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Effect of intravenous nitroglycerin therapy on erythrocyte antioxidant enzymes

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Abstract
Intravenous nitroglycerin (GTN) has been used as an anti-ischemic agent for the therapy of unstable and post-infarction angina. Nitric oxide ($^{\cdot}$NO) and S-nitrosothiols constitute the biologically active species formed via nitroglycerin bioactivation. Increased levels of reactive oxygen species can diminish the therapeutic action of organic nitrates by scavenging donated $^{\cdot}$NO and oxidizing tissue thiols important in nitrate biotransformation. Studies reported here show that the red cell activity of antioxidant enzymes, catalase and glutathione peroxidase, are significantly decreased after intravenous nitroglycerin treatment. Catalase activity (739.6 ± 92.3 k/g Hb) decreased to 440.1 ± 111.9 and 459.8 ± 130.7 k/g Hb after 1 and 24 hr GTN infusion, respectively. Similarly, glutathione peroxidase activity (5.8 ± 1.8 U/g Hb) decreased to 3.2 ± 1.7 and 3.8 ± 1.1 U/g Hb after 1 and 24 hr GTN infusion, respectively. The reported decrease in antioxidant enzyme activities can lead to an oxidant milieu and contribute to the generation of nitrate tolerance.

Keywords: *Glyceryl Trinitrate, erythrocyte, antioxidant enzymes, reactive oxygen species, ROS*

Introduction

Intravenous nitroglycerin (GTN) has been the foremost anti-ischemic therapy used in unstable angina, acute myocardial infarction and post-infarction angina [1–3]. Although venous effects predominate, GTN produces dilation of both arterial and venous beds [4]. Nitroglycerin bioactivation is reported to be tissue and cell specific and dose-dependent, yielding 1,2-glyceryl dinitrate (1,2-GDN), 1,3-GDN, inorganic nitrite, $^{\cdot}$NO and S-nitrosothiols (SNO) [5–7]. It is generally assumed that GTN is converted in vascular smooth muscle cells to SNO or to $^{\cdot}$NO, which constitute the biologically active species [7].

The mechanisms underlying nitrate tolerance are not fully understood. There is a growing body of evidence that development of tolerance is, at least in part, secondary to enhanced production of oxygen-derived free radicals. It has been proposed that decreased bioavailability of $^{\cdot}$NO, derived from nitroglycerin, occurs as a consequence of the interaction of $^{\cdot}$NO with superoxide ($O_2^{\cdot-}$) and contributes to the development of tolerance. Indeed, nitroglycerin has been reported to stimulate the production of oxygen-derived free radicals within vascular tissue [8,9]. The generation of oxidants also causes oxidation of tissue thiols important in nitrate biotransformation. Multiple studies have demonstrated that changes in the redox state or increased free radical formation inhibits glutathione-S-transferases, a family of enzymes potentially involved in GTN biotransformation [10].

Enhancing antioxidant status and scavenging of $O_2^{\cdot-}$ has been shown to attenuate the development of hemodynamic tolerance accompanying continuous nitrate administration [11–13]. In view of previous studies reporting nitroglycerin-induced generation of reactive oxygen species, this study aimed to

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determine the effect of intravenous nitroglycerin therapy on antioxidant enzymes. *In vivo* and *in vitro* studies reported here show that the red cell activity of antioxidant enzymes, catalase (CAT) and selenium-glutathione peroxidase (selenium-GPx), are significantly decreased after intravenous nitroglycerin treatment and incubation with GTN. The observed decrease in antioxidant enzyme activities can contribute to GTN-induced oxidative stress and lead to oxidation of tissue thiols important in nitrate biotransformation.

Patients and methods

Patients

In vivo studies were conducted on twenty two patients, aged 45–65 years (5 female, 17 male), who were admitted to Akdeniz University Cardiology and Emergency Clinic with a diagnosis of unstable angina. Unstable angina was defined as recurrent or increased frequency of chest pain lasting over 5 min at rest or with minimal exertion within 24 h before randomization. Intravenous nitroglycerin was diluted in 5% dextrose in water and infused at an initial rate of 10 μg/min via an infusion pump. The infusion rate was lowered for systolic pressure below 100 mmHg and was temporarily interrupted for systolic pressure below 80 mmHg.

Red blood cell (RBC) antioxidant enzyme activities for the in vivo studies were determined from blood collected before, 60 min and 24 h after the administration of intravenous nitroglycerin. In vitro experiments were carried out on RBCs obtained from 30 healthy volunteers aged 35–66 years (14 female, 16 male). Cells were incubated at 37°C in PBS buffer containing 4×10^{-5} M GTN for 1 and 3 h. All experimental protocols were approved by the Institutional Review Board, and all patients gave informed consent.

Measurement of erythrocyte antioxidant enzyme activities

Blood samples for both in vivo and in vitro studies were drawn from the cubital vein into heparinized test tubes and erythrocyte fractions were separated by centrifugation. Hemoglobin concentration was determined with Drabkin's reagent at 540 nm [14]. Glucose-6-phosphate dehydrogenase (G6PD) activity was measured by a modification of Zinkham's method recommended by WHO [15]. The activity of catalase (CAT), Cu/Zn-superoxide dismutase (Cu/Zn-SOD) and selenium-GPx were assayed by the methods of Aebi [16], Misra and Fridovich [17] and Paglia and Valentine [18], respectively. Erythrocyte glutathione (GSH) levels were measured by the method of Beutler [14].

Statistical analysis

Data are expressed as mean ± S.D. The statistical analysis of the obtained data was performed by Sigma Stat (version 2.03) software for windows. The differences among the different groups were analyzed via one way analysis of variance and all pairwise multiple comparisons were performed by Tukey Test. A *P*-value of less than 0.05 was considered significant. The calculated *P*-values by the Sigma Stat (version 2.03) statistical software program are given as $P < 0.001$.

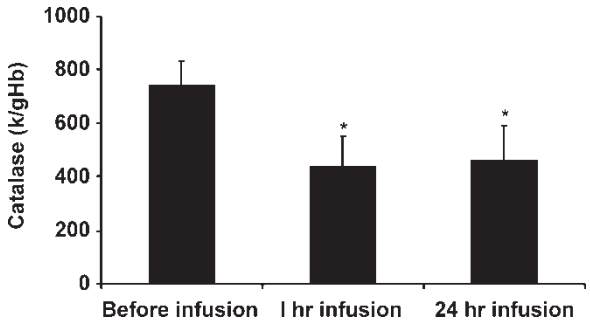


Figure 1. Effect of intravenous nitroglycerin treatment on erythrocyte catalase activity. Values are mean ± SD and $n = 13–22$ for each measurement. * $p < 0.001$ vs. control.

Results

Erythrocyte antioxidant enzyme activities after intravenous nitroglycerin infusion

Red cell CAT and selenium-GPx activities were significantly reduced after 1 and 24 h of 10 μg/min nitroglycerin infusion (Figures 1 and 2, respectively). Calculated CAT activity before infusion was 739.6 ± 92.3 k/g Hb, and decreased to 440.1 ± 111.9 and 459.8 ± 130.7 k/g Hb after 1 and 24 h infusion, respectively. Selenium-GPx activity before infusion was 5.8 ± 1.8 U/g Hb, and decreased to 3.2 ± 1.7 and 3.8 ± 1.1 U/g Hb after 1 and 24 h infusion, respectively. Intravenous nitroglycerin infusion had no significant effect on red cell GSH levels, Cu/Zn-SOD and G6PD activity (Table I).

Erythrocyte antioxidant enzyme activities after glyceryl trinitrate incubation

Incubation of RBCs in PBS buffer containing 4×10^{-5} M GTN for 1 and 3 h resulted in a significant



Figure 2. Effect of intravenous nitroglycerin treatment on erythrocyte selenium-glutathione peroxidase activity. Values are mean ± SD and $n = 13–22$ for each measurement. * $P < 0.001$ vs. control.

Table I. Erythrocyte antioxidant enzyme activities and glutathione levels after intravenous nitroglycerin treatment.

Measurement	Enzyme activity		
	Before infusion (*22*)	1 hr infusion (*22*)	24 hr infusion (*13*)
Cu/Zn-SOD (U/g protein)	80.00 ± 11.14	81.13 ± 16.22	82.42 ± 14.76
G6PD (U/g Hb)	7.67 ± 1.39	7.70 ± 1.35	7.70 ± 1.10
GSH (mg/g Hb)	23.36 ± 5.37	24.09 ± 5.46	23.92 ± 5.04

Values are mean ± SD.
n for each measurement is italicized in parentheses.

decrease in red cell CAT and selenium-GPx activity (Tables II and III). Nitroglycerin incubation had no significant effect on erythrocyte GSH levels, Cu/Zn-SOD and G6PD activity (Tables II and III).

Discussion

The elimination of GTN in humans is extremely rapid with a serum half life of about 3 minutes [19]. The observed clearance rates of GTN greatly exceed hepatic blood flow and as a result, extrahepatic metabolism of GTN takes place in RBCs and the vascular wall [20,21]. Due to drawbacks in obtaining vascular and liver tissue from human subjects enrolled in the study, we were unable to carry out enzyme activity measurements in the liver and vascular wall. Given this limitation, we focused on performing these experiments on the red blood cells of patients receiving intravenous nitroglycerin therapy. In view of the fact that both CAT and selenium-GPx are found in many cell types including hepatocytes and endothelial cells [22] it is probable that GTN-induced changes in antioxidant enzymes also occur during systemic metabolism of nitroglycerin.

Although enhancing antioxidant status and the scavenging of free radical species have been shown to attenuate the development of hemodynamic tolerance accompanying continuous nitrate administration [11–13], this is the first *in vivo* study to assess the inhibitory effect of intravenous nitroglycerin therapy on antioxidant enzymes. Data reported here show that the antioxidant enzymes CAT and selenium-GPx are significantly inhibited by intravenous infusion or incubation with GTN (Figures 1 and 2; Tables II and III, respectively). The observed decrease in red cell CAT and selenium-GPx activity following intravenous nitroglycerin treatment and incubation with GTN likely occurs through the molecular interaction of nitroglycerin-donated $\dot{N}O$ with the catalytic centers of these enzymes [23–26].

Intravenous nitroglycerin dosage in coronary patient groups, ranges from 10–120 μg/min [27–29]. In this study, intravenous nitroglycerin was infused at an initial rate of 10 μg/min. Although the analysis of antioxidant parameters with different dose ranges of intravenous GTN would be supportive of the obtained data, the study population included patients who were admitted to the emergency clinic with a diagnosis of unstable angina and thus a standard dosing regimen had to be followed. Overall, the reported low dose of GTN infusion caused minor fluctuations in heart rate and minimally affected blood pressure. In this context, it is important to note that dilatation of coronary stenosis plays an important role in the antianginal action of nitroglycerin, and that strong hemodynamic effects do not appear to be a prerequisite of the observed beneficial effects.

In summary, this paper reports the effect of intravenous administered GTN or *in vitro* added GTN on erythrocyte antioxidant enzymes. The observed inhibition of CAT and selenium-GPx activity can contribute to GTN-induced oxidative stress and nitrate tolerance reported during continuous nitroglycerin administration.

Table II. Erythrocyte antioxidant enzyme activities and glutathione levels after 1 hour glyceryl trinitrate incubation.

Measurement	Enzyme activity	
	Control (*30*)	GTN [4×10^{-5} M] (*30*)
CAT (k/g Hb)	552.3 ± 68.3	322.16 ± 62.32⋆
Selenium-GPx (U/g Hb)	4.35 ± 0.58	2.17 ± 0.57⋆
Cu/Zn-SOD (U/g protein)	47.36 ± 12.16	47.06 ± 11.46
G6PD (U/gr Hb)	6.98 ± 1.76	6.74 ± 1.86
GSH (mg/gr Hb)	25.66 ± 5.67	25.02 ± 5.83

Values are mean ± SD. ⋆ $p < 0.001$ from control.
n for each measurement is italicized in parentheses.

Table III. Erythrocyte antioxidant enzyme activities and glutathione levels after 3 hours glyceryl trinitrate incubation.

Measurement	Enzyme activity	
	Control (*30*)	GTN [4×10^{-5} M] (*30*)
CAT (k/g Hb)	550.03 ± 62.30	326.66 ± 60.50⋆
Selenium-GPx (U/g Hb)	4.00 ± 0.48	2.29 ± 0.5⋆
Cu/Zn-SOD (U/g protein)	49.86 ± 11.70	53.43 ± 11.80
G6PD (U/g Hb)	5.98 ± 1.87	6.84 ± 1.79
GSH (mg/g Hb)	24.98 ± 5.91	24.92 ± 4.91

Values are mean ± SD. ⋆ $p < 0.001$ from control.
n for each measurement is italicized in parentheses.

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