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Investigation of the vasorelaxant effects of moxonidine and its relaxation mechanism on the human radial artery when used as a coronary bypass graft

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Abstract

OBJECTIVES: In both low- and high-risk patients undergoing coronary artery bypass grafting, the internal mammary artery is the first choice of arterial graft, and the second choice is the radial artery (RA). Unfortunately, RA spasms are a significant problem for a surgical team to overcome in the perioperative and postoperative period. In current surgical practice, the use of vasodilator agents perioperatively in the pending graft preparation is generally accepted and these may be implemented topically, endoluminally or both ways. Moxonidine is the latest second-generation, centrally acting antihypertensive agent, and the intention in this paper is to investigate its direct vasorelaxant effects and relaxation mechanisms on the human radial artery *in vitro*.

METHODS: RA rings were mounted in an organ bath and tested for changes in isometric tension in its relaxation response to moxonidine in the presence and absence of NG-nitro-L-arginine methyl ester (L-NAME, non-specific inhibitor of nitric oxide synthase), idazoxan (non-selective I1 and α2-antagonist) and yohimbine (selective α2-antagonist).

RESULTS: Moxonidine induced concentration-dependent relaxations on the RA rings precontracted with phenylephrine ($P < 0.05$). L-NAME and idazoxan significantly reduced the relaxation caused by moxonidine ($P < 0.05$), while yohimbine significantly increased the relaxation by moxonidine ($P < 0.05$). In the presence of L-NAME + idazoxan, the relaxation by moxonidine was eliminated completely ($P < 0.05$).

CONCLUSIONS: We speculate that the relaxant effect of moxonidine may be attributed partly to the synthesis and/or release of nitric oxide, and partly to the stimulation of imidazoline I1 receptors. We suggest that moxonidine may help to prevent RA spasms during the preparation period in operation when used topically or/and endoluminally.

Keywords: Moxonidine • Human radial artery • Relaxation • Coronary artery bypass graft

INTRODUCTION

The radial artery (RA) has been demonstrated to provide superior long-term patency outcomes compared with saphenous veins for selected patients who undergo coronary artery bypass graft surgery (CABG) [1]. Although the popularity of using RA as a graft is increasing, due to its good length, suitable inner diameter, ease of handling and minimal donor site discomfort, graft spasm is one of the most important problems in RA grafts [2]. Graft spasm without any clinical signs may occur in the operation and shortly after the operation, due to maximal RA reactivity within days of the operation [2]. Reversible spasm is more frequent than believed, and unstable haemodynamic changes can be determined in grafts supplying critical coronary territories with blood [3]. Many vasodilator drugs are used topically or/and endoluminally to prohibit the RA vasospasm, such as calcium channel blockers, verapamil and nitroglycerine (VG solution), phenoxybenzamine and phosphodiesterase inhibitors such as papaverine, amrinone and milrinone [4–6]. However, an occasional lack of effectiveness of these drugs and their side effects limit usage; therefore, this stimulated the search for alternative vasodilator agents to prevent RA spasms [7].

Moxonidine is the newest, second-generation, centrally acting antihypertensive agent [8] that has not only selective agonist activity in the imidazoline I1 receptors and minor activity in the α2-adrenoceptors, but also less adverse effects than other

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centrally acting drugs [8, 9]. The direct effects of moxonidine on vascular tissue are not known in detail; however, what is known is that there are both post- and presynaptic imidazoline binding sites in vascular tissue [10].

Since graft spasms are one of the most important problems both during and after CABG surgery [2], new approaches in the prevention or treatment of these spasms seem essential. In the present study, we aimed to investigate if moxonidine has a vasorelaxant effect on the human RA, and if so, what is the mechanism of action? Thus, we will be able to reveal an important target for new antivasospastic drugs, which can be used topically during the preparation of the RA.

METHODS

Human RA segments were collected from 20 patients who have undergone selective CABG surgery. All experimental steps of the present study have been approved by the Human Ethics Committee of Cumhuriyet University. Written informed consent was obtained from all patients prior to surgery. Prior to surgical treatment, a modified Allen test was performed for all patients by using a pulse oximeter placed on the thumb. This test evaluates whether palmar arch flow is sufficient or not, by observing the pulse and oxygen saturation [11], for preoperative evaluation of ulnar collateral circulation to the hand. If collateral circulation is sufficient, the RA could be harvested without any ischaemic complications in the hand. In the operation, the RA was harvested via an open surgical procedure from the non-dominant hand. Before the distal anastomosis was performed, specimens of 1–2 cm in length were excised from the distal or proximal end of the RA and placed in a Krebs–Henseleit solution. All connective tissues were dissected, and the vessel was divided into rings measuring 3–4 mm in length.

RA rings were cut into four segments and each segment placed in a 10-ml organ bath which is already filled with Krebs–Henseleit solution of the following composition in millimolar concentrations/units: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 5.0 and glucose, 11.0. The solution was gassed continuously with a mixture of 95% O_2 and 5% CO_2 and the temperature was maintained at 37°C. The RA rings were equilibrated for 2 h with a resting tension of 2 g, which is chosen as the optimal tension from the knowledge gathered from preliminary studies. Smooth muscle tension was recorded using a Grass FT-03 force-displacement transducer and on a four-channel Grass polygraph (model 79 E; Grass Instruments, Quincy, MA, USA) [12]. After the period of equilibration, which is optimally 2 h, the RA rings were precontracted with a sub-maximal concentration of phenylephrine (10^{-6} mol/l). After phenylephrine-induced sub-maximal contraction took place in the chard, it was tested if moxonidine had a relaxant effect on RA rings by adding each concentration to the bath cumulatively (10^{-8}–10^{-4} mol/l). The relaxations caused by moxonidine were obtained again in the presence of NG-nitro-L-arginine methyl ester [L-NAME, 3×10^{-5} mol/l, non-specific inhibitor of nitric oxide synthase (NOS)], idazoxan (10^{-5} mol/l, non-selective I1 and α2-antagonist) and yohimbine (10^{-5} mol/l, selective α2-antagonist), which were added to the organ bath 15 min prior to the precontraction of phenylephrine. The relaxation responses caused by moxonidine were presented as a percentage of precontraction by phenylephrine. The maximum relaxant effect (E_{max}) and concentrations of moxonidine producing 50% of the E_{max} (EC50) were calculated for each concentration–response curve, while the pEC50 (pD_2) was calculated as the negative logarithm to base 10 of the EC50 for statistical analysis. Data are presented as mean ± the standard error of the mean, and were analysed by using a *t*-test, when appropriate. A *P* value of <0.05 was considered significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) ver. 14. The chemicals used in the current experiments were phenylephrine hydrochloride, moxonidine hydrochloride, L-NAME, idazoxan hydrochloride and yohimbine hydrochloride purchased from Sigma (St Louis, MO, USA). The chemicals were dissolved in distilled water and then further diluted with distilled water for the preparation of decreasing concentrations. The drug-containing solutions were prepared fresh on the day of the experiments.

RESULTS

Segments of the RA were obtained from 20 patients (12 male and 8 female, with an average age of 59.73 ± 4.89 years), whose characteristics, risk factors and medications are presented in Table 1. Moxonidine (10^{-8}–10^{-4} mol/l, $n = 20$) was seen to induce concentration-dependent relaxations on RA rings precontracted with phenylephrine (10^{-6} mol/l) (Figs 1–3), with moxonidine relaxation responses taken in the presence and absence of L-NAME (3×10^{-5} mol/l), idazoxan (10^{-5} mol/l) and yohimbine (10^{-5} mol/l). The relaxation responses induced by moxonidine in the isolated RA rings reached statistical significance beginning at a 10^{-7} mol/l concentration ($P < 0.05$, Fig. 1). At the end of the experiment, papaverine caused a complete relaxation of the sub-maximal phenylephrine contraction. Table 2 presents the values for pD_2 and the E_{max} values of moxonidine in RA rings precontracted

Table 1: Patient characteristics, risk factors and medication in radial artery segments obtained from 20 patients

Patient characteristics and risk factors	
Age (years)	59.73 ± 4.89
Male, *n* (%)	12/8 (60.0/40.0)
Smoker, *n* (%)	8 (40.0)
Hypertensives, *n* (%)	13 (65.0)
Diabetics, *n* (%)	5 (25.0)
Hyperlipidaemia, *n* (%)	6 (30.0)
Total cholesterol (mg/dl)	195.08 ± 48.83
Triglyceride (mg/dl)	160.99 ± 46.33
HDL (mg/dl)	47.55 ± 13.14
LDL (mg/dl)	128.27 ± 37.41
VLDL (mg/dl)	35.70 ± 20.30
Medication	
Nitrates, *n* (%)	10 (50.0)
Statins, *n* (%)	5 (25.0)
ACE inhibitors, *n* (%)	10 (50.0)
Ca^{2+} antagonists, *n* (%)	5 (25.0)
β-Blockers, *n* (%)	8 (40.0)
Oral antidiabetics, *n* (%)	4 (20.0)
Insulin, *n* (%)	1 (5.0)
Aspirin, *n* (%)	11 (55.0)

HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; ACE: angiotensin-converting enzyme.

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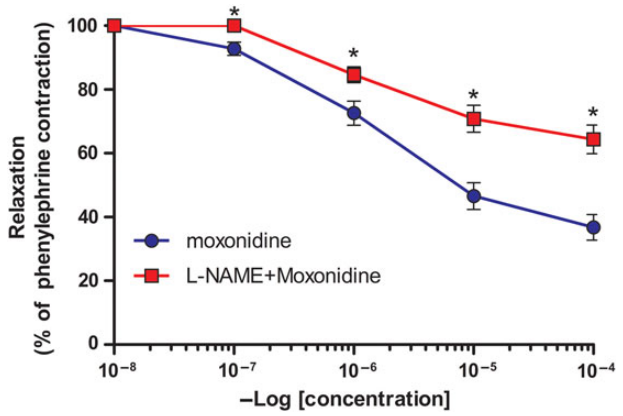

Figure 1: Relaxation responses induced by moxonidine alone, and in the presence of L-NAME in human RA. Relaxation responses are expressed as a percentage of phenylephrine (10^{-6} mol/l)-induced precontraction, and are shown as mean ± SEM. *Statistically different from moxonidine relaxations ($P < 0.05$). L-NAME: NG-nitro-L-arginine methyl ester; RA: radial artery; SEM: standard error of the mean.

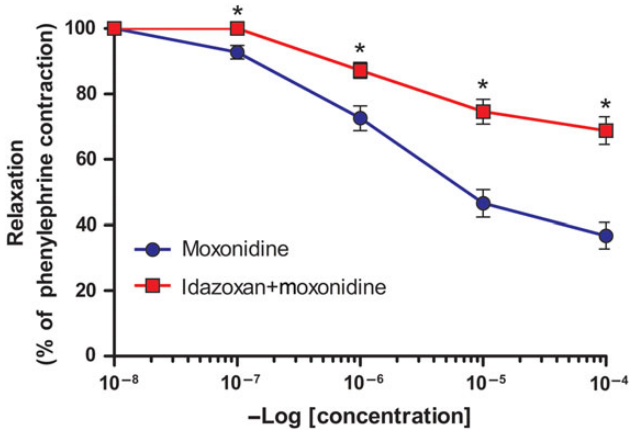

Figure 2: Relaxation responses induced by moxonidine alone and in the presence of idazoxan in human RA. Relaxation responses are expressed as a percentage of phenylephrine (10^{-6} mol/l)-induced precontraction and are shown as mean ± SEM. *Statistically significantly different from moxonidine relaxations ($P < 0.05$). RA: radial artery; SEM: standard error of the mean.

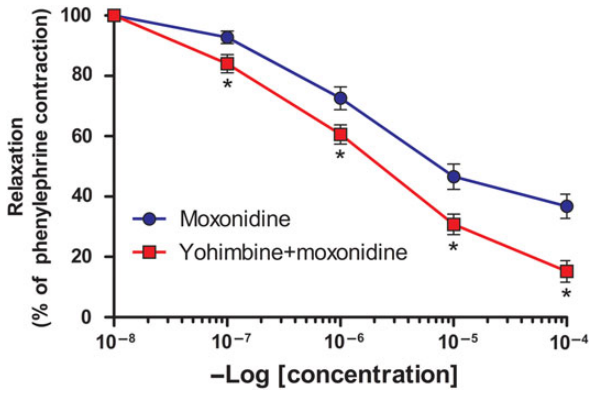

Figure 3: Relaxation responses induced by moxonidine alone and in the presence of yohimbine in human RA. Relaxation responses are expressed as percentage of phenylephrine (10^{-6} mol/l)-induced precontraction and shown as mean ± SEM. *Statistically different from moxonidine relaxations ($P < 0.05$). RA: radial artery; SEM: standard error of the mean.

Table 2: pD_2 and E_{max} values of moxonidine in RA rings precontracted with phenylephrine

	pD_2	E_{max}
Moxonidine	5.90 ± 0.10	63.2 ± 4.0
L-NAME + moxonidine	5.94 ± 0.08	35. ± 3.6
Moxonidine	5.90 ± 0.10	63.2 ± 4.0
Idazoxan + moxonidine	6.06 ± 0.12	31.2 ± 4.2
Moxonidine	5.90 ± 0.10	63.2 ± 4.0
Yohimbine + moxonidine	6.02 ± 0.09	84.8 ± 3.6

L-NAME: NG-nitro-L-arginine methyl ester; RA: radial artery.

with phenylephrine. The administration of L-NAME, idazoxan and L-NAME + idazoxan significantly reduced the relaxation by moxonidine ($P < 0.05$, Figs 1 and 2), whereas yohimbine significantly increased the relaxation by moxonidine ($P < 0.05$, Fig. 3). In the presence of L-NAME + idazoxan, relaxation by moxonidine was eliminated completely ($P < 0.05$).

DISCUSSION

The RA, as a graft in coronary artery bypass surgery, has many benefits with its unique features including adequate length to reach any coronary vessel, easy to manipulate surgically and having a more superficial anatomical location. Furthermore, in addition to these features, it has less risk for sternal wound infection and mediastinitis [13]. Although the RA graft was first used in 1973, its use was soon abandoned due to the unacceptably high early graft failure attributed to spontaneous contractions or spasms. However, it has gained popularity in the last decades [14]. After its reintroduction as an arterial graft in coronary artery surgery, the RA is now well established as an alternative arterial conduit, but vasodilator strategies still have been needed due to concerns about its vasospasticity [15]. Additionally, improvements in surgical techniques and handling have reduced the incidence of vasospasm. However, these improvements are not enough to reduce RA graft spasm; hence, many drugs, both experimentally and clinically, have been monitored to cope with this problem.

Many previous *in vitro* studies have shown that the RA has dominant alpha-adrenergic receptor activity and strong contractions were attained in response to alpha-receptor agonists [16, 17]. Unfortunately, vasospasms of the artery were observed in 4–10% of patients, despite intraoperative and postoperative treatment with such antispastic agents such as diltiazem and verapamil [18]. During perioperative and early postoperative periods, circulating catecholamines and exogenous sympathomimetic agents can lead to serious RA spasm. However, in order to counteract the RA spasm, vasodilators may be used at the time of harvesting, for a short period between harvesting and implantation, with topical application after implantation. Generally, calcium channel blockers, verapamil and nitroglycerine (VG solution), phenoxybenzamine and phosphodiesterase inhibitors such as papaverine, amrinone and milrinone are used topically for preventing vasospasms [4–6]. The topical or intraluminal use of these drugs has some limitations. Several studies have demonstrated that papaverine damages the endothelium, and the most likely mechanism for this is through a

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decrease in pH [19, 20]. In addition, nitroglycerine acts with a slow onset and in a short duration [6]. According to Taggart *et al.*, phenoxybenzamine prevents RA vasospasms for at least 6 h without damage to the endothelium [21], but papaverine does so only for a maximum of 30 min, and also appeared to compromise endothelium-dependent relaxation. Moreover, nitroglycerine inhibits only thromboxane-mediated radial spasm. He and Yang warned that radial arteries pretreated with nitroglycerine developed tolerance to the successive doses of the drug [14]. However, currently used vasodilators have side effects, with inefficacy revealed in some cases and, hence, there is the necessity to find new alternatives.

Moxonidine is a mixed α2-adrenergic and imidazoline receptor agonist with an improved side-effect profile when compared with clonidine [22], based on moxonidine's reduced activity in the α2-adrenergic receptors that mediate its undesirable actions [23]. Previous literature has claimed that moxonidine shows its effects partly via the nitric oxide (NO) pathway, and it has been reported that the effect of centrally acting drugs (i.e. moxonidine or clonidine) is closely related to the NO mechanism within the central nervous system [24]. In addition, further studies suggest that some drugs that affect the imidazoline receptors may cause relaxations in various vessels by using NO-dependent or independent pathways [25].

In this study, moxonidine was found to have concentration-dependent relaxant effects in human RA that has been pre-contracted with phenylephrine. The administration of L-NAME, idazoxan and L-NAME + idazoxan significantly reduced the relaxation of moxonidine, whereas yohimbine significantly increased the relaxation of moxonidine. In the presence of L-NAME+ idazoxan, the moxonidine relaxation was completely eliminated.

From these results, it can be speculated that the relaxant effects of moxonidine are partially mediated with NO synthesis and/or release, and partially related to stimulation of the imidazoline I1 receptors by moxonidine. Furthermore, stimulation of the α2-adrenoceptors by moxonidine may cause a reduction in relaxation. The NO synthesis and/or release enhancing effects of moxonidine are independent of the imidazoline I1 receptors; otherwise, idazoxan (antagonist of imidazoline I1 receptor) or L-NAME (non-specific inhibitor of NOS) should have completely eliminated the relaxation caused by moxonidine. As such, it can be speculated that the relaxation responses caused by the imidazoline I1 receptors are independent of the synthesis and/or release of NO. Further studies may reveal how moxonidine increases synthesis and/or the release of NO, and how the imidazoline I1 receptors that are stimulated by moxonidine cause relaxation in the RA. In light of these findings, moxonidine may be a safe and effective alternative for topical or/and intraluminal usage during the preparation period.

CONCLUSION

In conclusion, moxonidine causes relaxation in the human RA, and we suggest that these relaxant effects of moxonidine are partially mediated with NO synthesis and/or release, and partially related to the stimulation of the imidazoline I1 receptors by moxonidine. Our results suggest that moxonidine may be used topically in the future to prevent spasms in the human RA.

Conflict of interest: none declared.

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