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A novel dextran 40-based preservation solution

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Abstract Although the University of Wisconsin (UW) solution has become the standard solution for the preservation of kidneys for transplantation, the importance of the colloid hydroxyethylstarch (HES), one of the key compounds of the UW solution, has been questioned repeatedly. It is now established that HES is not necessary for routine kidney preservation. However, colloids may still be advantageous in UW like solutions for the purpose of multiorgan procurements and the preservation of organs from marginal donors. It has been shown in various experimental models that dextran 40 may successfully substitute for HES. Dextran 40 is not only cheaper but also has a variety of biological effects that may be beneficial during the graft reperfusion phase. The aim of this clinical study was to examine the efficacy of a dextran 40-based preservation solution (Dex-PS) for its use in human kidney graft preservation and to compare the transplantation results with kidneys preserved with UW solution. A total of 87 kidneys were preserved with Dex-PS and matched with 87 kidneys preserved with UW solution. Both groups were comparable in terms of donor and recipient characteristics and both had a high proportion of kidneys from non-heart-beating donors. Patient survival and graft survival after 1 year were 95 % and 86 % for the Dex-PS group and 94 % and 90 % for the UW group, respectively (P = NS). Primary nonfunction, delayed graft function, postoperative need for dialysis, and follow-up of serum creatinine were statistically comparable between these two groups. We conclude that dextran 40 can safely replace HES in UW solution for the purpose of clinical kidney preservation. There were no statistically detectable differences in graft performance between the kidneys preserved with UW and those preserved with Dex-PS.

Key words Kidney preservation, dextran 40 · Dextran 40, kidney preservation · UW solution, dextran 40, kidney preservation

Introduction

Since the early days of organ preservation for transplantation, the potential benefits of dextrans on organ preservation have been recognized [10]. Although the beneficial effect of dextrans has been shown repeatedly in different organ systems [3, 29], dextrans have not been used routinely in clinical organ preservation. In the meantime, the preservation of cadaveric liver and pancreas grafts has dramatically improved with the introduction of the lactobionate/hydroxyethylstarch (HES)-based University of Wisconsin (UW) solution. Because of its clinically tested safety, UW solution has become the single flush solution of choice for multiorgan pro-

Table 1 Comparison of the main characteristics of cadaveric kidney donors in the Dex-PS and UW groups. Values represent mean ± standard deviation (range)

	Dex-PS ($n = 48$)	UW ($n = 74$)	P
Age (years)	38.5 ± 15.8	33.5 ± 15.5	0.08
Diuresis during last hour (ml/h)	277 (0–2000)	354 (0–2500)	0.5
Serum creatinine (μmol/l)	96 ± 28.1	96.7 ± 37.4	0.95
Cold ischemia time (hours)	15.1 (3–32)	15.7 (3.5–32)	0.5

curement of all intra-abdominal organs. However, the importance of several components of UW solution, including HES, has been questioned repeatedly.

Colloidal substances in a preservation solution are used to prevent interstitial edema during the cold storage time of an organ [5]. However, experimental and clinical data in routine kidney and liver transplantation suggest that the colloid HES can be safely omitted without replacement of another colloid [4, 6, 11, 12]. Although a colloid may not be essential for short-term preservation of the kidney and liver, it appears to be an important factor in successful pancreas preservation [21]. There is little published data on the safety of preservation fluids without colloids in the context of multiple organ procurement, the use of marginal donors, and conditions of prolonged storage.

Under these circumstances it would be useful to have effective substitutes for HES, given the expense of the patented pentafraction of the HES in the parent UW solution. Under the hypothesis that colloids are advantageous for multiorgan procurements and for the preservation of marginal donor organs such as kidneys from nonheart-beating donors, we have evaluated dextran 40 as a substitute for HES. It has been shown in various experimental models that a modified UW preservation solution containing dextran 40 instead of HES can be used effectively for canine kidney, pancreas, and small bowel preservation [9, 16, 26]. Given the biological properties of dextrans and the fact that dextrans are cheap, there is considerable interest in launching an experimentally proven dextran 40-based preservation solution for clinical use.

The aim of this pilot study was to examine the efficacy of Dex-PS in its clinical use for kidney preservation and to compare the transplantation results with kidneys preserved with UW solution.

Materials and methods

Between January 1990 and August 1993, a total of 262 kidney transplantations were performed at the University of Zurich Hospital, Switzerland. One hundred fifty-nine kidneys were preserved with UW solution, 87 with Dex-PS, and 16 with Euro Collins. Dex-PS was used for kidney and pancreas preservation exclusively and consistently by two members of the retrieving team while three other members of the team used UW solution for kidney and pancreas preservation. This method of allocation was chosen over randomization because of the simplicity of the logistics involved. (Until recently our institution had no full-time transplantation coordinator.) The possibility of a systematic error by choosing this design was deemed minimal since all members of the retrieval team use a uniform, standardized procurement technique. Furthermore, the schedule for retrieval teams was organized in advance on a monthly basis, thereby minimizing the chance of a systematic bias in donor allocation to a study group.

Out of a total of 96 kidneys (48 donor operations) procured with Dex-PS, 9 kidneys were shipped to other institutions and excluded from analysis. The remaining 87 kidneys were transplanted at the University of Zurich Hospital and their recipients represent the study population (Dex-PS group). In eight cases (9.2 %) the kidney was transplanted simultaneously with a pancreas graft, which was also preserved with Dex-PS. In order to create a valid comparison group, each recipient in the Dex-PS group was retrospectively matched to a transplant kidney recipient whose graft was preserved with UW solution (UW group). The matched UW control group ($n = 87$) was selected from 159 consecutive kidney transplant recipients according to the following criteria: same sex, same age group (± 5 years), same number of previous grafts, same period (± 6 months) of transplantation, same type of circulatory status of the donor (heart beating or nonheart-beating). The control group was not matched in terms of side of the transplant or whether the transplanted kidney came from a multiorgan donor. The matching process was performed by using blinded lists of both patient groups consisting only of a patient number and the matching criteria. Thus, 87 kidney recipients whose kidneys were preserved with Dex-PS were matched with 87 recipients whose grafts were preserved with UW solution. Because the matching procedure was primarily based on recipient criteria, there was a higher number of donors in the UW group (74 donors) than in the Dex-PS group (48 donors).

This study was approved by the institutional ethics committee and was performed in accordance with the ethical standards set down in the 1964 Declaration of Helsinki.

Donors

An overview of the main donor parameters is given in Table 1. The procurement technique for heart-beating donors (HBD) and nonheart-beating donors (NHBD) was performed as described previously in detail [7].

Out of 48 kidney donors in the Dex-PS group, 23 had died of head injury, 23 had had a cerebrovascular event, and 2 had suffered brain death after intoxication. A multiorgan procurement was performed in 22 donors; 15 kidneys in this group were procured from NHBD.

In the UW group, 45 out of 74 kidney donors had died of head injury, 27 had had a cerebrovascular event, and 2 had suffered from intoxication. A multiorgan procurement was performed in 45 donors and 10 kidneys were procured from NHBD ($P = 0.28$ for the proportion of NHBD between the two groups).

Recipients

In the 87 patients (28 women) in the Dex-PS group, chronic renal failure was caused by: chronic glomerulonephritis ($n = 41$), diabetes mellitus ($n = 12$), polycystic kidney disease ($n = 6$), chronic pyelonephritis ($n = 5$), glomerulosclerosis ($n = 5$), analgesic nephr-

Table 2 Comparison of the main characteristics of recipients of cadaveric kidneys preserved with Dex-PS and UW solution. Values represent mean ± standard deviation

	Dex-PS ($n = 87$)	UW ($n = 87$)	P
Recipient age (years)	42.3 ± 14.4	38.3 ± 14.5	0.06
First graft / regraft	77/10	76/11	1
Previous hemodialysis / CAPD	67/18	72/14	0.53
Duration of preoperative dialysis (months)	32.2 ± 27.2	35.8 ± 32.5	0.43
Diabetes mellitus / recipients with simultaneous pancreas graft	12/8	13/10	1
Mean HLA – A,B mismatch	2.4 ± 1	2.6 ± 1	0.20
Mean HLA – DR mismatch	0.8 ± 0.6	0.7 ± 0.6	0.43
Maximal titer of panel reactive antibodies (%)	6.7 ± 16.3	15.8 ± 25.9	0.007

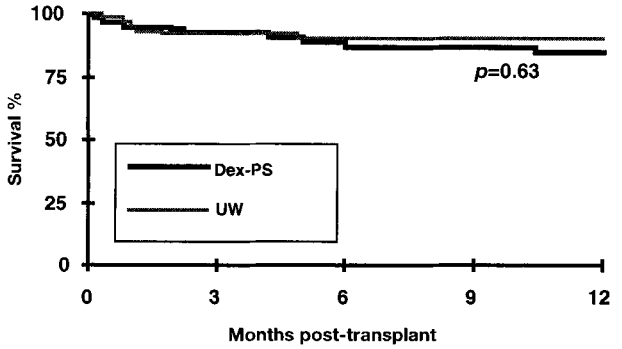

Fig. 1 Comparison of actual graft survival

opathy ($n = 4$), renal hypoplasia ($n = 3$), and various other causes ($n = 11$). Seventy-seven patients received a first graft, nine had a second graft, and one patient had a third transplant.

In the 87 patients (27 women) in the UW group, chronic renal failure was caused by: chronic glomerulonephritis ($n = 25$), diabetes mellitus ($n = 13$), polycystic kidney disease ($n = 9$), chronic pyelonephritis ($n = 7$), Alport's syndrome ($n = 4$), renal hypoplasia ($n = 3$), analgesic nephropathy ($n = 2$), and various other causes ($n = 24$). Seventy-six patients received first grafts, 9 second grafts, and 2 third grafts. . More data comparing the two groups are shown in Table 2. Except for the maximal titer of panel reactive antibodies, there was no statistically significant difference between the two groups.

Recipients of a graft from a HBD received triple immunosuppressive therapy consisting of prednisone, azathioprine, and cyclosporin, and recipients of an NHBD kidney received a modified regimen with induction therapy consisting of ATG, azathioprine, and predisone [9]. In all groups rejection was treated according to the severity with high doses of steroids or monoclonal antibodies.

Preservation solutions

UW solution was purchased from Dupont Pharmaceuticals, Switzerland. Dex-PS was first produced by the local hospital pharmacy and was later produced and generously donated by Pharmacia, Switzerland. The composition of Dex-PS has been described elsewhere in detail [26]. Briefly, Dex-PS is identical to UW solution except that: (1) Dextran 40 (7 g%) replaces HES (5 g%); (2) the Ca^{+} + concentration is 0.5 mmol/l instead of nil, according to a suggestion by McAnulty et al.[15]; and (3) insulin, dexamethasone, and penicillin are not added to it. In order to prevent the oxidation of glutathione, Dex-PS was kept in light, impermeable bags that were also equipped with a carbon absorber. Stability testing showed that this helped significantly to prevent oxidation of glutathione and also to keep lactobionate and allopurinol at stable concentrations, thereby prolonging the shelf life of Dex-PS (Schlumpf R, Habilitation Thesis, University of Zurich, 1993).

Statistics

Renal graft and patient survival were the primary end points. Graft loss was determined by the irreversible loss of transplant function with subsequent permanent need for dialysis. The date of the first permanent postoperative dialysis was defined as the day of graft loss. Primary nonfunction (PNF) was defined as the continued need for postoperative dialysis and nonestablishment of graft function. Delayed graft function (DGF) was determined by the postoperative need for temporary dialysis and eventual establishment of graft function. The diagnosis of rejection was based on such clinical signs as oliguria, hypertension, and fever and was confirmed by histology.

Patient and graft survival rates were calculated using the Kaplan-Meier method. Survival data were compared using the log-rank test. The significance of differences was determined by Student's t-test for comparison of means and by the chi-square test for comparison of proportions. Two-sided P values below 0.05 were considered statistically significant.

Results

Patient survival and graft survival after 1 year were 95 % and 86 % for the Dex-PS group and 94 % and 90 % for the UW group, respectively (Figs. 1, 2). There was no statistical difference in patient survival ($P = 0.43$) or graft survival ($P = 0.63$) between these two groups. A summary of post-transplant follow-up data is given in Table 3. Primary nonfunction, delayed graft function, postoperative need for dialysis, and follow-up of serum creatinine were without significant differences between these two groups (Fig. 3). Major technical complications requiring reintervention were ureteral obstruction ($n = 2$ and 3), iliac lymphoceles ($n = 1$ and 3), and hematoma ($n = 1$ and 1) in the Dex-PS and UW groups, respectively.

In the Dex-PS group, PNF occurred in none of the 87 transplants and DGF was observed in 13 recipients. Seven grafts were lost (after 7, 9, and 24 days and 2, 4, 5, and 11 months) due to irreversible acute rejection.

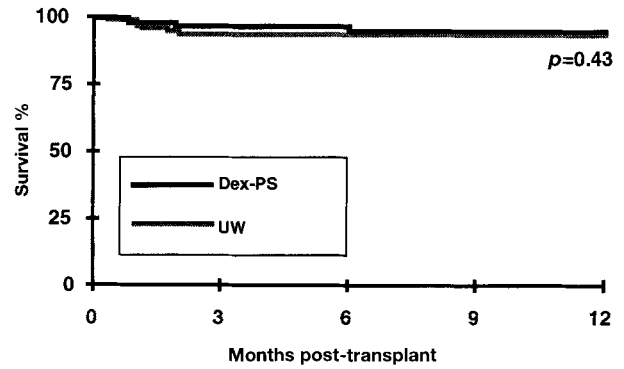

Fig. 2 Comparison of actual patient survival

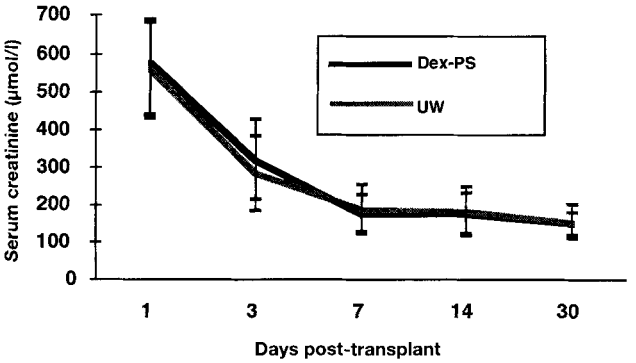

Fig. 3 Comparison of postoperative serum creatinine

Three patients died with a functioning graft (one suicide, one accidental drowning, and one fulminant hepatitis B infection after combined kidney and pancreas transplantation). Of the remaining seven simultaneously transplanted pancreases, six are currently functioning well; one pancreas had to be removed after good initial function was followed by graft thrombosis on day 7 postoperatively.

In the UW group, no PNF was observed and DGF occurred in 17 kidney grafts. Four grafts were lost due to acute rejection (14 and 21 days and 1 and 5 months after transplantation). Five patients died with a functioning graft (three myocardial infarctions, one suicide, and one septic graft infection). Of the ten simultaneously transplanted pancreases, six are currently functioning while one graft had to be removed for graft thrombosis and three recipients died.

Discussion

The introduction of UW solution for organ preservation into clinical practice has contributed considerably to the safe prolongation of cold ischemia time for organ transplants [18, 22, 31]. In particular, the clinical use of UW solution for kidney preservation has shown a positive effect on early transplant function and has reduced the post-transplant dialysis rate [20, 25]. However, UW solution is expensive and it has never been substantiated that all components of UW solution are necessary for its function. It has further been shown that it is possible to omit the additives insulin, penicillin, and dexamethasone from UW solution without any harmful effects [12, 30].

Table 3 Comparison of the outcome after renal transplantation between grafts preserved with Dex-PS and those preserved with UW solution

	Dex-PS ($n = 87$)	UW ($n = 87$)	P
Delayed graft function	13 (15%)	17 (20%)	0.54
Primary nonfunction	0 (0%)	0 (0%)	–
Graft failure due to rejection	7 (8%)	4 (4.6%)	0.53
Patient death with functioning graft	3 (3.4%)	5 (5.7%)	0.71

The value of a colloid in the preservation solution has been judged controversial [1, 2, 12, 26, 27, 30]. On the one hand, the theoretical role of colloids is to prevent leakage of fluid into the interstitial space by counteracting the intravascular pressure [2]. We have previously reported that dog kidneys preserved with colloid-containing solutions showed less edema after unclamping than kidneys preserved with a modified UW solution lacking a colloid [27].

On the other hand, it has been suggested in experimental kidney transplantation in rats [6] and in cold preservation of rabbit livers [12] that colloids are not an essential compound of UW solution. Clinically, this was confirmed by a recent trial in kidney transplantation in which a colloid-free UW solution proved as effective as the original HES-containing UW solution, at least when cold ischemia time remained within 48 h [4]. Thus, the theoretical advantage of colloid-containing solutions may only have an impact in the context of considerably prolonged cold ischemia time or when organs from marginal donors are used. The latter situation applies to our study, where a relatively high proportion of nonheart-beating donor kidneys were transplanted. Moreover, an "all in one solution", i.e., a preservation solution that has proven efficacy for at least all abdominal organs, is desirable in clinical organ transplantation for obvious logistic reasons. It is noteworthy that the pancreas appears to be very susceptible to preservation related edema, and it has therefore been suggested that colloids might be of special importance for pancreas preservation [16].

The mechanism by which dextrans act is thought to be nonspecific in terms of its function as a colloid (i.e., to prevent excessive passage of fluid from the capillaries into the extracellular space). Beyond this function, dextrans have additional properties that might be beneficial for organ preservation and during the subsequent graft

reperfusion. Dextrans are known to reduce erythrocyte aggregation [10] and to improve the removal of blood during the organ washout procedure [13]. Dextrans interfere with platelet adhesion [17, 19, 24] and thereby help to counteract the prothrombotic status that is promoted by organ reperfusion. Furthermore, carbohydrate interactions are involved in the aggregation of neutrophils, and it has been shown that high molecular dextran is able to compete with monoclonal antibody binding to a number of leukocyte adhesion proteins such as L-selectin [23].

There is ample experimental data supporting the use of dextran 40 in preservation solutions. In the canine kidney model, dextran 40 successfully replaced UW solution for 72-h simple cold storage [26]. Additional experimental trials have also shown Dex-PS to be safe and effective in simple cold storage of the canine pancreas [16] and the canine small bowel [9]. A dextran 40-based preservation solution has also been shown to be effective in rat and rabbit liver preservation [2, 3], and various groups have reported safe experimental lung preservation with modified dextran 40-based preservation solutions [8, 14].

Finally, economical aspects may also be important for a preservation solution. In a recent cost effectiveness study in renal transplantation, it was shown that the superior outcome associated with UW preservation compared to Euro-Collins preservation resulted in considerable long-term savings [25]. The question arises, however, of whether the same goal could not be accomplished using a cheap colloid like dextran 40. In our clinical study the experimentally proven properties of Dex-PS were fully confirmed, and Dex-PS proved to be as safe and effective as UW for the clinical preservation of kidney allografts. In the case of preservation of kidneys from NHBD, it is essential that the preservation solution be able to prevent additional damage, in particular to the endothelium, which becomes activated due to the prolonged warm ischemia time [28]. With regard to this crucial test, no difference in outcome was observed between grafts preserved with Dex-PS and those preserved with UW.

Although this study was not specifically designed to examine the effect of Dex-PS on pancreas preservation, our preliminary results show that Dex-PS can also safely be used to preserve pancreatic grafts. These positive findings have encouraged us to further promote the use of Dex-PS in a larger clinical trial. Moreover, a comparative clinical trial between Dex-PS and UW solution in liver transplantation has recently been started.

In conclusion, we proved with this study that dextran 40 can safely replace HES in UW solution for the purpose of human kidney preservation for transplantation. There were no statistically significant differences in graft performance between the kidneys preserved with UW and those preserved with Dex-PS. Encouraged by these findings, the application of Dex-PS in more extensive trials and preservation of other solid organs is proposed.

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