Functional Aftereffects of Intraparenchymatous Injection of Human Fetal Stem and Progenitor Cells to Rats with Chronic and Acute Renal Failure

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Chronic renal insufficiency was modeled in rats by unilateral nephrectomy and electrocoagulation of both poles of the remaining kidney; acute renal failure was induced by 90-min clamping of the vascular pedicle of the only kidney. Injection of unfractionated culture of human fetal kidney cells or bone marrow mesenchymal stem cells into damaged kidney restored its function in rats with chronic renal insufficiency (observation period up to 2 months). After 2.5 months a relapse of chronic renal insufficiency was observed in 1 of 3 rats receiving human fetal kidney cells and in 1 of 2 animals receiving bone marrow mesenchymal stem cell culture. Injection of bone marrow mesenchymal stem cell culture to rats with acute renal failure improved recovery of renal function and prevented the death from uremia, while injection of total culture of human fetal kidney cells had virtually no effect on the course of acute renal failure.

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

Acute and chronic renal dysfunctions are prevalent life-threatening conditions. Chronic renal insufficiency (CRI) necessitates dialysis therapy or kidney transplantation for the maintenance or complete replacement of renal functions. However, potentialities of modern medicine lag behind the need in these treatments in Russia and in other countries with well-developed economy [2]. Unfortunately, despite new drugs, whose efficiency was demonstrated experimentally, recent decades did not witness an appreciable improvement in the treatment of patients with acute renal failure (ARF) [5]. Alternative methods of support or replacement renal therapy are now investigated.

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Cell therapy, specifically, the use of various types of stem cells, is a promising approach to improving the function of the damaged kidney [4, 13]. We studied various aspects of cell therapy with stem and progenitor cells of neural origin. For example, the efficiency of transplantation of neural stem and progenitor cells for restoration of the nervous system function was demonstrated in rats with spinal injury [1]. In nephrology many authors showed that host bone marrow hemopoietic and mesenchymal cells participate in regeneration of the damaged kidney; these cells are detected in various kidney structures, *e. g.*, in the glomerular capillary endothelium, mesangium, renal tubular epithelium [7,8,11, 13]. Exogenous injection of bone marrow cells to animals with ARF of different origin stimulates recovery of disordered functional parameters [9,12,14]. Good prospects of this therapy for CRI were shown [10,15].

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However, many problems remain unclear. For example, the optimal sources of stem cells (embryonal, fetal, or adult tissue), efficiency of organ-specific precursor cells and routes of their transplantation, the efficiency of cell therapy in CRI, *etc.*, are still to be investigated.

We studied the time course of parameters characterizing renal function in rats with CRI and postischemic ARF after injection of cultured human fetal kidney cells and bone marrow mesenchymal stem cells (BM-MSC).

MATERIALS AND METHODS

Experiments were carried out on 34 outbred albino rats (290-440 g). CRI was induced in 18 animals and postischemic ARF in 16. Three experimental series were carried out: control (injection of saline: 5 rats with CRI and 5 with ARF), injection of summary human fetal kidney cells (7 rats with CRI and 6 with ARF), and of human fetal BM-MSC (6 rats with CRI and 5 with ARF).

Model of chronic renal insufficiency. For CRI modeling, functional renal parenchyma was sharply reduced. To this end right-side nephrectomy and electrocoagulation of both poles of the only (left) kidney were carried out under ether narcosis; about $^1/_5$ of functioning parenchyma was left. The rats were observed over 1.5-2 months. Functional efficiency of the remaining renal tissue was evaluated 2 and 6 weeks after the intervention. The rats were placed into metabolic cages for 24 h in order to collect the urine; at the end of this term the blood was collected from the caudal vein. The animals with stable elevated concentrations of creatinine and urea in the blood and reduced creatinine clearance and sodium reabsorption were used in further experiments.

Model of ischemic acute renal failure. The left kidney, renal vessels, and the ureter were mobilized under ether narcosis. The vascular pedicle was clamped with a microsurgical clamp for 90 min. Right-side nephrectomy was carried out.

Preparation of human fetal stem and progenitor cell culture. Summary culture of fetal somatic renal cells, containing cells of epithelial and mesenchymal origin, was isolated from human fetal kidneys of different terms of gestation, obtained as a result of medical abortions. (Abortion material was obtained from institutions licensed by the Ministry of Health of the Russian Federation and functioning within the framework of legislation of the Russian Federation, Order of the Ministry of Health No. 302 of 28.12.1993 and supplement No. 3 of 05.04.1994). Nonviable human fetuses obtained from women as a result of medical abortions served as the donor material. Viable cells were counted using Trypan Blue and propidium iodide; cell viability was ≥90%.

The cells were cultured in DMSI and F-12 (1:1) media with 10% fetal calf serum (FCS) and 0.02% gentamicin. The total culture of renal cells was cultured in culture flasks (25 cm^2, Corning) at 37°C in humid atmosphere with 5% CO_2 until the formation of a monolayer.

BM-MSC were derived from human fetal tubular bones. The cells were washed from the bone marrow with DMSI containing 2 mM EDTA (anticoagulant). Cell suspension was then layered onto Ficoll-verograffin (1.077 g/ml density) and centrifuged for 30 min at 2000*g*. Mononuclear cell fraction at the interphase was collected, resuspended in the medium, and repeatedly centrifuged for 5 min at 1500*g*. The resultant precipitate was resuspended in complete nutrient medium (DMSI and F-12, 1:1) supplemented with 10% FCS and 0.02% gentamicin) and inoculated into culture flasks (25 cm^2, Corning). After BM-MSC adhesion to plastic, nonadherent cells were washed out and adherent cells were cultured until the formation of a monolayer.

Primary (not passaged) renal cell and BM-MSC cultures were used in experiments.

Before injection to animals adherent cells were dissociated with 0.25% trypsin-EDTA solution. The resultant suspension was washed from dissociating agents by centrifugation in DMSI (5 min at 2000*g*). The precipitate was resuspended in DMSI and viable cells were counted (viability ≥98%). The suspension was recentrifuged (5 min at 2000*g*) and diluted with saline to a needed concentration.

Injection of fetal stem and progenitor cells into the kidney. Damaged left kidney was mobilized and released from adhesions under ether narcosis in rats with signs of CRI. Stem cell suspension (1×10^6 cells/0.1 ml saline) was punctured with an insulin syringe (needle diameter 0.33 mm; 29 G) into the upper and lower segments of the kidney along the saggittal line at the interphase with the zone of previous thermal injury.

Similar procedure was carried out in rats with postischemic ARF. The cells were injected directly after removal of the clamp from the renal vascular pedicle.

The animals were observed for 1.5-2.5 months; functional parameters of the kidneys were monitored.

Biochemical parameters of the blood and urine were measured by standard methods using an automated analyzer.

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

RESULTS

Effect of stem cell transplantation on the course of CRI. In rats with drastically reduced volume of functioning renal parenchyma signs of functional insufficiency of the kidney persisted during the entire period of observation (1.5 months; Table 1). Blood concentrations of creatinine, urea, and potassium increased significantly, while the clearance of endogenous creatinine and sodium reabsorption in the renal tubules decreased.

In controls injection of saline into the damaged kidney led to a significant deterioration of all functional parameters by day 4 after injection. A slight improvement was observed by day 11, but later all parameters indicated persisting CRI (Table 2).

Injection of cultured fetal kidney cells led to a decrease in the blood concentrations of creatinine and urea, increase of creatinine clearance, and normalization of sodium reabsorption after 4 days. By days 11-12 all parameters of renal function returned to normal and remained at normal or subnormal levels until days 39-40 (Table 2). Later (days 61-72) normal parameters of renal function was observed in 3 of 4 rats, while one rat developed a relapse of CRI.

On day 4 after injection of BM-MSC we observed a certain increase in the blood levels of creatinine and urea, despite increased creatinine clearance and sodium reabsorption. However, the increase in these values by this term was less pronounced than after injection of renal cell culture. Later parameters of renal function gradually improved, the majority of them returned to normal by days 11-12 and remained at normal or subnormal levels until days 39-40 (Table 2). One of 2 rats developed a relapse of CRI during the delayed period (day 72), while the other retained normal parameters of renal function. The time course of blood creatinine and creatinine clearance in rats with CRI injected with stem and progenitor cells is presented in Fig. 1.

In rats receiving fetal stem and progenitor cells diuresis stable increased, which correlated with the increase in glomerular filtration (evaluated by endogenous creatinine clearance).

Blood sodium concentration was in general stable, but significant hyponatremia developed on days 11-12 after injection of stem cells, though sodium reabsorption virtually returned to normal by this period. These changes seemed to be due to a sharp increase in filtered sodium fraction due to increased diuresis and hence, increased sodium excretion, despite normal percentage of its reabsorption. Filtered sodium fraction increased from 267±16 to 417±22 μmol/min ($p<0.01$) after injection of fetal kidney cells, *vs.* from 284±18 to 677±35 μmol/min after injection of BM-MSC ($p<0.001$). Excreted sodium fraction increased from 0.52±0.04 to 0.69± 0.05 μmol/min ($p<0.05$) in the former case and from 0.78±0.05 to 1.01± 0.06 μmol/min ($p<0.05$) in the latter case, that is, by 33 and 29%, respectively.

A rapid increase in creatinine clearance reflecting increased glomerular filtration after injection of stem and progenitor cells together with the diuretic effect can be caused by hemodynamic restructuring of the vascular system in the organ, primarily in the glomerular vessels, which seemed to be due to humoral factors released by transplanted cells. It is hardly possible to explain rapid shifts by structural rearrangement of the renal tissue of any kind.

Comparative analysis of the time course of the main parameters characterizing the excretory function of the kidney indicates that recovery of the renal function after transplantation of fetal kidney cell culture is more rapid than after transplantation of BM-MSC, but later the difference leveled.

Effect of stem cell transplantation on the course of postischemic ARF. In control experiments, when rats were injected with saline into the parenchyma after 90-min thermal ischemia of the only kidney, all animals died from uremia on days 2-3 after ARF

TABLE 1. Renal Function Parameters in Rats before and after Induction of CRI

Parameter	Intact rats	Rats with CRI	
		after 2 weeks	after 6 weeks
Blood creatinine, μmol/liter	46±2	106±8***	76±5**
Blood urea, mmol/liter	4.4±0.1	5.3±0.4*	5.9±0.4*
Blood sodium, mol/liter	141±1	141±2	142±1
Blood potassium, mol/liter	6.5±0.2	7.8±0.4*	8.2±0.5*
Creatinine clearance, ml/min/kg	3.32±0.12	1.77±0.05***	1.93±0.07***
Sodium reabsorption, %	99.64±0.05	99.35±0.12*	99.24±0.10*

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

TABLE 2. Effect of Human Fetal Cell Transplantation on Renal Function in Rats with CRI

Parameter	Before injection	Day after injection				
		4	11-12	20-21	39-40	61-72
Blood creatinine, μmol/liter						
control	72±4	164±7	149±5	112±5	86±3	—
fetal kidney cells	80±4	72±3	55±2	60±2	63±2	64±3
BM-MSC	74±3	107±4	68±2	50±1	56±2	80±7
Blood urea, mmol/liter						
control	8.0±0.2	9.4±0.3	5.7±0.2	8.1±0.3	7.2±0.1	—
fetal kidney cells	6.7±0.2	5.0±0.1	3.9±0.1	4.6±0.1	4.6±0.1	8.8±2.3
BM-MSC	5.2±0.1	5.7±0.1	5.0±0.1	3.6±0.1	4.2±0.1	6.0±0.9
Blood sodium, mmol/liter						
control	142±1	143±2	136±1	142±1	142±1	—
fetal kidney cells	140±1	141±2	123±8	142±2	141±1	143±1
BM-MSC	142±1	142±1	134±5	143±1	142±2	142±1
Blood potassium, mmol/liter						
control	8.6±0.2	8.2±0.2	9.6±0.3	8.5±0.2	9.2±0.3	—
fetal kidney cells	8.3±0.2	8.5±0.3	8.0±0.2	8.5±0.3	9.3±0.3	8.7±0.2
BM-MSC	8.0±0.4	8.3±0.3	7.9±0.2	8.4±0.3	8.2±0.2	9.1±0.1
Diuresis, ml						
control	22.1±4.2	28.2±3.6	43.5±7.4	45.3±4.2	25.4±3.2	—
fetal kidney cells	15.4±3.1	15.5±3.9	20.5±3.1	16.9±2.2	27.5±3.3	20.4±1.4
BM-MSC	18.6±3.7	18.5±5.1	25.1±5.9	28.3±2.8	27.3±4.1	10.5±2.1
Creatinine clearance, ml/min						
control	1.74±0.06	0.96±0.23	3.92±0.36	1.66±0.19	1.54±0.16	—
fetal kidney cells	1.88±0.08	2.45±0.37	3.39±0.55	2.63±0.11	2.76±0.17	3.09±0.51
BM-MSC	2.09±0.12	2.54±0.71	5.05±0.43	2.78±0.36	3.37±0.32	1.77±0.69
Sodium reabsorption, %						
control	99.41±0.03	99.54±0.02	99.50±0.02	99.23±0.02	99.07±0.02	—
fetal kidney cells	99.37±0.04	99.76±0.05	99.63±0.04	99.60±0.02	99.36±0.04	98.96±0.42
BM-MSC	99.11±0.21	99.31±0.22	99.78±0.01	99.51±0.02	99.57±0.02	99.40±0.08
Creatinine excretion, μmol/day						
control	232±27	274±34	277±31	188±1	192±13	—
fetal kidney cells	197±54	249±25	269±23	201±10	249±11	318±32
BM-MSC	204±31	242±23	286±39	269±35	270±24	188±51

Note. Here and in Table 3: "—" parameter was not measured.

induction. Of 6 animals receiving injection of fetal kidney culture 5 rats (83%) died on days 1-2. After transplantation of fetal BM-MSC none of 5 rats died from uremia; one rat died on day 9 from an unknown cause in the presence of improving characteristics of renal function.

The parameters of the functional state of ischemic kidney in the only surviving rat after intraparenchymatous transplantation of fetal kidney cell culture remained abnormal until day 39 of observation. By this term creatinine clearance and sodium reabsorption remained at a subnormal level. By day 80 renal function returned to normal.

After injection of BM-MSC significant improvement of the function of ischemic kidney was observed starting from early period of observation (day 4; Table 3). Later (after 20-21 days) the function of ischemic kidney returned to normal in 1 of 3 survivors, and by day 39 in the rest 2 rats. Normal parameters persisted until day 80 (maximum period of observation). The dynamics of blood creatinine and creatinine clearance in rats with post-

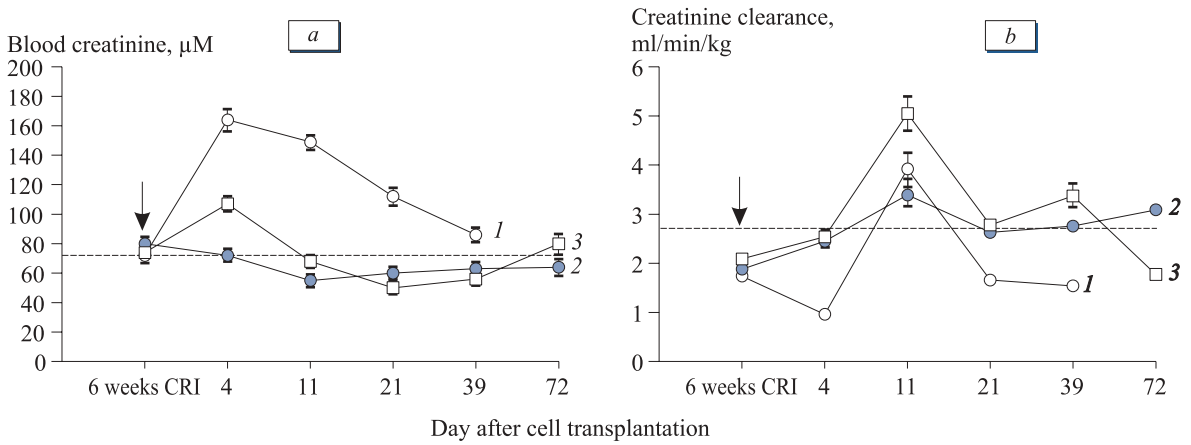

Fig. 1. Effect of transplantation of human fetal stem and progenitor cells into the renal parenchyma on the dynamics of blood creatinine concentration (*a*) and creatinine clearance (*b*) in rats with chronic renal insufficiency. Here and in Fig. 2: interrupted line: upper (*a*) or lower (*b*) threshold normal values. Arrow shows the moment of cell transplantation. *1*) control (saline); *2*) renal cells; *3*) bone marrow mesenchymal stem cells (BM-MSC).

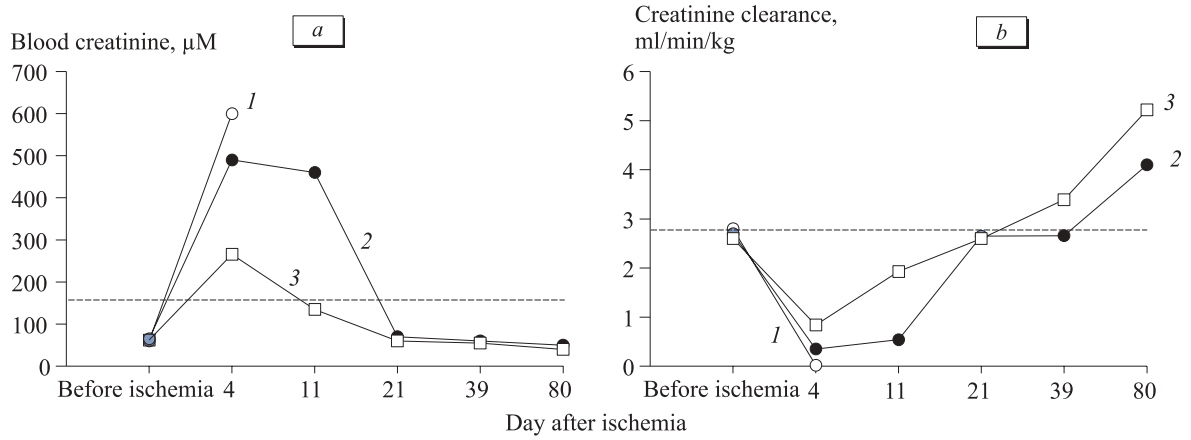

Fig. 2. Relationship between injections of human fetal stem and progenitor cells into the renal parenchyma and dynamics of blood creatinine concentration (*a*) and creatinine clearance (*b*) in rats with acute renal insufficiency.

ischemic ARF, recipients of stem and progenitor cells, is presented (Fig. 2).

Similarly as in experiments with CRI, blood sodium concentration decreased on days 11-12 of the postischemic period in rats with ARF, but in this case it was due to decreased sodium reabsorption in the renal tubules against the background of polyuria.

Intraparenchymatous injection of cultured human fetal stem and progenitor cells (summary culture of renal cells and BM-MSC) led to a significant improvement of renal function in rats with CRI and normalization of the functional parameters no later than after 2 weeks. The stimulatory effect persisted for more than 1 month in all animals, in 50-66% up to 72 days (maximum period of observation). No appreciable difference in the dynamics of functional recovery of the damaged kidney in animals receiving renal cell culture and BM-MSC were detected.

Injection of fetal kidney cells into the renal parenchyma of animals with postischemic ARF virtually did not modify the course of the postischemic period, while injection of BM-MSC stimulated the recovery of functional activity of ischemic kidney preventing the death of animals from renal failure.

The mechanism of the stimulatory effect of transplanted cells deserves further investigation. Some authors reported incorporation of intravenously injected bone marrow stem cells into various structures of the kidney, specifically, into glomerular capillary endothelium, mesangium, and renal tubular epithelium, but the percentage of "incorporated" cells did not surpass 1.5% [6]. Other authors detected no transplanted cells after 24 h [14]. We cannot rule out the possibility that these intensely proliferating cells create a population replacing dead cells of the vascular bed, interstitium, and even tubular epithelium, though these cells belong to different histogenetic lineages [7,12]. On the other hand, the version of paracrine stimulation

TABLE 3. Effect of Fetal Stem Cell Transplantation on the Time Course of Renal Function in Rats with Postischemic ARF

Parameter	Day after cell injection				
	4	11-12	20-21	39	80
Blood creatinine, μmol/liter					
control	600	—	—	—	—
fetal kidney cells	490	460	70	60	50
BM-MSC	266±64	135±8	60±3	55±2	40
Blood urea, mmol/liter					
control	24.2	—	—	—	—
fetal kidney cells	20.1	18.4	6.3	4.5	4.0
BM-MSC	14.0±2.1	10.4±1.1	4.6±0.5	3.6±0.1	3.1
Blood sodium, mmol/liter					
control	137	—	—	—	—
fetal kidney cells	139	99	141	141	144
BM-MSC	140±1	125±3*	142±1	141±1	145
Blood potassium, mmol/liter					
control	9.8	—	—	—	—
fetal kidney cells	7.8	—	9.5	9.9	9.0
BM-MSC	8.6±0.3	7.6±0.3	6.1±0.4	7.2±0.1	9.2
Diuresis, ml control	21	—	—	—	—
fetal kidney cells	22.5	23.5	22.5	17.0	12.5
BM-MSC	26.1±3.7	29.2±0.9	29.5±2.6	15.3±1.1	14.0
Creatinine clearance, ml/min					
control	0.02	—	—	—	—
fetal kidney cells	0.35	0.54	2.65	2.66	4.10
BM-MSC	0.84±0.11	1.93±0.09	2.60±0.18	3.39±0.05	5.22
Sodium reabsorption, %					
control	75.34	—	—	—	—
fetal kidney cells	—	97.87	99.05	99.35	99.76
BM-MSC	97.06±0.88	99.72±0.02	99.32±0.04	99.56±0.01	99.79
Creatinine excretion, μmol/kg/day					
control	17	—	—	—	—
fetal kidney cells	248	345	249	230	276
BM-MSC	297±33	350±39	208±27	269±16	288

of "resident" (endogenous) stem cells of the kidney by a complex of factors released by transplanted cells [6,14] seems to be interesting. Possible dedifferentiation of mature differentiated cells under the effect of paracrine stimulation and their transformation into cells of other tissues and fusion of the transplanted stem cell genome with the genome of mature somatic cells as mechanisms of dedifferentiation and transformation of these cells are discussed [3,13].

Our experiments demonstrated the important role of the paracrine mechanism of the stimulatory effect of fetal stem cells, because improvement of functional parameters of the damaged kidney was observed during the early period after transplantation. This hypothesis is confirmed by the fact that 33-50% rats with CRI developed a relapse of renal insufficiency after 2.5 months, presumably because of exhaustion of the pool of transplanted cell and cessation of their stimulatory effect.

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