Nephrol Dial Transplant (2004) 1 of 5
DOI: 10.1093/ndt/gfh261

Nephrology Dialysis Transplantation

Original Article

Renal effects of *N*-acetylcysteine in patients at risk for contrast nephropathy: decrease in oxidant stress-mediated renal tubular injury

Luciano F. Drager[1], Lúcia Andrade[2], João Felipe Barros de Toledo[1], Francisco R. M. Laurindo[1], Luiz Antônio Machado César[1] and Antonio Carlos Seguro[2]

[1]Heart Institute (InCor) and [2]Department of Nephrology, Laboratory of Basic Research, School of Medicine, University of São Paulo, São Paulo, Brazil

Abstract

Background. *N*-Acetylcysteine has been shown to protect against contrast nephropathy, although the mechanisms underlying such an effect are unclear. Surprisingly, studies have shown that post-radiocontrast renal function actually improves in chronic renal failure patients receiving *N*-acetylcysteine. However, there have been no studies investigating the cause of this improvement.

Methods. In a double-blind, placebo-controlled study, 24 patients (aged 65 ± 2 years) suffering from stable mild-to-moderate renal insufficiency and undergoing elective coronary angiography were randomized to receive either placebo or *N*-acetylcysteine. All received similar hydration. Renal function parameters were assessed 48 h before and 48 h after radiocontrast administration. Urinary 15-isoprostane $F2_t$, a specific marker of oxidative stress, was measured immediately before and after the procedure. Expression of urinary α-glutathione *S*-transferase protein, a specific proximal tubular injury marker, was assessed after the procedure.

Results. Comparing creatinine clearance values before and after angiography, a significant increase was seen in *N*-acetylcysteine patients (44.7 ± 4.2 *vs* 57.2 ± 6.3 ml/min/1.73 m^2; $P = 0.02$), whereas placebo patients presented no change (46.6 ± 5.0 *vs* 46.9 ± 4.3 ml/min/1.73 m^2; $P = 0.90$). After radiocontrast, urinary 15-isoprostane $F2_t$ levels in placebo patients increased significantly over baseline values (2.9 ± 0.7 *vs* 10.3 ± 2.1 ng/mg creatinine; $P = 0.007$), whereas urinary 15-isoprostane $F2_t$ levels in *N*-acetylcysteine patients remained basically unchanged (3.5 ± 0.5 *vs* 4.1 ± 0.9 ng/mg creatinine; $P = 0.63$). Furthermore, *N*-acetylcysteine treatment led to lower levels of α-glutathione *S*-transferase than did placebo treatment (0.8 ± 0.2 *vs* 2.4 ± 0.7 μg/g; $P = 0.046$).

Conclusions. In chronic renal failure patients, the improvement in renal function induced by post-radiocontrast administration of *N*-acetylcysteine is strongly associated with suppression of oxidant stress-mediated proximal tubular injury.

Keywords: *N*-acetylcysteine; α-glutathione *S*-transferase; 15-isoprostane $F2_t$; oxidative stress; radiocontrast; renal failure

Introduction

Acute renal failure (ARF) induced by radiocontrast administration is a common cause of acquired in-hospital renal injury, contributing to greater morbidity and prolonged hospitalization [1]. Studies have demonstrated that age, diabetic nephropathy, volume depletion, dehydration, hypercholesterolaemia and, in particular, pre-existing renal insufficiency are risk factors for radiocontrast-induced ARF [2,3]. The pathogenesis of radiocontrast nephropathy is multifactorial. Indirect experimental evidence suggests that oxidative stress occurs after intrarenal radiocontrast injection and its antagonism with allopurinol or superoxide dismutase leads to improved glomerular filtration rate (GFR) [4]. In addition, both oxidative stress and inflammation may contribute to chronic renal disease pathophysiology [5].

Recently, a protective effect of *N*-acetylcysteine (NAC) against contrast nephropathy has been demonstrated [6–8]. A meta-analysis including these data revealed that NAC with hydration reduces by 56% the relative risk of contrast nephropathy in chronic renal insufficiency patients [9]. In fact, NAC has been shown to improve post-radiocontrast renal function in chronic renal failure (CRF) patients [6–8]. Nevertheless,

Correspondence and offprint requests to: Antonio Carlos Seguro, MD, PhD, Faculdade de Medicina da USP, Av Doutor Arnaldo 455, sala 3310, 01246-903 São Paulo, Brazil. Email: trulu@usp.br

NAC-mediated protection mechanisms are not completely understood [10]. Although correction of redox imbalance associated with decreased reactive oxygen species (ROS) generation might induce these changes, there have been no human studies supporting this mechanism.

This double-blind, placebo-controlled study assessed pre- and post-radiocontrast NAC effects on specific oxidative stress and renal tubular injury markers in patients with stable mild-to-moderate chronic renal insufficiency. Increased production of ROS is a feature of CRF and may be worsened by radiocontrast administration. Administration of NAC to CRF patients after coronary angiography increases GFRs. We hypothesise that this increase is caused by decreased ROS as well as by protection against proximal tubular cell injury.

Fluimucil

Subjects ~~and~~ methods

Patients

Patients aged 18–80 years scheduled for angiography and with serum creatinine concentrations >1.4 and <5.0 mg/dl and creatinine clearance rates <70 ml/min/1.73 m^2 (based on 24 h creatinine clearance) were enrolled. Only patients who were known to have a history of CRF and who presented stable serum creatinine concentrations were included. Exclusion criteria were acute myocardial infarction, ARF, renovascular hypertension, prior vasopressor usage, cardiogenic shock and current peritoneal or haemodialysis. The local ethics committee approved the protocol. All patients gave written informed consent.

Study protocol

Figure 1 depicts the double-blind study design. Patients were randomized to receive, pre- and post-contrast, either NAC or placebo. In both cases, intravenous saline was administered (2 ml/kg body weight/h from 4 h pre- until 4 h post-angiography). An oral dose (600 mg) of NAC was given twice daily on four consecutive days, beginning 2 days prior to the angiography. Orange-flavoured NAC was purchased from Zambon Group (Vicenza, Italy). Identical orange flavouring was added to the placebo and patients returned all of the empty envelopes at the end of the study to prove compliance. No patient received theophylline, dopamine, mannitol or any non-steroidal anti-inflammatory drugs during the study. Diuretics were discontinued 48 h before angiography and resumed only after the protocol endpoint.

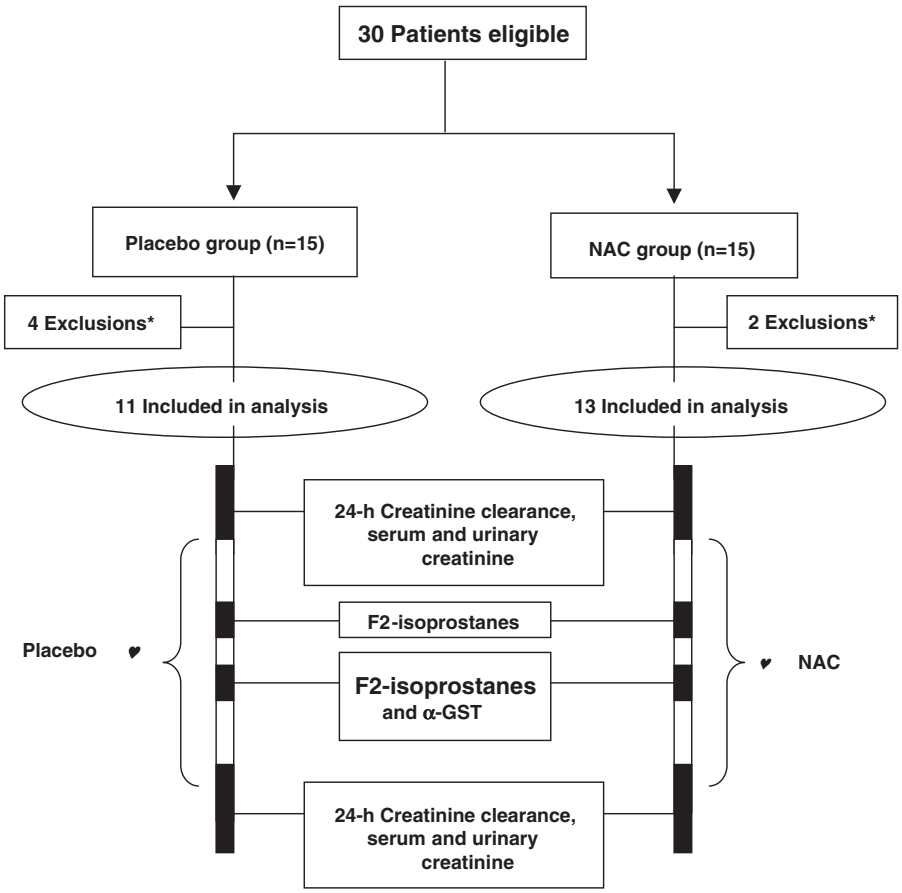

Fig. 1. Study design. *Six subjects were excluded: two patients (one from the placebo group and one from the NAC group) suspected of inadequate collection of urine for creatinine clearance study; one patient from the placebo group with acute pulmonary oedema during saline infusion; and three patients (two from the placebo group and one from the NAC group) with emergency indications of coronary angiography for acute coronary syndrome. Filled heart, coronary angiography.

Coronary procedures

Angiography was performed according to standard clinical practice. Patients received a low-osmolality, non-ionic contrast agent (iopamidol).

Biochemical analysis

Both before and 48 h after angiography, 24 h creatinine clearance was measured. Serum and urinary creatinine were measured colorimetrically and clearance was normalized to 1.73 m^2 body surface area (ml/min/1.73 m^2). In addition, urine samples were collected over ice immediately before and after catheterization and stored at –80°C until F2-isoprostane measurement. Urinary α-glutathione *S*-transferase (α-GST) was measured in samples collected after the procedure.

Urinary F2-isoprostane was extracted and quantitated using enzyme-linked immunosorbent assay to determine levels of 15-isoprostane $F2_t$ (Oxford Biomedical Research, Inc., Oxford, UK), following manufacturer's instructions.

An enzyme immunoassay (NEPHKIT-Alpha; Biotrin, Dublin, Irish Republic) was used according to manufacturer's instructions to quantitatively determine human α-GST expression.

Statistical analysis

Data are reported as means ± SEM. Categorical variables were analysed with Fisher's exact and chi-square tests. Non-paired variables were analysed with the non-parametric Mann–Whitney test. Analyses were performed with GraphPad Prism software (version 3.0; GraphPad Software, San Diego, CA, USA). All statistical tests were two-sided and a *P*-value of <0.05 was considered statistically significant.

Results

Table 1 displays baseline characteristics of the study population. The two groups were similar regarding demographics (age and sex), body mass index, clinical conditions, medication and volume of radiocontrast administered.

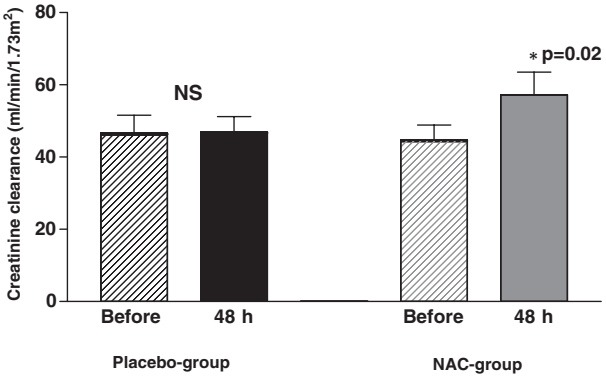

Fig. 2. Creatinine clearance at baseline (before) and at 48 h after radiocontrast administration in patients treated with hydration and placebo alone (placebo group) and in those treated with NAC and hydration (NAC group). Results are expressed as means ± SEM.

Figure 2 shows increased post-angiography mean creatinine clearance in the NAC group (44.7 ± 4.2 *vs* 57.2 ± 6.3 ml/min/1.73 m^2; $P = 0.02$) and no change in the placebo group (46.6 ± 5 *vs* 46.9 ± 4.3 ml/min/1.73 m^2; $P = 0.90$).

Compared with baseline values, post-radiocontrast urinary F2-isoprostane increased significantly in the placebo group (2.9 ± 0.7 *vs* 10.3 ± 2.1 ng/mg creatinine; $P = 0.007$). However, isoprostane formation was blocked in the NAC group (3.5 ± 0.5 *vs* 4.1 ± 0.9 ng/mg creatinine; $P = 0.63$; Figure 3).

Post-angiography proximal tubular injury was lessened by NAC administration, evidenced by higher levels of urinary α-GST in the placebo group (0.8 ± 0.2 *vs* 2.4 ± 0.7 μg/g; $P = 0.046$; Figure 4).

Table 1. Patients' characteristics

	Placebo ($n = 11$)	NAC ($n = 13$)	*P*-value
Age (years)[a]	65.8 ± 2.5	64.4 ± 3	0.82
Males (%)	9 (81.9%)	11 (84.6%)	0.85
Body mass index (kg/m^2)[a]	25.7 ± 1	27.2 ± 2.0	0.82
Serum creatinine (mg/dl)[a]	1.76 ± 0.2	1.79 ± 0.2	0.52
Creatinine clearance (ml/min)[a]	46.6 ± 5	44.65 ± 4.2	0.61
Cholesterol (mg/dl)[a]	187 ± 11	196 ± 13	0.61
Diabetes mellitus [*n* (%)][b]	5 (45%)	4 (31%)	0.75
Hypertension [*n* (%)][c]	10 (91%)	11 (85%)	0.64
Heart failure [*n* (%)][d]	4 (37%)	6 (46%)	0.94
Previous myocardial infarction [*n* (%)][e]	4 (37%)	3 (23%)	0.8
Angiotensin-converting enzyme [*n* (%)]	6 (54.5%)	6 (46.1%)	0.7
Calcium channel blockers [*n* (%)]	5 (45.5%)	3 (23%)	0.47
Beta-blockers [*n* (%)]	6 (54.5%)	7 (54%)	0.97
Aspirin [*n* (%)]	9 (82%)	11 (84%)	0.86
Statins [*n* (%)]	7 (63%)	4 (31%)	0.23
Volume of radiocontrast (ml)[a]	98.2 ± 12	103.6 ± 10	0.77

[a]Values are means ± SEM.
[b]Two measurements of fasting plasma glucose ≥126 mg/dl or concurrent use of insulin or hypoglycaemic therapy.
[c]Arterial pressure ≥140/90 mmHg or current use of antihypertensive therapy.
[d]Symptomatic heart failure according NYHA classes.
[e]Previous history documented in medical records of chest pain, electrocardiographic changes and levels of cardiac enzymes.

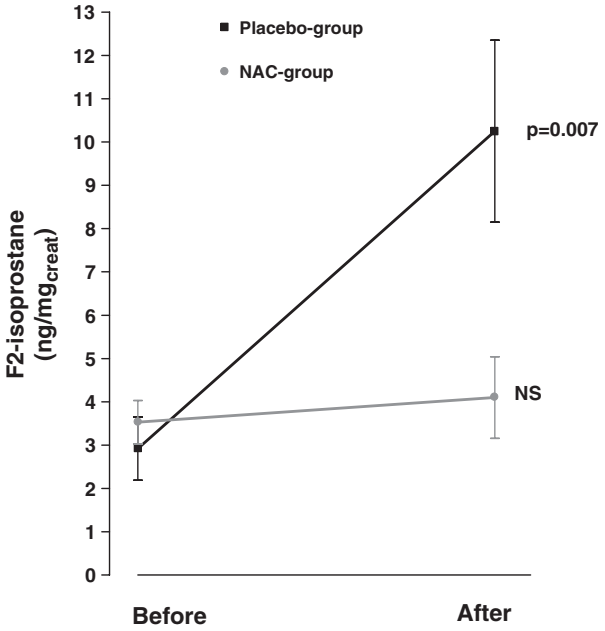

Fig. 3. Levels of F2-isoprostane in urine obtained before and immediately after radiocontrast administration in patients treated with hydration and placebo alone (placebo group) and in those treated with hydration and NAC (NAC group). Results are expressed as means ± SEM. Creat, creatinine.

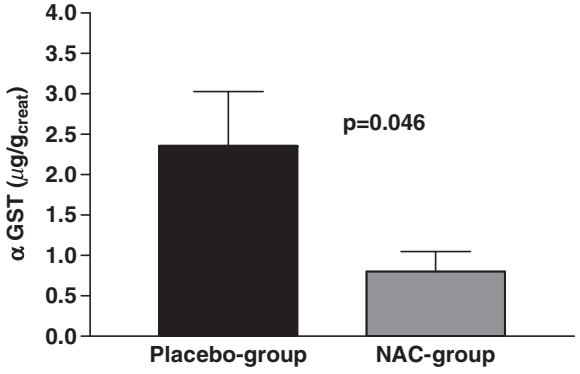

Fig. 4. Levels of α-GST in urine obtained immediately after radiocontrast administration in patients treated with hydration and placebo alone (placebo group) and in those treated with hydration and NAC (NAC group). Results are expressed as means ± SEM. Creat, creatinine.

Discussion

There are two novel findings in the present study. First, radiocontrast increased oxidative stress, as assessed by F2-isoprostane [11], and NAC completely blocked that increase. Second, post-radiocontrast levels of α-GST, a specific tubular proximal enzyme [12], were significantly lower in the NAC group.

Improved renal function with NAC in CRF was remarkable, but consistent with previous data [6–8]. However, none of those authors chose to investigate the mechanism of such effects.

It is not surprising that the control group presented no change in renal function. Previous studies have demonstrated the effectiveness of using hydration in CRF patients to prevent radiocontrast-induced reduction in renal function [6].

It is known that plasma and urinary excretion rates of the lipid peroxidation biomarker malondialdehyde are elevated in animal models and CRF patients [13]. In our study, elevated basal isoprostane levels support the hypothesis that the redox state plays an important role in renal fibrosis and progressive kidney damage [14]. In accordance with the findings of those authors, we found that pre-radiocontrast administration levels of F2-isoprostane in our chronic renal patients were higher than in normal individuals [11]. These elevated F2-isoprostane levels strongly indicate a role for free radicals in CRF pathogenesis. A recent study by Shimizu *et al.* [15] showed a significant decrease in malondialdehyde, a marker of oxidative stress, in NAC-treated rats in the remnant kidney model. Their results demonstrated that NAC attenuated the GFR drop and lowered proteinuria in 5/6-nephrectomized rats and they concluded that, in the remnant kidney model, NAC has a protective effect associated with a decrease in plasma aldosterone and a reduction in oxidative stress.

F2-Isoprostanes are a unique series of prostaglandin-like compounds formed *in vivo* from the free radical-initiated peroxidation of arachidonic acid, independent of cyclooxygenase enzymes, and allow better assessment of oxidative stress [11].

Bakris *et al.* [4] demonstrated that radiocontrast injection promotes transient vasoconstriction and a persistent decline in GFR, creating an ideal scenario for an increase in oxidative stress levels in CRF patients. The authors also reported that intrarenal contrast injection in dogs results in increased ROS production and that intrarenal superoxide dismutase, a scavenger of oxygen free radicals, attenuates the effects of contrast-induced GFR reduction.

It has been postulated that radiocontrast-induced oxidative stress has severe consequences, including direct renal tubular epithelial cell toxicity and renal medullar ischaemia [16], as well as altered renal dynamics caused by imbalances between vasodilator and vasoconstrictor factors.

Marked protective effects of NAC against oxidative stress-induced tubular damage might involve various chemical mechanisms. First, NAC directly scavenges superoxide radicals [17]. Second, as a precursor of glutathione synthesis, NAC significantly increases intracellular redox potential and, likely, improves the reductive status of critical regulatory protein thiol groups [18]. Third, NAC may combine with nitric oxide (NO), leading to the formation of *S*-nitrosothiols, which are a more stable storage form of NO and display potent vasodilator properties [19]. It is well known that NO inhibition is involved in the pathogenesis of CRF. In addition, NO plays an important post-radiocontrast role by increasing medullary oxygenation and regulating blood flow [20]. Previously, we demonstrated that administration of L-arginine (an endogenous substrate for NO synthesis) prevents the lower GFR and reduced

renal blood flow seen in contrast nephropathy [2]. It has also been shown that NAC increases the expression of NO synthase [21]. These biological NO effects facilitate reversal of contrast-induced renal haemodynamic alterations, thereby limiting peroxynitrite anion production.

In light of the fact that α-GST is a sensitive and specific proximal tubular marker protein for monitoring tubular damage under various clinical conditions [13], our data clearly suggest that NAC administration is associated with preservation of tubular cell viability.

In conclusion, we found that the marked protective effects of NAC against radiocontrast-induced tubular injury in CRF patients are strongly associated with amelioration of the effects of oxidative stress-induced tubular injury. Furthermore, our data underscore the role of redox imbalance in the pathophysiology of chronic renal disease and suggest that improved understanding of the pathophysiology of such redox changes might lead to the development of novel therapeutic techniques.

Acknowledgements. We thank the patients who participated in this study, as well as the nursing and pharmacology staff of InCor. We would also like to thank Magali de Araújo for her technical assistance.

Conflict of interest statement. None declared.

References

1. Safirstein R, Andrade L, Vieira JM. Acetylcysteine and nephrotoxic effects of radiographic contrast agents – a new use for an old drug. *N Engl J Med* 2000; 343: 210–212
2. Andrade L, Campos SB, Seguro AC. Hypercholesterolemia aggravates radiocontrast nephrotoxicity: protective role of L-arginine. *Kidney Int* 1998; 53: 1736–1742
3. Parfrey PS, Griffiths SM, Barrett BJ *et al*. Contrast material-induced renal failure in patients with diabetes mellitus, renal insufficiency, or both. A prospective controlled study. *N Engl J Med* 1989; 320: 143–149
4. Bakris GL, Lass N, Gaber AO, Jones JD, Burnett JC, Jr. Radiocontrast medium-induced declines in renal function: a role for oxygen free radicals. *Am J Physiol* 1990; 258: F115–F120
5. Handelman GJ, Walter MF, Adhikarla R *et al*. Elevated plasma F2-isoprostanes in patients on long-term hemodialysis. *Kidney Int* 2001; 59: 1960–1966
6. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. *N Engl J Med* 2000; 343: 180–184
7. Diaz-Sandoval LJ, Kosowsky BD, Losordo DW. Acetylcysteine to prevent angiography-related renal tissue injury (the APART Trial). *Am J Cardiol* 2002; 89: 356–358
8. Kay J, Chow WH, Chan TM *et al*. Acetylcysteine for prevention of acute deterioration of renal function following elective coronary angiography and intervention. A randomized controlled trial. *JAMA* 2003; 289: 553–558
9. Birck R, Krzossok S, Markowetz F, Schnülle P, van der Woude FJ, Braun C. Acetylcysteine for prevention of contrast nephropathy: meta-analysis. *Lancet* 2003; 362: 598–603
10. Bagshaw SM, Culleton BF. Acetylcysteine and renal function following coronary angiographic procedures. *JAMA* 2003; 289: 2795
11. Morrow JD. The isoprostanes: their quantification as an index of oxidant stress status *in vivo*. *Drug Metab Rev* 2000; 32: 377–385
12. Branten AJ, Mulder TP, Peters WH, Assmann KJ, Wetzels JF. Urinary excretion of glutathione *S* transferases alpha and pi in patients with proteinuria: reflection of the site of tubular injury. *Nephron* 2000; 85: 120–126
13. Nath KA, Croatt AJ, Hostetter TH. Oxygen consumption and oxidant stress in surviving nephrons. *Am J Physiol* 1990; 258: F1354–F1362
14. Siems W, Quast S, Carluccio F *et al*. Oxidative stress in chronic renal failure as a cardiovascular risk factor. *Clin Nephrol* 2002; 58 [Suppl 1]: S12–S19
15. Shimizu MHM, Menezes LFC, Seguro AC. *N*-Acetylcysteine protects against progressive chronic kidney disease. *J Am Soc Nephrol* 2003; 14 [Suppl]: 535A
16. Hizoh I, Haller C. Radiocontrast-induced renal tubular cell apoptosis: hypertonic versus oxidative stress. *Invest Radiol* 2002; 37: 428–434
17. Winterbourn CC, Metodiewa D. Reaction of superoxide with glutathione and other thiols. *Methods Enzymol* 1995; 251: 81–86
18. Schaffer FC, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001; 30: 1191–1212
19. Alencar JL, Lobysheva I, Geffard M *et al*. Role of *S*-nitrosation of cysteine residues in long-lasting inhibitory effect of nitric oxide on arterial tone. *Mol Pharmacol* 2003; 63: 1148–1158
20. Brezis Y, Heyman SN, Dinour D, Epstein FH, Rosen S. Role of nitric oxide in renal medullary oxygenation. *J Clin Invest* 1991; 88: 390–395
21. Arstall MA, Yang J, Stafford I, Betts WH, Horowitz JD. *N*-Acetylcysteine in combination with nitroglycerin and streptokinase for treatment of evolving acute myocardial infarction: safety and biochemical effects. *Circulation* 1995; 92: 2855–2862

Received for publication: 7.01.04
Accepted in revised form: 12.03.04