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SECONDARY FAILURE OF ISLETS AUTOTRANSPLANTATION IN TOTALLY PANCREATECTOMIZED DOGS: A MULTIFACTORIAL STUDY

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ABSTRACT

Even without any immunologic barrier, as in autografts, reversal of diabetes induced by islets transplantation is not definitive and a secondary failure generally occurs. Factors involved in this failure were investigated in 13 male dogs undergoing total pancreatectomy and extemporaneous islets reinjection in the portal circulation. Five dogs died from postoperatory complications, three remained diabetic, and five were normoglycemic without insulin therapy for a duration of two to 26 weeks. Factors associated with failure appeared to be: (a) *hemorrhage during dissection of pancreas prior to islets isolation,* (b) *imperfect collagenase digestion. Moreover, duration of this period of normoglycemia was negatively correlated with glycemia on the 20th postoperatory hour ($r = 0.759$, $p < 0.05$), suggesting a possible relationship between glycemia and further graft outcome. Therefore, such a multifactorial statistical analysis seems to be helpful for the study of multiple technical parameters associated with the prognosis of islets transplantations.*

ABRÉGÉ

La correction d'un diabète expérimental par greffe d'îlots de Langerhans, même lorsque n'existe aucune barrière immunologique (comme dans les autogreffes) a généralement une durée limitée, le greffon cessant de fonctionner au bout de quelques semaines ou mois. Nous avons étudié cet échec secondaire chez 13 chiens mâles totalement pancréatectomisés et recevant extemporanément une autogreffe d'îlots de Langerhans intraportale. Cinq chiens décèdent de complications opératoires, trois deviennent immédiatement diabétiques et cinq restent normoglycémiques durant 2 à 26 semaines. Les facteurs associés avec la précocité de cet échec secondaire semblent être : (a) *le caractère hémorragique de la dissection du pancréas avant isolement des îlots,* (b) *un isolement par la collagénase considéré* a posteriori *comme imparfait. La durée de la période de normoglycémie est négativement corrélée ($r = -0.759$, $p < 0,05$) à la glycémie à la 20e heure post-opératoire, suggérant une relation entre la glycémie post-opératoire et le succès ultérieur de la greffe. De telles études multifactorielles pourraient aider à mieux comprendre les échecs de ce type de greffes, où de nombreux paramètres semblent être impliqués.*

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INTRODUCTION

Although immune rejection is a major problem in transplantations of insulin-producing tissue, additional factors have been shown to influence the fate of islets transplanted into a diabetic recipient. Autografts, which successfully prevent pancreatectomy-induced diabetes in animals and humans (4, 7, 9, 11), may represent a model for the study of non-immunologic aspects of islets transplantations. In this work, we investigated a possible influence of some technical factors on the outcome of islets autotransplantations in totally pancreatectomized dogs, which represent a classical, reliable model for experimental insulin dependent diabetes mellitus (3).

MATERIALS AND METHODS

Animals

Thirteen mongrel dogs (weight 11 to 20 kg) underwent islets autotransplantation after total removal of the pancreas. All remained fasting for 36 hours before they were anesthetized with pentobarbital sodium. Total pancreatectomy was performed with the technique of Hédon (3). Briefly, pancreatic gland was dissociated from duodenum by careful dissection using finger nails. Only pancreatic duct and vascular supply of the splenic part of the gland were ligated, whereas dissected duodenal vessels remained without any ligature to avoid duodenal necrosis. When all the duodenal part (25 to 45 % of the total mass of the gland) had been dissected, it was distended with a cold Hanks solution, removed, and fragmented with scissors. Islets isolation was then performed as previously described (8) with Worthington Collagenase (type IV, 202 U/ml) in digestion-filtration chambers, under regular mechanical shaking. Five incubation periods at 37° C were performed: 6 minutes with 1,000 U/ml; 3 minutes with 500 U/ml; 3 minutes with 250 U/ml; 3 minutes with 125 U/ml; 3 minutes with 62.5 U/ml. Then, digestion product was carefully washed with a cold Hanks solution. In order to avoid islets loss, no Ficoll purification was performed. During this isolation pancreatectomy was achieved by careful removal of the splenic part of the gland. Islets were extemporareously reinjected in a vein of the portal system. Follow-up of the dogs of both groups was performed with daily venepunctures and urine glucose determinations. In the dogs in which long term normoglycemia was obtained, both oral (2 g of sucrose/kg) and intravenous (0.33 g/kg) glucose tolerance tests were performed.

Measurements

Glucose concentrations were measured with a colorimetric method using orthotoluidine. Insulinemia was determined by radioimmunoassay with a double antibody technique using the kit « SB-INSI 1 » (Commissariat à l'Énergie Atomique, BP 21, 91120 Gif-sur-Yvette, France). The sensitivity of this assay is 2 μU/ml. Intraassay coefficients of variation range between 8.7 and 9.7 % whereas between-assay ones range between 12.5 and 14.4 %.

Statistics

Correlations were determined by least square fitting. Statistical significance was defined as $p < 0.05$. A correspondance factor analysis was performed for multivariate determination of parameters associated with failure or successs of islets transplant. This statistical method gives a synthetic graphic representation of multiple subjects and parameters, in so a manner that proximity on the graph indicates a « profile similarity » and suggests a possible relationships between two factors. The algorithm mathematically defines synthetic axes on which the coordinates of each subject and parameter can be plotted. Therefore, distance among points in the graph indicates a difference, whereas closeness suggests association or similarity (6). A fortran software developped on the central computer of the Centre Universitaire Sud-Calcul (CNUSC), Montpellier, was used for this analysis. For the 13 dogs, numeric and nonnumeric items were included in the analysis. Numeric data were: weight of the dog, whole weight of the pancreas, percentage of the gland used for islet isolation (*i. e.* the duodenal part and the processus uncinatus), the weight of this part of the gland, insulinemia and glycemia measured before pancreatectomy, just after it had been completed, and on the 1st, 2nd and 3rd morning after operation (at 9 a. m.), and the number of days of normoglycemia before secondary failure. Non numeric items were defined as indicated below. The importance of warm ischemia was classified into three degrees: (*a*) « no warm ischemia » means that the removal of dissected part of the pancreas was rapidly done (less than twenty minutes), without any modification in the aspect of the gland (*b*) « warm ischemia » indicates that either a change in color or a lack of vascular supply for more than 20 minutes; (*c*) « warm ischemia −+ » indicates either a marked modification in aspect and color of the tissue during removal, or a duration of withdrawal from vascular supply of more than 30 minutes. Liver swelling (yes or no) indicates that a modification in hepatic volume was (or not) clearly observed just after reinjection of islets. Hemorrage during the dissection of duodenal part was defined by three degrees: (*a*) « no hemorrage », which indicate that bleeding was easily avoided with a unique compress so that it seemed almost inexistant; (*b*) « hemorrage + », which indicates that moderate bleeding was noticed and needed the use of several (up to 5) compresses; (*c*) « hemorrage +++ », which indicates that more than 5 compresses were needed for obtaining hemostasis. Gland distension by direct venous catheterism was classified in two categories: (*a*) « good distension », with homogeneous swelling of the gland which became translucent; (*b*) « bad distension » if this aspect was not totally achieved. Digestion of the gland by collagenase was described as « good », if perfectly isolated islets were visible among exocrine fragments or « incorrect » if there was either islets still attached to exocrine fragments or « overdigested » nearly translucid islets (« ghost islets »). The part of the portal system in which the catheter was set for reinjection of islets towards the liver was also indicated: « portal vein », « jejunal vein » or « splenic vein ». The items « failure » (definitive diabetes following the operation), « immediate success » (no need for insulin after the operation) or « delayed success » (after one week of insulin dependent diabetes) were also included in the factor analysis.

RESULTS

1. *Fate of the dogs*

In routinely performed pancreatectomies according to the technique of Hédon (3), insulin dependent diabetes is always obtained. Glycemic values always reach more than 300 mg/dl on day 3, whereas insulinemia falls to undetectable levels (5 μU/ml). Insulin therapy is definitely required.

In contrast, for the 13 dogs of our study, an important variability was observed in the outcome of islets transplantation. Five dogs died within the first week from postoperatory complications. In three cases, obvious signs of duodenal ischemic necrosis were found during necropsy. The others survived more than six months with exocrine pancreatic deficiency, which was treated with pancreatic extracts. In five dogs, an effective glycoregulation was maintained several weeks: the best results were obtained in three animals which remained normoglycemic during more than 90 days: 93, 105 and 184 days (Fig. 1). In each case, fasting glycemia remained lower than 200 mg/dl, whereas both intravenous and oral glucose tolerance tests showed impaired carbohydrate tolerance. Results of these tests (Fig. 2) indicate that insulin fails to increase after oral sucrose loading. Insulin response after intravenous glucose appears to be less abnormal, though the peak values are lowered. This period of normoglycemia ended suddenly, while a delayed onset insulin dependent diabetes mellitus occurred. When assaying insulinemia, it was shown that this secondary failure of autotransplantation was announced by abnormally high values of insulin (> 100 μU/ml) (Fig. 1). Attempts to find histologically grafted islets in hepatic tissue were made in these five dogs, but gave only negative results.

2. *Statistical studies*

Making the assumption that the considerable differences among individual dogs in this experiment resulted from the variability of several experimental parameters which were not easy to keep constant, we analyzed numeric data and non numeric items concerning islets isolation and reinjection by the means of a correspondence factor analysis (Fig. 3). Axis No. 1 represents 60.88 % of the total variance and seems to opposite failure (on the left) and success and duration of normoglycemia (on the right). On this axis, factors linked with success seem to be: (*a*) absence of hemorrhage; (*b*) absence of warm ischemia; (*c*) good distension of the pancreas. To a lesser extent, the location of the items « liver swelling » and « percentage of the gland used for isolation » also suggests their association with success. In contrast,

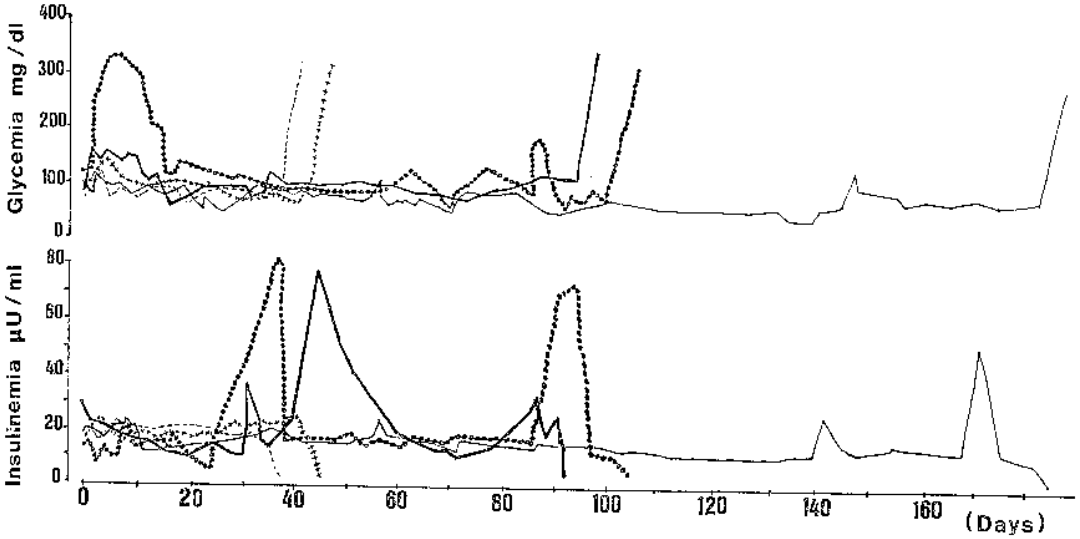

FIG. 1.

Individual evolution of insulinemia and glycemia of the five dogs in which successful islets autotransplantation prevented diabetes occurrence after pancreatectomy.

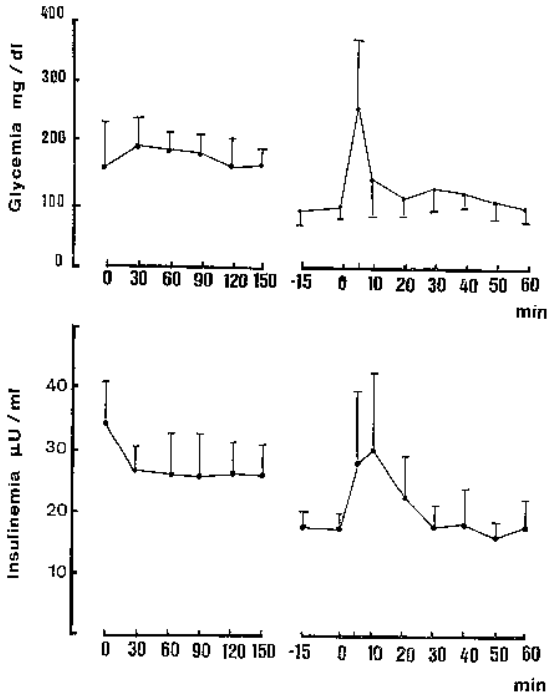

FIG. 2.

Mean values (± SD) of glycemia and insulinemia after oral glucose tolerance test (2 g/kg of sucrose, left pannel) and intravenous glucose tolerance test (0.33 g/kg, right pannel). Tests were performed during the 1st postoperatory month in the 5 dogs which underwent successful islets transplantation.

failure seems to be associated with the following items: (*a*) marked hemorrhage during the dissection and removal of pancreas; (*b*) imperfect results of extemporaneous collagenase digestion, showing either underdigested or overdigested tissue fragments; (*c*) warm ischemia during removal of the duodenal part of the gland before islets isolation; (*d*) imperfect distension of the gland obtained by venous injection of Hanks medium; (*e*) lack of liver swelling during islets reinjection. Axis No. 2 represented only 25 % of the total variance. It seems to separate the diverse modes of failure, but does not provide additional information.

Duration of the period of normoglycemia preceding secondary failure is negatively correlated with glycemia measured on the morning of the first postoperatory day, *i. e.* 20 hours after transplantation ($r = -0.759$; $p < 0.05$), in the 8 dogs in which no postoperatory complication occurred. All the successes were found in dogs with glycemia lower than 180 mg/dl on day 1 (individual values: 156, 119, 130, 153, 153 mg/dl). When glycemia was higher than this treshold (individual values: 240, 196, 183 mg/dl for the three dogs with no postoperatory complication), failure was always obtained. The values in the dogs which died from postoperatory complications were the following: 363, 200, 301, 250, 145 mg/dl. Neither glycemia measured at other times nor insulinemia were found to be statistically related to the duration of normoglycemia.

No relationship between peroperatory hemorrhage and these early glycemic values was detected.

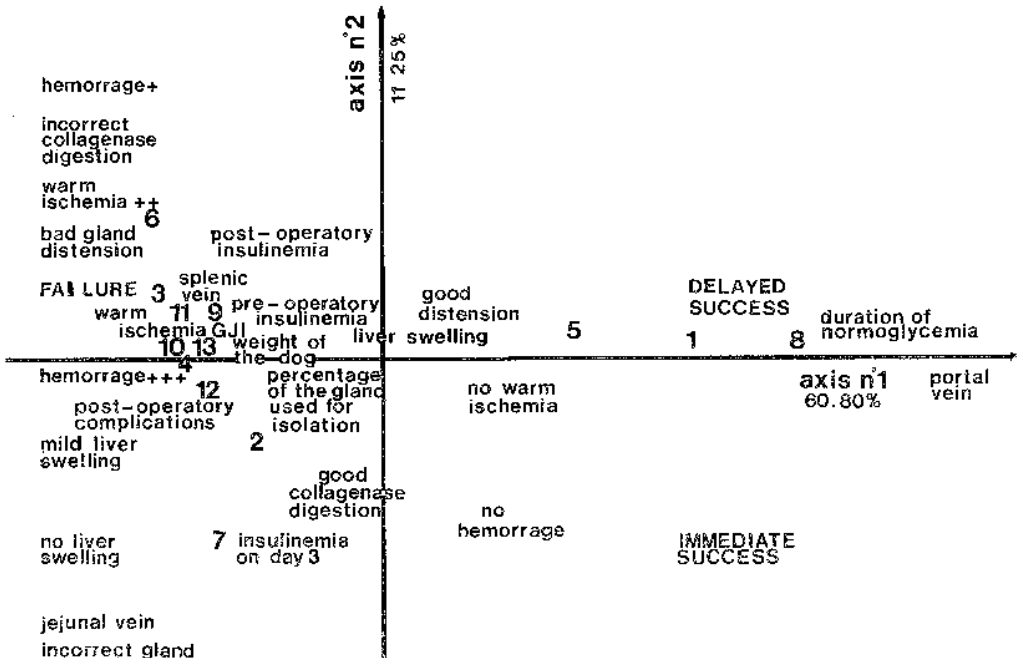

FIG. 3.

Correspondence factor analysis plotting parameters associated with failure of islets autotransplantation. Axis No. 1 opposes success (on the right) and failure (on the left).

DISCUSSION

Pancreatectomy with the technique of Hédon is a classical model of diabetes mellitus in which beta cell function is totally suppressed (3). Consistently with numerous previous reports (4, 7, 9, 10), we find in this study that islets autotransplantation can provide an efficient glycoregulation in dogs totally pancreatectomized with this procedure. Nevertheless, it was clearly found, in our experimental conditions (with reinjection of a reduced mass of endocrine tissue), that successful islet transplantation does not provide a complete prevention of diabetes. Firstly, blood glucose levels, although generally normal in fasting animals, reach pathologic values after carbohydrate intake (Fig. 2). Carbohydrate tolerance does not appear to be normal, mainly when studied with oral glucose tolerance tests. We suggest that such experimental dogs might provide to investigators an original model of diabetes, with markedly fragilized islets, which could be helpful for the study of some diabetogenic factors. However, such a model would be interesting only during the limited period of normoglycemia which follows successful autotransplantation. The second critical point in the metabolic effects of this autograft is that a secondary failure always occurs after several weeks or months, indicating that the pancreatic function remained « fragilized » in those experimental conditions. Notwithstanding the first reports of islets transplantations mainly insisted on feasability and efficiency of this technique (4, 11), number of further studies have pointed out this abnormal function of autografted islets, as well as their increased fragility to stress (8). Loss of innervation probably represents a critical factor for the poor functional quality of this grafted tissue, explaining to some extent its fragility (8). The reduced mass of endocrine tissue reinjected to the subject is probably also an important factor in this process (11). The location of this item on the graph (Fig. 3) is consistent with such an hypothesis.

Other critical points, however, are suggested by our study. The importance of an adequate collagenase digestion has been already pointed out by others (9). Hemorrhage during the dissection of pancreas prior to its removal for islets isolation seems, in our experiments, to be also important. The reasons for this

are unclear, since hemorrhage never exceeded 150 ml and was not likely to induce shock. Was bleeding associated with islets peroperatory damage, or with a lesser ability to receive the graft? This point remains to be elucidated by further investigations. We found no relationship between peroperatory bleeding and postoperatory glycemia.

The inverse relationship between duration of normoglycemia and glycemia on the first postoperatory day could be interpreted as an early manifestation of the functional efficiency of the grafted tissue. However, in one dog, the beginning of a long term normoglycemia occurred with delay, after one week of hyperglycemia ($>$ 200 mg/dl after the 2nd postoperatory day), as shown on Figure 1. Such an evolution was never seen in control dogs. Therefore, it seems more likely that early glycemic levels are a factor influencing islets graft success, or a marker of such factors. For instance, excessive postoperatory stress might be associated with higher glucose levels, and might result by itself in lesser graft success. Concerning a role of glucose by its own on the outcome of islets transplantation, conflicting opinions could be found in the literature. Mosimann (5) postulates that hyperglycemia might represent a functional requirement, therefore stimulating intrasplenic transplanted islets and improving graft results. In contrast, it has been suggested that hyperglycemia could be deleterious for islets function and finally result in beta cell damage (12, 13). Short term hyperglycemia can cause relative unresponsiveness of the beta cell to further increments in glucose concentration (2). Chronic hyperglycemia (4 weeks) reduces the ability of the beta cell to respond to an acute glucose stimulus and induces a state of beta cell « potentiation » to non glucose secretagogues such as arginine (10). However, the current results of our study are by no means conclusive on this point.

In summary, this study shows that autotransplantations of a limited amount of islets tissue in totally pancreatectomized dogs are able to temporarily suppress insulin requirements, but result in a precarious glycoregulation which finally leads into secondary failure. Critical factors in this outcome seem to be manifold, including (*a*) collagenase digestion; (*b*) hemorrhage during pancreas dissection. In addition, glycemia on day 1, which maybe indicates a stress situation during graft fitting, could also, consistently with recent reports, be by its own a factor resulting in beta cell damage. We postulate that such autografts represent a model for the study of nonimmunologic aspects of islets transplantation, and that the problems discussed above can be also relevant, to some extent, when islets are transplanted across an immunologic barrier. Multifactorial studies will possibly be helpful in clarifying some aspects of this complex interactive process. Nonetheless, futher experiments with a greater number of dogs will be necessary to confirm these preliminary findings.

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