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Suppression of Angioplasty-Related Inflammation by Pre-Procedural Treatment with Trimetazidine

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Kuralay, F., Altekin, E., Yazlar, A.S., Onvural, B. and Goldeli, O. *Suppression of Angioplasty-Related Inflammation by Pre-Procedural Treatment with Trimetazidine*. Tohoku J. Exp. Med., 2006, **208** (3), 203-212 —— Percutaneous transluminal coronary angioplasty (PTCA) has been recognized as a reliable treatment procedure for acute reversible ischemia and reperfusion. Ischemic reperfusion cycle in PTCA leads to the systemic inflammation and extensive tissue injury by the production of reactive oxygen species including nitric oxide (NO) radicals. In patients with coronary artery disease, undergoing PTCA, the effects of trimetazidine (TMZ), a piperazine-derivative anti-anginal drug, were studied on several indirect markers of systemic inflammatory response: tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and NO products (nitrite and nitrate). Patients (n = 11 each group) were untreated or pre-treated with TMZ (20 mg per orally three times a day), begun three days prior to PTCA, and marker levels were measured before the start of TMZ therapy (baseline), just before PTCA (0 hr), and 4, 24, and 48 hrs after PTCA. The baseline levels of markers were not significantly different between the untreated and pre-treated patients. In contrast, all parameters were lower in the TMZ-treated group than those in the matched control group in the pre- and post-angioplasty periods. Interestingly, in the TMZ group, CRP and nitrite levels were significantly lower than in the control group at each time point of the pre- and post-angioplasty periods, but the TNF-α levels were significantly decreased only in the post-angioplasty period. Pre-procedural treatment with oral TMZ for three days significantly suppressed the elevation of inflammatory markers before and shortly after PTCA. We suggest the usefulness of TMZ in preventing inflammatory cardiovascular events after PTCA. ———— trimetazidine; percutaneous transluminal coronary angioplasty; C-reactive protein; nitric oxide; tumor necrosis factor-α.

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Trimetazidine (1-[2,3,4-trimethoxy-benzyl] piperazine HCl; TMZ) has been found to have cytoprotective effects on ischemic cardiac tissue. During ischemic episodes, TMZ maintains cellular ATP levels, limits intracellular acidosis and accumulation of inorganic phosphate, Na^+ and Ca^{++} in cardiac tissue. These changes are independent of oxygen supply or demand alterations (Demaison et al. 1995; Kay et al. 1995; Cross 2000; Kantor et al. 2000). Trimetazidine selec-

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tively inhibits the fatty acid β-oxidation enzyme: 3 ketoacyl-CoA-thiolase, and lacks direct hemodynamic effects (Stanley and Marzilli 2003; Tabbi-Anneni et al. 2003; Stanley 2004; Folmes et al. 2005; Grynberg 2005). TMZ also limits membrane damage induced by reactive oxygen species (ROS) and protects tissue from free radicals with its antioxidant effects (Guarnieri and Muscari 1993; Doehner 1999; Tikhaze et al. 2000; Kaminski et al. 2002). It has been suggested that ROS- and nitric oxide (NO)-mediated damage enhances the release of proinflammatory mediators such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-8 from macrophages both in inflammation and ischemia (Camussi et al. 1991; Seo et al. 1995; Halliwil et al. 2000; Kuralay et al. 2003; Tamam et al. 2005).

In patients with acute and chronic coronary artery disease, TMZ is safe and has antianginal and antiischemic clinical effects (Opie and Boucher 1995; Veitch et al. 1995; Fantini et al. 1997; El Banani et al. 2000). Experimentally, it reduces infarct size, decreases platelet aggregation and limits leucocyte influx into the infarct zone (Belcher et al. 1993; Williams et al. 1993; Noble et al. 1995). In double-blind placebo-controlled trials, trimetazidine significantly improved symptom-limited exercise performance in stable angina patients when used either as monotherapy or in combination with traditional cardiovascular drugs such as nitrates, β-blockers or Ca^{++} channel antagonists (Levy 1995; Manchanda and Krishnaswami 1997; Szwed et al. 2001a). Moreover, TMZ has been shown to be an excellent alternative to classic hemodynamic agents, and is able to reduce symptoms of angina in patients resistant to vasodilators, β-blockers or calcium channel antagonists (Michaelides et al. 1989; Michaelides and Dimopoulos 1997; Szwed et al. 2001b).

Percutaneous transluminal coronary angioplasty (PTCA) is a reproducible and reliable treatment procedure for acute reversible ischemia and reperfusion. PTCA triggers a systemic inflammatory response and extensive tissue injury by inducing an ischemic reperfusion cycle through repeated balloon inflations (Kober et al. 1993; Kobayashi et al. 1997; French 2000; Gluckman et al. 2000; Heindland et al. 2000). This reperfusion leads to ROS production of hydrogen peroxide and hydroxyl, superoxide, and NO radicals, which are associated with a larger infarct area and worse prognosis (Fox 2000). So, tissue levels of these radicals, including NO and subsequent proinflammatory mediators, are the best direct indicators of systemic inflammatory response, while serum levels could be used as indirect measures of inflammation. Although TMZ is a relatively widely studied drug, only a few studies have been undertaken on its effects in PTCA patients (Fabiani et al. 1992; Steg et al. 2001). In this study on stable coronary artery disease patients, we examined the effects of pre-PTCA treatment with TMZ, a compound having various cell protective effects on ischemic cardiac tissue, on the subsequent levels of serum TNF-α, CRP and NO products.

Materials and Methods

The study protocol was approved by the Clinical Ethics Committee of Dokuz Eylul University Medical School, and ethical guidelines of the Declaration of Helsinki were followed.

Patients

Patients were considered for inclusion into the study if they had stable coronary artery disease with at least > 70% blockage in at least one coronary artery who were scheduled for PTCA. Fifty-four patients met inclusion criteria but 32 were excluded due to cardiac failure, liver failure, renal failure, clinical signs of acute coronary syndrome, and side branch occlusion during or after PTCA. Thus, 22 patients were randomized for inclusion into the study. Informed consent was obtained from all patients. Medical history (hypertension, diabetes, smoking, duration of coronary artery disease), angiographical findings before PTCA and properties of the process (size of the balloon, highest pressure and time for total balloon inflation, stent diameter) were similar for all patients. Criteria for hypertension were systolic and diastolic blood pressures of $\geqq$ 135 mmHg and $\geqq$85 mmHg, respectively; for diabetes, clinical manifestions of diabetes mellitus and a fasting glucose concentration of $\geqq$126 mg/dl; for smoking, cigarette smoking $\geqq$10 pack-years (Pauly and

Pepine 2004). Because serum NO levels can be heavily influenced by dietary nitrogen intake, we fixed the dietary nitrogen intake to 1 g/kg/day of protein per meal during the week prior to PTCA.

Patients (n = 11 each group) were untreated or pre-treated with TMZ (20 mg per orally three times a day), begun three days prior to PTCA. Blood samples were collected from all cases just before starting TMZ (baseline), just before the PTCA procedure (0 hrs) and 4 hrs, 24 hrs, and 48 hrs after PTCA. Blood samples were drawn through antecubital vein, and sera were separated and stored at –70°C until analysis.

CRP measurement

CRP was measured by particle-enhanced immunonephelometry (N Latex CRP mono, Behring Diagnostics, Marburg, Germany) by using a 1:400 sample dilution; a range of 2.4-220 mg/ml was covered (normal values < 5 mg/ml). The intra- and interassay coefficients of variation were < 1.5% and < 2.7%, respectively.

TNF-α measurement

A human TNF-α cytoscreen ELISA kit (Biosource, Cat No: KRC 3012, Camarillo, CA, USA) was used according to manufacturer's instructions and the resulting yellow to blue colour intensity was recorded at 450 nm by a Sorin-Biomedica microplate reader. The intra- and interassay coefficients of variations were < 4.2% and < 7.5%, respectively.

Measurement of NO products

NO was determined by quantitating the stable NO oxidation products, nitrite and nitrate. First, nitrite concentrations were measured by the Griess reaction (Green et al. 1982). Then, samples were incubated with nitrate reductase (0.1 U/ml, Boehringer Mannheim Gmbh, Mannheim, Germany) for 30 min, and nitrate was reduced to nitrite (Bories and Bories 1995). The nitrate level was determined by subtracting the nitrite concentration from total (nitrite + nitrate) concentrations. The intra- and interassay coefficients of variations were < 5% and < 7%, respectively.

All chemicals were purchased from Sigma (Steinheim, Germany).

Statistical analysis

Data were analyzed by using with Mann-Whitney's U-test and Pearson correlation analysis in SPSS for MS Windows Release 10.0® (Redmond, WA, USA), and $p <$ 0.05 was accepted as the level of statistical significance.

Results

Demographic data about cardiovascular risk factors and medications of the patient groups are shown in Table 1. The medications are listed (statins, ACE inhibitors), because these may heavily influence the levels of inflammatory markers. No significant differences were found between the groups regarding risk factors or medications used. The coronary artery characteristics of the patients before PTCA are shown in Table 2 in order to describe the type of ischemic heart disease. There were no significant differences between the male and female patients regarding risk factors, coronary artery characteristics or biochemical data.

Pre- and post-PTCA biochemical data are shown in Table 3. The baseline levels of markers were not significantly different between the untreated and pre-treated patients. In contrast, all parameters were lower in the TMZ group than those in the matched control group in the pre- and post-angioplasty periods. Interestingly, in the TMZ group, CRP and nitrite levels were significantly lower than in the control group at each time point in the pre- and post-angioplasty periods. TNF-α levels were significantly lower than the control group only in the post-angioplasty period. Additionally, total NO levels were significantly lower at 0, 24 and 48 hrs, while nitrate levels were significantly lower in the TMZ group at only 48 hrs.

When the time course levels of the biochemical parameters in untreated and pre-treated patients were compared; significantly lower levels of CRP were detected at all measurement points in the pre-treated group. The peak levels for CRP were reached at 48 hrs in both groups ($p < 0.0001$; for each) (Fig. 1). TNF-α levels at 4, 24, and 48 hrs were significantly lower ($p < 0.0001$ at all measurement points) in patients pre-treated with TMZ (Table 3). In both groups, TNF-α reached its peak at 24 hrs (Fig. 2).

NO activity peaked at 24 and 48 hrs in the pre-treated and untreated groups, respectively. Significant positive correlations were found between TNF-α and CRP ($p < 0.001$; $r = 0.620$),

TABLE 1. *Demographic data, cardiovascular risk factors, medications, and vital signs of study patients. Groups were not different statistically ($p > 0.05$) in any category.*

	Pre-treated with TMZ group ($n = 11$)	Untreated group ($n = 11$)
Age (years)	56.3 ± 10.8	53.8 ± 8.6
Male	5 (45%)	3 (27%)
> 10 pack years cigarettes	4 (36%)	5 (45%)
Diabetes	6 (55%)	6 (55%)
Hypertension	6 (55%)	5 (45%)
Past myocardial infarction	2 (18%)	2 (18%)
Past CABG 40-49%	1 (9%)	2 (18%)
Past CABG ≧ 50%	10 (91%)	9 (82%)
Aortic systolic pressure (mmHg)	144 ± 28	136 ± 18
Aortic diastolic pressure (mmHg)	76 ± 11	78 ± 10
Pulse pressure (mmHg)	68 ± 26	57 ± 16
ACE inhibitors	5 (45%)	5 (45%)
β-blockers	8 (73%)	7 (64%)
Angiotensin II receptor blockers	2 (18%)	1 (9%)
Calcium channel blockers	1 (9%)	2 (18%)
Aspirin	10 (91%)	11 (100%)
Nitrates	7 (64%)	8 (73%)
Statins	8 (73%)	7 (64%)
Serum total cholesterol (mg/dl)	198.6 ± 45.2	216 ± 37.7
Serum triglyceride (mg/dl)	176.7 ± 89.1	187.9 ± 83.5
Serum LDL (mg/dl)	129.8 ± 46.4	140.8 ± 37.3
Serum HDL cholesterol (mg/dl)	37.4 ± 11.7	38 ± 12.6

CABG, coronary artery by-pass grafting; ACE, Angiotensin Converting Enzyme.

TNF-α and nitrite ($p < 0.001$, $r = 0.472$), TNF-α and nitrate ($p < 0.001$, $r = 0.470$), CRP and nitrite ($p < 0.001$, $r = 0.569$), and CRP and nitrate ($p < 0.05$, $r = 0.268$).

DISCUSSION

PTCA triggers a systemic inflammatory response by inducing an ischemic reperfusion cycle through repeated balloon inflations, which can be followed by tissue injury and impaired antioxidant status (Kloner et al. 1991; Liuzzo et al. 1994; Azar et al. 1997; Ridker et al. 1997; Ambrosio and Tritto 1998). Inflammatory marker levels, such as CRP, IL-1 and IL-6, increase after PTCA (Kloner et al. 1991; Balligand et al. 1994; Azar et al. 1997; Matsumara et al. 1998; Wildhirt et al. 1999).

Nitric oxide has diverse effects on cardiac tissue. An increase in NO synthesis and inducible nitric oxide synthase (iNOS) activity decreases myocardial blood flow and tissue oxygenation, causing ischemia in the myocytes (Balligand et al. 1994; Wildhirt et al. 1999; Sumeray et al. 2000). In experimental ischemia models, inhibition of NOS was shown to prevent tissue damage (Camussi et al. 1991; Seo et al. 1995; Halliwil et al. 2000; Szwed et al. 2001a, 2001b). Some investigators however, have suggested that the release of NO is beneficial to the ischemic heart. A recent study in humans found that a decrease in NO levels could cause ischemia through vasoconstriction (Josephy et al. 2003). Recent studies in

TABLE 2. *Coronary artery characteristics of patients before PTCA*[*]

Coronary artery characteristics		Pre-treated with TMZ group (n = 11)	Untreated group (n = 11)
≧ 70% Coronary Artery stenosis Location	Left anterior Descending artery	8 (73%)	10 (91%)
	Circumflex artery	7 (64%)	4 (36%)
	Right coronary artery	8 (73%)	8 (73%)
Number of diseased Coronary arteries	One	4 (36%)	3 (27%)
	Two	4 (36%)	3 (27%)
	Three	3 (27%)	5 (45%)
Left ventricular wall motion defects		6 (54%)	6 (54%)
Collateral circulation		3 (27%)	2 (18%)
Lesion type[*]	A	3 (27%)	2 (18%)
	B1	4 (36%)	3 (27%)
	B2	2 (18%)	3 (27%)
	C	2 (18%)	3 (27%)
Lesion length (mm)		10.8 ± 4.6	11.7 ± 5.4

[*] American Heart Association/American College of Cardiology (AHA/ACC) Lesion Classification (Pauly and Pepine 2004)

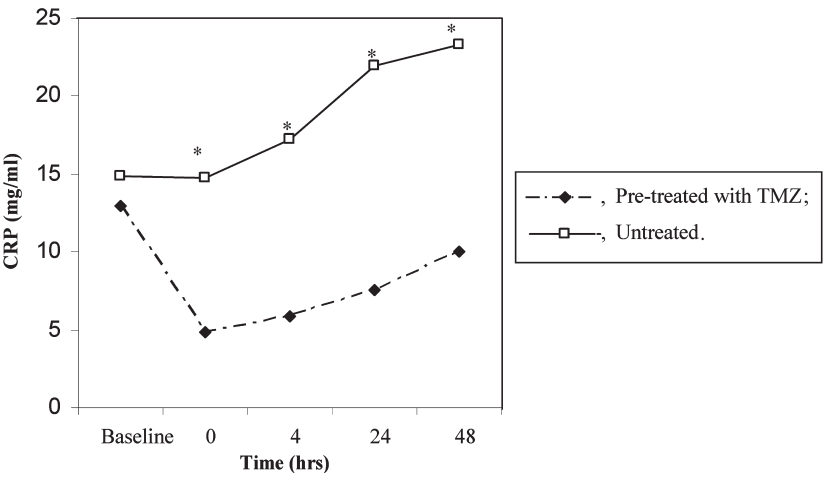


Fig. 1. CRP levels at various time intervals in the pre-treated with TMZ and untreated groups.
[*] $p < 0.0001$ significantly higher when compared with pre-treated patients at the same hour.

rats have shown that chronic administration of L-arginine analogues, such as N-nitro-L-arginine methyl ester (L-NAME), cause systemic arterial hypertension, decrease arterial wall cGMP (the second messenger of NO) levels (Arnal et al. 1992; Baylis et al. 1992; Numaguchi et al. 1995), decrease urinary secretion of the stable products of NO (Bank et al. 1994), and cause vascular inflammation including increased tumor growth factor-β 1 (Koyanagi et al. 2000a, b; Kataoka et

TABLE 3. *Levels of inflammatory markers at various time intervals in the pre-treated with TMZ*

	Baseline		0 hrs		4 hrs	
	Pre-treated	Untreated	Pre-treated	Untreated	Pre-treated	Untreated
CRP (mg/ml)	13.0 ± 5.9	14.9 ± 4.5	4.8 ± 3.1[a]	14.7 ± 5.8	5.8 ± 1.2[a]	17.2 ± 2.4
TNF-α (pg/ml)	9.7 ± 7.3	9.2 ± 6.8	4.9 ± 2.9	9.5 ± 7.5	9.5 ± 2.2[a]	31.7 ± 9.6
Nitrite (nmol/ml)	60.2 ± 24.3	58.0 ± 26.2	19.4 ± 7.0[a]	61.5 ± 25.7	30.4 ± 14.1[b]	60.7 ± 9.8
Nitrate (nmol/ml)	28.0 ± 12.4	28.0 ± 16.9	28.4 ± 14.6	29.2 ± 14.3	31.7 ± 11.7	38.2 ± 12.6
Total NO (nmol/ml)	98.4 ± 46.7	97.6 ± 44.1	57.9 ± 15.1[a]	96.4 ± 45.0	61.6 ± 18.6	68.9 ± 17.0

[a] $p < 0.0001$, [b] $p < 0.005$, [c] $p < 0.01$ (when compared with untreated patients at the same hour).

al. 2003) and vascular endothelial growth factor production (Zhao et al. 2002).

Reactive oxygen species and neutrophils are the key players in reperfusion injury, interacting in multiple ways with other elements, such as the complement system, endothelial cells, lymphocytes, macrophages and myocytes. The production of ROS increases considerably during tissue ischemia due dissociation of oxidative phosphorylation, which results in univalent reduction of oxygen, catabolism of ATP into hypoxanthine and uric acid and infiltration of damaged tissues by polymorphonuclear leukocytes (Rogacka et al. 2000; Kanko et al. 2005). Zhao et al. (2000) demonstrated the peak of neutrophil accumulation to occur between 6 and 24 hrs after myocardial infarction. Reperfusion injury has been shown in some studies to occur after 4-6 hours (Lucchesi 1990; Albertine et al. 1994; Di Pasquale et al. 1999). Kaminski et al. (2002) suggested that increased CRP and NO levels in the setting of ischemia are usually related to myocyte death, causing tissue edema, decreased microcirculation, and myocardial perfusion in the first 24 hrs. Similarly, in our study, CRP levels started to increase at four hours and continued to increase for 48 hrs, particularly in the untreated group. The levels of TNF-α increased up to 24 hrs, and then decreased in both the untreated and pre-treated with TMZ groups, indicating that the damage takes place in the first few hours, and continues over the first 24 hrs.

TMZ infusion during (Fabiani et al. 1992) and 48 hrs after (Di Pasquale et al. 1999; The EMIP-FR Group 2000; Steg et al. 2001) coronary artery bypass graft operation significantly reduced

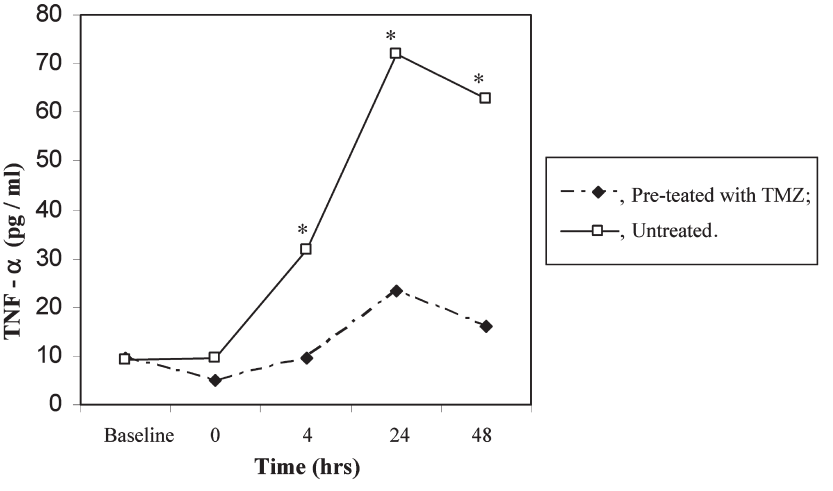


Fig. 2. TNF-α levels at various time intervals in the pre-treated with TMZ and untreated groups.
* $p < 0.0001$ significantly higher when compared with pre-treated patients at the same hour.

and untreated groups (mean ± S.D.).

24 hrs		48 hrs	
Pre-treated	Untreated	Pre-treated	Untreated
7.5 ± 4.3[a]	22.0 ± 4.1	10.0 ± 6.2[a]	23.3 ± 6.4
23.2 ± 7.4[a]	71.8 ± 12.3	15.9 ± 5.0[a]	6 2.8 ± 21.7
26.6 ± 6.1[b]	62.7 ± 22.2	19.4 ± 7.5[a]	150.5 ± 70.9
43.8 ± 18.6	51.4 ± 14.5	27.6 ± 9.4[a]	5 3.2 ± 12.2
68.3 ± 18.0[c]	114.1 ± 22.5	47.0 ± 13.9[a]	203.7 ± 20.4

CRP, myosin and malondialdehyde levels. Our results are also similar, in that TMZ significantly reduced CRP, TNF-α, and NO levels. CRP levels in the pre-treated group were two to three times less than the untreated group at all measurement points. Looking at the increases in NO and TNF-α, these markers/mediators may stimulate the production of each other, that is, NO may be enhancing the inflammatory alterations induced by TNF-α or vice versa.

While we used TMZ orally (60 mg qd for three days), TMZ treatment protocols in the literature differ in their doses, routes, and duration of treatment (Fabiani et al. 1992; Di Pasquale et al. 1999; Girgin et al. 1999; The EMIP-FR Group 2000; Kowalski et al. 2000; Steg et al. 2001; Kuralay et al. 2003; Baumert et al. 2004). A variation in our protocol may have given different results.

Many in-vitro and animal experiments have found cardioprotective effects of TMZ on different parameters of cell metabolism via its antioxidant properties and its causing a reduction in cellular acidosis (Demaison et al. 1995; Kay et al. 1995; Di Pasquale et al. 1999; Kantor et al. 2000; Cross et al. 2000; El Banani et al. 2000; Rogacka et al. 2000; Szwed et al. 2001a). When given before cardiologic interventions, TMZ has been found to improve ventricular function without altering hemodynamics, limit infarct area, and reduce both platelet aggregation and leukocyte migration to the area (Fabiani et al. 1992; Steg et al. 2001). Preliminary clinical studies tend to confirm a direct benefit of a metabolic approach with TMZ in left ventricular dysfunction (Wildhirt et al. 1999; Zhao et al. 2000; Belardinelli and Purcaro 2001). A significant improvement in ejection fraction was also found with TMZ, compared with placebo, in a group of patients undergoing PTCA (Brottier et al. 1990; Birand et al. 1998). Recent studies have found that TMZ protects from dobutamine-induced ischemia and improves resting ventricular function (Lu et al. 1998; Belardinelli and Purcaro 2001).

Optimising energy metabolism in the heart is a novel approach to use in managing a patient with ischemic heart disease. In particular, promoting myocardial glucose metabolism can enhance

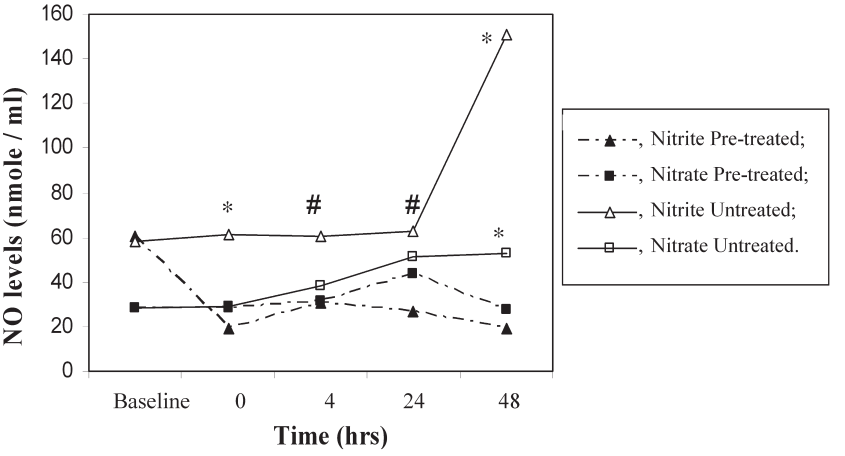


Fig. 3. NO levels at various time intervals in the pre-treated with TMZ and untreated groups.
*$p < 0.0001$ significantly higher when compared with pre-treated patients at the same hour.
#$p < 0.005$ significantly higher when compared with pre-treated patients at the same hour.

heart function, lessen injury to tissue, or both. TMZ partially reduces fatty acid β-oxidation secondary to the selective inhibition of a mitochondrial enzyme, 3 ketoacyl-CoA-thiolase. This results in a shift of cardiac metabolism from fatty acids to glucose oxidation, which in turn restores ATP production and protects the heart from ischemia-related damage (Lopaschuk 2001). This may have been the beneficial mechanism of TMZ that we observed.

Patients receiving three days of oral TMZ prior to PTCA had significantly decreased levels of CRP, TNF-α and NO during the initial 48 hrs after PTCA. In conclusion, TMZ should be studied clinically on larger study populations to determine its usefulness for preventing inflammatory cardiovascular events after PTCA.

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