N-acetylcysteine and magnesium improve biochemical abnormalities associated with myocardial ischaemic reperfusion in South Indian patients undergoing coronary artery bypass grafting: a comparative analysis

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ABSTRACT

Introduction: The clinical presentation of ischaemic reperfusion in postoperative patients correlates with oxidative stress. The limited clinical success of anti-ischaemic reperfusion agents has prompted a comparison of the efficacy of N-acetylcysteine (NAC) and magnesium (Mg) in South Indian patients undergoing coronary artery bypass grafting (CABG).

Methods: In Clinical Trial I, 52 South Indian patients who had undergone CABG surgery (with intraoperative Mg supplementation) and 40 controls (without Mg supplementation) were selected and matched. The control patients underwent the same protocol without Mg. In Clinical Trial II, the study population consisted of 50 patients, where 25 patients received NAC just before the release of the aortic cross clamp. In the NAC untreated group, dextrose solution was administered at the same time as the placebo. Six blood samples were taken at different times during the cardiac surgery and the antioxidant enzymes, ATPase and cardiac markers from the coronary sinus blood samples were analysed.

Results: Increased blood lipid peroxidation was observed in patients who were not treated with Mg/NAC. The administration of Mg/NAC just before the release of the aortic cross clamp reduced the lipid peroxidation significantly (p-value is less than 0.05). The above observations were supported by the antioxidant enzyme levels. Significant improvements to the erythrocyte ATPase and cardiac markers in patients treated with Mg/NAC correlated with a reduction in postoperative abnormalities. Based on the biochemical status of the patients, Mg was shown to mediate better recovery from postoperative changes.

Conclusion: NAC and Mg decreased pump-induced oxidative stress during cardiopulmonary bypass (CPB), suggesting that it could be a novel therapy for assisting in the prevention of CPB-induced oxidative stress.

Keywords: coronary artery bypass graft, free radicals, N-acetylcysteine, reperfusion injury

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INTRODUCTION

Coronary artery bypass grafting (CABG) is a very effective procedure for reducing angina and stabilising ventricular function. Although revascularisation of the heart may be successful in CABG, the surgical procedure may be associated with clinically relevant problems such as an ischaemic reperfusion injury. Post ischaemic reperfusion injury is associated with oxidative stress and its subsequent lipid peroxidation.[1]

The release of free oxygen radicals, such as the superoxide anion (O^{2-}), the hydroxyl radical, hydrogen peroxide and the peroxynitrite radical,[2] seems to occur during the re-introduction of oxygen into ischaemic myocardial tissue, which can lead to the formation of lipid peroxides and hydroperoxides via a chain of reactions that result in decreased membrane fluidity, increased membrane permeability and finally, the disruption of membranes.[3] Indeed, the likelihood of these events occurring is higher during the CABG procedure.[4]

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Table I. Demographic data for Clinical Trial I.

Variable	No. of patients	
	Received magnesium (n = 52)	Did not receive magnesium (n = 40)
Gender		
Male	42	30
Female	10	10
Mean age ± SD (years)	63.7 ± 12.7	62.1 ± 11.4
Hypertension	25	18
Diabetes mellitus	20	12
Angina class		
I	12	10
II	33	20
III–IV	7	10
Coronary lesions (stenosis ≥ 70)		
Left anterior descending artery	49	38
Left circumflex artery	27	23
Right coronary artery	30	13
Posterior descending artery	20	15
Preoperative medicines		
Beta blockers	36	24
Calcium channel blocker	10	9
Diuretics	2	2
Angiotensin converting enzyme inhibitors	4	5
Postoperative magnesium level ± SD		
Initial magnesium (mg/100ml)	2.37 ± 0.54	1.86 ± 0.40
Initial potassium (mEq/L)	4.17 ± 0.50	4.22 ± 0.40
Cardiopulmonary bypass time ± SD (min)	84.4 ± 25.5	83.8 ± 24.7
Aortic cross clamp time ± SD (min)	58.8 ± 18.9	57.2 ±19.5

SD: standard deviation

Hypomagnesaemia, one of the significant changes that occurs in CABG patients after surgery, has been reported to be a trigger for coronary artery spasm and can lead to life-threatening arrhythmias, cardiac collapse and even death.[5] The attenuation of increased free radical and calcium overload may limit reperfusion mediated injuries such as cardiac arrhythmias, as has been reported by others.[6] Interestingly, magnesium (Mg) can reduce free radicals after a brief coronary occlusion-reperfusion sequence.[7] Countless reports have been published regarding the efficacy of both pharmacologic and non-pharmacologic interventions in experimental animal models over the past 30 years; however, with the exception of early reperfusion, none have been translated into clinical practice. Despite this, there are a handful of recent studies that provide hope.

One such trial utilised N-acetylcysteine (NAC) as the antioxidant that targets reactive oxygen species (ROS) release during the revascularisation procedure and the administration of Mg^{2+} in reducing the incidence of adverse outcomes.[8,9] However, evidence from some studies that utilised these drugs found limited positive outcomes for postoperative patients, and prompted the authors to design a comparative study on the prophylactic use of Mg^{2+} and NAC on CABG patients. Moreover, no study to date has reported the effect of Mg^{2+} or NAC as anti-ischaemic reperfusion agents in the Indian population.

METHODS

Patients undergoing a CABG operation in which full revisualisation was expected were studied. Ethical approval was provided by the ethics committee of the Institute of Cardiovascular Diseases, Madras Medical Mission. Written consent was obtained from each patient.

In Clinical Trial I, a total of 92 patients (72 male and 20 female, mean age 62.6 ± 11.2 years) who underwent elective CABG for stable angina pectoris between January 2003 and December 2004 were included in the study. Patients were randomly assigned to the Mg treated and Mg untreated groups. 52 patients (42 male and ten female) received Mg, while 40 patients (30 male and ten female) did not receive Mg. The demographic data of the patients is provided in Table I.

In Clinical Trial II, conducted between January 2003 and December 2004, 50 patients (mean age 60.8 ± 11.2 years) with coronary artery disease, who underwent elective CABG for the first time, were enrolled in the study. Patients were randomly assigned to the NAC

Table II. Demographic data for Clinical Trial II.

Variable	No. of patients	
	Received NAC (n = 25)	Did not receive NAC (n = 25)
Gender		
Male	15	17
Female	10	8
Mean age ± SD (years)	61.1 ± 10.3	60.1 ± 9.4
Hypertension	12	10
Diabetes mellitus	10	9
Angina class		
I	5	4
II	13	17
III–IV	7	4
Coronary lesions (stenosis ≥ 70)		
Left anterior descending artery	23	15
Left circumflex artery	12	9
Right coronary artery	14	3
Posterior descending artery	9	6
Preoperative medicines		
Beta blockers	17	9
Calcium channel blocker	4	3
Diuretics	1	1
Angiotensin converting enzyme inhibitors	2	2
None	1	10
Cardiopulmonary bypass time ± SD (min)	82.4 ± 15.5	84.8 ± 14.7
Aortic cross clamp time ± SD (min)	51.8 ±18.9	55.2 ±18.5

SD: standard deviation; NAC: N-acetylcysteine

treated and NAC untreated groups. 25 patients (15 male and ten female) received NAC, while the other 25 patients (17 male and eight female) did not receive NAC. The demographic data of these patients is provided in Table II.

Patients who used antioxidants such as captopril and allopurinol were excluded from the study. Patients who received a blood transfusion or blood products during the operation were also excluded, since the antioxidant properties of such products have not been established yet. None of the patients were taking vitamins or dietary supplements with established antioxidant properties prior to the study. None of the controls had a history of cerebrovascular disease.

A standard cardiopulmonary bypass (CPB) technique was used throughout the study. The extracorporeal circuit was primed with 1.5l Ringer's lactate solution and 100 ml mannitol. In 25 patients (n = 25, age 61.1 ± 10.3 years), myocyte preservation was effected with NAC (20 mg/kg body weight) administration just before the release of the aortic cross clamp. In the NAC untreated group (n = 25, age 60.1 ± 9.4 years), dextrose solution was administered at the same time as the placebo. Similarly, in 52 patients (n = 52, age 63.7 ± 12.7 years), myocyte preservation was effected with Mg (2 g/kg body weight) administration just before the release of the aortic cross clamp. In the Mg untreated group (n = 52, age 62.1 ± 11.4 years) dextrose solution was administered at the same time as the placebo. The perfusion pressure was maintained between 50 mm Hg and 70 mm Hg during the bypass.

On the day of the surgery, the patient was pre-medicated with morphine (0.2 mg/kg) and promethazine (0.5 mg/kg) intramuscularly about 40 minutes prior to the induction of anaesthesia. Anaesthesia was induced with thiopentone (5 mg/kg), and vecuronium was used to accomplish endotracheal intubation with an appropriately sized tube (generally, 9.0 mm for males and 7.5 mm for females). Anaesthesia was maintained with 50% nitrous oxide (N_2O) along with 0.5%–1% halothane. Morphine (0.05 mg/kg) was administered before incision, and 0.15 mg/kg was added to the pump prime. Additional morphine (0.1 mg/kg) and vecuronium (0.1 mg/kg) were administered during re-warming. Post CPB anaesthesia was maintained with 50% O_2, 50% N_2O, 0.5%–1% halothane and vecuronium (¼ of the induction dose).

The bypass circuit was primed with a mixture of Ringer's lactate and hydroxyethyl starch-steril to make the priming volume 1,500 ml. Standard bypass techniques with normothermia (> 32°C) were employed. The mean arterial pressure was continuously monitored and maintained at 50–60 mmHg. The haematocrit was maintained above 22%. Urine output was monitored throughout the procedure. Blood sugar was monitored

Table III. Comparison of the erythrocyte antioxidants measured among bypass patients who received and those who did not receive Mg/NAC.

Antioxidant enzyme	Received Mg	Did not receive Mg	Received NAC	Did not receive NAC
TBARS				
T1	^{a}1.055 ± 0.11	^{a}0.99 ± 0.11	^{a}0.955 ± 0.10	^{a}0.90 ± 0.11
T2	^{b}1.523 ± 0.14	^{b}1.453 ± 0.11	^{b}1.373 ± 0.15	^{b}1.308 ± 0.12
T3	^{c}2.153 ± 0.17	^{c}2.087 ± 0.18	^{c}1.938 ± 0.17	^{c}1.879 ± 0.17
T4	^{d}2.975 ± 0.19	^{d}3.302 ± 0.19	^{d}2.685 ± 0.19	^{d}3.379 ± 0.18
T5	^{e}2.438 ± 0.12 *	^{d}3.111 ± 0.17	^{e}2.198 ± 0.11 *	^{d}3.121 ± 0.18
T6	^{b}1.661 ± 0.13 *	^{e}2.334 ± 0.14	^{b}1.501 ± 0.12 *	^{e}2.324 ± 0.14
Catalase				
T1	^{a}688.54 ± 20.93	^{a}695.22 ± 20.1	^{a}620.44 ± 21.73	^{a}625.72 ± 20.5
T2	^{b}978.89 ± 36.12	^{b}987.68 ± 35.9	^{b}881.09 ± 35.12	^{b}888.98 ± 34.9
T3	^{c}1047.41 ± 38.61	^{c}1058.92 ± 39.1	^{c}943.31 ± 37.61	^{c}953.92 ± 39.4
T4	^{c}1062.07 ± 38.14	^{c}1094.67 ± 38.6	^{c}955.87 ± 39.14	^{c}985.27 ± 37.6
T5	^{b}954.87 ± 36.12 *	^{c}1001.12 ± 37.1	^{b}859.47 ± 35.22 *	^{c}901.02 ± 36.1
T6	^{d}823.68 ± 34.23 *	^{d}869.93 ± 33.7	^{d}741.38 ± 34.23 *	^{d}869.93 ± 33.7
Glutathione peroxidase				
T1	^{a}7.13 ± 1.1	^{a}7.01 ± 1.2	^{a}6.41 ± 1.1	^{a}6.30 ± 1.2
T2	^{b}5.85 ± 0.9	^{b}5.74 ± 0.9	^{b}5.26 ± 0.9	^{b}5.17 ± 0.9
T3	^{c}4.79 ± 0.8	^{c}4.70 ± 0.8	^{c}4.31 ± 0.8	^{c}4.23 ± 0.8
T4	^{b}6.45 ± 1.0	^{a}7.03 ± 1.1	^{b}5.41 ± 1.0	^{a}6.33 ± 1.1
T5	^{c}4.96 ± 0.8 *	^{a}6.55 ± 0.9	^{c}4.36 ± 0.8 *	^{a}5.89 ± 0.9
T6	^{b}5.77 ± 0.9	^{b}5.84 ± 0.9	^{b}5.26 ± 0.9	^{b}5.26 ± 0.9
Superoxide dismutase				
T1	^{a}4372.8 ± 340	^{a}4254.1 ± 323	^{a}3938.8 ± 340	^{a}3829.1 ± 323
T2	^{b}2697.8 ± 235	^{b}2621.6 ± 243	^{b}2429.8 ± 235	^{b}2359.6 ± 243
T3	^{b}2834.8 ± 255	^{b}2761.3 ± 233	^{b}2551.8 ± 255	^{b}2485.3 ± 233
T4	^{c}1426.7 ± 241	^{c}1353.6 ± 255	^{c}1264.7 ± 241	^{c}1218.6 ± 255
T5	^{c}1504.1 ± 254 *	^{c}1397.9 ± 213	^{c}1334.1 ± 254 *	^{c}1258.9 ± 213
T6	^{c}1634.8 ± 222 *	^{c}1527.9 ± 221	^{c}1461.8 ± 222 *	^{c}1375.9 ± 221
Glutathione reductase				
T1	^{a}1.228 ± 0.18	^{a}1.20 ± 0.17	^{a}1.106 ± 0.16	^{a}1.08 ± 0.16
T2	^{b}1.702 ± 0.21	^{b}1.70 ± 0.20	^{b}1.531 ± 0.20	^{b}1.53 ± 0.19
T3	^{c}1.009 ± 0.10	^{c}0.987 ± 0.10	^{c}0.909 ± 0.09	^{c}0.88 ± 0.09
T4	^{d}0.473 ± 0.08	^{d}0.444 ± 0.08	^{d}0.426 ± 0.07	^{d}0.41 ± 0.07
T5	^{d}0.551 ± 0.09 *	^{d}0.476 ± 0.09	^{d}0.496 ± 0.08 *	^{d}0.42 ± 0.08
T6	^{e}0.831 ± 0.09 *	^{e}0.756 ± 0.09	^{e}0.747 ± 0.08 *	^{e}0.68 ± 0.08

*Significantly different from the control group (those who did not receive NAC/Mg) ($p < 0.05$).
NB Values not sharing a common superscript (a, b, c, d, e) differed significantly (at $p < 0.05$) when compared between the groups. Each data point represents the mean ± SD. Activity is expressed as nM/g haemoglobin for TBARS, μM of H_2O_2 utilised/min/g haemoglobin for catalase, unit/g haemoglobin for glutathione peroxidase, superoxide dismutase and glutathione reductase.
T1: before aortic cross clamp was on; T2: 10 min after aortic cross clamp was on; T3: 30 min after aortic cross clamp was on; T4: 10 min after aortic cross clamp was off; T5: 30 min after aortic cross clamp was off; T6: during rewarming; TBARS: thiobarbituric acid reactive substances; NAC: N-acetylcysteine; Mg: magnesium; SD: standard deviation

using a glucometer intraoperatively, and sugar levels were maintained at 180–240 mg.

Post surgery, CPB was discontinued and heparin was neutralised with protamine. The patient then received ionotropic support in the form of dopamine and adrenaline was added, if required, to attain the desired haemodynamic stability. Coronary sinus blood samples were taken at different time intervals, namely, T1: before the aortic cross clamp was on; T2: 10 minutes after the aortic cross clamp was on; T3: 30 minutes after the aortic cross clamp was on; T4: 10 minutes after the aortic cross clamp was off; T5: 30 minutes after the aortic cross clamp was off; and T6: during rewarming. Blood samples taken at T2 and T3 referred to the ischaemic state of the heart, while that taken at T4 referred to the ischaemic reperfused (revascularisation) state. Thiobarbituric acid reactive substances (TBARS) were measured on plasma samples anticoagulated with EDTA using the technique described by Yagi.[10] The antioxidant enzymes, such as superoxide dismutase (SOD),[11] catalase,[12] glutathione peroxidase[13] and glutathione reductase,[14] were also measured. The serum troponin, creatinine phosphokinase (CPK) MB, lactate dehydrogenase (LDH) and calcium levels were analysed using Sigma diagnostic kits (Sigma, St Louis, MO, USA). The protein concentrations were determined using the method described by Bradford.[15] The erythrocyte membrane ATPase, such as Na^+K^+ATPase,[16] Ca^{2+}ATPase,[17] Mg^{2+}ATPase[18] and 5'-nucleotidase,[19] were estimated using ultraviolet-visible spectroscopy.

Approximately 5 ml of blood was drawn and rinsed

Table IV. Comparison of cardiac enzyme activity among bypass patients who received and those who did not receive Mg/NAC.

Cardiac enzyme	Received Mg	Did not receive Mg	Received NAC	Did not receive NAC
CPK MB (ng/ml)				
T1	^{a}0.90 ± 0.10	^{a}1.60 ± 0.50	^{a}0.85 ± 0.10	^{a}1.52 ± 0.40
T2	^{a}0.70 ± 0.10	^{a}1.40 ± 0.30	^{a}0.66 ± 0.1	^{a}1.33 ± 0.30
T3	^{b}4.07 ± 1.40	^{b}9.94 ± 2.10	^{b}3.90 ± 1.30	^{b}9.44 ± 2.02
T4	^{c}17.28 ± 2.10	^{c}15.43 ± 3.14	^{c}16.41 ± 2.02	^{c}14.63 ± 3.04
T5	^{d}21.10 ± 3.60	^{d}25.66 ± 4.50	^{d}20.04 ± 3.41	^{d}24.36 ± 4.30
T6	^{d}20.60 ± 3.13	^{d}24.80 ± 5.13	^{d}19.60 ± 3.02	^{d}23.56 ± 5.03
24 hr postoperative	^{e}11.80 ± 2.17	^{d}21.27 ± 5.11	^{e}10.80 ± 2.04	^{d}20.21 ± 5.01
48 hr postoperative	^{b}2.90 ± 0.80	^{e}4.32 ± 1.12	^{b}2.50 ± 0.70	^{e}4.12 ± 1.11
Troponin I (ng/ml)				
T1	^{a}0.09 ± 0.10	^{a}0.02 ± 0.10	^{a}0.08 ± 0.13	^{a}0.02 ± 0.10
T2	^{a}0.07 ± 0.20	^{a}0.04 ± 0.10	^{a}0.06 ± 0.22	^{a}0.03 ± 0.12
T3	^{b}0.85 ± 0.20	^{b}0.92 ± 1.20	^{b}0.73 ± 0.20	^{b}0.95 ± 1.25
T4	^{c}3.21 ± 1.20	^{c}7.5 ± 2.20	^{c}3.16 ± 1.21	^{c}7.52 ± 2.18
T5	^{c}3.22 ± 1.10	^{c}7.8 ± 2.10	^{c}3.14 ± 1.14	^{c}7.88 ± 2.05
T6	^{c}2.81 ± 0.90	^{c}7.4 ± 2.50	^{c}2.54 ± 0.95	^{c}7.47 ± 2.42
24 hr postoperative	^{d}1.9 ± 0.70	^{d}6.5 ± 2.20	^{d}1.80 ± 0.73	^{d}6.53 ± 2.08
48 hr postoperative	^{b}1.1 ± 0.60	^{e}5.6 ± 2.10	^{b}1.13 ± 0.62	^{e}5.62 ± 2.07
LDH (U/L)				
T1	^{a}595 ± 132	^{a}613 ± 213	^{a}574 ± 130	^{a}604 ± 210
T2	^{a}485 ± 130	^{a}598 ± 256	^{a}464 ± 125	^{a}587 ± 247
T3	^{b}716 ± 231	^{b}734 ± 287	^{b}705 ± 228	^{b}723 ± 275
T4	^{b}786 ± 243	^{c}833 ± 312	^{b}758 ± 251	^{c}822 ± 308
T5	^{c}931 ± 345	^{d}996 ± 333	^{c}919 ± 352	^{d}985 ± 325
T6	^{c}1080 ± 376	^{d}1166 ± 398	^{c}1030 ± 366	^{d}1154 ± 376
24 hr postoperative	^{d}1741 ± 413	^{e}1954 ± 399	^{d}1671 ± 425	^{e}1942 ± 382
48 hr postoperative	^{d}2314 ± 412	^{f}2561 ± 411	^{d}2304 ± 422	^{f}2551 ± 402

*Significantly different from the control group (those who did not receive NAC/Mg) ($p < 0.05$).
NB Values not sharing a common superscript (a, b, c, d, e, f) differed significantly (at $p < 0.05$) when compared between the groups. Each data point represents the mean ± SD.
T1: before aortic cross clamp was on; T2: 10 min after aortic cross clamp was on; T3: 30 min after aortic cross clamp was on; T4: 10 min after aortic cross clamp was off; T5: 30 min after aortic cross clamp was off; T6: during rewarming; CPK MB: creatinine phosphokinase MB; LDH: lactate dehydrogenase; NAC: N-acetylcysteine; Mg: magnesium; SD: standard deviation

with heparin (106 U/L of phosphate buffered saline [PBS]) to serve as an anticoagulant. Erythrocytes were separated from the plasma through centrifugation at 4,000 × g for 10 minutes at room temperature. After the removal of the buffy coat, they were transferred to another tube and washed twice with 10 vol of PBS (150 mmol/L NaCl in 5 mmol/L phosphate, pH 7.4) and collected through centrifugation at 10,000 × g for 10 minutes at 4°C. At this stage, they were referred to as washed erythrocytes.

Erythrocyte membranes, or ghosts, were prepared as described by Johanning and O'Dell,[20] as well as Steck and Kant.[21] Briefly, the washed erythrocytes were lysed with 15 vol of 5 mmol/L phosphate buffer (pH 8.0) and subsequently washed five additional times with the same lysing buffer. Membranes were collected after each wash by centrifugation at 4°C for 10 minutes at 10,000 × g. This procedure yielded approximately 1 mg of protein per 1 ml of blood, as measured by the Lowry method,[22] using bovine serum albumin as the standard.

The data is presented as mean ± standard deviation. Comparisons within groups were made using repeated measures through one way ANOVA, followed by Duncan's Multiple Range Test using the Statistical Package for the Social Sciences version 12 (SPSS, Chicago, IL, USA). The limit of statistical significance was set at $p < 0.05$. Comparisons between groups (preoperative and surgical data) were carried out using the chi-square test. Continuous normally distributed data was analysed by *t*-test (single comparisons). Continuous non-normal data was analysed with the Mann-Whitney U test.

RESULTS

The clinical profiles and intraoperative data of the patients are summarised in Tables I and II. There were no significant peri- and postoperative complications in any group of patients who were operated upon. There were no deaths in this series, probably due to the high quality of the care provided. Moreover, severely ill patients were excluded from the study.

The iron-catalysed oxygen free radical production and subsequent lipid peroxidation, which were assessed by measuring the erythrocyte and erythrocyte membrane levels of TBARS, are shown in Table III and Figs. 1 and 2,

Table V. The activity of the erythrocyte membrane ATPase among CABG patients who received and those who did not receive Mg/NAC.

Erythrocyte ATPase	Received Mg	Did not receive Mg	Received NAC	Did not receive NAC
Na^+K^+ ATPase				
T1	^{a}3.745 ± 0.76	^{a}3.645 ± 0.63	^{a}3.371 ± 0.74	^{a}3.281 ± 0.61
T2	^{b}2.557 ± 0.86	^{b}2.507 ± 0.65	^{b}2.302 ± 0.84	^{b}2.257 ± 0.63
T3	^{b}2.657 ± 0.90	^{b}2.617 ± 0.76	^{b}2.392 ± 0.88	^{b}2.355 ± 0.74
T4	^{b}2.931 ± 0.92	^{b}2.961 ± 0.71	^{b}2.638 ± 0.90	^{b}2.665 ± 0.69
T5	^{b}3.052 ± 0.91	^{a}3.152 ± 0.84	^{b}3.017 ± 0.89	^{a}2.787 ± 0.82
T6	^{c}4.55 ± 0.80	^{c}4.75 ± 0.96	^{c}4.095 ± 0.78	^{c}4.275 ± 0.94
Ca^{2+} ATPase				
T1	^{a}32.71 ± 3.17	^{a}33.71 ± 3.27	^{a}32.38 ± 3.15	^{a}33.37 ± 3.25
T2	^{b}21.62 ± 3.06	^{b}22.62 ± 3.46	^{b}21.40 ± 3.04	^{b}22.39 ± 3.44
T3	^{c}29.25 ± 3.98	^{c}29.55 ± 3.78	^{c}28.95 ± 3.96	^{c}29.25 ± 3.76
T4	^{b}24.82 ± 3.34	^{d}17.72 ± 2.64	^{b}21.60 ± 3.32	^{d}17.55 ± 2.62
T5	^{b}25.18 ± 3.92	^{b}20.85 ± 2.32	^{b}21.95 ± 3.90	^{b}20.64 ± 2.30
T6	^{c}28.64 ± 3.18	^{e}25.64 ± 3.91	^{c}28.35 ± 3.16	^{c}25.38 ± 3.89
Mg^{2+} ATPase				
T1	^{a}5.05 ± 1.76	^{a}5.15 ± 1.36	^{a}4.55 ± 1.74	^{a}4.63 ± 1.34
T2	^{b}3.40 ± 1.77	^{b}3.52 ± 1.47	^{b}3.06 ± 1.75	^{b}3.16 ± 1.45
T3	^{a}5.06 ± 1.61	^{a}5.09 ± 1.32	^{a}4.55 ± 1.59	^{a}4.58 ± 1.30
T4	^{b}3.91 ± 1.22	^{b}3.21 ± 1.22	^{b}3.16 ± 1.20	^{b}2.88 ± 1.20
T5	^{c}4.52 ± 1.21	^{b}3.82 ± 1.43	^{c}4.07 ± 1.19	^{b}3.43 ± 1.41
T6	^{d}6.24 ± 1.17	^{a}5.64 ± 1.57	^{d}5.61 ± 1.15	^{a}5.07 ± 1.55
5' nucleotidase				
T1	^{a}0.8974 ± 0.07	^{a}0.8951 ± 0.06	^{a}0.807 ± 0.06	^{a}0.805 ± 0.05
T2	^{b}0.8138 ± 0.05	^{b}0.8115 ± 0.05	^{b}0.731 ± 0.04	^{b}0.730 ± 0.04
T3	^{a}0.9013 ± 0.06	^{a}0.8901 ± 0.06	^{a}0.810 ± 0.05	^{a}0.801 ± 0.05
T4	^{b}0.8234 ± 0.06	^{b}0.8222 ± 0.05	^{b}0.740 ± 0.05	^{b}0.740 ± 0.04
T5	^{b}0.8142 ± 0.05	^{b}0.8129 ± 0.05	^{b}0.732 ± 0.04	^{b}0.730 ± 0.04
T6	^{a}0.8919 ± 0.07	^{a}0.8709 ± 0.06	^{a}0.801 ± 0.06	^{a}0.783 ± 0.05

*Significantly different from the control group (those who did not receive NAC/Mg) ($p < 0.05$).
NB Values not sharing a common superscript (a, b, c, d, e,f) differed significantly (at $p < 0.05$) when compared between the groups. Each data point represents the mean ± SD. Activity is expressed as μM of inorganic phosphorus liberated/min/mg protein for Na^+K^+ ATPase, nM of inorganic phosphorus liberated/min/mg protein for Ca^{2+} ATPase, Mg^{2+} ATPase and 5' nucleotidase.
T1: before aortic cross clamp was on; T2: 10 minutes after aortic cross clamp was on; T3: 30 minutes after aortic cross clamp was on; T4: 10 minutes after aortic cross clamp was off; T5: 30 minutes after aortic cross clamp was off; T6: during rewarming; NAC: N-acetylcysteine; Mg: magnesium; SD: standard deviation

respectively. No significant difference in erythrocyte and erythrocyte membrane TBARS concentration was found between the groups that were treated and not treated with drugs before revascularisation. However, as expected, an increased production of oxygen free radicals leading to lipid peroxidation was noted after reperfusion in both groups. In fact, in the late phase of revascularisation, significant differences were observed between the subject and control groups (erythrocyte TBARS levels increased from 2.198 ± 0.11 nmol/mg Hb to 3.121 ± 0.18 nmol/mg Hb, $p < 0.05$). In comparison with the NAC treated patients, reduced levels of lipid peroxidation in erythrocytes and the erythrocyte membrane were observed in Mg^{2+} supplemented subjects during the early phase of revascularisation.

Table III shows the activity of different antioxidant enzymes such as glutathione peroxidase, SOD, catalase and glutathione reductase. The number of erythrocyte antioxidant enzymes, with the exception of catalase, was found to have decreased during the late phase of myocardial ischaemia and even in the early ischaemic reperfusion stage. The level of these enzyme activities was significantly different between the control and subject groups for both drugs.

Some of the cardiac markers, including creatinine kinase MB isoenzyme and troponin I, are more specific and sensitive to cardiac damage, and are thus useful for diagnosing myocardial injury. The activity of these enzymes, along with LDH activity, is shown in Table IV. The abovementioned enzymes were observed to have a similar pattern of changes in serum obtained from both NAC and Mg^{2+} CABG patients. During re-warming, with the exception of LDH, the other enzymes were observed to have better recovery.

The activity of erythrocyte membrane ATPase, such as Na^+K^+ ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and 5'-nucleotidase, in CABG patients who received and did not receive NAC are shown in Table V. No significant difference was observed between the subject and control groups in the activities of ATPase. With the exception of 5'-

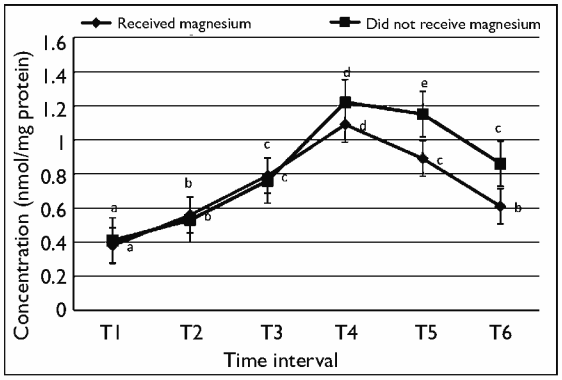

Fig. 1 Comparison of measured thiobarbituric acid reactive substances in the erythrocyte membrane of total bypass patients.

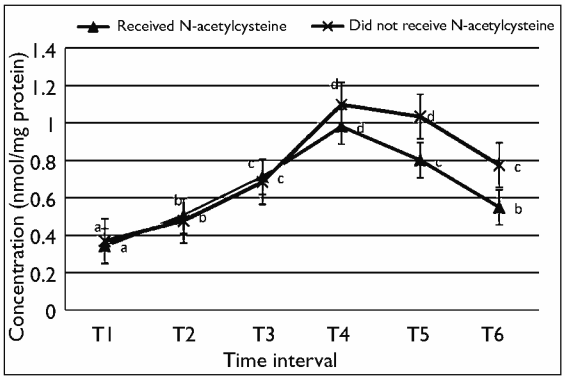

Fig. 2 Comparison of measured thiobarbituric acid reactive substances in the erythrocyte membrane of total bypass patients.

nucleotidase, all other enzymes showed a similar pattern of change during the ischaemic and revascularisation stages in both the NAC and Mg^{2+} treated groups.

DISCUSSION

The present study describes the changes in several circulating antioxidants and a lipid peroxidation index in a group of patients undergoing CABG. The main finding of this study is that NAC pre-treatment causes an improvement in early postoperative function and a reduction in perioperative lipid peroxidation. Although NAC improved membrane-bound ionic pumps in erythrocytes, the Mg^{2+} treated patients in our study showed ideal improvement. Moreover, the number of days of intensive care unit and hospital stay was less for Mg^{2+} treated patients.

Table III as well as Figs. 1 and 2 show the changes in the TBARS and antioxidant capacity of erythrocytes in response to CPB, which is generally used in on-pump CABG. The increased oxidative stress observed in the current study in patients undergoing open heart surgery after CPB had begun complements the findings of other studies.[23] In fact, NAC/Mg^{2+} administration improved the antioxidant status of patients during the revascularisation procedure. NAC can reduce lipid peroxidation via increased glutathione concentrations and subsequent suppression of tumour necrosis factor production.[24] The existence of a direct correlation between Mg deficiency and ROS formation in myocardium has already been well established.[25]

A substantial reduction in the activity of SOD in control patients (Table II) may induce endothelial dysfunction in subjects and can delay early recovery from postoperative trauma.[26] Importantly, the NAC/Mg^{2+} treatment of patients improved the SOD activity (Table III). Catalase, an important hydrogen peroxide scavenger in erythrocytes,[27] was found to be elevated during the ischaemic and revascularisation phases. However, in the physiological condition, glutathione peroxidase is the major route for H_2O_2 breakdown.[28] Thus, a significant ($p < 0.05$) improvement in the activity of both catalase and glutathione peroxidase among NAC/Mg^{2+} treated patients complements the results reported by other studies that focused on the antioxidant property possessed by NAC and Mg^{2+}.[24]

Glutathione reductase plays a very important role in maintaining the reduced to oxidised glutathione (GSH/GSSG) ratio that can, in turn, act as an oxidative stress index experienced by the left ventricle during a surgical procedure.[29] In the current study, an improved glutathione reductase activity level after NAC/Mg^{2+} treatment (Table III, Figs. 1 & 2) predicted a reduction in oxidative stress and subsequent early recovery.

Numerous studies have emphasised the extended release of the cardiac markers in the on-pump CABG procedure and partially due to reperfusion-induced abnormalities.[31] The serum levels of cardiac enzymes and isoenzymes have quite possibly been the most reliable parameter by which myocardial damage is diagnosed or excluded. TnI measurements are highly sensitive in the diagnosis of myocardial injury.[32] Our results on the cardiac marker enzymes such as LDH, CPK MB and troponin I showed that myocardial cell injury was significantly reduced in patients administered with NAC/Mg^{2+} during CABG. On the other hand, increased LDH activity during the late period of surgery and postoperative periods in the control groups predicted the anaerobic metabolic state in the myocardium, which can aggravate the metabolic abnormalities that exist with an ischaemic reperfused heart.

Erythrocyte membrane ATPase in our study decreased significantly during the revascularisation procedure and recovered in the late phase (Table V). The inhibition of red cell membrane ATPase activity in

the revascularisation procedure reflects the probability of intracellular calcium release and may inhibit the cell membrane function. The diminished activity of Ca^{2+} ATPase would lead to intracellular calcium accumulation in vascular smooth muscle cells,[30] and this may be of primary importance in the origination of increased peripheral vascular resistance, which is a characteristic feature of ischaemic reperfusion injury. The improved activities in Na^+K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in NAC/Mg^{2+} treated patients predict the possible correlation of free radicals and erythrocyte ATPase with the early recovery of patients. In fact, Mg^{2+} was reported to influence the activity of enzymes by acting as a co-factor for several ATP requiring enzymes such as Na^+, K^+ATPase.

It can thus be concluded that both NAC and Mg^{2+} attenuate ischaemic reperfusion-induced lipid peroxidation when administered in the therapeutic dose just before the release of the aortic cross clamp during CABG. The multi-spectral activity of Mg^{2+} may ensure a more significant recovery for CABG patients as compared to NAC treated patients, even though the latter imparts a better antioxidant status. Based on the biochemical status of patients in the present study, it was concluded that Mg^{2+} administration results in the rapid recovery of patients from ischaemic reperfusion mediated anomalies as compared to NAC in CABG patients.

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