Influence of low molecular weight heparin preparations on human internal thoracic artery contraction

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Abstract

Objective: Low molecular weight heparins (LMWHs) offer practical and potential pharmacological advantages over unfractionated heparin in multiple applications but have not been studied as vasoactive agents. The purpose of this study was to investigate the effects of two commercial preparations of LMWHs, enoxaparin sodium and nadroparin calcium, on vasoconstriction in the human internal thoracic artery (ITA) in vitro.

Methods: Samples of redundant ITA segments obtained from 36 patients who underwent coronary artery bypass surgery were cut into 3 mm wide rings and suspended in 20 ml organ bath. Activity of ITA rings precontracted with 80 mM KCl, 0.1 mM endothelin-1 (ET-1) and 1 mM norepinephrine (NE) after administration of enoxaparin and nadroparin in accumulative concentration ranging from 0.1 to 13.2 UI AXa/ml were recorded under isometric conditions by means of force transducers with digital output. The contraction after 80 mmol KCl, 0.1 mM ET-1 and 1 mM NE administration was treated as a control.

Results: Both studied LMWHs in concentration ranging from 0.12 to 13.2 UI AXa/ml did not change basal tonus and KCl precontracted ITA rings. When used in concentrations higher than 13.2 UI AXa/ml nadroparin but not enoxaparin significantly increased the tension in KCl precontracted arterial rings. In NE and ET-1 precontracted rings enoxaparin and nadroparin caused dose dependent relaxation without significant differences between both preparations. Incubation with nitric oxide blocker - Nω-NITRO-L-ARGININE (L-NNA) in concentration 0.2 mM caused a significant attenuation of relaxant responses to both studied LMWHs in NE and ET-1 precontracted rings.

Conclusion: LMWHs can have vasorelaxant effects on the receptor-mediated ITA vasoconstriction. The results suggest that LMWHs-induced relaxation in the human ITA is at least partially caused by nitric oxide release. Although the vasoactive effects are not the primary advantage of these drugs used as antithrombotics, such effects might have some clinical importance in the treatment and prophylaxis of graft spasm.

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1. Introduction

The internal thoracic artery (ITA) is the commonly used graft for myocardial revascularization in surgical treatment of coronary artery disease. Peri- and postoperative treatment in coronary artery bypass grafting (CABG) consist of variety of pharmacological agents having influence on blood vessels [1–4], among them unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) are routinely used before, during and after surgery.

UFH is a heterogeneous mixture of polysaccharide chains (glycosaminoglycans) ranging in molecular weight from about 3000 to 30000. LMWHs are fragments of UFH produced by controlled enzymatic or chemical depolymerization processes that yield chains with a mean molecular weight of about 5000. Both UFH and LMWHs exert their anticoagulant activity by activating antithrombin [5,6]. LMWHs compared with UFH have some significant advantages e.g. improved bioavailability, a longer half-life and more predictable dose response [7] which make their use, also in cardiac surgery, increasingly common in the treatment and prophylaxis. Vasorelaxant properties of UFH have been widely studied. By its calcium chelating ability and inhibition of intracellular calcium mobilization,
UFH has the potential to down-regulate the vascular smooth muscle cells (VSMCs) contractile pathway and thereby cause vascular relaxation [8–11]. Other studies have also linked heparin to the production and release of several endothelial vasoactive mediators including nitric oxide and endothelin [2,9,11,12]. There are recent experimental data on animal model concerning vascular effects of one of LMWHs preparation of enoxaparin, which has been found to diminish the contractile effect of noradrenaline and K+ [13]. Possible vasorelaxing effects of LMWHs on the arterial wall, different from their anticoagulant activity may play a certain role in the prevention and/or treatment of grafts vasospasm, regulation of their early and midterm function and thereby influence the arterial conduits long term patency. To our knowledge, the effect of LMWHs on the vascular activity of human ITA has not been studied yet. Therefore, the purpose of this in vitro study was to evaluate the contribution of two commercial preparations of LMWHs—enoxaparin sodium and nadroparin calcium to vasoactive reactions of isolated human ITA.

2. Materials and methods

2.1. Sampling and preparation of human arteries

This study was reviewed and approved by the local committee of the ethics on human research. Because the ITA obtained during coronary artery bypass operation was classified as a surgical specimen, its use was exempted by the committee from required patient consent. Samples of redundant ITA obtained from 36 patients who underwent coronary artery bypass graft surgery were included in this study. During the operation, distal ITA segments were collected—a total number of 180 ITA rings were investigated.

2.2. Organ chamber experiments

The rings were mounted in an organ bath containing the physiological salt solution of pH 7.4 and a temperature 37 °C, and bubbled with 95% O2 and 5% CO2. The details of the technique were published by other investigators [14–16]. Particular attention was paid to avoid damage to inner surface by carefully mounting the ITA rings on the wire hooks in the organ bath.

In all experiments, the presence of functional endothelium was confirmed by determining the relaxation response to 10−5 mol/l acetylcholine. The preparations were allowed to equilibrate for 2–4 h. During the equilibration period the passive tension was adjusted several times until the resting tension became stable at 6 mN. Activity of the ITA rings was recorded under isometric conditions by means of force transducers with digital output.

In all experiments both LMWH preparations were used in accumulative doses: 0.12; 0.36; 0.72; 1.2; 2.4; 4.8; 8.4; 13.2 UI AXa/ml, increased every 10 min.

In a first series of experiments the influence of enoxaparin and nadroparin on baseline tension of ITA rings was investigated (n=12 for each LMWH, respectively).

Next, responses of ITA rings precontracted with KCl (K+ , 80 mM) to both studied LMWHs were determined (n=12, respectively).

Then enoxaparin and nadroparin-induced responses were studied in ITA rings precontracted with norepinephrine (NE, 1 µM, n=12, respectively) and endothelin-1 (ET-1, 0.1 µM, n=12, respectively). In another set of experiments the effects of enoxaparin and nadroparin following 30 min L-NNA (0.2 mM) incubation on norepinephrine (NE, 1 µM, n=12, respectively), and endothelin-1 (ET-1, 0.1 µM, n=12, respectively) precontracted ITA rings was investigated.

There were no reactions observed when mentioned above LMWHs concentration range was used during experiments on ITA rings baseline tension and KCl precontracted. Thus, additionally, in a separate series of experiments we increased concentrations of both enoxaparin and nadroparin (doses: 12; 36; 72; 118 UI AXa/ml) in order to determine their influence on baseline tension (n=10, respectively) and KCl precontracted (n=10, respectively) ITA rings.

Contractions after KCl (K+ , 80 mM), norepinephrine (NE, 1 µM), and endothelin-1 (ET-1, 0.1 µM) administration were treated as control reactions, respectively.

Quantification of the response was done by calculation of the area under the curve (AUC). The effects were evaluated by comparing experimental responses with the controls.

2.3. Drugs

The following pharmacological agents were used: nadroparin calcium (Sanofi-Winthrop, Gentilly Cedex, France), enoxaparin natrium (Laboratorie Aventis, Paris Cedex, France), endothelin-1 (ET-1, Novabiochem, Laufelingen, Switzerland), norepinephrine (NE, Sigma, St Louis, MO, USA), Nω-NITRO-L-ARGININE (L-NNA, Sigma, St Louis, MO, USA). All other reagents used were of analytical grade.

2.4. Statistical analysis

The data were analyzed with ANOVA, Student’s unpaired t-test or Wilcoxon matched pairs signed rank test, where appropriate. The statistical significance was
considered when probability value was \( P < 0.05 \). Throughout the paper the results are expressed as mean \( \pm \) SEM.

### 3. Results

Administration of both the studied LMWHs in concentration ranging from 0.12 to 13.2 UI AXa/ml did not cause a statistically significant alteration either to the basal tonus of ITA rings or the tension of KCl-precontracted.

Neither enoxaparin nor nadroparin caused any changes in the baseline tension of ITA rings after increasing their concentration up to 118 UI AXa/ml.

Nadroparin used in accumulative concentration ranging from 12 to 118 UI AXa/ml, was found to increase the tension of KCl-precontracted arterial rings. Contractile response calculated as AUC for the maximal concentration of nadroparin (118 UI AXa/ml) was 291.9 \( \pm \) 76.14% of control. In the same range of concentrations enoxaparin did not change the tension of arterial rings precontracted with 80 mM KCl.

Original recording of the effects of high concentration LMWHs on KCl precontracted ITA rings is shown in Fig. 1.

We observed dose-dependent relaxation of precontracted with 0.1 mM ET-1 and 1 mM NE ITA rings in response to accumulative concentrations of nadroparin (Fig. 2) and enoxaparin (Fig. 3). Relaxation responses of ITA rings precontracted with 0.1 mM ET-1 for the maximal concentration (13.2 UI AXa/ml) were 65.7 \( \pm \) 6.4 and 69.1 \( \pm \) 13.8% of control for nadroparin and enoxaparin, respectively. Relaxation responses of ITA rings precontracted by 1 mM NE for the maximal concentration (13.2 UI AXa/ml) were 70.9 \( \pm \) 12.4 and 66.45 \( \pm \) 12.2% of control for nadroparin and enoxaparin, respectively. The differences in relaxation for both LMWH preparations were not statistically significant in any concentration used.

Nadroparin- and enoxaparin-induced relaxations were significantly \( (P < 0.05) \) attenuated by the pre-treatment of nitric oxide blocker L-NNA (0.2 mM) (Figs. 2 and 3, respectively). Differences were statistically significant with all LMWHs concentrations used except the lowest one.

![Fig. 1. Original recording of nadroparin (a) and enoxaparin (b) high concentrations (12–118 UI AXa/ml) effects on ITA segment precontracted by KCl (KCl, 80 mM).](image)

![Fig. 2. (A) Effects of 30 min LNNNA (0.2 mM) incubation on nadroparin-induced relaxations (percent reversal of the agonist-induced contraction) in human internal thoracic artery rings precontracted by norepinephrine (NE, 1 \( \mu \)M) and endothelin-1 (ET-1, 0.1 \( \mu \)M). (B) Nadroparin-induced relaxations (percent reversal of the agonist-induced contraction) in human internal thoracic artery rings precontracted by norepinephrine (NE, 1 \( \mu \)M) and endothelin-1 (ET-1, 0.1 \( \mu \)M). Contractions after norepinephrine (NE, 1 mM) and endothelin-1 (ET-1, 0.1 mM) administration were treated as control reactions, respectively. Each point represents the mean with SEM shown by vertical bars, \( n = 12 \) for all groups. \( *P < 0.05 \) as compared between A and B.](image)

![Fig. 3. (A) Effects of 30 min LNNNA (0.2 mM) incubation on enoxaparin-induced relaxations (percent reversal of the agonist-induced contraction) in human internal thoracic artery rings precontracted by norepinephrine (NE, 1 \( \mu \)M) and endothelin-1 (ET-1, 0.1 \( \mu \)M). (B) Nadoxaparin-induced relaxations (percent reversal of the agonist-induced contraction) in human internal thoracic artery rings precontracted by norepinephrine (NE, 1 \( \mu \)M) and endothelin-1 (ET-1, 0.1 \( \mu \)M) administration were treated as control reactions, respectively. Each point represents the mean with SEM shown by vertical bars, \( n = 12 \) for all groups. \( *P < 0.05 \) as compared between A and B.](image)
With the maximum concentration (13.2 UI AXa/ml) used, nadroparin- and enoxaparin-induced relaxations of ITA rings precontracted with 0.1 μM. ET-1 after incubation with LNNA, were 30.1 ± 2.84 and 29.5 ± 2.3% of control, respectively. With the same concentration, nadroparin- and enoxaparin-induced relaxations of ITA rings precontracted by 1 μM NE after incubation with LNNA, were 27.3 ± 6.3 and 28.4 ± 4.2% of control, respectively.

4. Discussion

Spasm of arterial grafts used for coronary artery surgery is a factor determining the survival of both the graft and the patient. This raises concern about graft’s reactivity to different stimulants; physical (e.g. mechanical stimulation, temperature changes) or pharmacologic (endogenous and exogenous vasoconstrictors) [16]. Several substances, acting either in a synergistic or additive manner induce alterations in vascular tone which can result in graft spasm [4]. Vascular endothelium serves an important role in maintaining vascular tone by releasing substances that modulate the balance between vasoconstriction and vasodilatation. The endothelial function of the coronary bypass grafts is crucial to long-term graft patency. In response to different stimulants endothelium produce and release endothelium-derived relaxing factors: nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) [17]. Nitric oxide has been identified as a key component in the interactions between endothelium and underlying smooth muscle [18]. In our study, the presence of functional endothelium was always confirmed.

ITA is a very responsive graft material to many vasoactive agents, however, for many drugs used during and after CABG, their adjunctive vasoactive mode of action is not fully verified so far.

UFH has been shown to affect the vascular wall by acting on both the endothelium and VSMCs [8–11,19]. Its vasorelaxant endothelium mediated effect involves a series of secondary signaling pathways, such as suppression of endothelin production and stimulation of endothelium derived nitric oxide release and/or cGMP stimulation [2,20].

There are some experimental data in single VSMCs that LMWHs applied intracellularly, inhibit IP₃ induced Ca²⁺ release from sarcoplasmic reticulum [21]. Also recent study on animal model showed the favorable effects of enoxaparin on vascular reactivity; diminishment of the contractile effect of NE and of K⁺, and augmentation of the endothelium-dependent relaxation to ACh [13]. However, in vivo enoxaparin did not show vasodilatatory properties in human hand veins when compared with UFH [22].

In the present study, we have found in the human ITA, the most commonly used arterial graft for coronary artery bypass surgery, that: (1) two LMWH preparations, nadroparin calcium and enoxaparin sodium have dose-dependent vasorelaxant effect on receptor mediated (NE, ET-1) vasoconstriction. This effect was significantly, but not totally, inhibited by the NO synthase inhibitor LNNA; (2) studied LMWHs have not any effect on basal tonus of ITA rings even with high concentrations; and (3) high concentrations of nadroparin increase KCl-induced ITA rings vasoconstriction.

LMWHs concentration range used in our study contained levels achieved in vivo for thromboembolic prophylaxis and cardiovascular disease treatment from 0.1 to 1.5 UI AXa/ml [7,23]. Much higher concentrations were used in order to evaluate the differences between two preparations: sodium salt-enoxaparin and calcium salt-nadroparin.

Nadroparin used in our study in high concentrations was found to increase the tension in KCl precontracted ITA rings. Potassium ions were used as a membrane depolarizing agents opening voltage-operated calcium channels. Our finding suggests that nadroparin might serve as the additional source of calcium ions entering the arterial cells through the voltage-operated calcium channels. Second preparation studied enoxaparin—a sodium salt, had no influence on arterial wall reactivity under the circumstances of our experiments. Since, such high concentrations are unlikely to achieve in vivo, this effect seems not to have any clinical importance.

Binding of UFH and LMWHs to vascular endothelium was demonstrated in vivo and in vitro [24,25], therefore it could be expected that LMWHs—the depolimerization products of parent heparin material—UFH, would exert similar biological activity (e.g. stimulation of nitric oxide release and/or cGMP stimulation) during receptor-mediated vascular contraction (ET-1 and NE in our study).

The main finding of this study is the observation that enoxaparin and nadroparin induced relaxation is mainly due to NO release. This effect was observed within the concentrations correlating with levels achievable in vivo. The residual relaxation of the ITA rings treated with LNNA may be caused by LMWHs stimulation of endothelial cells to release other vasorelaxing factors, like EDHF and/or PGI₂, which should be a subject of further investigation.

In conclusion, our study suggest that LMWHs can have beneficial effects in management of arterial graft spasm. Although their vasorelaxant effect is not the primary advantage of that group of drugs when used as antithrombotics, they may provide clinically useful antispasmodic effect in patients who also need graft spasm treatment after coronary artery bypass surgery.

References


