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HETEROGRAFTING CULTURES OF HUMAN PANCREATIC ISLET CELLS IN RATS WITH EXPERIMENTAL DIABETES MELLITUS

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Cultures of human fetal pancreatic islet cells were transplanted into the liver of rats with diabetes induced by alloxan. This heterografting led to a prolonged fall of the blood sugar. Histological examination of the recipients' liver revealed groups of implanted islet cells.

KEY WORDS: cell cultures; pancreatic islet cells; experimental diabetes; blood sugar.

It has now been shown that allografting of the pancreas into patients with severe diabetes mellitus as a rule gives unsatisfactory results, not only because of a rejection reaction, but also because of the high proteolytic activity of the exocrine part of the human cadaveric pancreas, leading to enzymic destruction of the transplanted organ [4, 5]. Accordingly in recent years considerable attention has been paid to the development of methods of isolation and culture of endocrine (islet) tissue of the human pancreas [10, 12]. Furthermore, the first reports of allografting of islet tissue into patients with severe diabetes have appeared. However, the results indicate an incomplete and brief effect of such an operation, which may be due either to rejection of the implanted B cells or to their insufficient number [4, 5, 13, 14].

The question of the use of human embryonic and fetal pancreas as the source of islet tissue for culture and subsequent transplantation has attracted special attention. In the fetus the endocrine portion accounts for up to 30% of the weight of the pancreas [7] and the exocrine portion has virtually no proteolytic activity. Moreover, B cells of the pancreatic islets of Langerhans are immunologically immature until a certain moment, a fact which must facilitate their survival in the recipient's body.

A new technique of obtaining cultures of human embryonic and fetal pancreatic islet cells has been developed in the writers' institute, with the use of the Soviet enzyme preparation collalytin [1, 3], and their insulin-forming activity has been studied during culture under various conditions [2-3]. Experiments have been carried out to study the effect of heterografting of cultures of human fetal pancreatic islet cells on the course of experimental diabetes in rats. These experiments also were regarded as an essential stage in the preparations for the clinical allografting of human pancreatic islet cell cultures.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar rats weighing 180-240 g. Experimental diabetes was induced by subcutaneous injection of alloxan (from Sigma, USA) in a dose of 200 mg/kg body weight. Rats with a blood glucose level of over 350 mg% for not less than 2 weeks after induction of diabetes were used as recipients. The blood sugar was determined by the orthotoluidine method using the "Bio-Lab-Test" kit (from Lachema, Czechoslovakia). Cultures of pancreatic islet cells from 16-20-week human fetuses were

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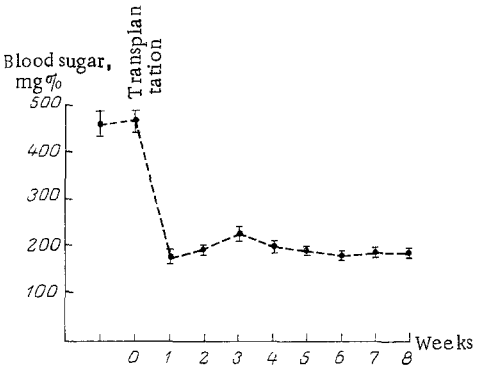

Fig. 1

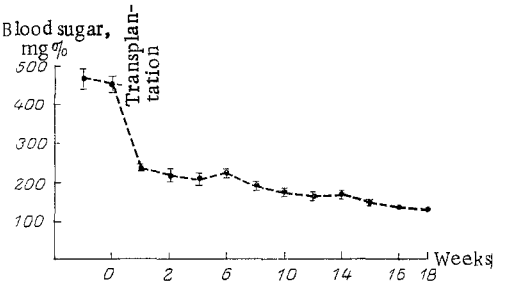

Fig. 2

Fig. 1. Changes in blood sugar of rats with alloxan diabetes undergoing intraportal transplantation of human fetal pancreatic islet cell cultures. Here and in Fig. 2: abscissa, time of investigation (in weeks); ordinate, blood glucose concentration (in mg%).

Fig. 2. Changes in blood sugar of rats with alloxan diabetes undergoing intrahepatic (intraparenchymal) transplantation of human fetal pancreatic islet cell cultures.

prepared by the method described previously [1, 3]. For each transplantation a 5-8-day culture of islet cells obtained from one pancreas was used. The cell suspension was injected into rats with alloxan diabetes into the liver through the portal vein [11] or directly into the parenchyma of the anterior lobe [8]. Rats with untreated alloxan diabetes served as the control. The liver of the recipient rats was examined histologically for the presence of implanted islet cells. Histological changes in the pancreas of the experimental and control rats also were investigated. The material was fixed in 10% neutral formalin and embedded in paraffin wax; sections 5-7μ thick were stained with hematoxylin–eosin and with aldehyde-fuchsin.

EXPERIMENTAL RESULTS

The rats developed hyperglycemia in the course of 24-48 h after injection of alloxan. Their symptoms were lethargy, polydipsia, and polyuria and they lost weight. Marked hyperglycemia in the untreated rats lasted throughout the period of observation; animals whose blood glucose concentration exceeded 600 mg% died soonest.

A suspension of previously cultured pancreatic islet cells from 16-20-week human fetuses was injected into the portal vein of eight rats with alloxan diabetes. In all cases this was followed by a fall in the blood glucose concentration of the recipients approximately to the normal level (Fig. 1). Correction of the blood glucose concentration was accompanied by disappearance of the lethargy, polydipsia, and polyuria and by an increase in the recipient's body weight.

A suspension of previously cultured human fetal pancreatic islet cells was injected from a syringe directly into the parenchyma of the anterior lobe of the liver. The fall in the recipients' blood glucose observed after this treatment took place more gradually than after intraportal injection of the islet cell cultures (Fig. 2). After stabilization the blood sugar level remained low throughout the period of observation (8-18 weeks). Histological investigation of the pancreas of the recipient rats after intrahepatic intraparenchymal transplantation revealed death of the B cells of the islets of Langerhans characteristic of alloxan diabetes. Meanwhile, groups of cells penetrated by blood capillaries, containing numerous B cells with normal structure and a varied number of B cells in different stages of destruction could be found in the liver of these rats. The cellular immune response in the zone of the clusters of islet cells varied in degree.

No information could be found in the literature on transplantation of human fetal pancreatic islet cell cultures into heterologous immunologically active recipients with experimental diabetes. The results of the present investigations show that heterografting of this type is possible. Moreover, it leads to a prolonged fall in the blood sugar and abolishes the diabetic symptoms. The fact that the therapeutic effect in these experiments was due to the functioning of the implanted islet cells is shown not only by the laboratory data and clinical observations on the animals, but also by the results of histological investigations. The successful results of these experiments was probably due to survival of immunologically immature human fetal pancreatic B cells in the heterologous recipient.

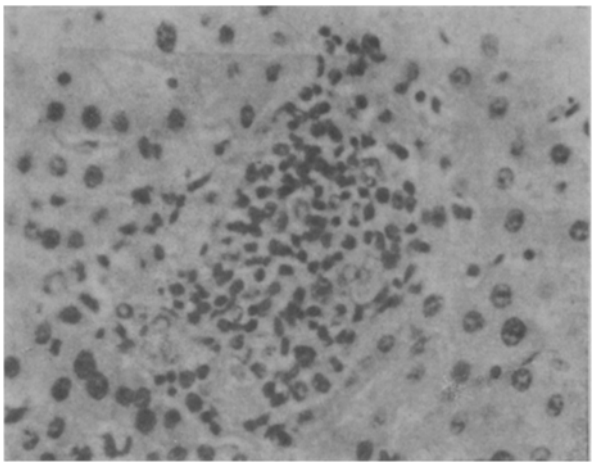

Fig. 3. Group of pancreatic islet cells of 20-week human fetus implanted in parenchyma of rat liver. Part of implant is infiltrated by lymphocytes. Hematoxylin–eosin, 120×.

Attention is drawn to the agreement between these results and those obtained by a group of French workers [6, 9], who carried out effective heterografting of pancreatic endocrine tissue from 15-17-day chick embryos into the hepatic parenchyma of rats with streptozotocin diabetes.

The results of the present experiments are hopeful as regards prospects for allografting of human pancreatic islet cell cultures into patients with diabetes mellitus. Further investigations are required to determine optimal conditions of heterografting human fetal pancreatic islet cell cultures: the "ideal age" of the donor's tissue, the quantity of it required, the most acceptable method of introducing cultures of islet cells into the recipient.

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