Transplantation of Fetal and Xenogeneic Nervous Tissue in Parkinson's Disease

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A mixture of fetal human brain tissue and of neural primordia of Notch *Drosophila melanogaster* is transplanted to the ventrolateral thalamic nucleus of patients with Parkinson's disease in stage 3-4. Two kinds of operation are performed. In the first case cryodestruction of the zone of tissue implantation is performed before transplant, and in the second case this procedure is not performed. A stable effect manifested in the disappearance of tremor and rigidity is achieved for both kinds of operation. When a transplant is performed without cryodestruction, the effect does not manifest itself until 2 months postoperation. The patients are followed up for 7-12 months postoperation. No side effects or relapses of Parkinson's disease are observed in this period.

Key Words: *transplantation; nervous system; fetal tissue; xenografts*

Analysis of results of transplantation of human fetal tissue to the brain of patients with Parkinson's disease has shown a relatively low efficacy of this approach [11,15,17]. The use of fetal tissue isolated from the adrenal cortex and mesencephalon of human fetuses resulted in a manifest therapeutic effect in only 3-5% of cases, although changes in the patient's condition posttransplant were noted much more often [9,10]. Numerous attempts to obtain better results after transplantation of human fetal tissue have been based on minor changes in the surgical techniques proposed back in 1982 [10,15,17]. The general strategy of the clinical approach to the transplantation of fetal tissue to the brain of patients with Parkinson's disease includes the following: stringent choice of patients; seeking the optimal zones for transplant to the brain; the development of new techniques of tissue transplantation; the use of short-term cell cultures; the use of new tissues for transplantation; and the stimulation of engraftment with different growth factors [5-7,17,18]. However, neither experimental nor clinical modification of surgical techniques of transplantation of human fetal tissue to the brain of patients with Parkinson's disease has yielded the expected results. The follow up of patients during the postoperative period has shown that after 6-12 months the effect of fetal tissue transplantation gradually diminishes [4,16,20]. Meanwhile, it is extremely difficult to assess the results of transplantation objectively, due to individual specificities of case histories and to differences in surgical methods [4,12,13,16,20].

The results of more than 200 cases of human fetal tissue transplant in Parkinson's disease invite two main conclusions. First, the likelihood of a stable therapeutic effect of fetal tissue transplant is extremely low and does not exceed 2.5%. Second, modification of already known methods of human

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fetal tissue transplantation seems unlikely to result in considerable progress in the surgical treatment of parkinsonism. These conclusions are corroborated by the attempts of some authorities to evoke the interest of researchers and clinicians in designing genetically modified cell strains with specified properties, in stimulating the differentiation of grafted cells with diverse growth factors, in using pure dopaminergic transplants obtained by human fetal tissue culturing, and in seeking animal brain tissues suitable for xenotransplantation to the human brain [11,15,19].

While researching methods to treat parkinsonism, we came to the conclusion that mixed fetal-xenogeneic transplants can be used to raise the efficacy of treatment. The present paper is devoted to the first results of transplantation of fetal and xenogeneic tissues to patients with Parkinson's disease who were followed-up from the 6th to the 12th month postoperation.

MATERIALS AND METHODS

Human fetal tissues derived from abortions between weeks 14 and 18 of gestation were used as the fetal component of the transplant. Tissues from the ventral mesencephalon and from the basal ganglia were used for transplantation. Before the operation the isolated tissues were stored, following a special technique, in liquid nitrogen for 1-3 days, which did not alter the morphological structure or biochemical properties of the tissue [14]. The neurogenic primordium of Notch *Drosophila melanogaster* mutants was used as the xenograft. The choice of xenograft was determined by the fact that the structure of the *Drosophila* genome has been studied in detail, and a large number of laboratory and natural mutations are known, which makes it possible to obtain cells with predictable properties. Preliminary studies of the transplantation of cells of drosophila neurogenic primordia to the brain of laboratory animals showed that they increase the rate of neuroblast differentiation, promote the growth of the new host's neuronal processes toward the transplant, and stimulate vascularization of donor and recipient tissues [1-3,8]. In mammals xenografts of such a kind preserve their viability for at least 2-3 months, which makes them useful, at any rate, as a cell stimulator promoting differentiation and vascularization of fetal human brain tissue transplanted to the recipient brain. Before surgery the fetal brain tissue was mixed with the xenograft prepared as described earlier [3,8]. The weight ratio between fetal tissue and xenograft was from 1000:20 to 1000:1. The cell mixture was injected in the ventrolateral thalamic nuclei via a microcannula mounted on a stereotactic apparatus. The site of injection of the transplant was located precisely, since a cavity had been preliminarily created in the thalamus by a special method which atraumatically and briefly separated the brain tissue with a small balloon with changeable geometry. This device was mounted on the stereotactic apparatus and inserted in the brain via the microcannula prepared for the transplant (Fig. 1). Injection of the transplant was x-ray monitored during the operation.

RESULTS

The first operation was performed on a female patient aged 43 suffering from the bilateral rigid-tremor form of parkinsonism, stage 3-4. The patient could not move about on her own and was unable to take care of her daily needs. She had been suffering from parkinsonism for 8 years. A right-side cryotomy of the ventrolateral thalamic nucleus was performed during the operation. This topology of cryotomy was chosen because the disease had started in the left extremities. During the operation, ten minutes after cryotomy 2 ml of a mixture of human fetal and xenogeneic tissue (1000:2) was injected into this zone. Disappearance of tremor and rigidity in the left extremities was observed while the patient was still on the operating table. After the surgery, the patient was able to move by herself and to take care of her needs. Nine months later a similar operation with cryotomy of the left ventrolateral thalamic nucleus was performed. A 12-month follow up did not reveal any signs of recurrence of parkinsonism or any side effects from the graft.

The second operation was performed on a male patient aged 49 who had had the bilateral rigid-tremor form of parkinsonism, stage 3-4, for 7 years. Before the transplantation of human fetal and xenogeneic tissue, he had been twice operated on for parkinsonism. Cryotomy of the left ventrolateral thalamic nucleus had been performed during the first operation. The postoperative effect had been preserved for one month. The patient's condition had then deteriorated to the preoperative state. Three years later a similar operation had been performed, to no avail. Six months after the second operation transplantation of the cell mixture was performed following the scheme described for the first case. Examination of the patient after the operation showed the absence of tremor and rigidity. During 6 months, the follow up revealed no evidence of a recurrence of parkinsonism and no side effects.

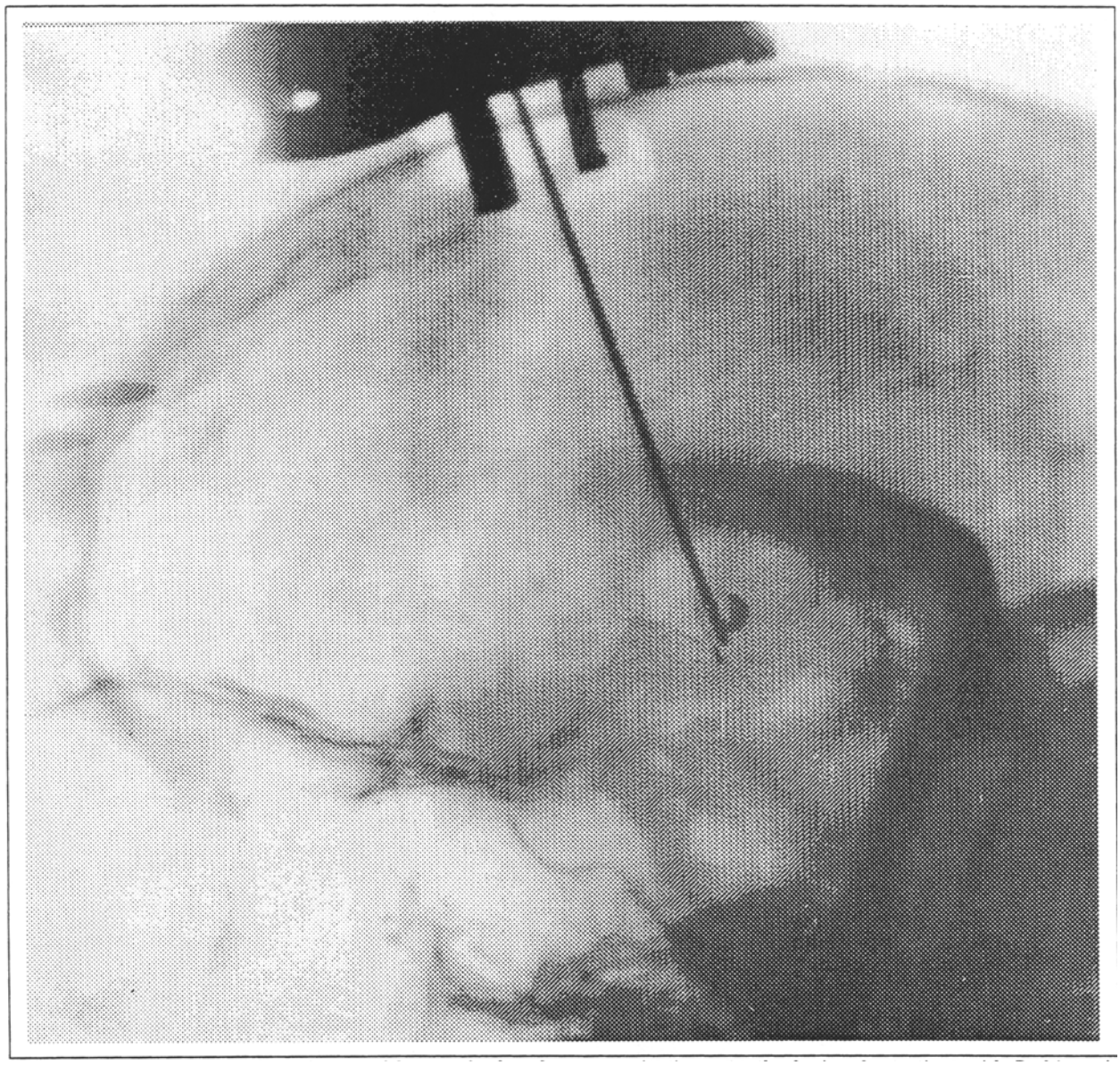

Fig. 1. Transplantation of a mixture of human fetal and xenogeneic tissue to the brain of a patient with Parkinson's disease. Roentgenogram of atraumatic formation of cavity prior to tissue implantation.

The third operation was performed on a female patient aged 60 suffering from the bilateral rigid-tremor form of parkinsonism, stage 3-4, for 10 years. Cryodestruction of the ventrolateral thalamic nucleus was performed on the left side without injecting a graft. Transplantation of a mixture of fetal and xenogeneic tissues to the right ventrolateral thalamic nucleus was performed in parallel without preliminary cryodestruction. Tremor and rigidity disappeared on the right side immediately after cryodestruction of the left nucleus. The effect of cryodestruction started to diminish 4 months postoperation. After transplantation of the mixture of human fetal and xenogeneic tissues, all signs typical of parkinsonism were preserved on the left side of the body. However, 2 months postoperation tremor and rigidity disappeared on the left side. Thus, the effect of the transplant did not manifest itself until 2 months postoperation and was preserved for 7 months.

These results demonstrate possible success with transplanting a mixture of human fetal and xenogeneic tissues in the treatment of parkinsonism. Two types of operation which differ with respect to the grafting techniques were performed. When cryodestruction of brain tissues is used in combination with the transplant, the effect of the operation manifests itself immediately after its completion. However, in this case it is the cryodestruction, rather than the transplantation of fetal and xenogeneic tissues, that is the cause of the rapid improvement in patient state. The results

of the third operation suggest that the effect of the transplant does not manifest itself until 1.5-2 months postoperation. During this time, differentiation of human fetal brain tissues occurs under the stimulating influence of the xenograft, and their activity starts to exert an effect on the state of the patient. Scant data obtained during autopsy of patients who had undergone transplantation of fetal tissue provide indirect evidence of this [21]. It was shown that 5 weeks after transplantation of dopaminergic cells to the head of the caudate nuclei the cells had survived, differentiated, and were exhibiting a positive immunohistochemical reaction to tyrosine hydroxylase. Evidently, it is logical to expect delayed therapeutic effects of transplants, resulting from differentiation of the tissue transplanted to the brain. As follows from our findings, it is the surgical intervention per se rather than the cellular activity of the transplant that underlies the majority of the effects observed.

Our study helped uncover both positive and negative aspects of the use of mixtures of fetal and xenogeneic tissues. One advantage of this approach is the possibility of treating the severe forms of parkinsonism, which relapse after routine stereotactic intervention. The use of a mixture of human fetal and xenogeneic tissues without cryodestruction of the implantation zone can be regarded as a key advance in transplantation. Minimized damage to the brain may be a factor of principal importance in the surgical treatment of parkinsonism, since this helps the brain functions to recover under the influence of the fetal-xenogeneic transplant. A mixture of cells derived from different animal species is a favorable combination of nondifferentiated structural elements of the human nervous system and of growth factors of nervous tissue secreted by the cells of the xenograft, which stimulate vascularization and the directed growth of neuronal processes in the recipient. In essence, the use of neurogenic primordia from different mutants and invertebrate strains offers the possibility of using ready-made cell lines with specified and well-known properties. However, there are a number of problems which demand further study. These have to do with the isolation of large amounts of neurogenic primordia and with the lack of techniques of their cryopreservation. When these problems are solved, it will be possible to gather the necessary data in order to assess this method of treatment of parkinsonism more comletely.

Our study provides grounds for believing that this new approach to the treatment of parkinsonism will be more effective than the earlier used methods. Encouraging signs are the absence of relapses in patients for 6-12 months, the pronounced effect in repeatedly operated patients, and the possibility of atraumatic introduction of the transplant without cryodestruction.

REFERENCES

1. S. V. Savel'ev, A. I. Ivanov, V. I. Gulimova, and L. I. Korochkin, *Dokl. Akad. Nauk SSSR*, **316**, 735-738 (1991).
2. S. V. Savel'ev, L. I. Korochkin, A. I. Ivanov, *et al.*, *Ibid.*, **305**, 1239-1241 (1989).
3. S. V. Savel'ev, L. I. Korochkin, A. I. Ivanov, *et al.*, *Ibid.*, **313**, 1491-1493 (1990).
4. C. R. Freed, R. E. Breeze, N. L. Rosenberg, *et al.*, *Prog. Brain Res.*, **82**, 715-721 (1990).
5. B. T. Henderson, C. G. Clough, R. C. Hughes, *et al.*, *Arch. Neurol.*, **48**, 822-827 (1991).
6. B. T. Henderson, B. G. Kenny, E. R. Hitchcock, *et al.*, *Acta Neurochir. Suppl. (Vienna)*, **52**, 48-50 (1991).
7. R. F. Iacono, Z. S. Tang, J. C. Mazziotta, *et al.*, *Stereotact. Funct. Neurosurg.*, **58**, 84-87 (1992).
8. L. Korochkin, S. Saveliev, A. Ivanov, *et al.*, *Genetica (Netherlands)*, **85**, 23-34 (1991).
9. A. Kupsch, H. Sauer, and W. H. Ortel, *Nervenarzt.*, **62**, 80-91 (1991).
10. O. Landvall, *Europ. Neurol.*, **31**, Suppl. 1, 17-27 (1991).
11. I. Madrazo, R. Franco-Bourland, M. Aguilera, *et al.*, *Neurosurgery*, **29**, 165-176 (1991).
12. I. Madrazo, R. Franco-Bourland, F. Ostrosky, *et al.*, *Arch. Invest. Med. (Mex)*, **21**, 201-207 (1990).
13. P. Menei, G. Guy, and A. Pouplard-Barthelaix, *Pres. Med.*, **20**, 513-517 (1991).
14. P. Nadvornik, O. Rozhold, J. Kolarik, *et al.*, *Acta Univ. Palacki Olomuc. Fac. Med.*, **130**, 125-129 (1991).
15. L. Olson, *Stereotact. Funct. Neurosurg.*, **55**, 250-267 (1990).
16. N. P. Quinn, *Prog. Brain Res.*, **82**, 619-625 (1990).
17. J. V. Rosenfeld, T. J. Kilpatrick, and P. F. Bartlett, *Aust.-N-Z-J-Med.*, **21**, 477-484 (1991).
18. G. V. Sawle, P. M. Bloomfield, A. Bjorklund, *et al.*, *Ann. Neurol.*, **31**, 166-173 (1992).
19. M. J. Staal, R. I. Hogen Esch, R. Tomasini, *et al.*, *Stereotact. Funct. Neurosurg.*, **54-55**, 290-296 (1990).
20. O. Subrt, M. Tichy, V. Vladyka, and K. Hurt, *Acta Neurochir. Suppl. (Vienna)*, **52**, 51-53 (1991).
21. M. Zabek, W. Mazurowski, J. Dymecki, *et al.*, *Neurol. Neurochir. Pol.*, Suppl. 1, 13-19 (1992).