Transpl Int (1996) 9: 201–207

ORIGINAL ARTICLE

Oğuzhan Büyükgebiz
A. Özdemir Aktan
Gonca Haklar
A. Süha Yalçin
Cumhur Yeğen
Rifat Yalin
Z. Sevim Ercan

BQ-123, a specific endothelin (ET_A) receptor antagonist, prevents ischemia-reperfusion injury in kidney transplantation

Received: 29 May 1995
Received after revision: 14 November 1995
Accepted: 21 November 1995

O. Büyükgebiz[1] · A. Ö. Aktan (✉)
C. Yeğen · R. Yalin
Department of General Surgery,
Marmara University, School of Medicine,
Marmara University Hospital,
Altunizade,
TR-81 190 Istanbul, Turkey
Fax: + 90 0216 325 0323

G. Haklar · A. S. Yalçin
Departments of Biochemistry,
Marmara University, School of Medicine,
Altunizade,
TR-81 190 Istanbul, Turkey

Z.S. Ercan
Department of Pharmacology,
Gazi University, School of Medicine,
Ankara, Turkey

[1] *Present address:* SOYAK SITESI,
44. Blok, D: 792, TR-81190 Istanbul,
Turkey

Abstract We studied the effects of the specific endothelin (ET_A) receptor antagonist, BQ-123, on reperfusion injury in a rat model of kidney transplantation. First, Sprague-Dawley rats were divided into three groups: a sham nephrectomy (SNEPH), an autotransplantation (AUTO-Tx), and an allotransplantation (ALLO-Tx) group. In a fourth group, ALLO-Tx + BQ, allografts were flushed with 20 μg BQ-123 containing cold Ringer's lactate before transplantation. For the allograft groups, kidneys from white Wistar albino rats were transplanted into allogeneic Sprague Dawley recipients. Grafts were allowed 120 min of reperfusion after 40 min of cold ischemia. ET-1,2 plasma concentrations in the renal venous blood, and kidney tissue prostaglandin (PG) E_2 and leukotriene (LT) B_4 levels were studied. Diene conjugates (DC), hydroxyalkanals (HAA), hydroxyalkenals (HAE) and malondialdehyde (MDA) levels, as the products of lipid peroxidation, and protein carbonyls (PC) and protein sulphydryls (PS), as the parameters of protein oxidation, were also analyzed in the kidney tissue. Plasma ET concentrations increased significantly in the AUTO-Tx and ALLO-Tx groups ($P < 0.05$ and $P < 0.01$, respectively) but this increase was reversed in the ALLO-Tx + BQ group. None of the lipid peroxidation products except DCs ($P < 0.05$) increased in the AUTO-Tx group, whereas they all increased in the ALLO-Tx group ($P < 0.01$). Protein oxidation parameters also changed significantly ($P < 0.01$) in the ALLO-Tx group but did not in the AUTO-Tx group ($P < 0.05$). The differences in PGE_2 and LTB_4 levels were not significant. Histopathologic examination revealed prominent glomerular and tubular injury in the AUTO-Tx and ALLO-Tx groups but less in the ALLO-Tx + BQ group. In the last group, all parameters of lipid peroxidation ($P < 0.001$ for all) and PCs decreased, and PSs were preserved ($P < 0.001$ for both) when compared with the AUTO-Tx and ALLO-Tx groups. We conclude that BQ-123, in addition to inhibiting the binding of ET-1,2 to the ET_A receptor, may also inhibit the release and/or synthesis of ET-1,2 and prevent reperfusion injury in kidney transplantation.

Key words BQ-123, reperfusion injury · Reperfusion injury, kidney, BQ-123 · Endothelin receptor antagonist, BQ-123 · Kidney, BQ-123, reperfusion injury

Introduction

Ischemic renal damage poses a serious problem for the subsequent fate of kidneys harvested for renal transplantation. Free oxygen radical generation has been implicated as a major mediator in ischemia-reperfusion injury [14, 31], which also occurs following revascularization of the cold-preserved and transplanted organs. Lipid peroxidation and protein oxidation products have been shown to be reliable indicators of free oxygen radical-generated injury [15, 47]. Lipid peroxidation and protein oxidation result in structural and functional cell damage [10, 16, 19, 23]. Endothelial cells produce free oxygen radicals with or without adherence of neutrophils to the vascular endothelium, which has been recognized as an important functional unit involved in the regulation of vascular smooth muscle tonus through the release of various local factors including arachidonic acid metabolites [i.e., prostacyclin (PGI_2), thromboxane (Tx) A_2 and leukotriene (LT) B_4], nitric oxide (NO), and endothelins (ET) [50]. Endothelial cells also play a significant role in the regulation of immunological reactivity [17] and modify the inflammatory response by regulating the expression of the adhesion molecules that bind to neutrophil integrins and mediate neutrophil infiltration [13]. In addition, previous work on the mechanisms of reperfusion injury has shown that ETs increase after warm ischemia-reperfusion injury [1, 11, 42] and that they have an important role in vascular rejection after kidney and liver transplantation [21, 32, 49]. Endothelins are a family of 21-amino-acid peptides composed of three isopeptides – ET-1, ET-2, and ET-3 – having profound cardiovascular, mitogenic and neuroregulatory functions [11]. The renal vasculature is several times more sensitive to the constrictor effects of ET peptides than other vascular regions [35]. At least two, and possibly three, types of ET receptors have thus far been identified and are referred to as ET_A, ET_B and ET_C [40]. A number of agonists and antagonists of ET receptors have been developed. The recently produced ET_A receptor antagonist BQ-123 has been shown to antagonize the vasoconstrictor effects of ET-1 in vitro and in vivo [20, 37].

The purpose of the present study was to investigate the role of ETs in a rat model of renal auto- and allotransplantation following cold ischemia. It was also hypothesized that the endothelin receptor antagonist (ETRA) BQ-123 might reverse the detrimental effects brought about by ETs after renal transplantation.

Materials and methods

Animals and surgical procedures

Studies were performed on 16- to 20-week-old male Sprague-Dawley rats as recipients and white Wistar albino rats weighing 280–350 g as donors (obtained from DETAM Research Center of Istanbul University). Animals were kept in a light- and temperature-controlled room. They were fed rat chow and had free access to water. After an overnight fast, the rats were anesthetized with intraperitoneal ketamine hydrochloride (10 mg/kg; Steropoverseas, Brussels) and the internal jugular vein was catheterized using a 24-gauge polytetrafluroethylene catheter (Angiocath). All subjects were treated with 80 U of heparin (Liquemine, Roche) in 3 ml of saline intravenously. After skin preparation with Betadine, a midline abdominal incision was made and the bowel was retracted to the right; the left kidney, with its artery and vein up to the junctions with the aorta and vena cava, was dissected free. The collaterals were ligated and cut. The ureter was ligated distally and cut at its entry into the bladder. The donor left kidney was flushed through the renal artery with 4 cc of ice-cold Ringer's lactate containing 1 % heparin until the kidney became uniformly pale and the perfusate clear before transplantation. The harvested kidney was transplanted orthotopically with the telescoping technique, as described previously [12]. The donor kidney was covered with swabs soaked in ice-cold saline and kept cool for the duration of the vascular anastomoses. All transplanted animals underwent right nephrectomy at the end of the procedure.

Animals were randomly placed into four experimental groups. The sham nephrectomy (SNEPH) group ($n = 10$) was subjected to denervation by mobilization of the left kidney; then, right nephrectomy was performed. The AUTO-Tx group ($n = 10$) underwent autotransplantation and, thus, the kidney was exposed to surgical trauma and cold ischemia-reperfusion injury. In the ALLO-Tx group ($n = 10$) and in the ALLO-Tx + BQ group ($n = 10$), left kidneys of donor Wistar albino rats were prepared as described above; then, orthotopic allogeneic transplantations were performed. In the ALLO-Tx + BQ group, 20 μg BQ-123 (gratefully obtained from Takeda Labs, Japan) was added to the 4-cc ice-cold Ringer's lactate flush solution prior to transplantation.

The transplanted kidney was allowed 120 min of reperfusion, during which time Ringer's lactate was infused (10 ml/kg) to keep the animals hemodynamically stable. Animals with incomplete reperfusion and bleeding or vascular complications and those exposed to cold ischemia for longer than 45 min were excluded from the study.

At the end of reperfusion, animals were anesthetized with ketamine (2 mg/kg) intravenously and relaparotomy was performed. After cannulation of the renal vein with 24-gauge Angiocath, blood was withdrawn and plasma was immediately separated for ET assay. Finally, animals were sacrificed by performing pneumothorax, and kidney tissue samples were obtained for the analysis of lipid peroxidation, protein oxidation, and prostaglandin (PG) E_2 and LTB_4 tissue levels, as well as for histopathologic examination. For the latter, tissue samples were kept in 10 % formalin solution and the rest were snap-frozen in liquid nitrogen immediately and stored at –78 °C until processing.

Histopathologic examination

Hematoxylin and eosin-stained sections were examined and graded systematically in a blinded fashion. Glomerular and tubulointerstitial changes were considered separately from vascular chan-

ges and graded as mild, moderate, or severe using a semiquantitative method based on the intensity of the infiltrate [22].

Plasma ET concentrations were analyzed by radioimmunoassay. An endothelin-1,2 assay kit was purchased from Amersham International (UK). Tissue PGE_2 and LTB_4 levels were determined as described previously [2, 46].

Lipid peroxidation assay

Lipids were extracted from the homogenates with chloroform/methanol and the liquid phases were removed. Lipid residues were then dissolved in cyclohexane in order to obtain a lipid concentration of 100 μg/ml, and their spectra (225–260 nm) were recorded against a cyclohexane blank using a Schimadzu UV 2100 double-beam scanning spectrophotometer. Second derivatives of the spectra were measured at 233 and 248 nm, denoting trans, trans and cis, and trans conjugates, and their sum was used for quantitation of diene conjugates, which were expressed as nmol/μM phospholipid (nmol/μM PL) [7].

Carbonyl content measurements were performed by incubating the lipid extracts with 2,3-dinitrophenylhydrazine for 2 h with occasional mixing. After centrifugation, the dinitrophenylhyrazones present in the supernatant were extracted with dichloromethane. The absorbances at 350 and 380 nm were recorded separately, each denoting different groups of aldehydes other than malondialdehyde (MDA), i.e., hydroxyalkanals (HAA) and hydroxyalkenals (HAE), respectively [8]. The results were expressed as nmol carbonyl/μM phospholipid (nmol/μM PL) using the extinction coefficient 25,500 M^{-1} cm^{-1}.

Thiobarbituric acid reactive substance and MDA of tissue homogenates were measured as described previously and were given as nmol/g tissue [6].

Protein oxidation assay

Protein carbonyls (PC) were determined by a modification of the procedure described by Levine et al. [29]. Protein precipitates were left to react with 2,4-dinitrophenylhyrazine for 1 h with occasional mixing. After the reaction, proteins were precipitated with 20% trichloroacetic acid and unreacted dye was washed out. The pellets were dissolved in 1 M NaOH and absorbances at 360 nm were recorded. The results were expressed as nmol/mg protein, using an extinction coefficient of 22,000 M^{-1} cm^{-1}. Protein sulfhydryls (PS) were determined according to a modification of the Ellman procedure and expressed as μmol/g tissue, assuming an extinction coefficient of 13,000 M^{-1} cm^{-1} at 412 nm [4].

Statistical analysis

Results were evaluated using a one-way ANOVA test, and the differences among the groups were analyzed with Dunn's test, except for ET values. For the latter, the nonparametric test was used and differences among the groups were analyzed with the Kruskal-Wallis test. All values are expressed as mean ± standard deviation.

Results

Histopathologic examination revealed no global necrosis, macrophage infiltration, or vascular inflammation in any of the kidney tissues in the experimental groups.

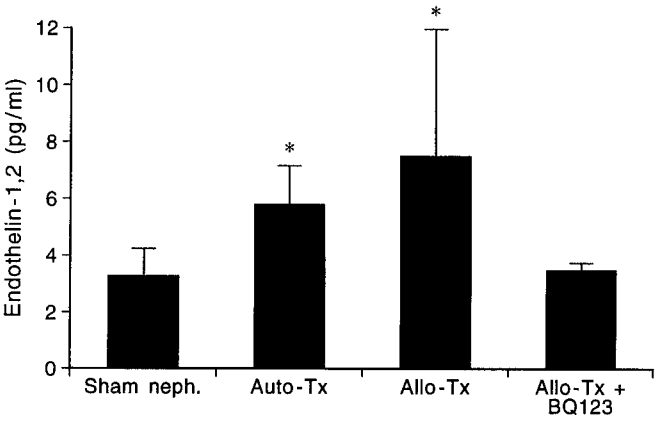

Fig. 1 Increased ET levels in the AUTO-Tx and ALLO-Tx groups ($P < 0.05$ and $P < 0.01$) significantly decreased in the ALLO-Tx + BQ group ($P < 0.05$ for both)

In the auto- and allotransplantation groups, mild to moderate glomerular polymorphonuclear leukocyte infiltration, glomerular congestion and, in some, patchy focal glomerular necrosis with morphological injury to the proximal and distal tubular lining cells in a spectrum up to cytolysis were determined. However, tubular changes, in particular, were quite mild in the allotransplantation group in which the donor kidneys were flushed with BQ-123.

Endothelin-1,-2

The mean ET levels were 3.2 ± 1.0, 5.7 ± 1.4, 7.4 ± 4.5, and 3.4 ± 0.3 pg/ml in the SNEPH, AUTO-Tx, ALLO-Tx, and ALLO-Tx + BQ groups, respectively (Fig. 1). The difference between AUTO-Tx and ALLO-Tx + BQ was not significant, but the ET levels were found to be significantly elevated in both when compared with the SNEPH group ($P < 0.05$ and $P < 0.01$, respectively). The decrease in the ET levels in the ALLO-Tx + BQ group was significant when compared with the AUTO-Tx and ALLO-Tx groups ($P < 0.05$) but was not different from that in the SNEPH group ($P > 0.05$).

Lipid peroxidation

Diene conjugates (DC), the first phase products, and hydroxyalkanals (HAA), hydroxyalkenals (HAE) and malondialdehyde (MDA) levels, the late phase products of the carboxylation, are presented in Figs. 2 and 3, respectively. The difference between the SNEPH and the AUTO-Tx groups was significant only in DC levels ($P < 0.05$). All lipid peroxidation parameters were significantly elevated in the ALLO-Tx group when com-

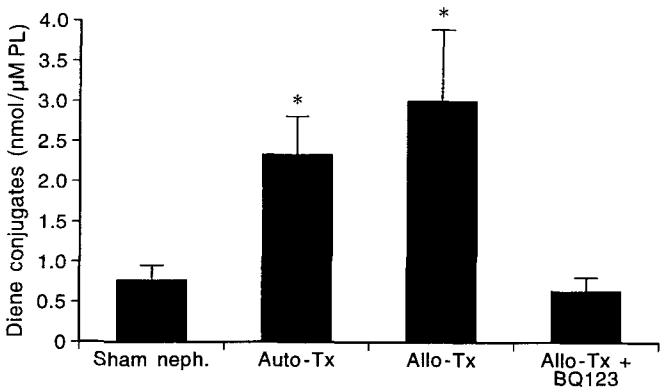

Fig. 2 As the beginning products of lipid peroxidation, DC levels significantly increased in the AUTO-Tx and ALLO-Tx groups ($P < 0.05$ and $P < 0.01$) compared to the SNEPH group. The decrease in the ALLO-Tx + BQ group was very significant when compared with the AUTO-Tx and ALLO-Tx groups ($P < 0.001$ for both)

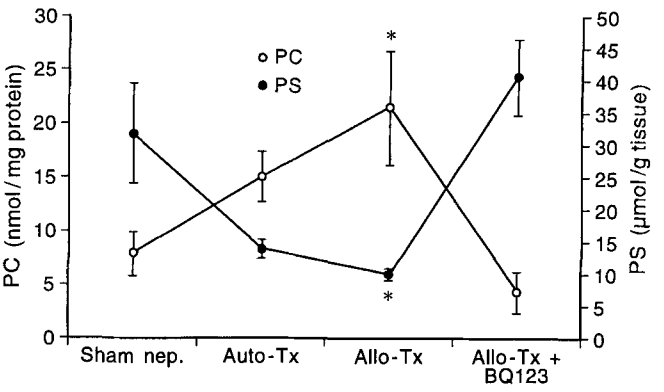

Fig. 4 The increased PC and the decreased PS levels in the ALLO-Tx group were significant when compared with the SNEPH group ($P < 0.01$). The reversed levels of both in the ALLO-Tx + BQ group were also very significant when compared with the AUTO-Tx and ALLO-Tx groups ($P < 0.001$)

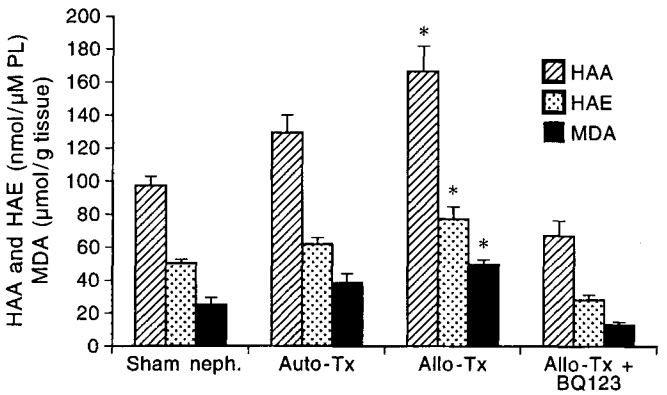

Fig. 3 As the end products of lipid peroxidation, HAA, HAE, and MDA levels increased significantly ($P < 0.01$) in the ALLO-Tx group when compared with the SNEPH group, and the decreases in the ALLO-Tx + BQ group were also significant when compared with the AUTO-Tx and ALLO-Tx groups ($P < 0.001$)

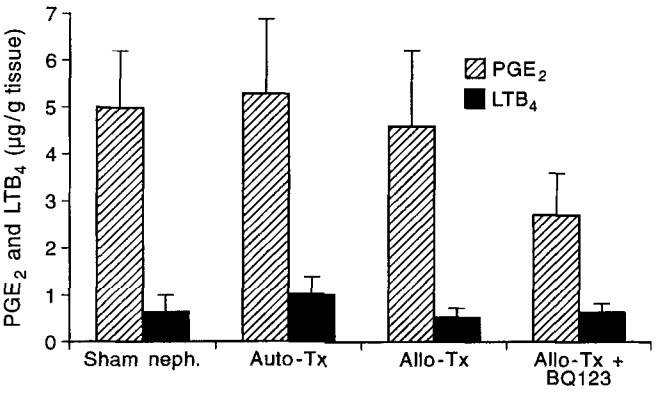

Fig. 5 Changes in PGE_2 and LTB_4 levels in all groups were found to be unrelated to cold ischemia-reperfusion injury at the end of the procedure ($P > 0.05$)

pared with the SNEPH group ($P < 0.01$), and those were not found to be significant when compared to the AUTO-Tx group ($P > 0.05$). In the allotransplanted kidneys flushed with BQ-123, all elements of lipid peroxidation were significantly lower than in the AUTO-Tx and ALLO-Tx groups ($P < 0.001$ in both cases).

Protein oxidation

Protein carbonyls (PC) and protein sulfhydryls (PS) were analyzed as the parameters of protein oxidation (Fig. 4). The PC levels were 7.8 ± 2.1, 15.0 ± 2.4, 21.4 ± 5.3, and 4.1 ± 1.9 nmol/mg protein, and the PS levels were 31.8 ± 7.7, 13.7 ± 1.5, 9.7 ± 0.8, and 40.5 ± 6.0 μmol/g tissue in the SNEPH, AUTO-Tx, ALLO-Tx, and ALLO-Tx + BQ groups, respectively. The differences between the SNEPH and AUTO-Tx groups were not significant ($P > 0.05$). The changes in ALLO-Tx when compared with those in the SNEPH group were significant ($P < 0.01$) but they were not significant when compared to the AUTO-Tx group ($P > 0.05$). The decreased levels of PCs and the elevated levels of PSs in the ALLO-Tx + BQ group were found to be highly significant when compared with the AUTO-Tx and ALLO-Tx groups ($P < 0.001$ in both cases), while the difference was not significant when compared to the SNEPH group ($P > 0.05$).

PGE_2 and LTB_4

Tissue PGE_2 levels were 5.0 ± 1.2, 5.3 ± 1.6, 4.6 ± 1.6, and 2.7 ± 0.9 μg/g, and LTB_4 levels were 0.6 ± 0.4, 1.0 ± 0.4, 0.5 ± 0.2, and 0.6 ± 0.2 μg/g in the SNEPH, AUTO-Tx, ALLO-Tx, and ALLO-Tx + BQ groups, respectively (Fig. 5). Neither the tissue levels of the two arachidonic acid metabolites nor the PGE_2/LTB_4 ratios were found to be significantly different among the groups ($P > 0.05$).

Discussion

This model was chosen primarily to allow evaluation of the different aspects of the relationship between reperfusion injury and ETs and their detrimental effects, as well as to see the change associated with the initiation of allograft reaction. The effects of BQ-123 on these events were also investigated in rat kidney transplantation. Experimental studies have established the important role of free radicals derived from activated xanthine oxidase at reperfusion as a substantial component of the postischemic injury [14, 31, 48]. Previous investigations have suggested that the reflow phenomenon caused by free oxygen radicals could be reversed with superoxide dismutase (SOD), catalase, allopurinol, captopril, and iloprost (a prostacyclin analog) [1, 5, 26, 34, 45]. Recently, Land et al. [28] performed a trial of intravenous use of recombinant human SOD (rh-SOD) with the immunosuppressive regimen in clinical renal transplantation and emphasized that reperfusion injury might be the initial event contributing to rejection. The results of this trial confirmed the beneficial effects of rh-SOD on acute rejection events and long term graft survival. In contrast, Pollak et al. [36] showed that rh-SOD failed to improve early post-transplant renal allograft functions.

Lipid peroxidation caused by free oxygen radicals is a complex phenomenon initiated by the abstraction of a hydrogen atom from a methylene group, and it results in the generation of lipid peroxides or lipid hydroperoxides. Further breakdown of the latter produces the relatively stable end-product MDA, which can be used as a marker of lipid peroxidation [44]. In the present study, lipid peroxidation was analyzed in a spectrum from the generation of the first product Dcs and mid-phase products HAA, HAE, and MDA. Our data showed that lipid peroxidation began in the AUTO-Tx group, as indicated by elevated DC levels only, but did not progress at the end of the 2-h period of reperfusion, while late products of lipid peroxidation were not elevated. In the ALLO-Tx group, however, lipid peroxidation was more severe and significantly higher than in the SNEPH group ($P < 0.01$).

Free oxygen radicals also cause degradation of the protein structures, especially those that contain unsaturated and sulphur-containing molecules and nucleic acids [29]. Protein carbonyls are released and sulphydryl-containing aminoacids are oxidized, resulting in a low level of PSs in free radical reactions. An increase in PC and also a decrease in PS levels in the ALLO-Tx group indicated severe protein oxidation, but no significant changes in PC and PS levels in the AUTO-Tx group were determined. The most striking findings of this study were obtained in the ALLO-Tx + BQ group, in which allografted kidneys had been flushed with BQ-123 just before transplantation. Lipid peroxidation products and PC levels were lowered, and PS levels were preserved and stayed high to a very significant degree ($P < 0.001$ for all), indicating that ETRA perfusion prevented free oxygen radical generation and/or their oxidative effects on protein and lipids.

Since the discovery of the first peptide, ET-1, in 1988 [52], studies have shown that synthesis of ETs is not confined to vascular endothelium but is common to many cell types including the lung, kidney, gut, and even macrophages and neutrophils [43]. Endothelins are a family of potent vasoactive peptides, and of the three ETs, ET-2 is the most potent vasoconstrictor, followed by ET-1 and ET-3 [30]. It is likely that distinct receptors – ET_A, ET_B, and ET_C receptors – the latter of which shows a higher affinity for ET-3, mediate the vasoactive effects of ET on vascular smooth muscle [51]. The affinity rank order of binding to the ET_A receptor is ET-13ET-23ET-3 [38], whereas that for the ET_B receptor is ET-1 ≥ ET-2 ≥ ET-3 [33, 39]. Endothelin can also stimulate the release and action of nitric oxide and prostacyclin via a distinct endothelial ET_B receptor and causes a transient vasodilation [9, 24]. The effects of ET-1 in the kidney include an increase in renal vascular resistance, a reduction of renal blood flow, and various effects on glomerular filtration rate [49]. Previous studies on the mechanisms of reperfusion injury have revealed that ETs increase after warm ischemia-reperfusion injury [1, 41, 42]; in humans, plasma ET concentrations increased transiently during acute rejection, particularly in cases of acute vascular rejection [32, 49]. Kusumoto et al. [27] have shown the protective effects of a new ET receptor antagonist in an experimental model of acute renal failure and suggested at least two different receptors in the rat kidney. Possibly both ET_A and ET_B are affected by their new agent, TAK-044.

BQ-123 is a competitive antagonist for the ET_A receptor [9]. This compound has been shown to antagonize the vasoconstrictor effects of ET-1 in vitro and in vivo [20, 37]. In the present study, the allografts were immediately flushed with BQ-123 containing ice-cold Ringer's lactate solution before transplantation into the ALLO-Tx + BQ group. Interestingly, ET-1,2 plasma concentrations did not increase in this group. We pre-

ferred to rinse the kidney just before transplantation rather than apply systemic treatment in order to minimize the effect on the kidney. Direct local infusion may also cause further damage of the endothelium and interruption of the blood flow during reperfusion. Since it is expected that BQ-123 will inhibit the binding of ET to the receptor, these findings suggest that the release and/or synthesis of ET-1,2 is also affected. However, in the kidney allograft, BQ-123 prevented lipid peroxidation and protein oxidation after reperfusion as well. This protective effect was also evident from the histopathologic findings. ET induces the release of $PGF_2\alpha$ and PGE_2 from rat glomeruli [51] as well as that of prostacyclin and thromboxane A_2 from isolated porcine and rat lungs [53]. Our data failed to show significant changes in the PGE_2 and LTB_4 levels, indicating that cycloxygenase and lipoxygenase pathways of the arachidonic acid cascade are not responsible for this effect, at least not at this stage.

ET receptors have distinct cell type/tissue distributions and thus may have different physiological roles. ET_A receptors, for example, generally reside in smooth muscle cells and mediate vasoconstrictor responses, whereas endothelial cells express the ET_B receptor, which mediates vasodilator effects via the endothelin-induced release of prostacyclin and nitric oxide [3, 38]. The cytopreservative effect of BQ-123 may be responsible for abolishing vasoconstrictor effects through ET_A receptors, thus releasing ET_B receptors, which increase nitric oxide and prostacyclin. It has been reported that PGI_2 release initially is decreased during reoxygenation but spontaneously returns to near normal levels in 24 h [18], so prostacyclin may not be the mediator.

In summary, on the basis of our data, it is difficult to draw definitive conclusions regarding acute rejection because of the lack of rejection markers and the short term of the study. However, it is obvious that the degree of reperfusion injury increased with transplantation and that BQ-123 was very effective in both reversing the increased ET-1,2 levels and abolishing ischemia-reperfusion injury. This agent would, therefore, appear to have substantial therapeutic potential for use in the routine practice of renal transplantation. However, further studies are necessary to clarify the causative role of ETs in the pathogenesis of rejection and to evaluate the clinical effects of BQ-123 in the prevention of reperfusion injury.

Acknowledgements This project was approved and supported by the Marmara University Research Foundation.

References

1. Aktan AÖ, Büyükgebiz O, Yeğen C, Yalin AS, Haklar G, Yalin R, Ercan S (1994) Does PGE_2 act as a mediator for endothelin release? Prostaglandins Leukot Essent Fatty Acids 50: 37–41
2. Andersen WH, O'Donnel M, Simco BA, Welton AF (1983) An in vivo model for measuring antigen induced SRS-A mediated broncoconstriction and plasma SRS-A levels in the guinea pigs. Br J Pharmacol 78: 67
3. Astaire-Dequeker C, Iouzalen L, Dufilho MD, Devynck MA (1992) In vitro inhibition by endothelins of thrombin-induced aggregation and Ca^{2+} mobilization in human platelets. Br J Pharmacol 106: 966
4. Ayka G, Uysal M, Yalin AS, Koak-Toker N, Sivas A, Öz H (1985) The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroksidase & glutathione transferase in rats. Toxicology 36: 71
5. Büyükgebiz O, Aktan AÖ, Yeğen C, Yalin AS, Haklar G, Yalin R, Ercan ZS (1994) Captopri increases endothelin release and preserves intestinal mucosa after mesenteric ischemia-reperfusion injury. Res Exp Med 194: 339–348
6. Casini A, Ferrali M, Pompella A, Maellaro E, Comporti M (1986) Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene intoxicated mice. Am J Pathol 123: 520–531
7. Corongiv FP, Banni S, Dessi MA (1994) Detection of conjugated dienes by second derivative ultraviolet spectrophotometry. Methods Enzymol 223: 303
8. Diazini MV, Cheeseman KH, Slater T, Lara S, Estebauer H (1985) Separation and characterization of aldehydic products of lipid peroxidation stimulated by CCl_4 and ADP-ion in isolated rat hepatocytes and rat liver microsomal suspensions. Biochem J 227: 629
9. Dohi Y, Lüscher TF (1991) Endothelin in hypertensive resistance arteries. Intraluminal and extraluminal dysfunction. Hypertension 18: 543
10. Döşlüoğlu HH, Aktan AÖ, Yeğen C, Okboy N, Yalin AS, Yalin R, Ercan S (1993) The cytoprotective effects of verapamil and iloprost (ZK 36374) on ischemia/reperfusion injury of kidneys. Transpl Int 6: 138–142
11. Elshourbagy NA, Korman DR, WU IL, Sylvester DR, Lee JA, Nuthalaganti P, Bergsma DJ, Kumar CS, Nambi P (1993) Molecular characterization and regulation of the human endothelin receptors. J Biol Chem 268: 3873–3879
12. Engelbrecht G, Delawir K, Duminy F, Hickman R (1992) New rapid technique for renal transplantation in the rat. Microsurgery 13: 340
13. Forman MB, Velasco CE, Jackson EK (1993) Adenosine attenuates reperfusion injury following regional myocardial ischemia. Cardiovasc Res 27: 9
14. Granger DN, Höllwarth ME, Parks DA (1986) Ischemia-reperfusion injury: role of oxygen derived free radicals. Acta Physiol Scand 548: 47
15. Green CJ, Healing G, Lunec J, Fuller BJ, Simpkin S (1986) Evidence of free-radical-induced damage in rabbit kidneys after simple hypothermic preservation and autotransplantation. Transplantation 41: 161
16. Hall NC, Carney JM, Cheng MS, Butterfield DA (1995) Ischemia-reperfusion induced changes in membrane proteins and lipids of gerbil cortical synaptosomes. Neuroscience 64: 81–89

17. Hayes WG, Webb CJ (1993) The endothelin family of peptides: local hormones with diverse roles in health and disease? Clin Sci 84: 485
18. Hempel SL, Haycraft DL, Hoak JC, Spector AA (1990) Reduced prostacyclin formation after reoxygenation of anoxic endothelium. Am J Physiol 259: C738
19. Hoshino T, Maley WR, Bulkley GB, Williams GM (1988) Ablation of free radical-mediated reperfusion injury for the salvage of kidneys taken from non-heart-beating donors. Transplantation 45: 284–289
20. Ihara M, Noguchi K, Saeki T, Fukuroda T, Tsuchida S, Kimura S, Fukami T, Ishikawa K, Nishikibe M, Yano M (1991) Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. Life Sci 50: 247
21. Ishikawa T, Yamamoto Y, Takeuchi K, Nakagawa H, Nagai Y, Ohira M, Yamashita T, Sowa M (1995) Relationship between peri- and postoperative plasma concentration of endothelin-1 and liver disfunction in rat liver transplantation Trans Proc 27: 524–527
22. Jablonski P, Howden BO, Rae DA (1983) An experimental model for assessment of renal recovery from warm ischemia. Transplantation 35: 198–204
23. Kellog EW (1975) Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 250: 8812–8817
24. Kiowski W, Lüscher TF, Linder L, Bühler FR (1991) Endothelin-1 induced vasoconstriction in humans. Reversal by calcium channel blockade but not by nitrovasodilators or endothelium derived relaxing factor. Circulation 83: 469
25. Kon V, Badr KF (1991) Biological actions and pathophysiologic significance of endothelin in kidney. Kidney Int 40: 1
26. Koyama I, Bulkley GB, Williams GM, Im MJ (1985) The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ishemic kidneys. Transplantation 40: 590
27. Kusumoto K, Kubo K, Kandori H, Kitayoshi T, Sato S, Wakimasu M, Watanabe T, Fujino M (1994) Effects of a new endothelin antagonist, TAK 044, on post-ischemic acute renal failure in rats. Life Sci 55: 301–310
28. Land W, Schneeberger H, Schleibner S, Illner WD, Abendroth D, Rutili G, Arfors KE, Messmer K (1994) The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. Transplantation 57: 211–217
29. Levine RL, Garland D, Oliver CV, Amici A, Climent I, Lenz AG, Ahr BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186: 464–473
30. Lüscher TF, Oemar BS, Boulanger CM, Hahn AWA (1993) Molecular and cellular biology of endothelin and its receptors. J Hypertens 11: 121
31. McCord JM (1985) Oxygen-derived free radicals in post ischemic tissue injury. N Engl J Med 312: 159–163
32. Mouquet C, Carayon A, Ourahma S, Chartier-Kastler E, Masson F, Bitker MO, Viars P (1994) Course of plasma endothelin levels during acute rejection in kidney transplantation. Transplant Proc 26: 279
33. Ogawa Y, Nakao K, Arai H, Nakagawa O, Hosoda K, Suga S (1991) Molecular cloning of a non- isopeptide-selective human endothelin receptor. Biochem Biophys Res Commun 178: 248
34. Ouriel K, Smedira NG, Ricotta JJ (1985) Protection of the kidney after temporary ischemia: free radical scavengers. J Vasc Surg 2: 49
35. Pernow J, Franco-Cereceda A, Matran R, Lundberg JM (1989) Effect of endothelin-1 on regional resistances in the pig. J Cardiovasc Pharmacol 13: 205–206
36. Pollak R, Andrisevic JH, Maddux MS, Gruber SA, Paller MS (1993) A randomised double-blind trial of the use of human recombinant superoxide dismutase in renal transplantation. Transplantation 55: 57
37. Pollock DM, Opgenorth TJ (1993) Evidence for endothelin induced renal vasoconstriction independent of ET_A receptor activation. Am J Physiol 264: R222
38. Sakamato A, Yanagisawa M, Sawamura T, Enoki T, Ohtani T, Sakurai T, Nakao K, Toyooka T, Masaki T (1993) Distinct subdomains of human endothelin receptors determine their selectivity to ET_A-selective antagonist and ET_B-selective agonists. J Biol Chem 268: 8547
39. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, Masaki T (1990) Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348: 732
40. Sakurai T, Yanagisawa M, Masaki T (1992) Molecular characterization of endothelin receptors. Trends Pharmacol Sci 13: 103
41. Satoshi N, Nishiyama R, Serizawa A, Yokoi Y, Suzuki S, Konno H, Baba S, Muro H (1995) Hepatic release of endothelin-1 after warm ischemia. Transplantation 59: 679–684
42. Shibouta Y, Suzuki N, Shino A, Matsumoto H, Terashita Z, Kondo K, Nihikawa K (1990) Pathophysiologic role of endothelin in acute renal failure. Life Sci 46: 1611–1618
43. Simonson MS, Duna MJ (1992) The molecular mechanisms of cardiovascular and renal regulation by endothelin peptides. J Lab Clin Med 119: 622
44. Slater TF (1984) Free radical mechanisms in tissue injury. Biochemistry 222: 1–15
45. Southard JH, Belzer FO (1993) New concepts in organ preservation. Clin Transplant 7: 134
46. Spurney RF, Ruiz P, Pisetsky DS, Coffman TM (1991) Enhanced leukotriene production in murine lupus: role of lipoxygenase metabolites. Kidney Int 39: 95
47. Stewart JR, Frist WH, Merrill WH (1990) Oxygen scavengers in myocardial preservation during transplantation. Methods Enzymol 186: 742
48. Takahashi S, Suzuki S, Takeuchi K, Hatanaka R, Narita J, Iwabuchi S, Yamada Y, Fukui K, Koyama K, Takaya S, Koie H (1993) Role of endothelium for tissue damage by active oxygen radicals in heart-lung transplantation. Transplant Proc 25: 1676–1677
49. Watschinger B, Vychval A, Schuller M (1991) The pathophysiologic role of endothelin in acute vascular rejection after renal transplantation. Transplantation 52: 743
50. Welbourn CRB, Goldman G, Paterson IS, Valeri CR, Shepro D, Hechtman HB (1991) Pathophysiology of ischemia reperfusion injury: central role of the neutrophil. Br J Surg 78: 651–655
51. Woodcock EA, Land S (1991) Endothelin receptors in rat renal papilla with a high affinity for endothelin-3. Eur J Pharmacol 208: 255
52. Yanagisava M, Kurihara H, Kimura E, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki K, Goto Y, Masaki T (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411
53. Yoshizumi M, Kurihara H, Morita T, Yamashita T, Ohhashi Y, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Yazaki Y (1990) Interleukin-1 increases the production of endothelin-1 by cultured endothelial cels. Biochem Biophys Res Commun 166: 324