

Effects of Levosimendan on Hydrogen Peroxide Induced Contraction in Human Saphenous Vein

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ABSTRACT

Aim: Increased oxidative stress plays important roles in vascular dysfunction in patients undergoing coronary artery bypass graft surgery. Hydrogen peroxide (H_2O_2) is used as an experimental model for oxidative stress. The present study was designed to assess the effects of levosimendan pretreatment on the contractile effects induced by H_2O_2 in human saphenous vein (HSV) segments. **Methods:** We studied H_2O_2 induced contractions of isolated HSV mounted in standard tissue baths. H_2O_2 (10^{-6} - 10^{-3} M) was added cumulatively to the organ bath. Concentration-response curves to H_2O_2 were repeated in the presence of levosimendan (10^{-8} M). In the second series of experiments, strips were contracted with 5-HT (10^{-5} M). When the contraction reached a stable plateau, H_2O_2 was administered cumulatively into the organ bath. The same procedure was conducted in the presence of levosimendan. **Results:** Pretreatment of the SV strips with levosimendan significantly reduced the contractile response to each concentration of H_2O_2 . 5-HT produced contractions in SV strips. Further treatment of these strips with H_2O_2 resulted in statistically significant concentration-dependent increases in tension. Preincubation of the tissues with levosimendan did not significantly influence the maximum amplitude of the 5-HT-induced tone but inhibited the contractile effect of H_2O_2 on the 5-HT-induced contraction. **Conclusion:** Pretreatment of HSV with clinical concentrations of levosimendan inhibits the vasoconstriction caused by oxidative stress, indicating its potential preventive effect against oxidative stress induced graft spasm.

Keywords: Hydrogen Peroxide, Human Saphenous Vein, Levosimendan

1. Introduction

Human saphenous vein is frequently used for coronary artery bypass grafting because of ready availability and suppleness [1]. Vasospasm of the graft following coronary artery bypass graft (CABG) surgery is a major problem and may cause perioperative and late failure of bypass conduits. There are ongoing researches on determining the mechanisms causing vasospasm and various vasodilators have been studied to prevent or reverse vasoconstriction in various grafts [2,3].

Increased oxidative stress plays important roles in myocardial and vascular dysfunction in patients undergoing CABG [4]. Hydrogen peroxide (H_2O_2) is an important derivative of oxidative metabolism and is a major contributor in oxidative stress-induced functional and metabolic dysfunction [5]. Although generation of H_2O_2 may occur under normal physiological conditions and is not restricted to pathological conditions, its formation in the endothelium of blood vessels under stress conditions

such as diabetes, hypertension, preeclampsia and extracorporeal membrane oxygenation holds an important place in CABG surgery [6]. H_2O_2 is already used as an experimental model for oxidative stress and it has been shown to induce concentration dependent increases in contraction in various blood vessels including saphenous veins [7,8] and potentiates the effects of vasoconstrictor agents [9].

Levosimendan is a new cardiac enhancer that exerts positive inotropic effects on heart failure by calcium sensitization of contractile proteins. Recent research has displayed that levosimendan also causes peripheral vasodilatation and may also have antioxidant properties [10]. The present study was designed to assess the effects of levosimendan pretreatment on the contractile effects of H_2O_2 in human saphenous vein (HSV) segments.

2. Methods

The study was approved by the ethics committee of Selcuk University, Meram Faculty of Medicine and in-

formed consent was obtained from all patients undergoing myocardial revascularization surgery. The discarded HSV segments were placed in cold Krebs-Henseleit solution (KHS: NaCl 119 mmol/L, KCl 4.7 mmol/L, MgSO₄ 1.5 mmol/L, KH₂PO₄ 1.2 mmol/L, CaCl₂ 2.5 mmol/L, NaHCO₃ 25 mmol/L, and glucose 11 mmol/L) and transported to the laboratory for the study in 20 minutes. After removal of the surrounding tissue, vein segments were cut into helical 12 - 15 mm strips and suspended in a 20 ml organ bath containing KHS at 37°C and continuously bubbled with 95% O₂ and 5% CO₂ gas mixture. Tissues were gradually stretched to a basal resting tension of 1.0 g and were allowed to equilibrate to their own resting tension for 60 minutes, and during this period the KHS was changed every 15 minutes.

After an equilibration period, strips (n = 8) were contracted using 10⁻⁵ M serotonin (5-HT). To standardize responses between strips, once maximal 5-HT contractions were recorded and used as a standard by which subsequent contractions of the tissue could be expressed (as a percentage of this contraction).

Two types of experiment were performed. In the first set of experiments, the effect of H₂O₂ was investigated in levosimendan-pretreated strips (n = 8). First the maximum contractions to 5-HT (10⁻⁵ M) were recorded in HSV strips. Tissues were then washed twice within 15 minutes of washout intervals and thereafter concentration-response curves to cumulative H₂O₂ (10⁻⁶ - 10⁻³ M) were recorded. After two 15 minutes of washout intervals concentration-response curves to cumulative H₂O₂ (10⁻⁶ - 10⁻³ M) were repeated in the presence of levosimendan (10⁻⁸ M).

In the second set of experiments, the effects of levosimendan on oxidative stress caused by H₂O₂ in the tissues contracted with 5-HT were investigated. Strips (n = 8) were contracted with 5-HT (10⁻⁵ M). When the contraction reached a stable plateau, H₂O₂ was administered cumulatively (10⁻⁶ - 10⁻³ M) into the organ baths in one-log increments. The same procedure was also conducted in the presence of levosimendan (10⁻⁸ M).

In all groups, isometric recording of tension changes were obtained with force transducers (Grass FTO4; Grass Instrument Co, W. Warwick, RI, USA) connected through amplifiers to a polygraph (Grass 7D).

The effects of H₂O₂ are expressed as the percentage of the control contractile response elicited at 10⁻⁵ M 5-HT. The maximum contraction (E_{max}) and the concentration required to achieve 50% of maximum contraction (EC₅₀) were calculated for H₂O₂.

All data were expressed as mean ± s.e. mean (SEM). The statistical significance of differences among groups was analyzed by using unpaired student's t-test. For all studies, p value less than 0.05 was considered significant.

All the drugs were prepared freshly at the day of the study. The following compounds were used: Hydrogen peroxide (H₂O₂) obtained from Merck, Darmstadt, Germany; levosimendan, Simdax, from Abbott Laboratories; serotonin (5-HT) from Sigma, St. Louis, MO, USA.

3. Results

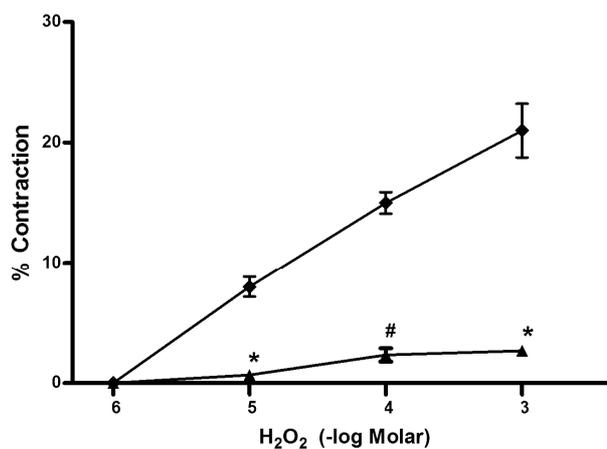
The effects of H₂O₂ (10⁻⁶ - 10⁻³ M) on HSV strips in the absence and presence of levosimendan are summarized in **Figure 1**. A maximum contraction of 32.4% ± 1.12% was obtained with H₂O₂ 10⁻³ M and the EC₅₀ value was 4.6 × 10⁻⁶ ± 0.3 M. Pretreatment of the HSV strips with levosimendan (10⁻⁸ M) significantly reduced the contractile response to each one concentration of H₂O₂ (p < 0.05). The EC₅₀ value for H₂O₂ was 4.5 × 10⁻⁵ ± 0.2 and E_{max} was 16.0% ± 1.0% in the presence of levosimendan.

5-HT (10⁻⁶ M) produced contractions in HSV strips. Further treatment of strips with H₂O₂ (10⁻⁶ - 10⁻³ M) resulted in statistically significant concentration-dependent increases in tension (E_{max}: 21.0 ± 2.2; EC₅₀: 2.5 × 10⁻⁶ ± 0.17). Preincubation of the tissues with levosimendan did not significantly influence the maximum amplitude of the 5-HT-induced tone but inhibited the contractile effect of H₂O₂ on the 5-HT-induced contraction (E_{max}: 2.67 ± 0.33) (**Figure 2**).

4. Discussion

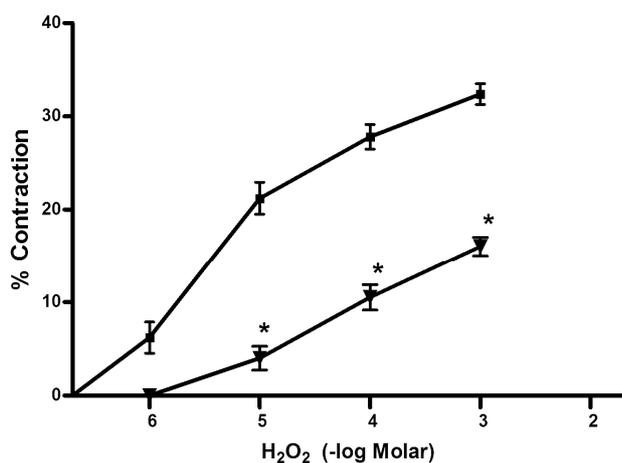
Results from our present *in vitro* model for oxidative stress shows that:

1) Pretreatment with clinical concentration of levosimendan (10⁻⁸ M) attenuates the contractile response elic-



* = p < 0.001 versus Levosimendan incubated group
= p < 0.005 versus Levosimendan incubated group

Figure 1. Concentration-response curves for contraction of HSV elicited by H₂O₂ under control conditions and after incubation with 10⁻⁸ M levosimendan. Each point represents the mean ± SEM expressed as percentage of the tension induced by 10⁻⁵ M 5-HT (n = 8).



* = $p < 0.001$ versus Levosimendan incubated group

Figure 2. Concentration-response curves for H_2O_2 on the vasoconstriction induced by 10^{-5} M 5-HT in HSV under control conditions and after incubation with 10^{-8} M levosimendan. Each point represents the mean \pm SEM expressed as percentage of the tension induced by 10^{-5} M 5-HT ($n = 8$).

ited by H_2O_2 .

2) Pretreatment with levosimendan (10^{-8} M) prevents contractile responses caused by H_2O_2 in the tissues precontracted with 5-HT but does not affect 5-HT elicited contractions.

H_2O_2 is found in the human plasma at micromolar concentrations and its concentration can increase up to millimolar levels in pathological states including myocardial ischemia and heart failure [11]. It can diffuse from its site of formation, easily crossing cell membranes and producing cellular oxidative damage [6]. However, previous studies have suggested that H_2O_2 may be an important mediator in the vasculature as a regulator of vasomotor tone [12]. In previous studies, 50 mM to 10 mM concentrations of H_2O_2 were used to induce experimental oxidative stress [13-15]. In the present study, we used 10^{-6} - 10^{-3} M concentrations of H_2O_2 . In order to evaluate the effect of levosimendan on vasoconstriction during oxidative stress, first we tested the effect of levosimendan with incubation on cumulative H_2O_2 concentration response curve in HSV strips at resting tension. Our results showed that the application of levosimendan inhibited contractions of H_2O_2 in HSV strips indicating its potential preventive effect against the graft spasm induced by oxidative stress. Active contraction induced by 5-HT is considered to be an important pathological mechanism for inducing arterial spasm and may cause perioperative and late failure of bypass conduits [1]. Since H_2O_2 also potentiates contractile responses to various agents [9], after 5-HT induced contractions we evaluated the effect with the same concentration of levo-

simendan incubation on cumulative H_2O_2 concentration response curve. Treatment of these strips with H_2O_2 potentiated contraction induced by 5-HT. Levosimendan 10^{-8} M prevented contractile responses caused by H_2O_2 in the tissues precontracted with 5-HT but does not affect 5-HT elicited contractions.

Plasma level of levosimendan during clinical practice is important to mention for proper interpretation of the experimental results. A single 0.5 mg oral dose of levosimendan produces a peak plasma concentration of 20 ng/ml (0.07 micromol) in patients with congestive heart failure [16]. Vasodilatation by levosimendan is believed to be achieved at a higher plasma concentration when compared to its positive inotropic effect. In patients with ischemic heart disease, 0.25 mg and 0.5 mg levosimendan increased left ventricular function, but a significant decrease in total peripheral resistance was seen only after 2 mg and 4 mg doses [17]. In the present study, the selected concentration (10^{-8} M) of levosimendan is elected as used for its inotropic effect. This concentration, which significantly inhibited the contractions to H_2O_2 in HSV strips, is much lower than previous *in vitro* studies, which reported that higher concentrations of levosimendan (in the micromolar range) required to elicit vasodilatation. Mirkhani *et al.* [16] reported a maximum relaxation of 45.4% with 10^{-4} M of levosimendan in norepinephrine-induced contraction. Similar to our result with 5-HT induced contraction, they observed no relaxation with 10^{-8} M of levosimendan in HSV precontracted with norepinephrine. In another study, levosimendan at a concentration of 10^{-8} M did not effect saphenous vein precontracted with 5-HT and the maximum relaxation induced by levosimendan (3×10^{-6} M) was $28.1 \pm 7.5\%$ with an EC_{50} of 0.32 ± 0.04 microM [17].

The present study design does not include the mechanisms of by which levosimendan attenuates H_2O_2 induced contractions or cause vasodilatation. Previous studies have shown that levosimendan causes positive inotropic and antistunning effects on the heart. This effect was shown to be mediated by calcium sensitization of contractile proteins and vasodilator and antiischemic effects mediated by the opening of ATP-sensitive potassium channels in vascular smooth muscle cells [18]. Levosimendan reduces plasma levels of malondialdehyde, a marker of oxidative stress, in patients with heart failure [19], inhibits H_2O_2 induced cardiomyocytes apoptotic cell death by activating K_{ATP} channels [20] and preconditioning with levosimendan prevents contractile dysfunction due to H_2O_2 -induced oxidative stress in human myocardium [21].

Although levosimendan has been shown to induce vasodilatation in human radial and internal mammary arteries and in HSV [22-24], this is the first study considering the effect of levosimendan on H_2O_2 -induced con-

traction in HSV.

In conclusion, according to our results we may speculate that pretreatment of HSV with clinical concentrations of levosimendan inhibit the vasoconstriction caused by oxidative stress, indicating its potential preventive effect against oxidative stress induced graft spasm. Further research is warranted to assess the exact mechanisms responsible for this effect of levosimendan.

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