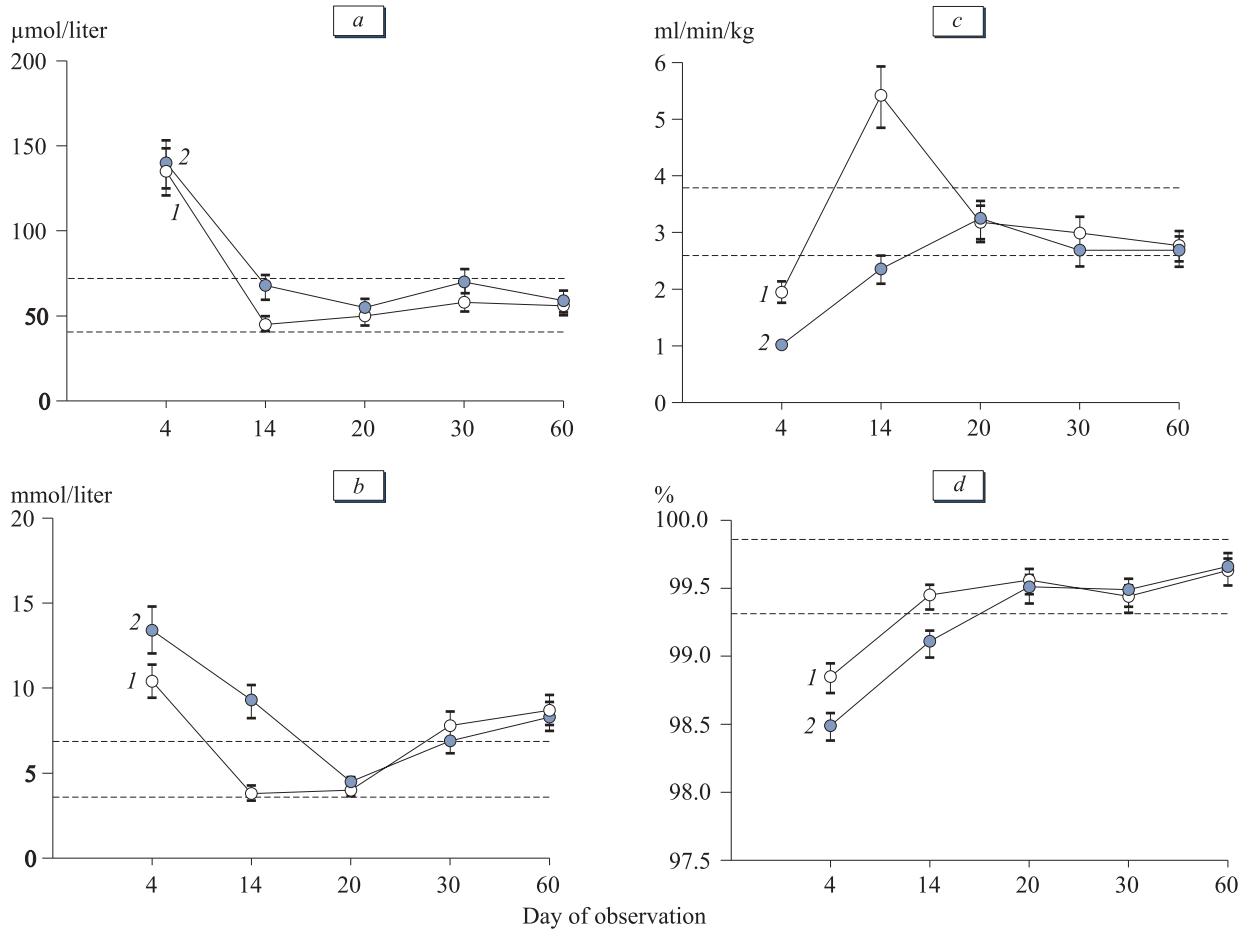

Fig. 3. Dynamics of blood creatinine (*a*) and urea (*b*) concentrations, creatinine clearance (*c*), and sodium reabsorption (*d*) in rats with postischemic ARF after intravenous injection of human fetal kidney cells (*1*) or BM-MSC (*2*).

Bearing in mind the delayed effect of cell therapy in ischemic exacerbation of CRI, in experimental series III we evaluated the effect on intravenous injection of stem cells in severe postischemic ARF.

In the control series, when 90-min thermal ischemia was followed by intravenous injection of 1 ml saline, all animals died from uremia within the first 3 days. Rat survival after injection of kidney cells and BM-MSC was 50 and 83%, respectively (deaths occurred on days 2-4 after ischemia).

Evaluation of the dynamics of renal functional parameters in survivors showed that normalization of renal function was observed 2 weeks after ischemia in animals intravenously injected with kidney cells and after 3 weeks in those injected with BM-MSC (Fig. 3). The decrease in blood creatinine concentration after injections of both cell types was similar in both groups, while the decrease in urea concentration was much slower in experiments with BM-MSC. Blood urea concentration somewhat increased during the delayed period in both groups and surpassed the corresponding value in intact animals. Clearance decreased less markedly on day 4 in animals receiving kidney cells, in comparison with the BM-MSC group, while after 2 weeks it sharply increased above the upper limit of normal and then stabilized within the normal range. The filtration function of the kidney gradually normalized after injection of BM-MSC. Sodium reabsorption in the renal tubules also decreased less markedly after injection of kidney cells compared to BM-MSC and more rapidly returned to normal.

Comparison of the efficiency of ARF therapy with two cell types demonstrates unambiguous effect of kidney cells: on the one hand, the improvement of animal survival was less demonstrative than after BM-MSC, on the other, these cells provided better functioning of the organ and more rapid normalization of functional parameters in survivors.

Our results prove the efficiency of intravenous therapy with stem and progenitor cells for ARF and CRI. The differences in the dynamics of improvement of damaged kidney function, for example, a slower manifestation of the therapeutic effect after

BM-MSC in comparison with kidney cells can be explained by different degree of differentiation of these cells. Organ-specific precursor cells presumably exhibit higher affinity for renal tissue and more rapidly penetrate from the circulatory system into the renal tissue, thus stimulating the regeneration of damaged renal structures or directly participating in these processes. Our previous studies, when human fetal kidney cells and BM-MSC were injected directly into the damaged kidneys of rats with CRI and ARF, indirectly confirm this hypothesis. In these experiments the dynamics of functional recovery in rats with CRI did not differ much after injection of two cell types [1].

There are different opinions on the mechanisms of therapeutic effects of stem cells on damaged kidney. Some authors admit their differentiation into the renal tubular epithelial cells, glomerular endothelium and mesangial cells, and direct replacement of damaged structures by these cells [6,7,9], while others attribute the stimulatory effect of stem cells to their capacity to release certain growth factors and cytokines [4,8,12]. Some data indicate that organogenesis is more intrinsic of embryonal and fetal stem cells [5,10], while stem cells isolated from an adult body possess a lower regeneratory potential and realize their therapeutic effect by predominantly paracrine route [3,4].

High plasticity of fetal stem cells used in our study and their capacity to differentiate into mature kidney cells admits their incorporation into renal structures and direct replacement of damaged tubular and/or glomerular cells. On the other hand, early improvement of some functional parameters in rats with CRI (a significant reduction of blood creatinine concentration and increase in creatinine clearance and sodium reabsorption on day 4) and better survival and less severe dysfunctions during the early periods in rats with ARF indicate an important role of the paracrine mechanism of their protective effect.

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