Urol Res (2002) 30: 164–168
DOI 10.1007/s00240-002-0256-3

ORIGINAL PAPER

R. Karabulut · K. Sönmez · B. Sancak · Z. Türkyılmaz
B. Demiroğulları · İ.O. Özen · G. Ekingen · S. Candan
A.C. Başaklar · N. Kale

Effects of amrinone on bilateral renal ischemia/reperfusion injury

Received: 16 October 2001 / Accepted: 20 March 2002 / Published online: 4 June 2002

Abstract Renal ischemia/reperfusion injury could arise as a consequence of clinical conditions such as renal transplantation, shock, cardiac arrest, hemorrhage and renal artery surgery. In this experimental study, we aimed to determine the preventive effects of amrinone on bilateral renal ischemia/reperfusion injury in rats. A total of 60 Wistar-albino rats were divided into six groups ($n = 10$). Midline laparotomies were made under ketamine anesthesia. In the sham, amrinone1 and amrinone2 without ischemia (AWI1 and AWI2) groups saline, 5 and 10 mg/kg of amrinone was infused, respectively. In the ischemia, ischemia plus amrinone1 (IPA1) and ischemia plus amrinone2 (IPA2) groups, saline and 5 and 10 mg/kg of amrinone was infused, respectively, at the beginning of reperfusion, subsequent to 45 min of bilateral renal artery occlusion. Following 6 h of reperfusion, blood was drawn to study serum BUN and creatinine and a bilateral nephrectomy was done to determine tissue malonyldialdehyde (MDA) and myeloperoxidase (MPO) levels. The results were analysed by Mann-Whitney U-test. The parameters studied were statistically higher in the ischemia group compared with the other groups ($P < 0.05$ for each comparison), indicating renal I/R injury. These parameters were lower in the amrinone without ischemia groups (AWI1 and AWI2) than in the sham group, however there were no significant differences between the groups ($P > 0.05$, for each comparison). The treatment groups IPA1 and IPA2 had statistically similar results compared with the sham group, showing the preventive effect of amrinone on renal I/R injury at the given doses. We conclude that amrinone prevented experimental renal ischemia/reperfusion injury in rats, independently of the administered doses. This preventive effect of the agent could depend on its effect of regulating the microcirculation, in decreasing intracellular calcium and in preventing neutrophil activation. We propose that this preventive effect of amrinone – which has gained clinical application especially in cases of cardiac insufficiency – could also be exploited in clinical conditions related with renal ischemia/reperfusion.

Keywords Renal ischemia · Amrinone

R. Karabulut (✉) · K. Sönmez · Z. Türkyılmaz · B. Demiroğulları
İ.O. Özen · G. Ekingen · A.C. Başaklar · N. Kale
Department of Pediatric Surgery, Faculty of Medicine,
Gazi University, Ankara, Turkey
E-mail: karabulutr@yahoo.com
Tel.: +90-312-4362197
Fax: +90-312-2134338

B. Sancak · S. Candan
Department of Biochemistry, Faculty of Medicine,
Gazi University, Ankara, Turkey

R. Karabulut
Gezegen Sokak, No.:1/10, Gaziosmanpaşa,
06670 Çankaya, Ankara, Turkey

Introduction

Advances in transplantation surgery and early intervention for acute myocardial ischemia has led to the realization and documentation of pathophysiology and treatment measures of ischemia/reperfusion (I/R) injury which has been studied intensely for the last two decades. The kidney, which is one of the most transplanted organs, could undergo ischemia not only following transplantation but also under clinical conditions such as cardiac arrest, hypotensive states, shock, hemorrhages and renal artery operations [15]. The correction of these clinical conditions could lead to reperfusion injury, further aggravating the renal ischemic damage. The superoxide radicals (SOR) which cause this damage have also been held responsible for various clinical disease states such as cataracts, diabetes, arteriosclerosis, rheumatic diseases, malignancies and inflammatory diseases, emphasizing the well known fact that I/R injury is not limited to the kidney [5].However, acute renal insufficiency due to ischemia is still one of the most common and important complications that physicians face. For example, acute tubular necrosis is seen in approximately 19–50% of cadavers and 10% of living donor

renal transplants [1, 15, 20]. We therefore consider it justified to study measures which could prevent such damage to renal tissue.

In this experiment the preventive role of amrinone in renal I/R injury is assessed. The drug is a phosphodiesterase III inhibitor, with reported effects of lowering intracellular calcium (Ca) and regulating the microcirculation [6, 28, 29]. It has inotropic and vasodilatatory activity and is being used in newborns, children and adults when these effects are needed clinically [17, 32, 34].

Materials and methods

A total of 60 Wistar-albino rats (210–280 g) were divided into six groups, each consisting of ten animals. Rats were fed standard food and fasted 12 h before being operated. All operations were done under ketamine HCl anesthesia (40 mg/kg, i.m.), using sterile techniques. In each animal, the abdomen was entered through midline incisions, the bowel was taken out of abdominal cavity and the renal arteries visualized. In the sham, AWI1 and AWI2 groups, saline and amrinone (5 and 10 mg/kg) were infused via the caudal caval vein, respectively. In the ischemia and IPA1 and IPA2 groups, both renal arteries were occluded using microvascular clamps. After 45 min the occlusions were removed. At the beginning of reperfusion, 5 and 10 mg/kg amrinone was infused via the caudal caval vein in the IPA1 and IPA2 groups, respectively. The bowel was returned to the abdomen and the incision was closed with 3–0 silk sutures in each group. At the end of 6 h of reperfusion, 3 ml of blood was drawn from each rat to study serum blood urea nitrogen (BUN) and creatinine (Cr) levels. The abdomen was reentered and bilateral nephrectomies were carried out. The kidneys were wrapped with aluminum foil, coded appropriately, placed in liquid nitrogen and kept at –30°C to determine tissue malonyldialdehyde (MDA) and myeloperoxidase (MPO) levels.

Serum Cr levels were determined by the Jaffe method which depends on the measurement of spectrophotometric absorbance of Janovski complex, produced as a result of the reaction of creatinine with the alkaline pitrate, at 520 nm [19].

Serum BUN levels were determined by the urease/glutamate dehydrogenase kinetic, spectrophotometric method. The decreasing absorbance of nicotinamide adenine dinucleotide (NADH) was determined in 340 nm [12].

MDA, which is the end product of lipid peroxidation, was used for the assessment of superoxide radical production. MDA levels were determined using the tiobarbituric acid (TBA) test based on the calorimetric measurement of the concentration of the pink colored end-product of the reaction between lipid peroxides and TBA. Briefly, renal tissues were weighed and homogenized in KCl at a ratio of 1/9 (w/v). Phosphoric acid and TBA were added to the homogenate and n-butanol was added to the solution which was then centrifuged. The absorbance of the supernatant was measured at 535 and 520 nm by spectrophotometry. 1,1,3,3-tetraetoxypropane was measured as standard. The difference between the two absorbance values was considered as the amount of MDA in nmoles/g-tissue [31].

MPO activity, which shows the degree of neutrophil infiltration, was determined after the tissues were prepared according to Schierwagen et al. [25] The MPO activity was assayed by measuring the H_2O_2-dependent oxidation of O-dianisidine. In its oxidized form o-dianisidine has a brown color. This was measured spectrophotometrically at 410 nm. The results are given as U/mg-protein. One unit of MPO activity was defined as the amount of enzyme caused absorbance change in 1 min at 410 nm [7].

The results were compared using Mann-Whitney U-tests. A value of $P < 0.05$ was considered significant.

Results

All results obtained are shown in Table 1.

Creatinine

The mean serum creatinine level was significantly higher in the ischemia group (0.79 ± 0.02 mg/dl) (mean ± SE) compared with other groups ($P < 0.05$, for each comparison). This value was lowest in group AWI1, followed by AWI2, sham, IPA2 and IPA1, respectively. Although some differences between the groups existed, nere was statistically significant ($P > 0.05$, for each comparison).

Blood urea nitrogen

The mean serum BUN level was significantly higher in the ischemia group (43.3 ± 1.3 mg/dl) compared with other groups ($P < 0.05$, for each comparison). This value was lowest in the sham group, followed by AWI2, AWI1, IPA2 and IPA1, respectively. Although some differences between the groups existed, none were statistically significant ($P > 0.05$, for each comparison).

Myeloperoxidase

The mean tissue MPO level was significantly higher in the ischemia group (1.24 ± 0.03 U/mg-protein) compared with other groups ($P < 0.05$, for each comparison). This value was lowest in group IPA2, followed by IPA1, AWI2, AW1 and sham, respectively. Although some differences between the groups existed, none was statistically significant ($P > 0.05$, for each comparison).

Table 1. MPO, MDA, BUN and creatinine values (mean ± SE). MPO = myeloperoxidase; MDA = malonyldialdehyde; BUN = blood urea nitrogen

Groups	MPO (U/mg-protein)	MDA (nmol/g-tissue)	BUN (mg/dl)	Creatinine (mg/dl)
Sham	0.64 ± 0.01	50.12 ± 1.5	19.8 ± 0.6	0.46 ± 0.01
Amrinone without ischemia1 (5 mg/kg)	0.44 ± 0.01	42.87 ± 1.3	19.9 ± 0.6	0.39 ± 0.01
Amrinone without ischemia2 (10 mg/kg)	0.43 ± 0.01	40.45 ± 1.2	19.5 ± 0.5	0.40 ± 0.02
Ischemia	1.24 ± 0.03	77.12 ± 2.3	43.3 ± 1.3	0.79 ± 0.02
Ischemia plus amrinone1 (5 mg/kg)	0.35 ± 0.01	48.37 ± 1.4	25.8 ± 0.7	0.50 ± 0.01
Ischemia plus amrinone2 (10 mg/kg)	0.31 ± 0.01	45.98 ± 1.2	25.1 ± 1.1	0.48 ± 0.02

Malonyldialdehyde

The mean tissue MDA level was significantly higher in the ischemia group (77.12 ± 2.3 nmol/g-tissue) compared with the other groups ($P < 0.05$, for each comparison). This value was lowest in AWI2, followed by AWI1, IPA2, IPA1 and sham, respectively. Differences between the groups were not statistically significant ($P > 0.05$, for each comparison).

Discussion

This experimental study on the effects of amrinone in renal I/R injury was done in the rat kidney with its very well known responses to such an injury [9, 13, 16].

Tissue injury subsequent to an I/R event has been attributed both to ischemia and to the production of SORs. Ischemia leads to adenosine triphosphate consumption which results in an increase in cytosolic calcium ion levels due to membrane instability. The influx of calcium has been shown to convert xanthine dehydrogenase to xanthine oxidase as a result of the activation of proteases and the formation of calcium-calmudulin complex, which would require as long as 30 min in the kidney [3, 5, 22, 26]. Xanthine oxidase thus formed, in turn produces superoxide radicals while converting hypoxanthine – accumulated due to ATP degradation – to xanthine with oxygen delivery during reperfusion. Several studies have shown the preventive effects of calcium channel blockers in renal I/R injury [26]. cAMP has also been reported to prevent I/R injury by inhibiting the increase in cytosolic calcium by means of activating membrane sodium/potassium ATPase [6, 28, 29]. High intracellular cAMP levels, sufficient to prevent I/R injury, can be reached by the inhibition of the enzyme phosphodiesterase III [6, 28]. Amrinone is a bipyrimidine derivative which effectively inhibits phosphodiesterase III. Tse et al. have shown a 24% inhibition of the enzyme activity in reperfused dog hearts. Replenishment of cAMP and high energy phosphates were also reported to be reached by amrinone administration during reperfusion [23, 30].

In a study evaluating the effects of amrinone on the suppression of intimal thickening in endothelial injury, the minumum doses of the drug to be effective probably due to a cAMP-mediated mechanism has been suggested as 3.0–10.0 mg/kg [14]. In another study, amrinone was reported to be effective in preventing muscle protein wasting during chronic sepsis in rats at a dose of 5 mg/kg [11]. Thus, in our study, 5 and 10 mg/kg of amrinone administered intravenously as single doses were chosen to evaluate its effects in renal I/R injury.

It has been reported that 45–60 min are enough for the experimental renal I/R injury to take effect [8, 21]. It was also shown that this injury could be documented by tissue and serum studies beginning at the 4th hour of reperfusion [21, 22]. In the present study, the period of ischemia was 45 min and the reperfusion period was finished in the 6th hour. The statistically higher levels of serum BUN, creatinine and tissue MDA and MPO in the ischemia group when compared with the sham group ($P < 0.05$) show that the procedure results in I/R injury in the kidney severe enough to document.

MPO activity is an indicator of neutrophil infiltration [8]. In a large number of studies related to renal ischemia reperfusion, it was shown that significant neutrophil infiltration occurs in the kidney, reaching a peak value at the 6th hour of reperfusion [35]. Therefore, the statistically higher MPO activity in the ischemia group compared to the Sham group ($P < 0.05$) is consistent with the literature. This infiltration is considered to be due to an increase in intracellular calcium, activating enzyme phospholipase A2 and causing neutrophil chemotaxis through leukotrienes [9, 10, 18]. In the present study amrinone prevented the neutrophilic infiltration as reflected in the significantly lower MPO values obtained in IPA1 (0.35 ± 0.01U/mg-protein) and IPA2 (0.31 ± 0.01U/mg-protein) compared to the ischemia group ($P < 0.05$). Although MPO levels obtained by 10 mg/kg of amrinone administration were lower than those obtained by 5 mg/kg of amrinone in renal I/R, these were not statistically significant ($P > 0.05$, when MPO levels were compared between IPA1 and IPA2 groups). This preventive effect could be attributed to amrinone's inhibitory activity on the enzyme phosphodiesterase III, causing cAMP to increase and intracellular calcium to decrease. The drug also prevents thrombocyte aggregation and regulates the microcirculation by inhibiting the intracellular calcium increments which results in lower neutrophils in the reperfused tissue [6, 28, 29]. The importance of the inhibition of neutrophilic infiltration to the kidney in preventing I/R injury is documented by a study demonstrating the reduction of SOR induced proteinuria in neutrophil-depleted animals [24]. As can be seen in the present study, amrinone exerts this effect in a dose-independent manner for the given doses.

MDA is an end-product of lipid peroxidation caused by superoxide radicals attacking of the lipid membranes of the cell, especially the hydroxyl ion [3, 4, 21, 22, 26]. Determination of its tissue levels is regarded as a reliable way of documenting the SOR production in an I/R event [2, 33]. SOR production in such an event is considered to begin as a result of occurrence of the calcium-calmudulin complex and proteolitic activity caused by increased intracellular calcium resulting in the conversion of the enzyme xantine dehydrogenase to xantine oxidase [6, 28, 29]. In a large number of studies, increased MDA levels in the renal tissue has been shown with the advent of reperfusion. In the present study, we were also able to obtain significantly increased levels of tissue MDA in the ischemia group compared with the sham group ($P < 0.05$). With amrinone administration in the IPA1 and IPA2 groups, statistically similar renal MDA levels were obtained compared with the sham group, exhibiting the totally preventive action of the drug against I/R injury in the kidney, at both administered doses. The

mean MDA value of the IPA2 group (45.98 ± 1.2 nmol/g-tissue) was lower than that of IPA1 group (48.37 ± 1.4 nmol/g-tissue); however these values were very close to each other and statistically insignificant between groups ($P > 0.05$). This protective effect is probably the result of the enzyme phosphodiesterase III inhibition leading to lower intracellular calcium levels and the consequent cessation of the proteolitic conversion of xanthine dehydrogenase to oxidase.

Amrinone has also been reported to inhibit the occurrence of the calcium-calmodulin complex – with vasoconstrictory effects through activation of myosine light-chain kinases – leading to regulation of the microcirculation [6, 28, 29, 32]. In the kidney, microcirculatory derangement results in erythrocyte congestion in the cortical and thrombus formation in the medullary capillaries.

In a large number of studies, serum BUN and creatinine have been used as a marker of functional renal ischemic injury that increases as a result of altered glomerular hemodynamics. In a time-sequence study in which the authors have assessed the protective role of ICAM-1 monoclonal antibody against renal ischemia-reperfusion injury, serum BUN and Cr were shown to increase as early as 2 h as the kidneys were perfused, reaching peak levels in the 24th hour [18, 35]. In the present study, statistically increased levels of both serum BUN and Cr were observed in the ischemia group when compared with the sham group following 6 h of reperfusion ($P < 0.05$). This observation is consistent with the studies that have documented an increase in arteriolar resistances – due to enhanced eicosanoid synthesis or degradation of nitric oxide – and a decrease in the glomerular ultrafiltration coefficient [27]. The statistically similar results of BUN and Cr in the IPA1 and IPA2 groups compared with the sham group again document the protective effects of amrinone in reperfusion injury ($P > 0.5$) by keeping the glomerular hemodynamics from alteration.

In conclusion, we show the protective role of amrinone in renal I/R injury, independently of the dose administered (5 or 10 mg/kg). The drug itself did not have an adverse effect in relation to the parameters studied in this experiment, as could be seen by the statistical similarity in the results obtained from the AWI1, AWI2 and sham groups ($P > 0.05$). Nevertheless, in the groups which received amrione all values of the studied parameters were lower than in the sham group. The reason for this could be related to the fact that there is a continuous production of SOR in the cells due to the "leakage" of electrons, mainly from the electron transport chain during normal metabolic activity. Other sources are the range of flavin oxidases and the autoxidation of certain compounds (such as ascorbic acid thiols) [4].

Amrinone is used successfully in newborns, children and adults in situations in which an increase in cardiac contractility is required. It decreases pulmonary vascular resistance if this is elevated and has been reported to improve cardiac index following cardiac surgery [17, 32, 34]. Because the agent has gained clinical application in humans, we propose that amrinone be used in situations in which renal I/R injury could arise. Furthermore, it could be of benefit to evaluate the preventive effects of amrinone in relation to renal damage at various time intervals after clamping the renal artery during renal surgery.

References

1. Al-Uzri A, Sullivan EK, Fine RN, Harmon WE (1998) Living-unrelated renal transplantation in children: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). Pediatr Transplant 2: 139
2. Baud L, Ardaillaou R (1993) Involvement of reactive oxygen species in kidney damage. Br Med Bull 49: 621
3. Bulkey GB (1987) Free radical mediated reperfusion injury. Br J Cancer 55: 66
4. Cheeseman KH, Slater TF (1993) An introduction to free radical biochemistry. Br Med Bull 49: 481
5. Cross CE, Halliewell B, Borish ET, Pryor WA (1987) Oxygen radicals and human disease. Ann Int Med 107: 526
6. Dale B (1989) Overview of cardiovasculer physiological and pharmacological aspect of selective PDE inhibition. Am J Cardiol 63: 9
7. Glowick SP, Kaplan SD (1988) Methods in enzymology. Am J Physiol 255: 1269
8. Günal Ö, Aktan AÖ, Yeğen C, Kurtel H, Yalin R (1997) Captopril prevents the oxidative damage to proteins after renal ischemia reperfusion injury: role of endotelin-1. Prostaglandins Leukot Essent Fatty Acids 56: 23
9. Hillegas LM, Griswold DE, Brickson B, Winslow C (1990) Assessment of myeloperoxidase activity in whole rat kidney. J Pharmacol Methods 24: 285
10. Hughes JD, Sammer MD, Mattar G, Chen C, Someren A, Noe B, Suwyn CR, Lumdsen AB (1996) Renal artery perfusion modifies isc/reper injury. J Surg Res 60: 321
11. Jurasinski CV, Kilpatrick L, Vary TC (1995) Amrinone prevents muscle protein wasting during chronic sepsis. Am J Physiol 268: E491
12. Kaplan SD, Pesce AJ (1996) Clinical chemistry, theory, analysis and correlation, 3rd edn. Mosby-Year Book, St.Louis, p 497
13. Karasawa A, Kubo K (1990) Protection by benidipine hydrochloride, a calcium antogonist of ischemic kidney in rats via inhibitions of Ca overload, ATP decline and lipid peroxidation. Japan J Pharmacol 52: 553
14. Kondo K, Umemura K, Miyaji M, Nakashima M (1999) Milrinone, a phosphodiesterase inhibitor, supresses intimal thickening after photochemically induced endothelial injury in the mouse femoral artery. Atherosclerosis 142: 133
15. Kumar V, Cotran RS, Robbin SL (1992) Basic pathology, 5th edn . W.B. Saunders, Philadelphia, p 593
16. Laight DW, Lad N, Woodward B, Waterfall JF (1994) Assessment of myeloperoxidase activity in renal tissue after i/r. Eur J Pharmacol 292: 81
17. Lawless S (1989) Amrinone in neonates and infants after cardiac surgery. Crit Care Med 17: 571
18. Lopez F, Toledo-Pereyra LH, Mirmiran R, Paez-Rollys AJ (1996) Time dependence of Na nitroprusside administration in the prevention of neutrophil infiltration in the rat ischemic kidney. Transplantation 61: 179
19. Miller SB, Martin D, Kıssane J, Hammerman R (1994) Rat models for clinical use of insulin-like growth factor in acute renal failure. Ren Fluid Electrolyte Physiol 35: 949
20. Morris-Stiff G, Jurewicz A, Balaji V, Babolal K, Moore R, Griffin P, Lord R (1997) Conversion from cylosporin to tacrolimus in a patient with prolonged acute tubular necrosis. Transplant Int 10: 398

21. Paller MS, Hebbel RP (1986) Ethane production as a measure of lipid peroxidation after renal ischemia. Am J Physiol 251: 839
22. Paller MS, Hoidal JR, Ferris TF (1984) Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 74: 1156
23. Pridjian AK, VanMenter CH, Mcfadden PM, Chung KC, Touchard CL, Ochsner JL (1996) Repletion of high energy phosphates in the postischemic neonatal heart. Am Surg 62: 494
24. Rehan A, Johnson KJ, Wiggins RC, Kunkel RG, Ward PA (1984) Evidence for the role of oxygen radicals in acute nephrotoxic nephritis. Lab Invest 51: 396
25. Schierwagen C, Bylund-Fellenius AC, Lundberg C (1990) Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. J Pharmacol Methods 23: 179
26. Schrier RW, Arnol PE, Putten VJV, Burke TJ (1987) Cellular calcium in ischemic acute renal failure: role of calcium entry blockers. Kidney Int 32: 313
27. Shah SV (1984) Effect of enzymatically generated reactive oxygen metabolites on the cyclic nucleotide content in isolated rat glomeruli. J Clin Invest 74: 393
28. Shigero I, Tashi M, Yukos, Mashiro S, Akira K, Kyinori H (1998) Amrinon selective PDE III inhibition improves microcirculation nad flap. J Surg Res 75: 42
29. Thierry H, Le Jemtel R, Scothini D, Levit B, Edward H (1989) Effects of phosphodiesterase inhibition on skeletal muscle vasculature. Am J Cardiol 63: 27
30. Tse J, Cimini C, Kedem J, Rodriquez E, Gonzalez M, Weiss HR (1993) Role or ischemia reperfusion on myocardial cAMP and cAMP phospodiesterase: effects of amrinone on regional myocardial force and shortening. J Cardiothorac Vasc Anesth 7: 566
31. Uchiama M, Mihara M (1987) Determination of malondehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86: 279
32. Ward A (1983) Amrinone: a preliminary review of its pharmacological properties and therapeutic use. Drugs 26: 468
33. Waz WR, Feld LG (1994) Reactive oxygen molecules in the kidney. Adv Exp Med Biol 366: 171
34. Wessel DL (1989) Pulmonary and systemic hemodynamics of amrinone in neonates following cardiopulmonary bypass. Circulation 80: 488
35. Williams P, Lopez H, Britt D, Chan C, Ezrin A, Hottenderf R (1997) Characterization of renal ischemia reperfusion injury in rats. J Pharmacol Toxicol Methods 37: 1