Insulin maintains plasma antioxidant capacity at an early phase of kidney transplantation

Matthieu Monge1, Nelly Ledeme2, Hakim Mazouz1, Jean-Daniel Lalau3, Mona Moubarak4, Claire Presne1, Albert Fournier1, Jean-Claude Maziere2, Gabriel Choukroun1 and Pierre-Francois Westeel1

1Department of Nephrology and Internal Medicine, 2Department of Biochemistry, 3Department of Endocrinology and 4Department of Anaesthesiology, CHU Amiens, INSERM ERI-12 and Jules Verne University, Amiens, France

Abstract

Background. Ischaemia-reperfusion and hyperglycaemia are two main sources of oxidative stress that plays an important role in the pathophysiology of tissue injury in transplant recipients. We hypothesized that controlling hyperglycaemia with insulin during the first hours following kidney transplantation could improve antioxidant defences and therefore decrease ischaemia-reperfusion-induced injury.

Method. We performed a prospective randomized study in non-diabetic dialysed patients receiving a first cadaveric renal allograft, and assigned them to receive either 200 g/day of glucose infusion (control group, n = 23) or the same glucose infusion and intravenous insulin to maintain blood glucose <10 mmol/l (insulin group, n = 20). Antioxidant defences were assessed by the plasma total radical-trapping antioxidant parameter (TRAP).

Results. TRAP values remained stable throughout the study in the Insulin group, whereas they decreased from admission to day 1 (−2.70 ± 0.16 vs −2.98 ± 0.26, P < 0.0001), and tended to retrieve the basal values at day 15 in the control group. TRAP values were significantly higher in the insulin group compared with the control group at days 1 (−2.80 ± 0.19 vs −2.98 ± 0.16, P < 0.05) and 4 (−2.80 ± 0.19 vs −2.95 ± 0.20, P < 0.05). No differences were found between the two groups on urinary malondialdehyde determination, two markers of oxidative damage, nor in graft function or patient outcome.

Conclusions. This is the first clinical trial to demonstrate improvement in insulin-induced antioxidant defences at the early stage of kidney transplantation. More extensive studies will tell if this strategy has beneficial impact in long-term graft outcome.

Keywords: insulin; ischemia-reperfusion injury; kidney transplantation; oxidative stress; reactive oxygen species; total radical-trapping antioxidant parameter (TRAP)

Introduction

Renal injury following the early phase of kidney transplantation may be caused by numerous factors, including ischaemia reperfusion, toxins and immunological processes [1]. Ischaemic damage is responsible for a large share of the high incidence of delayed graft function (DGF) and the increased incidence of acute rejection [2]. It also affects the risk of the development of chronic transplant nephropathy.

There is growing evidence in favour of the involvement of reactive oxygen species (ROS) in addition to a number of other factors, including vasoactive peptides, eicosanoid production and growth factors, in the pathophysiology of ischaemia-reperfusion-induced tissue injury (IRI) after transplantation [3]. During the ischaemic phase of the allograft, cells become ATP-depleted, leading to hypoxanthine accumulation, increased intracellular calcium concentration and changes in xanthine dehydrogenase determination, two markers of oxidative damage, nor in graft function or patient outcome.

Conclusions. This is the first clinical trial to demonstrate improvement in insulin-induced antioxidant defences at the early stage of kidney transplantation. More extensive studies will tell if this strategy has beneficial impact in long-term graft outcome.
nephropathy. Oxidative stress is present in diabetic patients, as reflected by the negative correlation between glycosylated haemoglobin and erythrocyte glutathione content [8], or the higher capacity of plasma from type 2 diabetes patients to peroxidize lipids [9]. Experimental studies have shown that inhibition of the mitochondrial respiratory chain in endothelial cells cultured in high-glucose media reduces ROS production and advanced glycation end-product (AGE) synthesis [10].

Renal transplant recipients frequently present hyperglycaemia during the first 72 h due to the use of high-dose steroid pulses and glucose infusions, and acute hyperglycaemia at the early stage of kidney transplantation is associated with an increased risk for allograft rejection whether recipients are diabetic [11] or not [12]. We therefore hypothesized that controlling acute hyperglycaemia by insulin infusion at the early stage of kidney transplantation could decrease oxidative stress and finally reduce ischaemic-reperfusion injury. To test this hypothesis, we conducted a single-centre, randomized, prospective, open-label, pilot study in which, immediately after the transplant surgical procedure, patients were randomized to receive glucose infusion only, as in our regular protocol (control group), or treatment with intravenous short-acting insulin in addition to glucose infusion (insulin group). Oxidative stress was assessed by the plasma total radical-trapping antioxidant parameter (TRAP) [13] and urinary malondialdehyde (uMDA) prior to surgery and at days 1, 4, 11 and 15 post-transplantation.

Patients and methods

Study design

All non-diabetic dialysed patients receiving cadaveric kidney transplantation, between July 2002 and September 2003, were included in this pilot study. After inclusion, patients were randomized according to a randomization table developed by the Biostatistical Department in Necker Hospital to receive 200 g/day of glucose infusion (11 of G20%) for 48 h following surgery as usually performed in our institution (control group) or the same glucose infusion and intravenous short-acting insulin (Actrapid®, NovoNordisk, Denmark) infusion in order to maintain blood glucose between 7 and 10 mmol/l (insulin group). This treatment was started at the time of surgery and was continued until patients returned to their normal diet. These amounts of glucose are used to avoid hypoglycaemia before a normal diet is allowed, and to provide 800 calories a day. The study was approved by our institutional ethics committee and all patients gave their written informed consent before entering the study.

Glycaemic control

Intravenous insulin infusion was adapted to maintain a glycaemic target of 7–10 mmol/l to avoid the risk of hypoglycaemia in the insulin group. Hourly capillary glycemic monitoring was performed in all patients during the first 18 h, then every 4 h until day 2, and finally three times a day, ensuring similar medical care in the two groups. When blood glucose values exceeded 22 mmol/l in the control group, the amount of glucose infused was decreased, but no insulin was administered to these patients.

Total radical-trapping antioxidant parameter (TRAP) determination

TRAP reflects the global plasma antioxidant capacity, resulting in the synergy of all extracellular antioxidants. The assay technique was described by Ghiselli et al. [13], and has been used elsewhere as a marker of oxidative stress in critically ill patients [14], type 2 diabetic patients [15] and dialysis patients [16]. Briefly, a blood sample was taken in an EDTA tube and immediately transferred to the laboratory for centrifugation or, if necessary, stored at −80°C for a maximum of 48 h. Thermal decomposition of 66 mM 2,2'-azobis-(2-aminopropane) dihydrochloride (ABAP, Sigma®, St Louis, MO, USA) leads to the production of peroxyl radicals that decrease spontaneous fluorescence emission of R-phycoerythrin (R-PE; Sigma®, St Louis, MO, USA), according to a first-degree slope of decay. After 1/10 dilution, the addition of plasma containing an unknown amount of antioxidants results in improvement of R-PE fluorescence decay. The slope of fluorescence decay is directly related to the total plasma antioxidant capacity, and a decreased value of this slope indicates a reduction in the global antioxidant capacity of plasma. Because of the possible bias induced by an additional experiment, we did not use Trolox to express TRAP as a concentration. TRAP was measured on the day of admission, and on the 1st, 4th, 11th and 15th postoperative days.

Malondialdehyde measurement

MDA is one of the main polyunsaturated fatty acid peroxidation end-products. Urine MDA determination was accessed by the technique described by Yagi [17], and referred to as the TBARS technique on 24 h urine samples at the same time as blood sampling for TRAP determination. MDA values at day 4 reflect MDA excretion from day 3 to day 4. MDA, when mixed with thiobarbituric acid in a stoichiometric proportion of 1:2 at a high temperature (90°C) and in an acidic medium, leads to the formation of spontaneously fluorescent adducts that can be quantified using a Shimadzu RF5301PC® spectrofluorometer (ex: 515 nm; em: 535 nm). Comparison of the fluorescence with a standard MDA curve allows the values to be expressed as μmol/l.

Immunosuppressive treatment

All randomized patients received induction treatment with either Thymoglobulins® (IMTIX SangSat, France), or basiliximab antiIL2 receptor antibody (Simulect®, Novartis Europharm, Basel, Switzerland), steroid infusion consisting of two 250 mg bolus injections before and after surgery followed by oral treatment tapered to 10 mg per day on day 60, calcineurin inhibitor ciclosporin A (CsA) (Neoral®, Novartis Europharm, Basel, Switzerland)
and mycophenolate mofetil (CellCept®, Roche, Basel, Switzerland). CsA was introduced when serum creatinine was <250 μmol/l, i.e. after an average of 3.3 days ± 1.9 in the overall population; this interval was similar in both groups.

**Statistical methods**

Continuous variables were compared using the Mann–Whitney U-test, and qualitative variables were compared by Fisher’s exact F-test. To evaluate the effects of blood glucose on TRAP values, we first correlated TRAP values by simple regression to the amounts and duration of glucose infusion the amounts and duration of insulin infusion, the mean blood glucose during the period before TRAP determination and the blood glucose at the time of TRAP determination. The initial population was separated retrospectively according to the observed median blood glucose (9.3 mmol/l) at the first two postoperative days. Two groups were defined as follows: group A, mean blood glucose ≤9.3 mmol/l, and group B, mean blood glucose >9.3 mmol/l. The effects of blood glucose on TRAP values were therefore evaluated irrespective of the use of insulin. A P-value ≤0.05 was considered statistically significant. All calculations were performed with StatView® 5.0 software, SAS Institute Inc., USA.

**Results**

**Patient characteristics and laboratory findings**

Forty-three consecutive transplanted patients (23 males, 20 females) were enrolled in this study with a mean age of 47 ± 15 years. After randomization, 23 patients received intravenous glucose (control group) and 20 received glucose infusion and short-acting intravenous insulin infusion (insulin group). Insulin and control groups were similar for all variables studied. Panel reactive antibodies (PRA) were 33% in one patient and 15% in another in the insulin group, and 10% in one patient in the control group, while all other patients presented a very low immunological risk with no detectable PRA before transplantation.

The initial renal disease consisted of chronic glomerulonephritis in 50% of cases, polycystic kidney disease in 16% of cases, malformative uropathy in 12% of cases, chronic interstitial nephropathy in 5% of cases, miscellaneous in 7% of cases and unknown in 9% of cases. Patients with diabetic nephropathy were not included in this study because of the frequent need for insulin at this stage of transplantation in these patients. Laboratory parameters on admission, including antioxidant defences (vitamins A and E, beta-carotene), pro-oxidant (serum iron), lipids (triglycerides and total cholesterol), HbA1c and PTH values were similar in the two groups. Three patients were treated with allopurinol while on dialysis, two in the control group, and one in the insulin group. Demographic, clinical and laboratory data are summarized in Table 1.

**Treatment**

The mean quantity of glucose infused in the whole population was 486 ± 121 g over 78 ± 26 h. Amounts and duration were similar in the control and insulin groups (472 ± 126 g over 80 ± 30 h vs 504 ± 116 g over 75 ± 20 h, P = NS). The mean insulin infusion was 102 ± 72 IU over 44 ± 23 h in the insulin group. In the control group, no patients received insulin. Besides, some patients had glycaemic values exceeding 22 mmol/l; therefore, glucose infusions were decreased, and so were the mean values for glucose infusion. In both the control and insulin groups, glucose was infused until the patient returned to a normal diet. These times were different for each patient, explaining

<p>| Table 1. Patients’ demographic characteristics and laboratories parameters on admission day |
|-----------------------------------------------|-----------------|-----------------|---------|</p>
<table>
<thead>
<tr>
<th>Control group (n = 23)</th>
<th>Insulin group (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient’s age (years)</td>
<td>46.2 ± 15.0</td>
<td>46.9 ± 15.0</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>12/11</td>
<td>12/8</td>
</tr>
<tr>
<td>Current smoker (n)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>69.1 ± 12.7</td>
<td>71.2 ± 13.1</td>
</tr>
<tr>
<td>Cold ischaemia time (h)</td>
<td>18.45 ± 4.6</td>
<td>19.23 ± 7.3</td>
</tr>
<tr>
<td>Warm ischaemia time (min)</td>
<td>48 ± 11</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>Duration on dialysis (weeks)</td>
<td>274 ± 223</td>
<td>217 ± 223</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>148 ± 25</td>
<td>141 ± 23</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 13</td>
<td>80 ± 15</td>
</tr>
<tr>
<td>HLA mismatches (n)</td>
<td>3.6 ± 1.4</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Donor’s age (years)</td>
<td>43 ± 15</td>
<td>42 ± 13</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.2 ± 1.6</td>
<td>11.8 ± 1.1</td>
</tr>
<tr>
<td>PTH (ng/ml)</td>
<td>296 ± 332</td>
<td>260 ± 204</td>
</tr>
<tr>
<td>Vitamin A (μmol/l)</td>
<td>9.74 ± 3.25</td>
<td>10.02 ± 3.05</td>
</tr>
<tr>
<td>Vitamin E (μmol/l)</td>
<td>30.16 ± 10.83</td>
<td>31.54 ± 8.06</td>
</tr>
<tr>
<td>Beta-carotene (μmol/l)</td>
<td>0.80 ± 0.56</td>
<td>0.77 ± 0.67</td>
</tr>
<tr>
<td>Serum iron (μmol/l)</td>
<td>11.8 ± 4.0</td>
<td>11.2 ± 5.6</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6 ± 1.5</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.47 ± 0.76</td>
<td>1.59 ± 0.56</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.91 ± 0.40</td>
<td>4.95 ± 0.64</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
the difference between theoretical and practical glucose infusions realized. Data on glucose and insulin infusions are summarized in Table 2.

On admission, mean blood glucose was 4.9 ± 0.6 mmol/l in the control group and 5.1 ± 0.6 mmol/l in the insulin group, but did not always correspond to fasting values. As expected, blood glucose values were significantly higher in the control group than in the insulin group during the first two days, and no hypoglycaemic episodes were observed in the insulin group. Data on blood glucose control are summarized in Table 3.

TRAP values

The TRAP was used as the main marker of oxidative stress in this study. All patients had a TRAP measurement during follow-up throughout the study at the expected time. Figure 1 shows the effect of insulin on TRAP values. In the insulin group, TRAP values remained unchanged throughout the study, whereas they decreased in the control group from admission to day 1 (−2.70 ± 0.16 vs −2.98 ± 0.26, \( P < 0.0001 \)) and tend to retrieve the basal values at day 15. Moreover, at days 1 and 4, TRAP values were significantly lower in patients not receiving insulin than in the group of insulin-treated patients (day 1: −2.98 ± 0.26 vs −2.80 ± 0.19, \( P < 0.05 \); day 4: −2.95 ± 0.20 vs −2.80 ± 0.19, \( P < 0.05 \)). We did not find any significant correlation between improvement of antioxidative defences and amounts and duration of insulin infusion, amounts and duration of glucose infusion and mean blood glucose before TRAP determination (data not shown). In order to confirm the effect of insulin on TRAP values, we subdivided the initial population according to the observed median blood glucose values on the first two postoperative days (9.3 mmol/l) (see ‘Patients and methods’). Amounts and duration of insulin infusion were similar between these two groups (data not shown). The observed difference at days 1 and 4 disappeared, indicating a non-glycaemic effect of insulin on TRAP values (Figure 2). Ciclosporin dosages, as well as residual and C2 values, were similar in both groups during the study period. We didn’t find any correlation between residual or C2 values and TRAP, therefore, it is unlikely that this drug could have influenced the TRAP values in our study.

Urinary malondialdehyde determination

As a marker of lipid oxidative damage, uMDA was measured on 24 h urine samples. In the overall population, uMDA was detected at day 1, and remained stable along the study period. No difference was observed between the two groups, according to the presence or absence of insulin therapy (Figure 3) or observed median blood glucose (data not shown).

Graft function and outcome

This pilot study was designed to evaluate the effects of blood glucose control by insulin during the early phase of kidney transplantation on plasma antioxidant capacity. We also recorded the plasma creatinine,
and glomerular filtration rate (GFR) estimated according to the Cockcroft and Gault formula. At 6 and 12 months posttransplantation, renal function was similar in both the groups (Table 4).

We also collected information on early complications and rejection. One patient experienced early and transient acute tubular necrosis, two patients presented posttransplantation diabetes mellitus, one developed CMV infection, one had an episode of biopsy-proven acute rejection during the study follow-up, and the need for haemodialysis concerned two patients in group 2 (three sessions for one patient and one session for the other one); none of these parameters were different between the two groups.

Discussion

This prospective study confirms that, following kidney transplantation, markers of oxidative stress increase during the first 24 h, and shows for the first time that insulin infusion was able to maintain the plasma antioxidant defences. This effect seems to be independent of glycaemic control, since no correlations were found between TRAP, a marker of oxidative stress, and blood glucose or the amounts of glucose infusion.

Reperfusion following cold ischaemia is known to play an important role in the pathophysiology of DGF and, in the long term, the development of chronic transplant nephropathy. Oxidative stress is usually assessed in clinical studies by measuring the activity of individual endogenous antioxidant parameters or plasma levels, or by evaluating the beneficial effect of an exogenous antioxidant. Due to the self-renewal capacity of plasma antioxidants [18], we quantified global plasma antioxidant power in this study, by measuring plasma TRAP.

TRAP determination has been previously used in various clinical settings, such as type 1 [19] and type 2 diabetes [15], haemodialysis [16] or systemic inflammatory response syndrome [14]. Two studies have assessed plasma antioxidant capacity in kidney transplantation [20,21]. In these studies, TRAP was measured once in each patient and the values were compared between groups, but this parameter was not evaluated prospectively. Oxidative stress was measured 6 months after transplantation and the authors found a correlation between oxidative stress and smoking, serum homocysteine, dyslipidaemia and chronic allograft nephropathy.

In our study, we found a decrease in TRAP values from day 1 after surgery, indicating consumption of plasma antioxidant defences following graft reperfusion. TRAP then slowly increased until day 15, without reaching the values observed before surgery. This decrease in TRAP was only observed in the control group, as TRAP values remained unchanged in patients receiving intravenous insulin. In our patients, blood glucose values peaked early after graft surgery in both groups, but at a significantly lower level on day 1 in patients treated with intravenous insulin. Blood glucose levels were higher than 5.5 mmol/l during the first four days of transplantation, and its control was not optimal in the insulin group, as we wanted to
avoid hypoglycaemia during this posttransplantation period. In an attempt to explain the difference in oxidative stress level between the two groups, we tried to correlate TRAP values with the blood glucose level at the time of TRAP determination, the mean blood glucose during the 24 h preceding TRAP determination, and the amount and duration of glucose infusion. No correlations were found between TRAP values and blood glucose, or amounts and duration of glucose infused during the first 72 h following surgery. Surprisingly, when the population was subdivided according to median blood glucose (9.3 mmol/l), TRAP values displayed a similar course, suggesting that the observed effect is probably linked to insulin infusion rather than glycaemic control.

There is growing evidence that hyperglycaemia, irrespective of the presence or absence of pre-existing diabetes, is associated with a poorer prognosis in acutely ill patients, after myocardial infarction [22] or stroke [23]. It is unclear whether the beneficial effects of achieving glycaemic control by insulin are related to the blood glucose level or to insulin itself. Van den Berghe et al. [24], in surgical intensive care patients, showed that glycaemic control had a protective effect on overall survival, irrespective of pre-existing diabetes. In diabetic patients, DIGAMI study also showed that tight glycaemic control at the acute phase of myocardial infarction resulted in a 21% reduction of total mortality [25]. In kidney transplantation, Thomas et al. showed a link between perioperative hyperglycaemia and acute rejection in diabetic [11] and non-diabetic [12] kidney recipients. In these studies, the authors did not use induction treatment and an abnormally high percentage of acute rejection was noted.

Apart from its effects on glycaemic control, insulin is known to regulate expression of various genes and has demonstrated antiapoptotic effects on cultured hepatocytes [26], and ischaemic mice hearts [27]. These effects are mediated by a decrease in oxidative stress and activation of PI-3 kinase and MAP kinase pathways [26]. In a rat model of cerebral ischaemia, Voll and Auer [28] showed attenuation of ischaemic brain damage (seizure rate and neuronal necrosis) after early insulin infusion, independently of its effects on blood glucose. Insulin is involved in the regulation of fatty acid metabolism. It has inhibitory effects on lipolysis and leads to a reduction of free fatty acid (FFA) production. FFA metabolism maintains free oxygen species generation, as their conjugated double bonds can interact with hydroxyl radicals and hydrogen peroxide [18]. Lowering plasma FFA levels might therefore decrease ROS production and improve antioxidant capacities. This effect could explain the benefit of glucose–insulin–potassium therapy in acute myocardial infarction [29]. The lack of correlation between blood glucose and TRAP values in our study is an argument in favour of the direct action of insulin on improvement of plasma antioxidant defences. Unfortunately, plasma insulin was not assessed in our study; this would have been an interesting parameter to correlate with antioxidant defences.

Because the oxidative stress associated with IRI is largely due to the xanthine oxidase pathway, previous prescription of allopurinol was taken into account in our population. An effect of allopurinol is unlikely to explain our results and the correlation between TRAP values, as a similar number of patients took this drug in the insulin and control groups.

The other parameter used in this study to assess oxidative stress was uMDA, which represents the final product of lipid peroxidation [18]. Previous studies have used uMDA to assess plasma oxidative stress [30]. However, to our knowledge, only one group has evaluated MDA in the urine of kidney transplant recipients [31]. These authors showed that uMDA peaked 10 days after an uneventful kidney transplantation. In our study, uMDA was detected on day 1, and uMDA excretion remained stable until the patient’s discharge at day 15. No peak was observed around day 10, but follow-up was different from that used in Romero’s study. No difference was observed in uMDA values according to insulin prescription. This might be explained by the method used for uMDA determination [17], as this technique takes into account not only lipid oxidative metabolism products but also amino acids and glucose. As all patients presented hyperglycaemia, glycosuria may have interfered with MDA determination.

Finally, in this study, no clinical differences, such as graft function were observed between the two groups. However, this was not the main purpose of our study. This prospective pilot study is the first clinical trial to suggest a beneficial effect of insulin on oxidative stress by maintaining plasma antioxidant defences at the early stage of kidney transplantation. This effect seems to be independent of glycaemic control. We believe that further studies are necessary to evaluate the exact mechanism of this protective effect and its clinical implications.

Acknowledgements. The authors are indebted to Prof. P.M. Ronco for his advise, Dr P. Levy for his help in statistical analysis, Ms N. Lyon, V. Marcheux and Mr J. L. Fillebeen for their technical assistance, and Dr A. S. Monge for recording data.

Conflict of interest statement. None declared.

References

2. Tilney NL, Guttmann RD. Effects of initial ischemia/reperfusion injury on the transplanted kidney. Transplantation 1997; 64: 945–947
Insulin as an antioxidant in renal transplantation


8. Jain SK, Mc Vie R. Effect of glycemic control, race (white versus black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. Metabolism 1994; 43: 306–309


Received for publication: 23.7.06
Accepted in revised form: 17.11.06