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Effect of different preservation solutions on adenine nucleotide content and metabolism in human kidney transplantation

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Abstract Differences in purine metabolism produced by three preservation solutions were studied by determining the adenine nucleotide (ATP, ADP, AMP, and IMP) and nucleoside (adenosine, inosine, and hypoxanthine) levels in human kidney cortical biopsies. Forty kidney allografts were studied using University of Wisconsin (UW) solution ($n = 20$), Euro-Collins (EC) solution ($n = 12$), and modified EC solution with mannitol (M; $n = 8$). No significant differences were found between the three solutions studied with regard to ATP, ADP, or AMP changes. The mean ATP level (nmol/mg prot ± SEM) at the end of preservation in the UW group was 2.7 ± 0.3 nmol/mg, in the EC group 3.8 ± 0.7 nmol/mg, and in the M group 2.3 ± 0.4 nmol/mg. ATP 30 min after reperfusion in the UW, EC, and M groups was 5.7 ± 0.8 nmol/mg, 6.4 ± 1.0 nmol/mg, and 4.6 ± 0.5 nmol/mg, respectively. However, an important difference appeared in the catabolic products determined. Kidneys perfused with UW solution had a significantly higher level of adenosine (2.6 ± 0.6 nmol/mg), inosine (11.8 ± 2.2 nmol/mg), and hypoxanthine (18.1 ± 2.1 nmol/mg) at the end of cold storage than those perfused with EC (0.4 ± 0.1 nmol/mg, 2.0 ± 0.8 nmol/mg, and 7.1 ± 1.4 nmol/mg) and M solutions (0.2 ± 0.05 nmol/mg, 0.5 ± 0.1 nmol/mg, and 5.2 ± 0.6 nmol/mg; $P < 0.05$). These levels returned to initial values 30 min postreperfusion and there were no differences with the EC or M solution groups at that time. Thus, the adenosine present in UW solution does not appear to be useful in recovering the adenine nucleotide pool at reperfusion. Moreover, it produces a marked increase in degradation products. Our findings do not support the beneficial metabolic effect of UW solution in terms of adenine nucleotide metabolism in comparison with simpler and less expensive preservation solutions like EC.

Key words Preservation, kidney, human · Kidney, preservation, human · UW solution, kidney Euro-Collins solution, kidney ATP, kidney, human

Introduction

The aim of renal preservation is to minimize organ damage during harvesting, cold storage, and blood reperfusion, especially in the early moments after implantation. The main modality for organ preservation, simple hypothermia, exerts its beneficial effects by curtailing the oxygen demand of the preserved organs [1, 2, 6, 13].

Different preservation media have been proposed and used on the basis of their theoretical capacity to preserve tissue energy stores and to prevent ischemic tissue dam-

Table 1 Characteristics of donors and recipients. Values indicate mean ± SD. No significant differences were found between the three groups of solutions. sCr, Serum creatinine

	M Solution ($n = 8$)	EC Solution ($n = 12$)	UW Solution ($n = 20$)
Donor			
Age (years)	26.0 ± 11.5	29.8 ± 11.0	28.2 ± 15.4
sCr (μmol/l)	115.6 ± 27.2	222.0 ± 103.7	158 ± 87.0
Recipient			
Cold ischemia time (h)	20.0 ± 6.2	27.5 ± 9.1	26.8 ± 7.7
Reanastomosis time (min)	54.6 ± 10.3	56.2 ± 11.5	56.6 ± 8.5

age [12]. Euro-Collins (EC) solution is one of the earliest and most extensively used in many transplant centers that preserve kidneys by cold storage. University of Wisconsin (UW) solution is now widely used for preservation of the liver, kidney, and pancreas in clinical transplantation. A reduced incidence of delayed graft function and increased graft survival in cadaveric kidney transplants perfused with UW solution rather than with EC solution have recently been reported [10]. However, to our knowledge, the potentially beneficial effect of UW solution in reducing the loss of energy content in human kidney allografts has not been studied directly. A modified EC solution containing mannitol (M solution) has been proposed to reduce the incidence of acute tubular necrosis in kidney transplantation [7, 11].

In order to assess the influence of these three solutions on energy metabolism during cold storage and after blood reperfusion, we studied the adenine nucleotide content and the accumulation of its degradation products in cortical biopsies obtained during human kidney transplantation.

Materials and methods

Surgery and human biopsies

Forty kidneys, transplanted in our hospital, were included in this study. Kidneys were obtained from cadaveric heart-beating donors and perfused with one of three different solutions: UW solution ($n = 20$), EC solution ($n = 12$), or M solution ($n = 8$). The characteristics of donors and recipients in the three groups are listed in Table 1.

After perfusion, kidneys were stored cold at 4 °C in their respective solutions until transplantation. The mean cold storage time was 25 h.

Three cortical biopsies were taken from each kidney during transplantation, as described previously [8, 9, 17]. The first (A) was taken during renal harvesting, just before cooling, the second (B) at the end of cold storage, and the last (C) 30 min after blood reperfusion. Biopsies were obtained with Tru-Cut needles (Travenol), obtaining a cylinder of tissue in sufficient quantity for determinations. No immediate or late surgical complications were attributed to these biopsies, and they produced minimal bleeding, stopped with compression or one stitch.

Composition of the media

The composition of the three solutions used was as follows:

Euro-Collins (EC) solution: 10 mM Na^+, 115 mM K^+, 15 mM Cl^-, 10 mM HCO_3^-, 15 mM HPO_42-, 195 mM glucose, pH 7.0, 355 mosmol

Mannitol (M) solution: 10 mM Na^+, 115 mM K^+, 15 mM Cl^-, 10 mM HCO_3^-, 50 mM HPO_42-, 220 mM mannitol, pH 7.4, 400 mosmol

University Wisconsin (UW) solution: 30 mM Na^+, 125 mM K^+, 5 mM Mg^{2+}, 25 mM HPO_42-, 5 mM SO_42-, 30 mM raffinose, 100 mM lactobionate, 5 mM adenosine, 3 mM glutathione, 1 mM allopurinol, 0.02 mM dexamethasone, 0.133 g/l penicillin, 100 U/l insulin, 50 g/l hydroxyethyl starch, pH 7.4, 320 mosmol.

Analytical procedures

Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C. The samples were weighed and homogenized in 120 μl (approximately 1 : 10 w/v) of ice-cooled 6% trifluoracetic acid (Merck) with a glass homogenizer. The homogenates were collected in Eppendorf tubes and centrifuged at 4 °C at 12,000 *g* for 10 min. The precipitates were kept for protein determination. The analysis of the supernatants was performed as described before [17] and according to Zakaria and Brown [19]. Nucleotides and nucleosides were identified and quantified with an HPLC system, using a Spherisorb SAX 5-μm column (25 × 0.4 cm, Tracer) for nucleotides (ATP, ADP, AMP, and IMP), and a Spherisorb ODS 5-μm column (25 × 0.4 cm, Pharmacia) for nucleosides (adenosine, inosine, and hypoxanthine). For protein analysis, the precipitates were digested

Table 2 Functional results. Values indicate mean ± SD. No significant differences were found between the three groups of solutions. DGF, Delayed graft function; sCr, serum creatinine

	M Solution	EC Solution	UW Solution
Incidence of DGF (%)	37.5 (3/8)	8.3 (1/12)	20 (4/20)
Urine volume 1st day (ml)	2495.5 ± 1922.0	3041.5 ± 1482.5	3777.2 ± 3111.6
Urine volume 7th day (ml)	2737.5 ± 1497.6	2505.8 ± 998.4	2402.2 ± 1135.3
sCr (μmol): 1st day	720.0 ± 211.2	623.6 ± 128.3	597.5 ± 178.7
7th day	409.7 ± 360.2	305.7 ± 298.0	264.3 ± 221.8
15th day	278.2 ± 212.8	231.2 ± 165.3	212.9 ± 156.0
30th day	148.4 ± 34.1	155.0 ± 28.3	183.9 ± 165.3
3rd month	140.3 ± 52.7	164.9 ± 68.3	160.2 ± 162.3
6th month	143.8 ± 61.5	150.6 ± 34.5	122.1 ± 29.4

overnight at room temperature with 1 ml of 1 M NaOH and determined according to the Bradford method using bovine serum albumin as standard [4].

Statistics

Statistical comparisons between the groups, depending on the preservation solution used, were performed with the chi-squared test for qualitative variables and with an analysis of variance (ANOVA) for quantitative variables followed, if significant, by Scheffé-based tests. Differences were considered significant when P was less than 0.05.

Results

As Table 2 summarizes, no functional differences were found between the kidneys perfused with UW and EC solutions. The group of kidneys perfused with the mannitol solution showed a higher incidence of posttransplant delayed graft function (DGF), but this was without statistical significance.

No significant differences were seen in serum creatinine levels, rejection incidence, or 6-month allograft survival (data not shown) between the three groups studied.

Levels of nucleotides (ATP, ADP, and AMP) in renal cortical biopsies performed at time of harvest (A), after cold storage (B), and 30 min after reperfusion (C) are shown in Fig. 1. The initial ATP, ADP, and AMP levels were similar in the three groups (A). No significant differences were found between these groups either in the decrease in ATP during cold storage (B) or in the partial recuperation of ATP 30 min after restoration of blood circulation (C). Moreover, there were no differences in the variations in ADP and AMP levels. The total nucleotide pool (ATP + ADP + AMP) fell significantly from A to B and was not recovered in C.

Considering all of the patients as one group, DGF kidneys ($n = 8$) had a significantly lower level of the total nucleotide pool at the end of preservation (B) than non-DGF kidneys ($n = 32$; data not shown). Due to the low incidence of DGF, these differences could not be assessed within the different groups.

During cold storage, cells fail to maintain ATP levels due to the lack of oxygen, and degradation of adenine nucleotides can be seen. This is shown in Fig. 2 (B), where a marked increase in IMP and other products of nucleotide catabolism (adenosine, inosine, and hypoxanthine) is evident. After blood reperfusion (C), tissue levels of these catabolic products returned to their initial values, in agreement with our previous results [17]. The variations in these catabolic products during the three steps of transplantation (renal harvesting, at the end of cold storage, and 30 min after blood reperfusion) are shown in Fig. 2. It is relevant to note that the kidneys perfused with UW solution had significantly higher levels of all of the degradation products, except AMP and IMP, at the end of cold storage than kidneys perfused with EC and M solutions. Xanthine was not detectable or only present in traces. After reperfusion, the levels of these products returned to their initial values and there were no significant differences between the three groups studied.

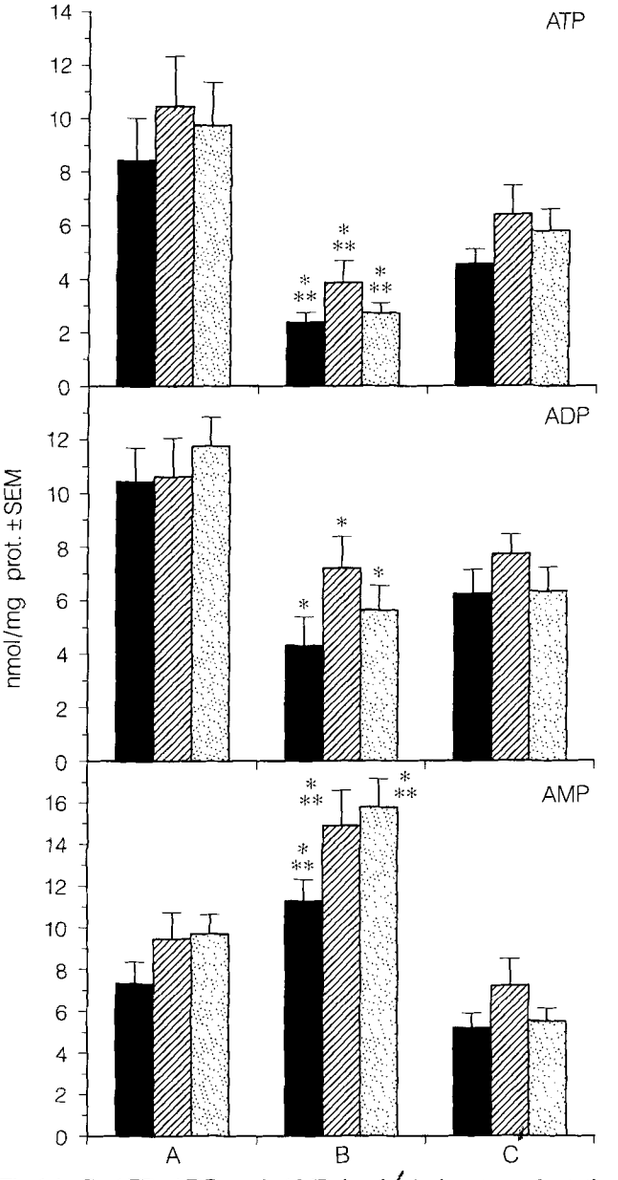

Fig. 1 A–C ATP, ADP, and AMP levels during transplantation using three preservation solutions – mannitol solution (■), Euro-Collins solution (▨), and University of Wisconsin solution (⬚). Biopsies were obtained as follows: **A** at renal harvesting; **B** at the end of cold storage; **C** 30 min after reperfusion. * $P < 0.05$ versus A; ** $P < 0.05$ versus C (both determined via ANOVA). No significant differences were found between the three groups of solutions

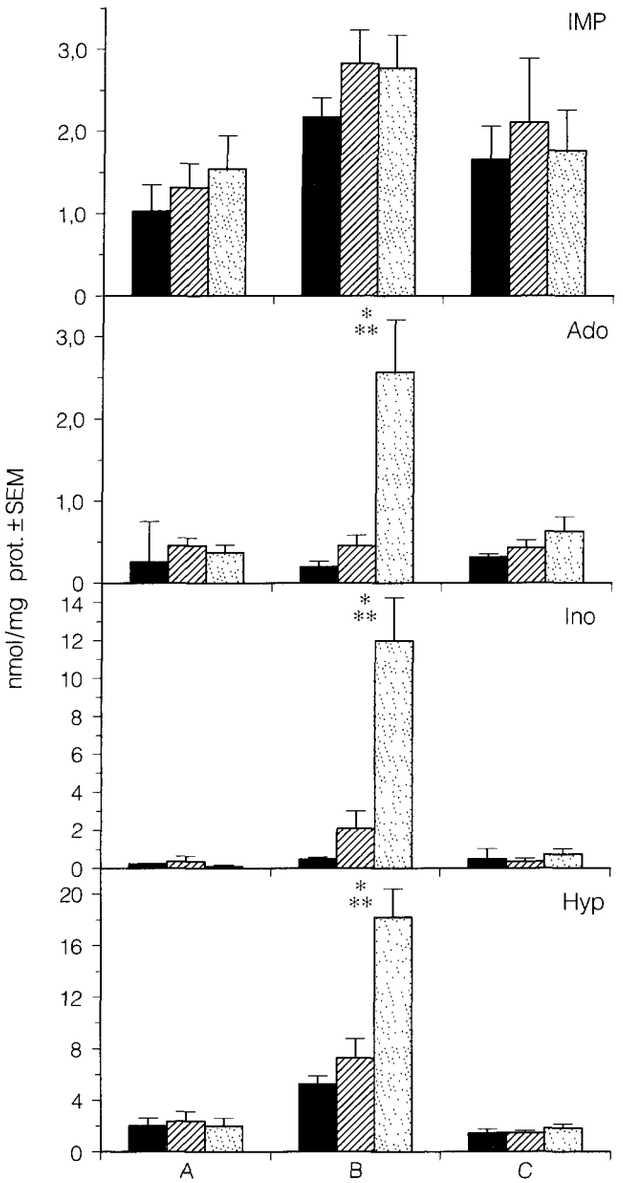

Fig. 2 A–C Changes in nucleotide catabolism products – IMP, adenosine *(Ado)*, inosine *(Ino)*, and Hypoxanthine *(Hyp)* – during transplantation with three different solutions – mannitol solution (■), Euro-Collins solution (▨), and University of Wisconsin solution (⬚). **A, B,** and **C** biopsies as in Fig. 1. * $P < 0.05$ versus EC solution; ** $P < 0.05$ versus M solution (both determined via ANOVA)

Discussion

The three solutions used in the present study represent three different approaches to organ preservation media. Compared to UW solution, EC and M solutions are simple, intracellular electrolyte solutions. EC solution represents a simple, physiological buffer with glucose at physiological intracellular pH and slight hyperosmolarity. As previously reported, there is general agreement that the composition of a preservation medium for kidney storage must prevent intracellular acidosis, minimize hypothermia-induced cell swelling, provide substrates for regenerating high-energy phosphate compounds, and prevent injury from oxygen-free radicals [2]. Thus, the substitution of glucose by mannitol may provide protection against acidosis by lactate. At the same time, mannitol is a potent hydroxyl radical scavenger and may prevent intracellular edema, especially under conditions of high osmolarity (400 mosmol). Indeed, a protective effect on post-transplant renal failure has been described with flush solutions containing mannitol [7, 11]. On the other hand, UW solution contains different components, each of which seems to be important, as suggested by experimental studies, in reducing injury due to simple hypothermic storage and reperfusion [15].

Adenosine has been reported to be an important component of UW solution because of its theoretical capacity to stimulate ATP synthesis by kidney cells after cold storage [3, 5, 15, 18]. Studies using the isolated renal tubular cell model suggest that adenosine enters the cell and can be used for ATP resynthesis after preservation, improving cellular viability [5, 14]. In addition, there is evidence that adenosine enters the cell during cold storage of preserved organs and can be utilized for the regeneration of ATP [15].

In UW solution, raffinose and lactobionate replace glucose, preventing lactate accumulation and maintaining physiological osmolarity. Hydroxyethyl starch is also added to protect the tissue architecture, as has been described for the liver. Glutathione acts as a scavenger of oxygen-free radical metabolites, since reperfusion injury in various organs seems to be related to the generation of oxygen-free radicals and since, during cold ischemia, there is a loss of glutathione from kidneys [15]. Phosphate is also a component of UW solution that is thought to act as an ATP substrate as well as a hydrogen ion buffer [14]. Allopurinol, another component of UW solution, acting as an inhibitor of xanthine oxidase, is included to reduce the reperfusion injury produced by oxygen-free radicals [15].

The results now presented show very little difference between the three media in maintaining and regenerating ATP levels (Fig. 1). The profiles for ATP, ADP, AMP, and IMP (Figs. 1, 2) are very similar; no significant differences were found in the recovery of adenine nucleotides using compounds that protect the tissue from acidosis (mannitol, raffinose) or from oxygen-free radical production (mannitol, glutathione, and allopurinol).

Adenosine is usually considered an essential component of UW solution because it is thought to preserve the energy content of kidney allografts during cold storage. Interestingly, nucleotide degradation products – hypoxan-

thine, inosine, and adenosine – sharply increased in kidneys stored in a solution containing adenosine (UW solution), whereas ATP behavior in the same samples did not differ from the solutions without adenosine (EC and M solutions). It is also important to note that the total nucleotide pool (ATP + ADP + AMP) remained unchanged from biopsies taken at the end of cold storage to biopsies taken 30 min after reperfusion in each of the three groups. This suggests that adenosine is incorporated into the cell in kidneys stored in UW solution, but that the mechanisms leading to its degradation, i.e., adenosine deaminase and nucleoside phosphorylase, are more active than the mechanisms for resynthesizing AMP. Indeed, recovery of nucleotides from nucleosides is energetically unfavorable, since it requires ATP at a time when the nucelotide pool is diminished [18].

The levels of degradation products returned to initial values after blood perfusion (Fig. 2), indicating that these compounds are quickly washed out of the blood circulation. If free radicals were involved in ischemic kidney tissue damage, the accumulation of inosine and hypoxanthine in these kidneys would involve an activation of xanthine oxidase which, under hypoxic conditions, might produce superoxide radicals. Thus, according to our results, the added adenosine may be dangerous for the stored organ rather than serve as a substrate to recover ATP. However, the role of oxygen-free radicals in kidney damage is questionable since the xanthine oxidase activity is very low in human kidneys, as indicated by the minimal production of uric acid, in contrast to other species such as the dog or the rat [9].

It should be borne in mind that other multiple renal effects, including alterations in hemodynamics, hormone and neurotransmitter release, and tubular reabsortion, could be explained by the action of adenosine through specific plasma membrane receptors [16].

In conclusion, our results suggest that UW solution does not represent an improvement in the preservation of human kidneys in terms of adenine nucleotide content in comparision with simpler and less expensive solutions. The metabolism of other abdominal organs, such as the liver and the pancreas, shows certain differences from that of the kidney [2], so the results obtained in this study cannot be extrapolated to the preservation of these organs. Further studies are needed to assess the utility of adenosine in such cases.

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