Prolongation of pancreatic islet transplant viability: comparison of preservation methods by phosphorus magnetic resonance spectroscopy

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Introduction: Minimizing pancreatic cold preservation injury is critical for successful islet of Langerhans transplantation. Islets are profoundly susceptible to cold ischaemia and successful functional outcome is associated with shorter hypothermic preservation times **[1]**. Perfluorocarbons (PFC) are biologically inert liquids with high oxygen solubility and release oxygen to surrounding tissues easily. In the two-layer method (TLM) of organ preservation **[2]**, developed to minimize the detrimental effect of cold ischaemia, the pancreas is maintained at the interface between PFC and a conventional preservation solution. This study aimed to develop an experimental model for dynamic assessment of pancreatic energy metabolism throughout preservation using phosphorus (^{31}P) magnetic resonance spectroscopy (MRS). This non-invasive technique has been previously applied to assessment of organ viability **[3, 4]** but only to a limited extent in pancreas **[5]**.

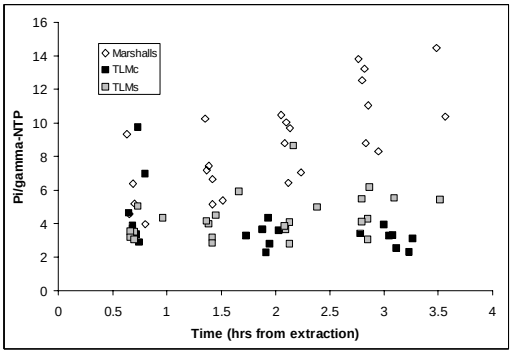
Methods: Pancreases attached to the spleen and duodenum (the latter securely ligated at both ends) were acquired from 64 male Sprague-Dawley rats. Using a 4.7 Tesla Bruker Biospec MRS system ^{31}P spectra were obtained from two pancreases at a time to increase signal-to-noise ratio. Thus the 64 pancreases were used in a total of 32 separate experiments (expts). Pancreases were procured either immediately following anaesthesia (19 expts - immediate cold preservation, ICP) or following 30 min warm ischaemia (13 expts - warm ischaemia, WI). Pancreases were then maintained between 4^{o}C and 6^{o}C in either Marshall's solution (M) alone or by the TLM (Marshall's solution and perfluorodecalin pre-saturated with oxygen). TLM pancreases were assigned to either of 2 further groups: TLM_s – no further oxygen; TLM_c – oxygen for 20 min at 1 hr intervals. Preservation method was randomly assigned immediately following pancreas excision and the numbers of experiments in each group were for ICP 6 M, 6 TLM_S, and 7 TLM_C; and for WI: 6 TLM_S, and 7 TLM_C. Single-pulse acquire ^{31}P MRS (repetition time 10 s, 256 summed free induction decays) was performed repeatedly using a 3-cm diameter surface coil, between 0.5 and 3 hr (ICP) or between 0.5 and 6 hr (WI) post pancreas excision. Spectra were analysed using AMARES **[6]** (jMRUI software **[7]**). The following metabolite peak-area ratios were calculated and plotted against time from extraction: inorganic phosphate (Pi)/γ- nucleotide triphosphate (γ-NTP) and Pi/β-NTP. Linear regressions to the data were performed and metabolite ratio rates of increase were calculated for each experiment.

Results: Mean Pi/γ-NTP and Pi/β-NTP between 0.5 hr and 1 hr from extraction were similar for both ICP (ANOVA; $p > 0.3$ and $p > 0.2$ respectively) and WI ($p > 0.1$ and $p > 0.1$ respectively). The rates of increase of Pi/γ-NTP and Pi/β-NTP are shown in the Table. The figure shows Pi/γ-NTP in the ICP experiments.

Conclusions: To our knowledge, this is the first ^{31}P-MRS study examining dynamic pancreatic NTP changes during cold preservation . Organs preserved in Marshall's solution alone showed rapid Pi/NTP increase: by 3 hr after pancreas excision NTP was almost completely depleted. TLM preservation maintained NTP levels for longer. Our results provide evidence that TLM prolongs rat pancreas viability better than Marshall's solution alone. The present study has shown that continuous preservation-medium oxygenation increases the duration of this beneficial effect and is thus superior to static oxygenation in TLM; Pi/NTP increase with TLM_C was significantly slower than for TLM_S.

	ICP: Rate of increase (hr^{-1})		WI: Rate of increase (hr^{-1})	
	[Pi]/[γ-NTP]	[Pi]/[β-NTP]	[Pi]/[γ-NTP]	[Pi]/[β-NTP]
TLM_C	-0.9 (1.1) ** #	-0.2 (1.9) *	-0.2 (0.2) ##	0.7 (0.9) #
TLM_S	0.5 (0.4) **	0.3 (0.8) *	0.8 (0.6)	5.1 (5.2)
Marshall's	2.6 (0.7)	15.7 (7.7)	---	---

Table: Rates of increase of metabolite ratios. * $P < 0.05$ and ** $P < 0.001$ compared to M. # $P < 0.02$ and ## $P < 0.001$ compared to TLM_S



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