Delayed Results of Transplantation of Fetal Neurogenic Tissue in Patients with Consequences of Spinal Cord Trauma

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We analyzed delayed effects of transplantation of nervous and hemopoietic fetal cells to patients with consequences of spinal trauma. A decrease in neurological deficit associated with pronounced improvement of functional independence was observed in 48.9% cases. The best results were observed in patients receiving cell transplantation within the first 2 years after trauma and in younger individuals. The pattern of morphological changes in the spinal cord at site of injury, severity of damage, and the method of transplantation had no appreciable effects on its delayed results.

Key Words: *spinal cord injury; fetal neurogenic cells; transplantation; functional deficit*

Severe spinal cord injury often triggers the development of complex irreversible structural and functional changes at the site of injury, which results in sustained functional disturbances and impairment of social adaptation of patients [6,12]. Rehabilitation measures can improve quality of life in these patients, but they remained disabled because of unrecoverable neurological deficit [1,5,14].

The perspectives of cardinal improvement of treatment results in these patients seem more optimistic with transplantation of live donor stem cells due to their unique properties such as integration with host tissues, modulation of the cytokine background, potentiation of regeneratory mechanisms, *etc*. [5,9,8,14]. Phase I-II clinical studies for the use of cell transplantation technologies in the treatment of patients with severe neurological disorders refractory to standard rehabilitation treatment were previously performed in our institute. Promising results were obtained in patients with severe cerebral palsy [3], consequences of brain damage [4], and brain stroke [2]. A special cell transplantation technology for the treatment of spinal cord trauma consequences was developed and the first clinical cases of its application were described [15]. Here we present the data characterizing the efficiency of the developed technology in 43 patients with follow-up period >3 years.

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MATERIALS AND METHODS

Clinical studies were performed in accordance with the protocol approved by Scientific Council and Ethical Committee of Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences. Delayed results of transplantation treatment were evaluated in 43 patients with consequences of spinal cord injury, in whom complex rehabilitation treatment did not led to significant neurological improvement. Demographic data, the level of injury, and anatomical peculiarities of the trauma focus (cyst or connective tissue and glial cicatrix) are shown in Table 1.

Cells for transplantation were isolated from brain and liver of 16-22-week's gestation fetuses after legal abortions as described previously [3,15], cryopreserved in 10% dimethylsulfoxide, and stored deeply frozen. For preparation of the transplant, fetal nervous and hemopoietic cells (9:1 ratio, total number 2-2.5×10^8 cells) were suspended in 5 ml plasma with

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TABLE 1. Characteristics of Patients with Spinal Trauma Consequences

Parameter		Number of patients
Sex	men	11
	women	32
Age	<20 years	3
	20-29 years	23
	30-39 years	13
	40-49 years	3
	>50 years	1
Localization of trauma	cervical (C_{IV}-C_{VII})	22
	upper thoracic (T_I-T_X)	12
	lower thoracic/lumbar (T_{XI}-L_{IV})	9
Type of changes at the site of injury	arachnoidal or intramedullary cyst	34
	glial cicatrix	9
Time from trauma to transplantation	<1 year	3
	1-2 years	14
	2-3 years	8
	3-4 years	7
	4-5 years	8
	>5 years	3

Allsphere solution. The plasma clot was formed by adding 2% $CaCl_2$. In cases with arachnoidal and/or intramedullary cysts (34 cases), laminectomy with meningoradiculolysis was performed, the cyst was opened, drained, and filled with the clot containing neurogenic and hemopoietic cells. After 10-14 days, repeated transplantation of fetal cell suspension was performed via lumbar puncture. In cases with predominance of cicatricial changes without cystic transformations (9 patients), only intralumbar transplantation at L_{IV}-L_V was performed.

The results were evaluated using FIM scale (functional independence measure) currently accepted for neurological status of spinal patients [1,10]. This scale evaluates functional independence of patients during their everyday life and control over pelvic functions. The scale consists of 13 questions, each is scored by a 7-poin scale, the maximum score is 91 (complete functional independence). Since the study included patients with different trauma levels and different degrees of conduction disturbances, we used not the absolute, but a relative parameter of recovery by FIM scale, the degree of restoration of functional deficit (DRFD), which was calculated by the formula:

$$\text{DRFD}=\frac{\text{final FIM score-initial FIM score}}{91}\times 100\%.$$

The questions were sent by post or asked during telephone conversation. Some additional questions about sensory disturbances, spasticity, and changes in the autonomic sphere were also asked during the telephone conversation.

RESULTS

The treatment was effective in 21 patients (48.9%). The subjective sensation of changes in the motor function (appearance of rocking motions of the toe, appearance of a sensation of shin muscle contraction without visible motor activity, *etc.*) and sensitivity not affecting the degree of functional independence and quality of life were not taken into account.

Of 21 patients with positive effects, a relatively insignificant improvement (increase in FIM score by ≤10%) was reported by 3 (7%) patients. The recorded changes primarily involved the function of pelvic organs (sensation of bladder filling or prolongation of the time of urine retention). The increase in functional independence by 10-50% was reported by 15 (35%) patients. These changes directly involved the locomotion and self-service spheres. The increase in functional independence by >50% was reported by 3 (7%) patients. On the whole, 18 (42%) patients reported improvement of pelvic function control, in 10 of them this improvement involved not only voiding, but also defecation control.

A significant decrease in pathologically elevated muscular tone in the paralyzed extremities and in the body was reported by 5 (14%) patients, in 2 of them spasmolytics were completely discontinued. The FIM scale does not include spasticity alleviation. However, these changes undoubtedly improve patient's quality of life, because they are associated with a decrease in pain syndrome severity.

Stable improvement in the sensory sphere was reported by 10 (27%) patients. These changes manifested in a decrease in anesthesia level by 2-4 segments. One female patient with paresis of the lower extremities examined by a neurologist reported reappearance of knee reflexes.

Three patients with injury to the cervical and upper thoracic segments had pronounced hypotension before transplantation treatment: they could not sit for more than 2-5 min. After treatment, the signs of orthostatic hypotension completely disappeared, which together with the decrease in neurological deficit, allowed these patients to make active physical exercises.

Analysis of the obtained results revealed a clear-cut dependence of the effect of treatment efficiency on the time elapsed after trauma (Fig. 1).

Improvement was noted in 71 and 78% patients, in whom the treatment was performed <1 year and 1-2 years after trauma, respectively. In patients receiving cell transplantation 2 and more years after trauma, this parameter decreased by more than 2 times.

The positive effect of treatment inversely correlated with patient's age (67% in patients aging <20 years, and 0% in patients over 50 years; Fig. 2).

There was no correlation between the treatment efficiency and level of injury. In all groups (injury to cervical, upper thoracic, lower thoracic, and lumbar portions of the spinal cord), the positive effects of treatment were observed in 46-54% patients. At the same time, spasticity reduction in paralyzed muscles and improvement of sensitivity were somewhat more pronounced in patients with thoracic traumas.

The type of morphological changes at the site of injury was inessential for the degree of recovery of motor functions (self-service and locomotion) and functions of pelvic organs. Improvement of FIM score was noted in 45% patients with cysts and in 55% patients with cicatricial changes. The decrease in pathological muscular tone was observed only in patients with cysts. This effect was probably associated with decompression of the spinal cord and meningoradiculolysis. Two of 9 patients with cicatricial changes at the site of injury reported increased spasticity. Nine patients (26.5%) with cysts reported improvement of

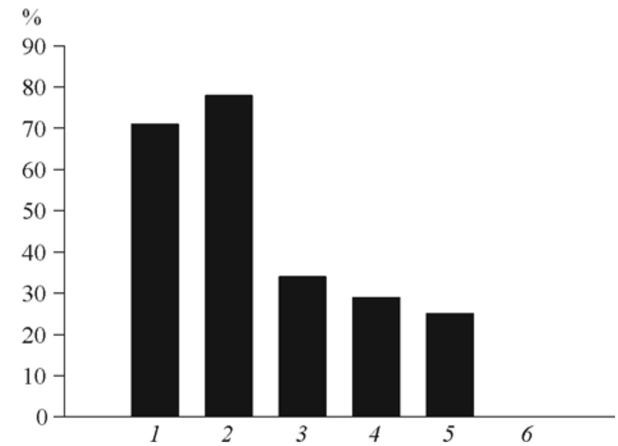

Fig. 1. Dependence of the efficiency of transplantation of fetal neurogenic cell on the time after trauma. Abscissa: time after trauma; ordinate: number of patients with improvement of functional independence. *1*) <1 year, *2*) 1-2 years, *3*) 2-3 years, *4*) 3-4 years, *5*) 4-5 years, *6*) >5 years.

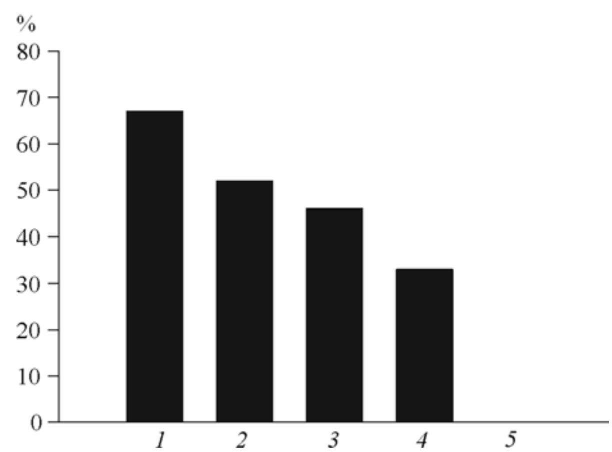

Fig. 2. Dependence of the efficiency of transplantation of fetal neurogenic cell on patient's age. Abscissa: patient's age; ordinate: number of patients with improvement of functional independence. *1*) <20 years, *2*) 20-30 years, *3*) 30-40 years, *4*) 40-50 years, *5*) >50 years.

sensory function. Similar changes were observed in only one patient with glial cicatrix.

Active experimental and clinical neurotransplantology studies with various cell types are now in progress [5,8,9,11,14]. Bearing in mind the tentative mechanism of the action of transplanted cells, we believe that fetal neurogenic cells have some advantages over autologous ones. The fetal neurotransplant used by us contains the whole spectrum of brain cells at different stages of differentiation, including blasts and neurogenic stem cells [6]. Neurons with formed axon and dendrites naturally die during cell isolation from the tissue [5]. However, relatively high (>10%) content of viable cells isolated from the fetal brain suggests that it contained considerable amounts of low-differentiated cells with high growth potential capable of integrating into the neuronal networks of recipient's spinal cord. Precursors of glial elements can produce neurogenic growth factors stimulating the growth of axons through the damaged zone and remyelination of the remaining axons [5,8,14].

Fetal liver cells at this gestation terms are primarily presented by hemopoietic blast cells and their use is determined by their capacity to stimulate angio- and vasculogenesis. Moreover, blast forms produce a wide spectrum of cytokines maintaining viability and growth of different types of cells and exhibit natural immunosuppressive activity [7].

In our study, the patients with spinal cord cyst received transplantation of fetal neurogenic cells in a plasma clot. The cells are embedded in a 3D fibrin net promoting axonal growth and neurogenesis. Moreover, fibrin clot facilitates diffusion of oxygen and nutrients and reverse transport of metabolites, thus improving survival of transplanted cells [13].

The best results obtained in patients receiving cell transplantation within 1-2 years after trauma are quite expected. At this term, the situation at the site of damage is not stable and inert. It is characterized by immaturity of the connective tissue-glial cicatrix, variable cytokine microenvironment, retained receptors to neurotrophic growth factors on nerve cells of the recipient, *etc.* [12] These conditions are quite appropriate for the realization of proliferative and differentiation potential of transplanted cells.

Our findings suggest that the best results of transplantation should be expected in young patients. Experimental studies showed that the growth and penetration of axons of transplanted neurons in old animals are inhibited by the microenvironment [11].

The fact that appreciable clinical effect in the recipient could be obtained rapidly attests to the involvement of humoral products into the mechanism of action of transplanted cells. Changes in the balance of neurogenic growth factors in the liquor should promote functional and regenerative activation of remaining neurons at the site of damage. However, the role of homing of donor cells in the focus of damage and their involvement into the formation of new nervous communications should not be underestimated. These effects might be responsible for neurological improvement in delayed post-transplantation periods.

The proposed protocol of transplantation treatment is safe. None complications related to unfavorable side activity of the transplanted cells were noted during the follow-up period. The appreciable clinical effect of treatment in our treatment group was noted in about half of patients. It should be noted that all patients were included in the treatment group after the end of the period of possible spontaneous recovery of neurological functions. From the viewpoint of standard medicine, the treatment of these patients is unpromising. Competent use of cell technologies now can cardinally improve treatment efficiency in patients with consequence of spinal cord injury. The most pronounced results should be expected in cases, when transplantation technologies are applied at early terms, when spontaneous recovery processes are not completed and relatively favorable conditions for axonal growth are retained at the focus of injury.

It should be emphasized that the methods of cell biotechnologies used in neurology and neurosurgery are not restricted to fetal cells. There is no conventional concept on the use of cell technologies in neurology. We hope that our study will contribute to the development of this concept in the nearest future.

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