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Lack of Effect of Atorvastatin or Pravastatin on the Endothelium-Dependent Relaxation in Segments of Human Vessels

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Abstract: Segments of radial artery and internal mammary artery were obtained from patients undergoing coronary artery bypass grafts, cut into two segments (≈ 5 mm in length) and placed in two organ chambers for isometric tension recording. Atorvastatin or pravastatin was added to one chamber and after a 2 h stabilization period, contractions to a plateau were elicited with 70 mM KCl. Then the rings were precontracted with 10^{-7} M noradrenaline and cumulative relaxation curves to 10^{-9} to 10^{-4} M acetylcholine and sodium nitroprusside (10^{-8} to 10^{-4} M) were then performed. Contraction to KCl was significantly higher in the radial artery than in the internal mammary and the opposite was obtained with NA-induced contractions. In both vessels, statins did not modify the KCl contraction. Atorvastatin reduced the response to NA in the radial artery. The radial artery and the internal mammary artery precontracted with NA, relaxed dose-dependently in response to ACh. The relaxation was significantly higher in the radial than in the internal mammary, both with and without pretreatment with atorvastatin or pravastatin. These findings demonstrate a lack of effect of acute treatment with atorvastatin or pravastatin on the modulation of vascular tone in segments of human radial and internal mammary artery as measured by endothelium-dependent relaxation induced by ACh.

Key words: Radial artery, internal mammary artery, endothelium-dependent relaxation, acetylcholine, noradrenaline

INTRODUCTION

The most common vessels used as a conduit in coronary artery bypass grafting for myocardial revascularization are the Internal Mammary Artery (IMA), the Saphenous Vein (SV) and the Radial Artery (RA). However, the endothelium heterogeneity among these vessels may be determinant for the immediate preoperative and the long term postoperative performance of the grafts. Arterial conduits are generally preferred because of their improved long-term patency rates as compared to venous conduits, even if the grafts supply the same coronary bed (Dzimiri *et al.*, 1996; Mills, 1997; Cable *et al.*, 1999).

Nitric Oxide (NO) is synthesized by three different NO synthase (NOS) isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) (Moncada *et al.*, 1997). Previous studies have demonstrated that statins regulate eNOS expression and subsequent NO synthesis and NO-

mediated endothelium dependent relaxation (Laufs *et al.*, 1998) and also upregulate vascular nNOS through an Akt/NF- κ B pathway (Nakata *et al.*, 2007). In addition, iNOS expression was upregulated in cytokine-stimulated vascular smooth by fluvastatin (Chen *et al.*, 2000). Furthermore, differences have been reported in NO-mediated vascular relaxation in vessels used in coronary revascularization (Shapira *et al.*, 1999).

The aim of this study was to assess the acute effect of atorvastatin and pravastatin in the acetylcholine (ACh) induced relaxation in human IMA and RA used in coronary revascularization.

MATERIALS AND METHODS

Segments of IMA and RA were obtained from patients undergoing coronary artery bypass grafts at the Clinical Hospital of the University of Chile from April to July 2007. Male and female patients were included with a

Table 1: Age and percentage of patients with pre-existing risk factors

Age (year)	61.1±1.2
Hypertension	74%
Dyslipidemia	51%
Diabetes mellitus	45%
Cigarette smoking	42%

mean age of 64.2±2.0 years. The clinical profile of these patients is shown in Table 1. The study was approved by the local ethical committee and written consent was obtained from each patient.

Experimental protocol: The arterial segments were harvested and immediately transferred to ice cold modified Tyrode solution (mM composition: NaCl 119.7; KCl 5.3; CaCl₂ 2.7; MgSO₄ 1.2; KH₂PO₄ 1.2; NaHCO₃ 23.8 and glucose 11.1). Each ring was carefully dissected with microsurgical instruments to remove excess fat tissue and cut into two segments (≈ 5 mm in length). Within 30 min of collection, the vessel segments were placed in two organ chambers containing modified Tyrode solution and aerated with 95% O₂/5% CO₂ at 37°C. Atorvastatin or pravastatin (10⁻⁴ M) was added to one of the chambers. Each vessel ring was suspended between two L-shaped stainless steel hooks for measurement of isometric tension with Grass FT-0.3 transducers. The resting tension of arterial segments was set to 1.5 g and the vessels were then allowed to equilibrate for 120 min before the introduction of vasoactive drugs. After the stabilization period, the rings were challenged twice with 70 mM KCl until the response reached a plateau followed by a complete return to the baseline after washing. Then the rings were precontracted with noradrenaline (NA) 10⁻⁷ M and a cumulative relaxation curve to ACh (10⁻⁹ to 10⁻⁴ M) was performed. A cumulative relaxation curve to sodium nitroprusside (10⁻⁸ to 10⁻⁴ M) was also obtained. Vessel relaxation was expressed as percent reduction in the maximal tension induced by NA.

Statistical analysis: Results are expressed as means±SEM and changes in tension were calculated as mg tension mg wet vessel weight⁻¹. Statistical comparisons were made using Student's t-test for independent means and p<0.05 was considered statistically significant.

RESULTS

Contractile response to KCl: Contraction was significantly higher in RA than in IMA, either with or without statins. Moreover, pravastatin pre-treatment of RA induced a higher KCl response than atorvastatin (Table 2).

Table 2: Contractile response of vessel segments to 70 mM KCl

	Radial artery tension (mg mg wet weight ⁻¹) (n)	Internal mammary artery tension (mg mg wet weight ⁻¹) (n)
Without statins	100.9±13.3 (13)	43.1±6.6 (23)*
Atorvastatin	77.1±13.6 (8)**	47.2±8.2 (14)*
Pravastatin	121.9±22.3 (5)	32.2±9.9 (9)*

*p<0.05 vs radial artery, **p<0.05 vs without statins

Table 3: Contractile response of vessel segments to 10⁻⁷ M noradrenaline

	Radial artery tension (mg mg wet weight ⁻¹) (n)	Internal mammary artery tension (mg mg wet weight ⁻¹) (n)
Without statins	31.2±7.2 (13)	80.6±5.8 (23)*
Atorvastatin	11.5±2.6 (8)**	60.8±9.0 (14)*
Pravastatin	38.0±7.8 (5)	63.9±2.5 (9)*

*p<0.05 vs radial artery, **p<0.05 vs without statins

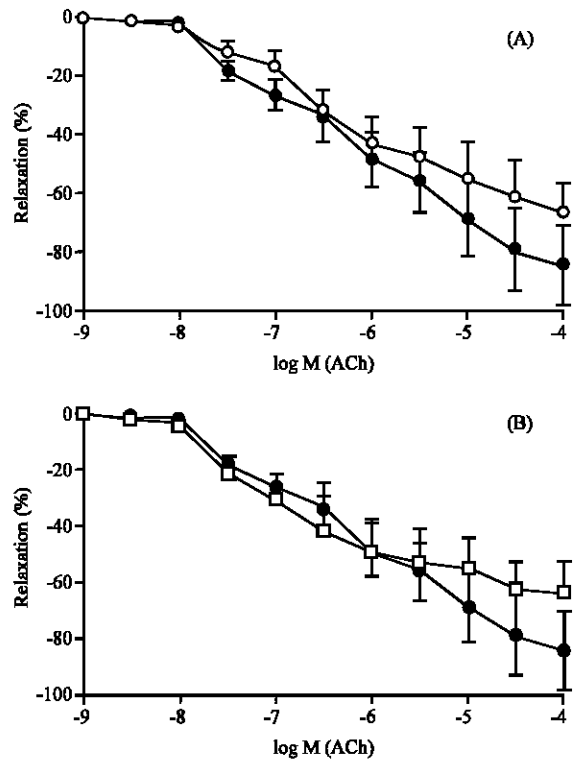


Fig. 1: Effect of atorvastatin (A, ○) and pravastatin (B, □) on the relaxation curve of the human radial artery to acetylcholine. (●) Control curve. Relaxation is expressed as % of the maximal contraction induced by noradrenaline (10⁻⁷ M)

Contractile response to NA: Contraction in IMA was significantly higher than in RA, with or without statins. However, pretreatment of RA with atorvastatin significantly reduced the contractile response of the vessels (Table 3).

Relaxant effect of ACh: RA and IMA precontracted with NA, relaxed dose-dependently in response to ACh.

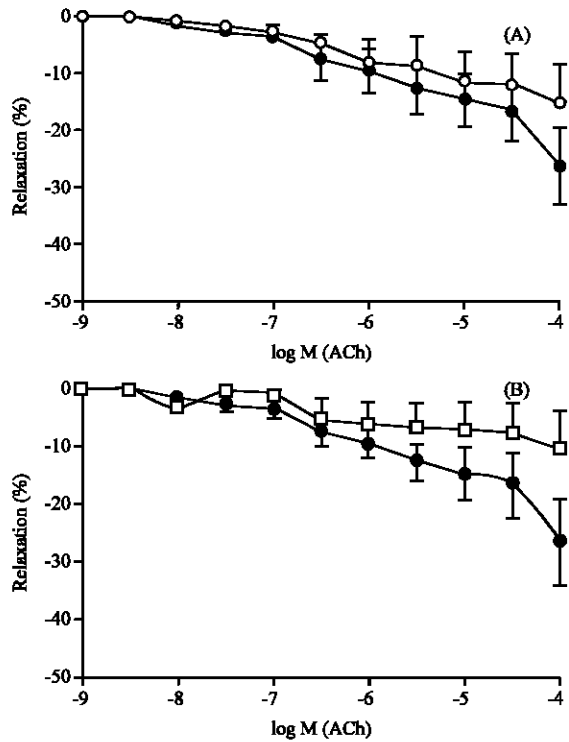


Fig. 2: Effect of atorvastatin (A, ○) and pravastatin (B, □) on the relaxation curve of the human internal mammary artery to acetylcholine. (●) Control curve. Relaxation is expressed as % of the maximal contraction induced by noradrenaline (10^{-7} M)

The relaxation was significantly higher in RA (Fig. 1A, B) than in IMA (Fig. 2A, B), both with and without pretreatment with atorvastatin or pravastatin, but statin pretreatment did not significantly change the control relaxation.

Relaxant effect of sodium nitroprusside: The endothelium-independent relaxation induced by sodium nitroprusside was not different in RA and IMA, either with or without exposure to both statins.

DISCUSSION

It is well known that ACh activates muscarinic receptors localized in the vascular smooth muscle and endothelium, inducing a vasodilatation mainly mediated by NO (Furchgott and Zawadzky, 1980; Holubarsch, 2000). Furthermore, it has been reported that statins are also able to induce a vasodilatation which may be endothelium dependent and induced by an increased production of NO (de Sotomayor *et al.*, 2000).

Theoretically, the coadministration of ACh and statins may induce additive or synergic responses, according to the drugs interaction theory (Chou, 2006).

In the present study, the acute (120 min) exposure to atorvastatin or pravastatin did not significantly modify the endothelium-dependent relaxation induced by ACh in human RA and IMA. These results are in agreement with those reported by Nakamura *et al.* (2002) who, after the incubation of human RA and IMA with cerivastatin for 120 min, did not find differences in maximal endothelium-dependent vasodilatation. In addition, in the present work, the relaxation induced by ACh was significantly higher in RA than in IMA, in agreement with the reports of Shapira *et al.* (1999) and Nakamura *et al.* (2003). This finding could be partially explained by differences in the regulation of NO synthesis, as well as in the basal production of other agents related to vasodilatation (i.e., prostacyclin, EDHF) (Chester *et al.*, 1998). Besides, RA is a thick-walled muscular vessel with a media width of approximately 500 μ m, as compared to close to 300 μ m for IMA (Van Son *et al.*, 1990). Endothelium-independent relaxation by nitroprusside was similar in both vessels.

It has been reported that simvastatin is able to induce relaxation of endothelium-intact aortic rings of rats (de Sotomayor *et al.*, 2000). In the present study on different vessels, relaxation by atorvastatin or pravastatin was not observed. On the other hand, it has been also reported that basal and stimulated NO production is reduced in human RA as opposed to IMA (Cable *et al.*, 1999; He and Liu, 2001; Gaudino *et al.*, 2003). Compared to these works, in the present experimental protocol precontraction was different (prostaglandin F 2α versus NA) and measurements of the molecular expression of NOS and NO levels were not performed, since only the pharmacological effect was considered.

The higher magnitude observed in the magnitude of KCl-induced contractile response of RA compared to IMA, is not concordant with previous results which demonstrated no significant difference in the contraction elicited by KCl (Arshad *et al.*, 2006; Rabbani *et al.*, 2007). This different finding could be due to the experimental protocol: basal passive tension of rings (1.5 vs 2 or 4 g); incubation physiological solution (Tyrode vs Krebs); KCl concentration (70 vs 123 mM). However, the exposure to statins did not modify the contractile response to KCl in both arteries.

The contraction induced by NA was higher in IMA than in RA, in agreement with reports using other α -adrenergic agonists (Arshad *et al.*, 2006; Rabbani *et al.*,

2007). The dependence of the effect of α -adrenergic agonists on extracellular Ca^{+2} concentration is greater in IMA than in RA (Rabbani *et al.*, 2007). The reduced contraction induced by NA in the atorvastatin-preincubated RA, could be explained by the fact that this statin, as simvastatin, affects Ca^{+2} release from intracellular pools and capacitative Ca^{+2} entry (de Sotomayor *et al.*, 2001). However, the lower contractile response of RA to NA is not concordant with the work of He and Liu (2001), who found that human RA has a higher receptor-mediated contractility since is an α -adrenoceptor dominant artery as compared to IMA.

In conclusion, this study demonstrated the lack of effect of short term exposure to atorvastatin or pravastatin on the modulation of vascular tone in segments of human RA and IMA, as measured by the endothelium-dependent relaxation induced by ACh.

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