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The effect of organ preservation solutions on kidney tubular and endothelial cells

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Abstract Organ preservation solutions have primarily been tested in whole organ animal models. In the current study, we have examined the effect of commonly used organ preservation solutions on both kidney tubular and endothelial cells. Primary human endothelial and kidney tubular cells were incubated at 4 °C in the following solutions: 0.9 % saline (NS), EuroCollins (EC), University of Wisconsin (UW), or Hank's balanced salts with 5 % polyethylene glycol (PEG). Cell viability was assessed by coloromet-ric measurement of mitochondrial reduction of 3 (4,5-dimethylthiazol-2-yl)-2-,5-diphenyltetrazolium bromide (MTT) to purple 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan. After hypothermic storage, cells were incubated at 37 °C in media with MTT, and the amount of reduced formazan present was quantified. Endothelial cells preserved in PEG displayed the best viability ($P < 0.05$). UW provided better cellular viability than EC or NS ($P < 0.05$). Control endothelial cells preserved in culture media at 37 °C displayed the highest absorbance values ($P < 0.01$).For kidney tubular cells, UW and PEG provided the best cellular protection ($P < 0.05$). Control kidney tubular cells cultured in complete media at 37 °C displayed the highest absorbance values ($P < 0.01$). Although the model presented here was not part of a truly morphological study, it may be more reliable for the rapid assessment of preservation-induced cell injury than models presented in previous morphological studies and may help in the development of improved preservation techniques.

Key words Preservation, tubular cells · Tubular cells, preservation · Endothelial cells, preservation

Introduction

The length of time for which an organ can be preserved plays a crucial role in the widespread sharing of organs. Organs such as the heart, liver, or pancreas cannot tolerate long periods of preservation [2], whereas the kidney has been successfully preserved for a period of up to 60 h. Monolayers of cells culture would provide a system for comparing the effectiveness of the different preservation solutions commonly used. This model may provide more information about events occurring at the cellular level than whole organ preparation. Kidney tubular cells play a major role in the elaboration of the final urine composition. Proximal tubular cells are the prime target in acute tubular necrosis after transplantation. Endothelial cells are in direct contact with the preservation solution during storage. Therefore, tubular and endothelial cells are very susceptible to injury during preservation. Damage to these cells leads to acute tubular necrosis with thrombosis of small vessels, compromising the function of the transplanted kidney. Only a little is known about the effect of hypothermic preser-

Table 1 Components of preservation solutions (*HBSS* Hank's balanced salt solution, *PEG* polyethylene glycol, *HBSS-PEG* HBSS with 5 % PEG)

Components (mM)	EuroCollins	UW	Saline	HBSS-PEG
Na^+	9.3	28	145	137.68
K^+	108	125		5.8
Mg^{++}		5		0.4
Cl–	14	145	145	144.92
HCO3–	9.3			
SO_4–		5		0.4
$P0_4^{-3}$	93	25		0.74
Ca^{++}				1.26
D-Glucose	182			5.5
Raffinose		30		
Lactobionate		100		
Allopurinol		1		
Gluthatione		3		
Adenosine		5		
Pentafraction g/l		50		
PEG				2.78
mosmol/l	310–320	310–320	310–320	310–320
pH	7.2–7.4	7.4	5	7.2–7.4

vation solutions on kidney tubular cells, and most of the studies focus on morphological changes [5].

In the present study, the sensitivity of kidney tubular cells and endothelial cells to different preservation solutions was assessed with an MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay, which has been widely used for testing the sensitivity of tumor cells to chemotherapeutic drugs [3].

Materials and methods

Preparation of cells

Human vascular umbilical vein endothelial cells (HUVEC)

These cells were isolated as described by Jaffe et al. [6] with some modifications. Briefly, the vein was distended with collagenase type IA (Sigma, St. Louis, Mo., USA) and incubated at 37 °C for 15 min. The collagenase solution containing cells was drained into a sterile centrifuge tube. The vein was flushed twice with M199 medium and the effluent collected, added to the cells, and centrifuged. The pellet of the endothelial cells was suspended in endothelial growth medium (EGM; Sanko Junyaku, Osaka, Japan). This medium consisted of MCDB131 supplemented with EGF 10 ng/ml, hydrocortisone, antibiotics (penicillin and amphotericin B), and 10 % fetal bovine serum (Hyclone, Utah, USA). Cells suspended in EGM were cultured on gelatin-coated dishes (Corning, N.Y., USA) at 37 °C in 5 % CO_2 until confluence, which is reached after 4–5 days. Cells were subcultured with trypsin (0.05 %)-EDTA (0.02 %) solution and seeded in gelatin-coated, 96-well plates (Corning). All experiments were performed on cells from second to third passage. These cells are of endothelial origin, as attested by positive immunostaining for factor willebrand (VIII).

Human kidney tubular cells (KTC)

These cells were isolated from normal kidneys that were removed due to renal carcinoma, as reported previously [11]. The cortex was minced in very small pieces in RPMI 1640 medium (Flow, Irvine, Scotland), which were digested using a collagenase type IA solution for 30 min at 37 °C. At the end of the incubation, this mixture was filtered through a 400-mesh stainless steel screen that retains all tissue debris and allows only passage of free cells obtained during collagenase digestion. The solution collected was centrifuged, the supernatant discarded, and the cell pellet washed twice with RPMI. The pellet of cells was suspended in kidney growth medium (KGM), which consists of RPMI 1640 supplemented with glutamine (Flow), hepes buffer (Gibco, N.Y., USA), antibiotics (penicillin and streptomycin solution; Gibco), EGF 10 ng/ml (Wako Chemical, Osaka, Japan), and 10 % FBS. The cells and the media were transferred to gelatin-coated dishes. After 4–5 days of culture at 37 °C in 5 % CO_2, these cells appear as a cobblestone, characteristic of epithelial cells, and become confluent after 8–10 days. Cells were subcultured as described above and seeded in gelatin-coated, 96-well plates. Confluent monolayer cells from third to fourth passage were used in this study. These cells are of epithelial origin, as attested by a positive staining for cytokeratin. These cells are unlikely tumorigenic cells because they cannot grow indefinitely under the culture conditions described above.

Preservation experiments

The media were removed from the 96-well plates containing the confluent monolayers of endothelial cells or kidney tubular cells, and the experimental solutions were applied. Each experimental group consisted of 10 wells of cells. The preservations solutions were applied and the plates, sealed in plastic, were incubated at 4 °C for varying lenghts of time. Control groups consisted of cells cultured in complete medium at 37 °C.

At the end of the incubation period, the preservation solutions were removed and new media (EGM in the case of endothelial cells and KGM in the case of kidney tubular cells) containing 1 mg/ml of MTT (Sigma) were applied. The plates were incubated at 37 °C in the dark for 4 h. At the end of the incubation period, the media and the unreacted MTT were removed, and the form-

azan crystals were solubilized by the addition of 100 µl of DMSO to each well, which gives a better solubilization of formazan crystals. The plates were agitated for 30–60 min and the absorbance was read by a micro-ELISA immunoreader (Nunc, Japan) at a wavelength of 492 nm, which gives an acceptable absorbance value for formazan.

Solutions tested

As shown in Table 1, the solutions tested included: University of Wisconsin (UW) solution (Dupont Pharmaceuticals, Wilmington, Del., USA), EuroCollins (EC) solution (Baxter Healthcare, Deerfield, Ill., USA), 0.9 % saline (NS), and a 5 % solution of polyethylene glycol (MW 15,000–20,000; Sigma) in Hank's balanced salt solution (PEG).

Expression of results and statistical analysis

Absorbances values were entered into a microcomputer and analyzed using a two-way analysis of variance. Values were considered significant when P was below 0.05. Results are the mean of absorbance values (viable cells) ± standard deviations.

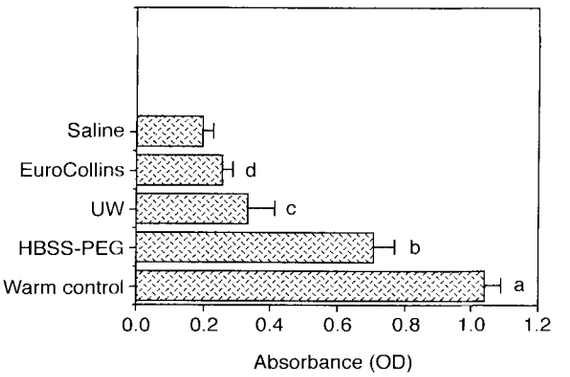

Fig. 1 Absorbance values of formazan crystals produced by endothelial cells after 60 h of preservation at 4 °C in different solutions and 4 h of rewarming. High viability is correlated with high absorbance. There was significantly higher cell viability ($P < 0.05$, ANOVA) of *a* warm control vs all tested solutions, *b* HBSS-PEG vs UW, EC, or NS, *c* UW vs EC or NS, and *d* EC vs NS

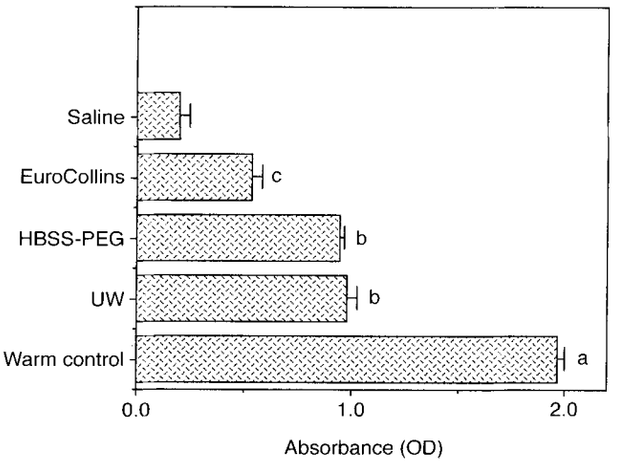

Fig. 2 Absorbance values of formazan crystals produced by kidney tubular cells after 60 h of storage in different solutions at 4 °C and 4 h of rewarming. Higher viability is correlated with higher absorbance. There was significantly higher cell viability ($P < 0.05$ ANOVA) of **a** warm control vs all tested solutions, **b** UW or PEG vs EC or NS. No significant difference was observed between UW and PEG. **c** There was significantly higher viability ($P < 0.05$ ANOVA) of EC vs NS

Results

Preservation of endothelial cells

As shown in Fig. 1, after 60 h of preservation, endothelial cells preserved in PEG solution showed the highest absorbance values (cell viability). The mean absorbance values for cells preserved in PEG were 0.708 ± 0.05, compared to 0.332 ± 0.079 for UW, 0.255 ± 0.03 for EC, and 0.197 ± 0.032 for saline. Endothelial cells cultured in complete medium at 37 °C (warm control) showed absorbance values of 1.037 ± 0.05.

The results, expressed as a percentage of viable cells (by matching with warm control), showed 68 % for PEG, 32 % for UW, 24.5 % for EC, and 19 % for saline. Thus, PEG solution resulted in significantly greater viability than the other solutions ($P < 0.05$, ANOVA). UW was superior to EC ($P < 0.05$, ANOVA). Cells preserved in NS showed the worst viability when compared with the other solutions ($P < 0.05$, ANOVA).

Preservation of kidney tubular cells

Kidney tubular cells cultured in optimal conditions showed very good viablity, as attested by a high absorbance of 1.97 ± 0.03 (warm control). In almost all experiments with the same number of cells, kidney tubular cells showed more mitotic activity than endothelial cells in vitro (1.05 vs 1.97 after 60 h of incubation).

As shown in Fig. 2, after 60 h of preservation, kidney tubular cells preserved in UW and PEG displayed the highest absorbance values: 0.987 ± 0.53 and 0.946 ± 0.02, respectively, compared with 0.532 ± 0.0049 for EC and 0.199 ± 0.039 for saline. The results, expressed as a percentage of cell viability (by matching with warm controls), showed 49.3 % for UW, 47.3 % for PEG, 26.6 % for EC, and 9.9 % for saline. Statistical analysis (ANOVA) revealed that cells preserved in UW or PEG displayed significantly higher viability than all other groups ($P < 0.05$). EC was significantly superior to NS ($P < 0.05$). Thus, cells preserved with NS showed a viability that was significantly worse than all other solutions tested.

Discussion

In this study we used an assay that is commonly used to assess the sensitivity of tumor cells to different chemotherapeutic drugs and as a rapid quantitated viability test for transplanted kidneys [3, 15]. This assay explores cell viability since it is a reduction of MTT to purple formazan, requiring an intact dehydrogenase system in the mitochondria. This attractive assay that screens for cytotoxicity was first used to assess cell viability in cold-preserved cells by Killinger et al. [9].

This study had two objectives. The first was to determine if the endothelial or kidney tubular cells could be used to differentiate injury caused by different preservation solutions. It is more appropriate to test the susceptibility of proximal tubular cells to a given preservation solution, but it was very difficult to separate proximal tubular cells from distal tubular cells using the methods described above. The finding described here demonstrated that UW was superior to EC in the preservation of both kidney tubular and endothelial cells. Similar findings have been reported in the preservation of hepatocytes [13] and myocytes [12]. Compared with Euro-Collins, UW solution prevents hepatocyte swelling, which is associated with endoplasmic reticulum fragmentation and the appearance of swollen mitochondria [7, 13, 14]. However, this solution can induce cellular rounding and shrinkage because it contains two impermeant substances: lactobionate and raffinose [7, 14]. These in vitro findings were confirmed by recent in vivo studies demonstrating the superiority of UW solution over all existing cold storage solutions for preservation of both animal and humans organs [4, 10, 13].

The second objective was to assess the effect of a very well-known cryoprotectant on the cell viabilty of kidney tubular cells compared to endothelial cells. The best cellular preservation in endothelial cells was observed with HBSS 5% PEG. These results are in agreement with data reported recently by Killinger et al. [9]. Marsh et al. [10] also reported that hepatocytes preserved in PEG displayed less edema and released less LDH than cells preserved in conventional organ storage solutions. Daniel and Wakerly [5] first reported in 1976 that kidney cells preserved in PEG displayed less morphological evidence of cytotoxicity.

The mechanism by which PEG improved cell preservation is not clear. PEG interacts with the cell membrane probably by association with its phospholipids, and this interaction may stabilize the membrane [1]. This stabilization may prevent membrane damage from swelling caused by unrestricted ion passage in the face of a nonfunctional sodium potassium-ATPase pump during cold storage.

We did not observe any superority of PEG over UW solution in kidney tubular cell viability, in contrast to endothelial cells in which PEG showed better preservation than UW. There is no clear explanation for this phenomenon, but one may attribute this difference to the structure of the cells. Tubular cells have microvilli that allow very high transmembrane transfers while endothelial cells are without such systems. UW, which may protect the microvilli systems efficiently, can preserve tubular cells better than endothelial cells.

This study certainly has clinical relevance to whole organ preservation since the data presented here are in agreement with results obtained in clinical preservation. Using the model reported in this study, it would be possible to define the mechanisms of preservation injury and to provide an easy method for the screening of agents useful in preservation. However, one should bear in mind that what works for a single cell does not necessarily work for an organ as whole.

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