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Preservation time dependent morphological changes in cold stored human donor pancreas

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Abstract. Pancreatic transplantation is being used to treat insulin-dependent diabetes. An intact structure of the graft is a prerequisite for preserved function and we therefore monitored the light microscopic and ultrastructural changes in 30 human donor pancreases stored in the cold in University of Wisconsin preservation solution. Twenty-three pancreases were stored for less than 24 h and 7 for more than 30 h. All glands stored longer than 30 h displayed cytoplasmic vacuolisation in a variable proportion of acinar cells. In addition, the glands stored over 40 h showed focal acinar necrosis. Endocrine tissue was only slightly affected, while duct cells showed no changes. It is concluded that cold preserved pancreases stored for less than 24 h are best for transplantation purposes and that acinar cells are more sensitive to ischaemic damage than endocrine and duct cells.

Key words: Donor pancreas – Cold preservation – Acinar cells – Islets – Transplantation

Introduction

Cold preservation of the human donor pancreas has increased the availability of this gland for the treatment of diabetes mellitus by whole organ transplantation. A donor gland is flushed in situ with a cold preservation solution prior to its storage at low temperature for periods up to 24 h (Florack 1988) and transplantated, often in conjunction with a kidney graft, into a diabetic recipient (Robertson 1992). A commonly used preservation solution for all abdominal organs was developed at the University of Wisconsin (UW) (Belzer et al. 1992). This UW solution, introduced clinically in 1987, was specifically designed for use in pancreas transplantation and was shown to allow succesfull grafting of whole glands up to 72 h of cold storage in animal models (Wahlberg et al. 1987). UW preserved pancreatic glands have also been used to make human endocrine islet preparations that were succesfully transplanted to the liver of diabetic recipients (Gores et al. 1993). Biopsies are not usually taken from human pancreatic glands prior to transplantation; as a result little is known about the morphological effects of cold preservation itself, nor is information available about cold storage time dependent changes in cellular morphology. The purpose of the present study was therefore to analyse and monitor the light and electron microscopic changes in a series of human donor pancreases cold preserved in UW solution for periods up to 61 h.

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Materials and methods

Thirty cold (0–4° C) preserved normal human pancreases from multi-organ donors were obtained from European clinical centres participating in the European Concerted Action 'Beta Cell Transplant' which aims at the isolation, purification and transplantation of human beta cell preparations. The organs were flushed and cold preserved with the UW cold storage medium via aortic perfusion before resection of the gland, according to local procedures, and transport on ice. Cold storage times ranged from 8 to 61 h with a mean of 21 ± 3 (SE) h (Table 1). Twenty-three glands were stored for less than 24 h and seven for more than 24 h. Age of the donors ranged from 7 to 53 years with a mean of 30 ± 3 (SE) years.

As part of the procedure for the isolation of pancreatic islet cells a single biopsy ($0.5 \times 0.5 \times 1.0$ cm) was taken from the body of the pancreas, fixed in Bouin's solution, and processed for light microscopy. For electron microscopic analysis, four UW preserved glands (two each with a storage time under and over 24 h) were perfusion fixed for 10 min with ice-cold 2% glutaraldehyde in 0.1 M sodium cacodylate pH 7.4 through a catheter inserted in the coeliac axis. Pieces of tissue from the region of the body were post-fixed in 1% osmium tetroxide, stained en-bloc with uranyl acetate and embedded in plastic.

A semi-quantitative light microscopic evaluation was performed on HE stained paraffin sections. Cytoplasmic vacuolisation of the exocrine tissue (acinar cells) was scored from 0 to 3 (0, no changes; 1, < 10% of cells affected; 2, 10–50% of cells affected; 3, > 50% of cells affected). Necrotic changes were scored from 0 to 3 (0, absent;

Table 1. Clinical data, cold storage time and histological preservation in human donor pancreases

Case no.	Age (years) / Sex	Cause of death	Cold storage time (h.)	Acinar vacuolisation	Acinar necrosis
1	20/M	MULT	10	3	0
2	45/M	SAB	8	0	0
3	32/F	SHT	12	0	0
4	21/F	SHT	11	1	0
5	7/F	SHT	EM–61	1	2
6	33/M	MULT	16	1	0
7	11/F	MULT	22	0	0
8	39/F	SAB	24	0	0
9	21/F	MULT	12	3	0
10	46/M	SHT	12	1	0
11	19/M	SHT	15	0	0
12	8/M	SHT	31	1	0
13	39/M	SHT	11	3	0
14	25/M	MULT	40	2	0
15	44/M	SAB	16	0	0
16	17/M	SHT	16	1	0
17	24/?	MULT	EM–55	3	0
18	32/M	SHT	36	1	0
19	43/F	MULT	42	3	1
20	53/M	Astrocytoma	18	3	0
21	25/M	MULT	23	1	0
22	52/?	SAB	9	1	0
23	49/F	SAB	19	2	0
24	33/F	SAB	38	2	0
25	50/F	SAB	20	0	0
26	40/M	SAB	11	0	0
27	29/F	SHT	15	0	0
28	8/M	Asphyxation	13	2	0
29	44/M	SHT	EM–15	–	–
30	11/M	SHT	EM–12	–	–

SAB, Subarachnoid bleeding; SHT, severe head trauma; MULT, not specified multiple traumata. Scoring system as explained in material and methods section. EM, material perfusion fixed for electron microscopy

1, single focal necrotic lesion; 2, multiple focal necrotic lesions; 3, generalized necrosis).

Results

All pancreases but 1 (29/30) appeared to be well flushed with the UW solution and presented a clear yellow colour on section, although significant haemorrages were occassionally present in peripancreatic fatty tissue. Only 1 organ contained macroscopically visible amounts of blood in the vessels.

Histological analysis of 28 pancreases revealed remaining red cells in the vasculature or thrombi in the acinar and islet tissue on few occasions. All glands showed mild to moderate intra and/or interlobular oedema. In 19/28 glands the parenchyma displayed changes characterized by cytoplasmic vacuolisation present in a variable proportion of the acinar cells. In 2/19 cases there were also necrotic changes of the acinar cells (Table 1). Almost half (9/21) of the glands that were preserved for a period under 24 h showed no morphological changes (Fig. 1a), except for intra or interlobular oedema. The other 12 presented acinar vacuolisation to a varying extent (Fig. 1b). In contrast, all glands that were stored longer than 24 h presented acinar vacuolisation (7/7), sometimes combined with focal necrotic changes (2/7).

Examination of the islets and the duct system revealed no clear cellular changes. Even in conditions in which acinar changes were apparent, the islets or ducts displayed no cytoplasmic vacuolisation of the cells or disruption of their architecture. Only in the two pancreases with foci of acinar necrosis did some of the islet cells (but none of the duct cells) exhibit cytoplasmic vacuolisation. All pancreases presented a normal distribution of insulin and glucagon immunoreactive cells in the islets, and there were no signs of previous islet lesions such as accumulation of extra-cellular amyloid deposits.

Electron microscopic examination of perfusion fixed pancreatic glands (Table 1) revealed no major acinar, duct or islet cell alterations in the two glands cold-preserved for less than 24 h. The exocrine tissue was well-preserved, with only minor vesiculation of the rough endoplasmic reticulum in a few acinar cells (Fig. 2a), while islet and duct cells appeared entirely normal (Fig. 2b). In contrast, in the 2 glands stored for 55 and 61 h respectively, severe vacuolisation of acinar cells was observed, with large vacuoles preferentially located at the apical pole of the cell (Fig. 2c). These vacuoles appeared to fuse with each other and sometimes contained floc-

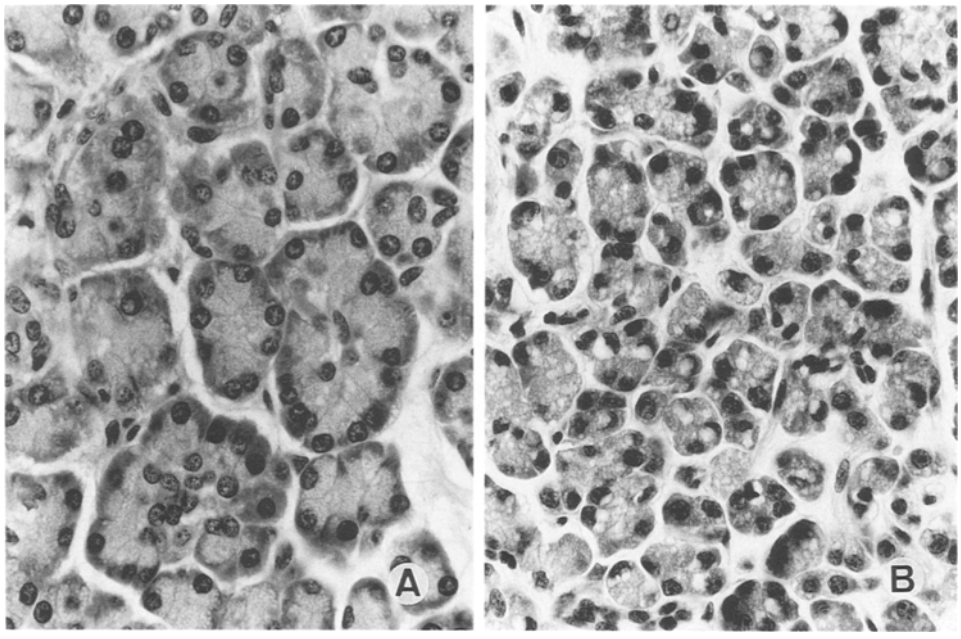

Fig. 1. Human pancreas from a 50-year-old donor is light microscopically well-preserved after 20 h cold storage in University of Wisconsin (UW) solution (**a**; × 250); cytoplasmic vacuolisation is present in more then 50% of acinar cells in a 40 h cold stored pancreas of a 20-year-old donor (**b**; × 250)

culent material and granules of moderate electron density suggesting that they derived from, or fused with, zymogen granules. There were no mitochondrial changes such as swelling and membrane disruption. A variable degree of islet cell damage was present, with some cells displaying a normal morphology and others displaying a discontinuous plasma membrane with cytoplasmic disruptions (Fig. 2d).

Discussion

This study showed that almost half of the human pancreata stored in UW solution up to 24 h are morphologically well-preserved, whereas all pancreases stored for 30 h and more revealed cytological changes. The changes found in the glands stored for less than 24 h were in principle the same as those in glands stored for more than 24 h, and consisted of cytoplasmic vacuolisation of the acinar cells. Endocrine cells remained intact morphologically, indicating that acinar cells are more sensitive to preservation related damage than endocrine cells, a finding also noted previously for the cold preserved canine pancreas (Baumgartner et al. 1980).

As no clear time dependent increase in the presence or degree of acinar vacuolisation was noted before 24 h storage, heterogeneity in pancreatic preservation seems to exist that is not primarily related to cold storage time itself, but is more likely due to other factors. These include the duration of warm ischaemia or differences in the explantation procedure between the various surgical centres; variables that could not be assessed in this study.

All glands stored over 30 h disclosed acinar vacuolisation. Moreover, the 2 glands stored longer than 40 h showed severe damage to the acinar cells with focal necrosis. This indicates that, from a morphological point of view, UW preservation times under 24 h should be suggested for transplantation purposes. It should be stressed, however, that no data exist that clearly link acinar vacuolisation to endocrine function or indeed pancreatic function. As the glands analysed in this study were not transplanted into human recipients, but were used for the isolation and purification of endocrine islet cells that were used experimentally, no correlation could be made between morphology and capacity for metabolic normalization.

The cytological changes in cold preserved glands (cytoplasmic vacuolisation, mitochondrial swelling and vesiculation of the endoplasmic reticulum of the acinar cells) resemble the pancreatic changes described in ischaemic shock (Jones et al. 1975). The significance of these changes for acinar function after transplantation is not clear. They may be reversible (Jones and Trump 1975; Jones et al. 1975) because mitochondrial changes indicating severe ischaemic cell injury (Donath et al. 1970; Nevalainen and Anttinen 1977; Hegewald et al. 1985), were absent. However, progress to irreversible ischaemic damage cannot be excluded, as both morphological and functional changes may become more pronounced after transplantation with exposure of the organ to physiological temperatures (Büsing et al. 1990). Functional changes in human donor pancreas due to cold storage in preservation solutions like UW are difficult to investigate. Studies that analysed the transplant success rate after various cold storage times indicate that no statistically significant difference exists before 24 h of storage. The number of glands transplanted after a storage period over 24 h was too small to draw adequate conclusions but both succesfull and unsuccesful transplants have been reported (Morel et al. 1990). Until more functional data become available, the results of our morphological study warrant caution in the transplantation of donor pancreases cold stored for over 40 h in UW solution and favours the use of glands stored for less than 24 h.

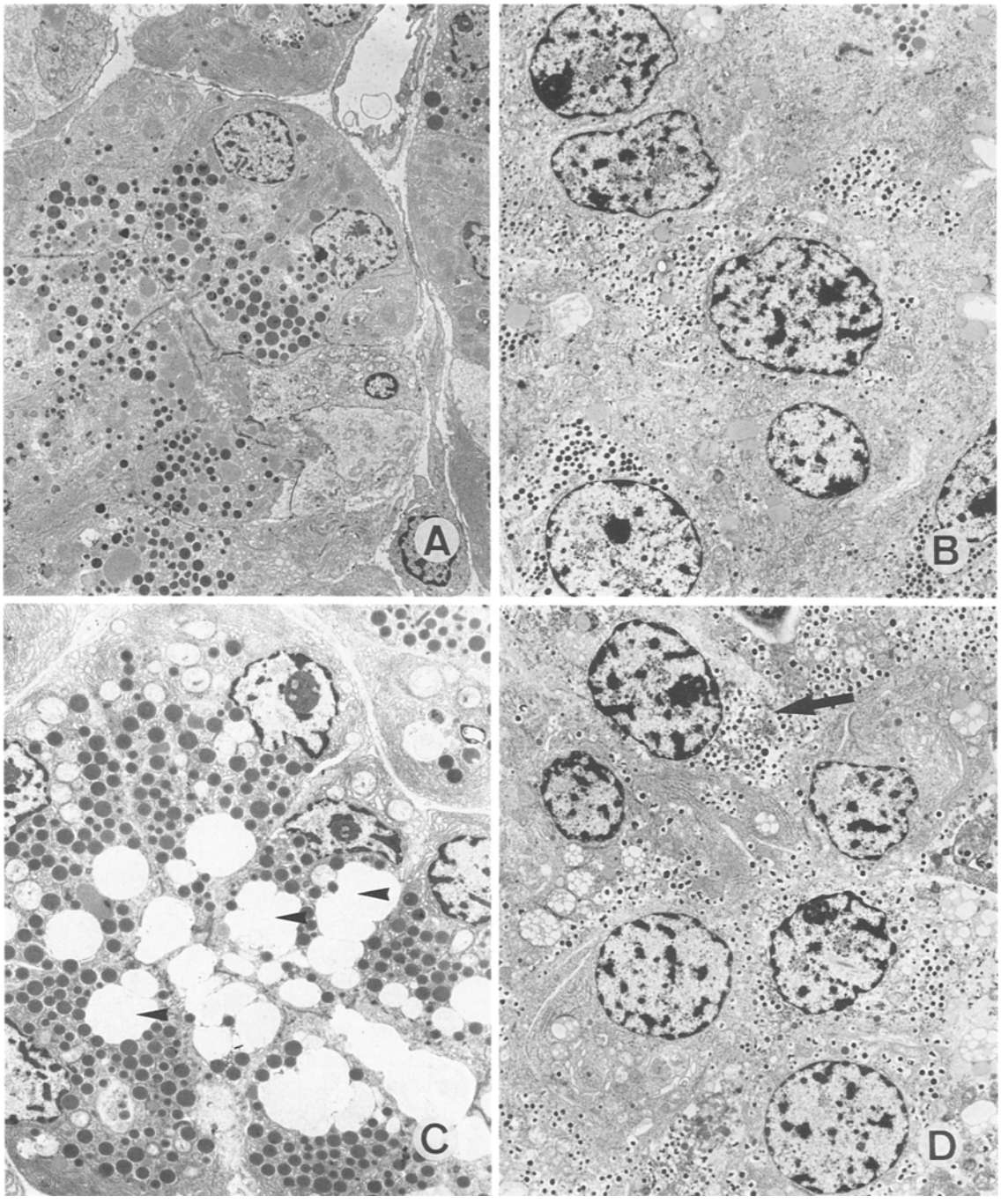

Fig. 2. Electron microscopy of human pancreas stored in UW solution: after 12 h in cold preservation both the acinar (**a**; ×1800) and endocrine (**b**; ×2,600) compartment are well-preserved in the pancreas of a 44-year-old donor. After 55 h of cold preservation the acinar tissue in a 24-year-old donor displays vesiculation and dilatation of the rough endoplasmic reticulum, a marked vacuolisation of the cytoplasm of exocrine cells and apparent fusion of some of the vacuoles *(arrowheads)* (**c**; ×2,200). Endocrine cells are mostly well-preserved but occasionally show signs of substantial cell damage *(arrow)* with discontinuous plasma membranes (**d**; ×2,600)

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