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Vasorelaxant effects of dobutamine and levosimendan on rat aorta rings

Sıçan aort halkasında dobutamin ve levosimendanın vazorelaksan etkileri

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ABSTRACT

Background: In this experimental study, the vasorelaxant effects of levosimendan and dobutamine on isolated rat thoracic aorta preparations were compared.

Methods: Sixteen Wistar albino type male rats were used. The thoracic aortas were removed carefully and were transferred to petri dishes containing Krebs solution. Aortic rings of approximately 5 mm in length were prepared and placed in the organ bath. Contraction and relaxation forces of the aortic rings were recorded. Contraction response was obtained by applying 10^{-5}M phenylephrine to aortic rings. Subsequently, cumulative doses of levosimendan (10^{-8}M-10^{-4}M) was applied to eight aortic rings and cumulative doses of dobutamine (10^{-8}M-10^{-3}M) was applied to the other eight aortic rings and dose-response curves were recorded. The EC_{50} and pD2 values were calculated by the computer program named Graph Pad Prisim 4.0. The Mann-Whitney U-test was performed for statistical analysis.

Results: The relaxation response of the aortic rings with levosimendan 10^{-4}M administration was %92.33 while the relaxation response with dobutamine 10^{-4}M administration was %82.48. It did not show a significant difference between both relaxation responses (p=0.059). The EC_{50} value was calculated as $6.605x10^{-6}$M for dobutamine and $5.093x10^{-5}$M for levosimendan. The pD2 value was found to be 5.2±0.4 for levosimendan and 4.3±0.2 for dobutamine.

Conclusion: Levosimendan and dobutamine molecules *in vitro* rat aortic rings have similar vasorelaxant effects.

Keywords: Dobutamine; levosimendan; organ bath; rat aorta; vasospasm.

ÖZ

Amaç: Bu deneysel çalışmada, izole sıçan torasik aort preparatlarında levosimendan ve dobutaminin vazorelaksan etkileri karşılaştırıldı.

Çalışma planı: Çalışmada 16 adet Wistar albino cinsi erkek sıçan kullanıldı. Sıçanların torasik aortu dikkatlice çıkarıldı ve Krebs solüsyonu içeren petri kaplarına konuldu. Aort halkaları 5 mm uzunluğunda olacak şekilde hazırlandı ve organ banyosuna yerleştirildi. Aort halkalarının kasılma ve gevşeme kuvvetleri kaydedildi. Aort halkalarına 10^{-5}M fenilefrin uygulanarak kasılma yanıtı elde edildi. Ardından sekiz aort halkasına artan dozlarda levosimendan (10^{-8}M-10^{-4}M), diğer sekiz aort halkasına ise yine artan dozlarda dobutamin (10^{-8}M-10^{-3}M) uygulanarak doz-yanıt eğrileri kayıt edildi. Graph Pad Prisim 4.0 bilgisayar programı ile EC_{50} ve pD2 değerleri hesaplandı. İstatistiksel analiz Mann-Whitney U testi ile yapıldı.

Bulgular: 10^{-4}M levosimendan uygulaması ile aort halkalarında %92.33 gevşeme yanıtı, 10^{-4}M dobutamin uygulaması ile %82.48 gevşeme yanıtı elde edildi. Her iki gevşeme yanıtı arasında anlamlı bir farklılık bulunmadı (p=0.059). EC_{50} değeri, levosimendan için $6.605x10^{-6}$M, dobutamin için $5.093x10^{-5}$M olarak hesaplandı. pD2 değeri levosimendan için 5.2±0.4, dobutamin için 4.3±0.2 bulundu.

Sonuç: Levosimendan ve dobutamin molekülleri *in vitro* sıçan aort halkalarında benzer vazorelaksan etkiye sahiptir.

Anahtar sözcükler: Dobutamin; levosimendan; organ banyosu; sıçan aortu; vazospazm.

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Vasospasms and their related problems are typically encountered in the perioperative period in cardiovascular surgery and during invasive cardiology interventions. Dobutamine is an inotropic agent that has been used for many years to treat this condition, but recently, a new-generation inotropic agent, levosimendan has also become a popular treatment option. In this study, we compared the effectiveness of levosimendan and dobutamine with respect to the problems associated with vasospasms. In spite of the number of clinical studies that have compared these two agents, only a few focused on vasorelaxant efficacy.

Levosimendan, a derivative of pyridazinone and dinitrile, is a positive inotropic agent[1] that strengthens myocardial contractility by increasing the sensitivity of cardiac troponin C to cytoplasmic calcium.[2-4] It is a drug with dual effects (inotropic and vasodilatory) and is used to treat low cardiac output that develops subsequent to open heart surgery.[5-7]

Dobutamine is an inotropic agent that has been prescribed for many years which also has dual effects. It acts as an agonist on the beta-1 (β1) adrenergic receptors,[8] and also has a slight stimulating effect on the beta-2 (β2) and alpha-1 (α1) receptors. Because the agonistic effects on the β1 adrenergic receptors are more powerful, dobutamine causes an increase in myocardial contractility and stroke volume while also displaying a moderate chronotropic effect, which leads to greater cardiac output.[9,10] A secondary hemodynamic effect of this drug is that it reduces systemic vascular resistance (afterload) and ventricular filling pressure (preload).

A vasospasm is defined as the narrowing of the vessel lumen where vasoconstrictor substances such as noradrenaline, angiotensin II, Ca +2, and endothelin play a dominant role.[11] Different groups of vasodilator agents are used to treat this condition, but no ideal vasospasmolytic agent has been discovered yet.[11] Vasospasms are most commonly seen as graft spasms in cardiac operations during the perioperative period of coronary artery bypass grafting (CABG) operations. In addition, the most important complication seen in the radial artery graft is a vasospasm that occurs in the early stages, with ratios of between 4 and 10% having been reported at different clinics.[12,13]

Intra-aortic balloon pumps (IABPs) and inotropes with dual effects have a life-saving role in low cardiac output syndrome (LCOS) that occurs subsequent to CABG or invasive cardiology procedures. Furthermore, low cardiac output along with the inotropic agents used in coronary bypass surgery may cause vasospasms in grafts and/or native coronary arteries, which could lead to morbidity and mortality.[11-15] Even though the effect mechanism of vasospasms is not clear, it has been shown that endothelium-derived vasoconstrictor agents, surgical trauma, neural factors, hypoxia, and ischemia play an important role.[11-17]

Vasospasms are associated with low cardiac output, acidosis, and hypothermia.[11] Dobutamine has been utilized for many years as an inotropic drug for patients with low cardiac output who are also receiving mechanical support, whereas levosimendan has only been used in the past 10 years as a myocardial protector with anti-ischemic properties. The vasorelaxant efficiency of both molecules is well known, but to our knowledge, there is no detailed study in the literature that compares the vasorelaxant properties of these agents in an organ bath. Therefore, in this study, we sought to examine and compare the vasorelaxant effects of dobutamine and levosimendan molecules in rat aortic rings in an organ bath.

MATERIALS AND METHODS

Sixteen male Wistar albino rats (eight-10-week-old; average weight 250 g) were used in this experimental study. The rats were kept in wire cages at a temperature of between 24 and 26 °C with 50-60% humidity prior to the experiment and received alternating 12-hour periods of light and darkness to maintain their circadian rhythms. The rats' diets were composed of standard commercial feed pellets and city drinking water, and they were not exposed to any processes or treatments prior to the organ bath experiments. In addition, the rats were cared for in accordance with the requirements of the Guide for the Care and Use of Laboratory Animals, and we obtained the approval of the local ethics committee to perform this experiment.

The rats were sacrificed using the cervical dislocation method. Their chest cavities were then opened, and the descending aorta of the thoracic aorta was removed from the cavity and placed in a Petri dish containing Krebs solution [sodium chloride (NaCl) 119 mM, potassium chloride (KCl) 4.7 mM, magnesium sulfate ($MgSO_4$) 1.5 mM, monopotassium phosphate (KH_2PO_4) 1.2 mM, calcium chloride ($CaCl_2$) 2.5 mM, sodium bicarbonate ($NaHCO_3$) 25 mM, and glucose 11 mM with a pH of 7.40±0.05/ liter]. Next, the surrounding fat and connective tissues were removed, and care was taken not to damage the aortic tissue and endothelial layer. Then one arterial ring measuring approximately 5 mm in diameter was obtained from each descending aorta. These rings were hung so that a different rat thoracic ring was placed in each of the four chambers of the organ bath (IOBS MAY 99 Isolated Tissue Bath

Stand Set, COMMAT Ltd., Ankara, Turkey), which all contained 20 ml of Krebs solution. The temperature of the organ bath was adjusted to 37 °C, and the solution was constantly vented throughout the experiment with a gas mixture composed of 95% oxygen (O_2) and 5% carbon dioxide (CO_2). In addition, the Krebs solutions in the organ baths were renewed every 15 minutes. Furthermore, we separated the rats into experimental groups and implemented the experiment protocols using a randomized, controlled, single-blind method.

After the isolated aortic rings were placed in the organ bath, they were kept at less than 1.5 grams resting tension for one hour until they reached a state of equilibrium, and then each vessel ring was contracted with 10^{-5} M phenylephrine hydrochloride (Sigma Chemical Co., St. Louis, USA). After receiving the maximum contractile response, increased cumulative doses (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) of levosimendan (Simdax®, Orion Corporation, Espoo, Finland) or dobutamine (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M) (Antigen Pharmaceuticals Ltd., Tipperary, Ireland) were applied to the vascular rings. If the endothelium in the rings did not contract or if it had no relaxation response, they were accepted as damaged and excluded from the study; hence, out of the original 10 aortic rings in each of the two drug groups, only eight remained after the exclusion criteria was applied. The contraction and relaxation responses based on isometric type were recorded using an FDT 10-A Force Displacement transducer and a BIOPAC MP30b-CE amplifier (BIOPAC Systems, Inc., Goleta, CA, USA). The BIOPAC Student Lab PRO Manual Professional Version 3.6.6 was then used to calculate the responses.

Statistical analysis of test results

The contractile response created through the use of 10^{-5} M phenylephrine in the aortic rings was considered to be 100% in both drug groups, and the relaxation percentage was then calculated using this value. The half maximal effective concentration (EC_{50}) and pD2 (- log EC_{50}) values were calculated using the GraphPad Prism 4.00 software program (GraphPad Software, Inc., La Jolla, CA, USA). Furthermore, a comparison of the vasorelaxant effects of levosimendan and dobutamine was performed using the EC_{50} value, and the sensitivity was expressed as pD2. All statistical analyses were performed using the SPSS for Windows version 13.0 software program (SPSS Inc., Chicago, IL, USA), and the Mann-Whitney U test was used to compare the parameters. The results were evaluated at a 95% confidence interval (CI), and a p value of <0.05 was considered to be significant.

RESULTS

Both levosimendan and dobutamine demonstrated a strong vasorelaxant effect on the rat aortic rings in the isolated organ bath. Furthermore, when the relaxation responses of the rat aortae that had been contracted by phenylephrine using the highest dose of levosimendan (10^{-4}M) were examined, there was more than 100% relaxation in some of the subjects. An exemplary dose-relaxation curve obtained for levosimendan is shown in Figure 1. The maximum relaxation response created by levosimendan with 10^{-4} M concentration was 92.33% on average while it was 82.48% for dobutamine using the same

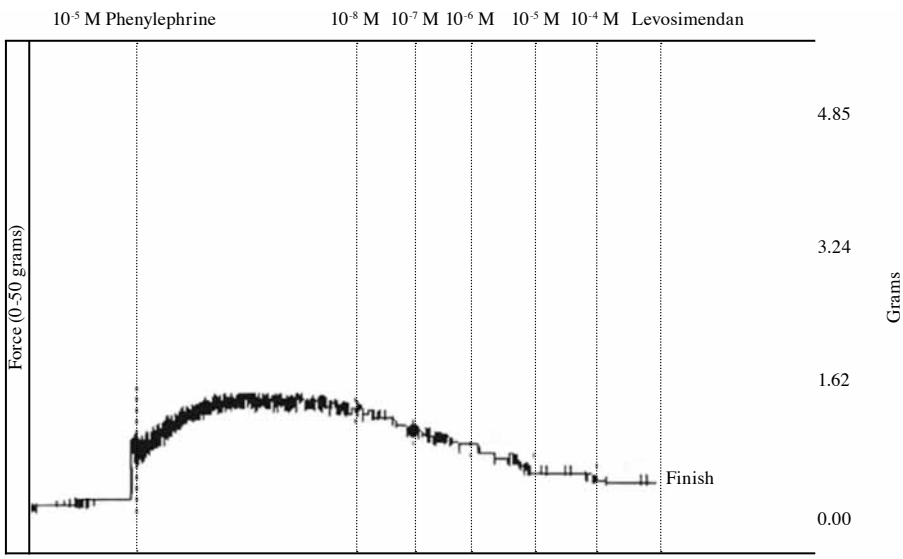

Figure 1. Dose-response curve for levosimendan in a rat ring sample.

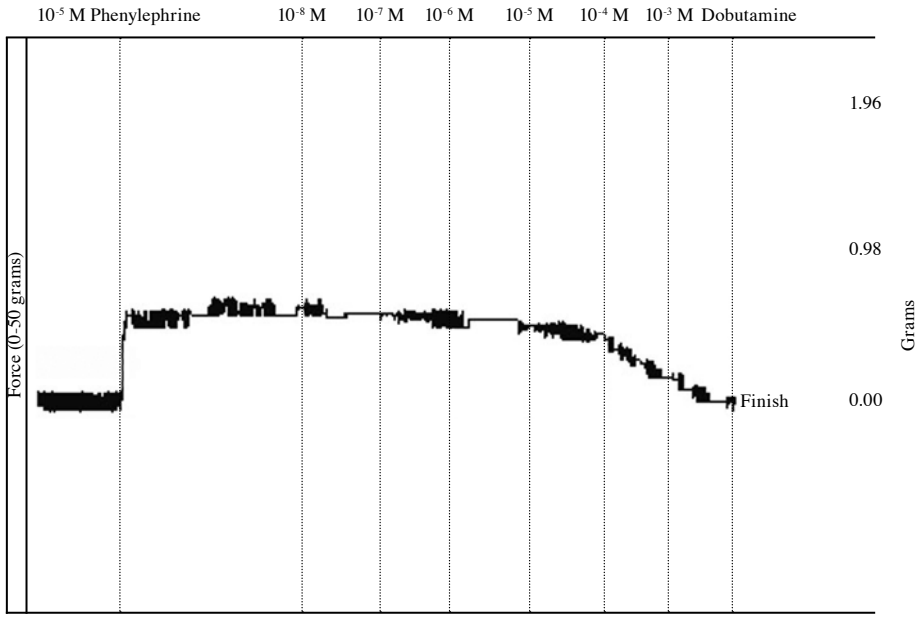

Figure 2. Dose-response curve for dobutamine in a rat ring sample.

concentration. An exemplary dose-relaxation curve obtained for the dobutamine group is shown in Figure 2, and a percentage of the maximum relaxation of both groups is shown in Table 1, with the EC_{50} and pD2 values given as mean ± standard deviation (SD).

The EC_{50} value for levosimendan was 6,605 x 10^{-6}±4.01 x 10^{-6} M, whereas for dobutamine, it was 5,093 x 10^{-5}±2.70 x 10^{-5} M (Figure 3). Moreover, the pD2 (sensitivity) value for levosimendan was 5.18±0.41, whereas it was 4.29±0.17 for dobutamine. When the relaxation responses of the two drugs were compared, no statistically significant differences were found (p=0.059).

DISCUSSION

In this study, strong relaxation responses were observed with both levosimendan and dobutamine molecules in rat thoracic aortic rings in an organ bath. We prepared the maximum concentrations of agents to be compared and reached a concentration of 10^{-4} M for levosimendan and 10^{-3} M for dobutamine. The values for both of the agents were higher than the reported therapeutic levels of the patients. While we found a relaxation rate of 92.33% for 10^{-4} M levosimendan and a relaxation rate of 82.48% for dobutamine in the aortic rings, Kivikko et al.[18] determined that a therapeutic dose of levosimendan for serious heart failure patients had an EC_{50} value of 1.24 x 10^{-7} M. In our study, we obtained a 20% relaxation response in the rat aortic rings when using this therapeutic dose of levosimendan.

In this study, the EC_{50} value for levosimendan was higher than for dobutamine, and as a vasorelaxant molecule, levosimendan was 10 times more potent

Table 1. Maximum relaxation, PD2 (neg. log. EC_{50}) and EC_{50} values of dobutamin and levosimendan

	Levosimendan (n=8)	Dobutamine (n=8)
	Mean±SD	Mean±SD
Maximum relaxation	92.3±17.8	82.5±22.3
95% Confidence interval	78.8-112.2	69.5-101.7
pD2 (-logEC_{50})	5.2±0.4	4.3±0.2
95% Confidence interval (-logEC_{50})	5.6-4.8	4.5-4.1
EC_{50}	6.60 x 10^{-6}±4.01 x 10^{-6} 5.09 x 10^{-5}±2.70 x 10^{-5}	

EC: Effective concentration; SD: Standard deviation.

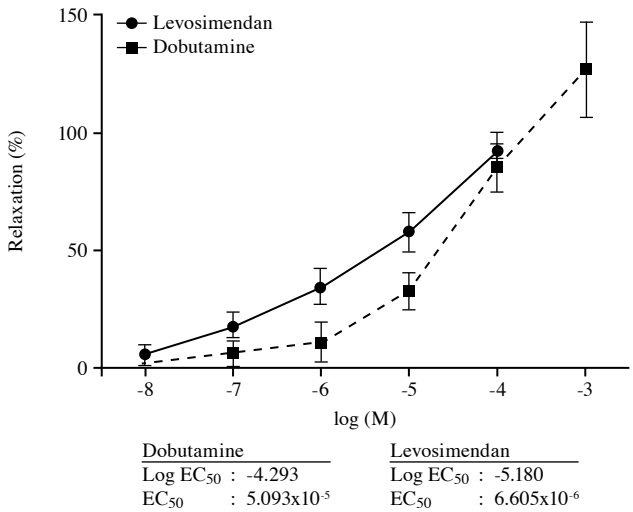

Figure 3. Dose-relaxation curves of levosimendan and dobutamine.

than dobutamine with respect to this value. However, no significant differences were found when the average relaxation percentages were compared.

Akar et al.[19] found that gender had a significant effect on the vasorelaxant properties of levosimendan and also determined that this agent had a significantly better vasorelaxant response in male internal mammary artery (IMA) rings. We used male Wistar albino rats in our study to avoid vascular tone differences resulting from hormonal cycle changes in female rats.

As previously noted, the rats in our study were sacrificed using the cervical dislocation method, which we chose because it does not require the use of anesthetic agents, thus eliminating the possibility of any negative impact to the vascular tone.

In addition, different agents were utilized in this study to create precontraction in the different organ bath experiments. Other studies have used serotonin, norepinephrine, PGF_{2a}, KCl, and barium chloride as contracting substances in these types of experiments,[18,19] with each of these agents affecting different receptors. We created precontraction with phenylephrine, a powerful vasoconstrictor that has both direct and indirect sympathomimetic effects, to create the dose-response curve.[8] Furthermore, this drug is a powerful stimulant with a direct selective effect on α-1 adrenergic receptors.

The literature also contained experiments conducted with human IMA rings, saphenous veins, and radial artery (RA) rings in organ baths.[16,19-21] However, we elected to use rat aortae because of our previous work experience with them.

Dobutamine is widely used as a perioperative inotropic agent in patients who undergo coronary artery bypass surgery. In addition to its inotropic effect, this agent is a β-adrenergic agonist that can produce peripheral vasodilation to some degree, and it has also been known to induce vasorelaxation in the IMA *in vitro*.[22-24]

The dobutamine infusion rate required to increase cardiac output ranges from 2.5-10 mg/kg/min. However, this rate may be increased according to the patient's hemodynamic responses (≤40 mg/kg/min). In patients with heart failure, it should be given with an infusion rate of between 2.5 and 10 mg/kg/min and a therapeutic plasma level of between 10^{-7} M and 5 x 10^{-7} M.[24-27] In our study, the vasorelaxation effect of between 20-30% with regard to the therapeutic doses of the levosimendan and dobutamine in the plasma supports the fact that these two agents alone are not strong enough to prevent vasospasms.

Montes et al.[1] examined the vasorelaxant effect of levosimendan in their organ bath experiment conducted on human IMA rings in 2006 and found a relaxation rate ranging from 80-100% in rings that were precontracted with levosimendan, nitroglycerin, milrinone, norepinephrine, and thromboxane A2 (TXA2) analog in the IMA segments. The most effective agent in terms of relaxant effects was nitroglycerin (EC_{50} of 2.7±2.4 x 10^{-8}). They determined that the EC_{50} value for levosimendan was 7: 07 x 10^{-6} M, and this value was similar to the EC_{50} value of levosimendan in our experiment.

Bowman et al.[28] studied the relaxant responses of levosimendan and milrinone in contraction created with TXA2 and U46619 analogues in their organ bath experiment using a porcine coronary artery. The maximum relaxant response to a levosimendan concentration of 10^{-6} M was 70% in the porcine coronary rings that had been divided into two groups.

One of the limitations of our study was that it was carried out using the thoracic aorta because it is a conducting artery. Furthermore, the oxidative status also affects the vascular tone. Another limitation was that we were only able to review and evaluate the vasomotor parameters related to endothelial function. In addition, hypothermia is also a factor that can trigger vasospasms, and an organ bath at 37 °C contains vasorelaxant responses.

Conclusion

Using inotropes with dual effects is more appropriate in the postoperative period where low

cardiac output; hypothermia, and vasospasms can be observed simultaneously. Our findings showed that levosimendan may be preferable to dobutamine because it demonstrated a more spasmolytic effect in the vasomotor tests. Our results also showed that dobutamine and levosimendan had similar vasorelaxant efficacy in rat aortic rings in organ baths. However, more research is needed regarding these two agents to obtain a definitive answer regarding whether one of these drugs should be preferred over the other.

Declaration of conflicting interests

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