[Frontiers in Bioscience E4, 1787-1794, January 1, 2012]

# Mechanism of the relaxant effect of rosuvastatin lactone on rat aortic rings

**Polanco-Ponce Ana Cecilia [1,2], Perez-Alvarez Victor Manuel[4], Wens-Flores Isabel[4], Castillo-Henkel Enrique[1], Lopez-Sanchez Pedro[1], Lopez-Canales Jorge Skiold[1,3], Castillo-Hernandez Maria del Carmen[1], Castillo-Henkel Carlos[1]**

*[1]Seccion de Estudios de Posgrado e Investigacion de la Escuela Superior de Medicina, I.P.N. Plan de San Luis y Diaz Miron, Col. Casco de Sto. Tomas, Mexico 11340, Mexico, D.F., [2]AstraZeneca, Mexico, [3]Instituto Nacional de Perinatología, Isidro Espinoza De Los Reyes, Mexico D.F., [4]Centro de investigacion y estudios avanzados, CINVESTAV, unidad zacatenco. Mexico, D.F.*

## TABLE OF CONTENTS



## 1. ABSTRACT

The relaxant effect of the lactone of rosuvastatin was evaluated on aortic rings from male Wistar rats (250-300 g) with and without endothelium, precontracted with 1.0 μM phenylephrine. The lactone presented a greater potency than rosuvastatin in relaxing aortic rings. Unlike rosuvastatin, the effect of its lactone was endothelium-independent. Pretreatment with either indomethacin (10 microM) or mevalonate (1 mM) did not inhibit the relaxant effect of the lactone. L-NAME (10 microM), 1400 W (10 μM), or tetraethylammonium (TEA, 10 mM) partially inhibited the relaxant effect of the lactone on endothelium-denuded aortic rings. However, cycloheximide (10 μM) or the combination of TEA plus L-NAME completely inhibited the relaxant effect. The NOS-2 was only present in endothelium-denuded aortic rings, as demonstrated by immunoblot with lactone treated rings. In conclusion, rosuvastatin was associated with a relaxant effect dependent on both endothelium and HMG-CoA reductase in rat aorta, whereas the lactone exhibited an endothelium and HMG-CoA reductase-independent relaxant effect. Both nitric oxide produced by NOS-2 and K+ channels are involved in the relaxant effect of the lactone.

## 2. INTRODUCTION

In addition to their beneficial lipid modulation effects, statins exert a variety of "pleiotropic" actions that may be clinically beneficial (1-3). Indeed, the pleiotropic effects of statins have been considered to be as important as their hypolipemiant effects in the prevention of cardiovascular diseases (4). Among the pleiotropic actions of statins such as pravastatin, cerivastatin, atorvastatin, simvastatin and rosuvastatin (5-7) are their direct acute vascular effects associated with increased production of either endothelial nitric oxide synthase (eNOS or NOS-3) in endothelial cells (8) or inducible nitric oxide synthase (iNOS or NOS-2) in smooth muscle cells (9). Other pleiotropic actions include antithrombotic effects, anti-inflammatory effects (10,11), and decreased nitrotyrosine production (12).

The favorable cardiovascular effects of rosuvastatin, one of the most commonly employed statins, have been demonstrated by a substantial number of experimental and clinical studies. These effects are generally associated with endothelial function. For instance, rosuvastatin-elicited vasodilatation is mediated by the production of nitric oxide and the opening of $Ca^{2+}$-

dependent $K^+$ channels of the slow subfamily (7). Several protective actions of this statin related to its vascular effects have been demonstrated, including the prevention of inflammation and endothelial glycocalyx damage elicited by metalloproteinases, the inhibition of smooth muscle cell proliferation, the production of oxidized low-density lipoprotein, and an increase in plaque stability (13,14).

The structure-activity relationship of statins regarding their hypolipemiant effect is clear (15). These drugs, through a mevalonic acid–like moiety, competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes an early, rate-limiting step in the cholesterol biosynthesis (15). Independent of cholesterol synthesis, an interaction of statins with HMG-CoA may also be associated with several of the pleiotropic effects of these compounds (16). On the other hand, since information is scarce about the structural characteristics of statins that are responsible for their pleiotropic effects, we compared the relaxant effect of rosuvastatin and its lactone derivative on aortic rings. Considering that lactone in rosuvastatin is a metabolite without hypolipemiant effects (17), it can be used to evaluate the possibility that a statin produces pleiotropic effects unrelated to the inhibition of HMG-CoA-reductase.

## 3. MATERIALS AND METHODS

### 3.1. Materials

All drugs were purchased from Sigma Chemical Co. (St. Louis MO, USA.). Rosuvastatin was a generous gift from Astra Zeneca, Mexico, and the lactone was synthesized in the Pharmacology Departament of the Centro de Investigacion y Estudios Avanzados, Instituto Politecnico Nacional, Mexico, by employing rosuvastatin, diciclohexilcorbodiimide and dichloromethane. The structure of the compound was confirmed by using $H^1$ and $C^{13}$ NMR.

### 3.2. Preparation of aortic rings

Experiments were conducted under protocols approved by the Animal Care Committee of our institution (Escuela Superior de Medicina), with local ethical approval and in agreement with the UK Animals Scientific Procedures Act of 1986) and received.

Male Wistar rats (250 - 300g) were kept in the animal colony until sacrifice. The animals were maintained on a 12-12 hr light-dark cycle in a special room at constant temperature (22 ± 2°C), with food and water freely available.

The animals were euthanized by decapitation and the aortas were immediately excised and placed in cold buffer, cleaned and freed from surrounding connective tissue. The isolated arteries were cut into rings (4-5 mm long) and placed in 10 ml tissue chambers filled with Krebs-Henseleit bicarbonate buffer ( 118 mM NaCl ;4.7 mM KCl ; 1.2 mM $KH_2$ $PO_4$; 1.2 mM $MgSO_4 \cdot 7H_2O$; 2.5 mM $CaCl_2 \cdot 2H_2O$; 25 mM $NaHCO_3$; 11.7 mM dextrose; and 0.026 mM calcium disodium EDTA**).** Tissue baths, maintained at 37 °C and pH 7.4, were bubbled with a mixture of 95% $O_2$ and 5% $CO_2$.

Aortic rings were mounted on two stainless steel hooks to fix them to the bottom of the chamber and to a Grass FTO3 force displacement transducer connected to a 7D Grass Polygraph (Grass Instrument Co., Quincy MA, USA) in order to record the isometric tension. Optimal tension, selected from preliminary experiments, was that which gave the greatest response to phenylephrine (Phen, 1 microM). The rings were given 2 g of initial tension and allowed to equilibrate for 2 hr. Thirty minutes after setting up the organ bath, tissues were first contracted with Phen to test their contractile responses. They were then rinsed three times with Krebs solution to restore tension to precontraction levels. We used denuded aortic rings in some experiments. Endothelium-denuded aortic strips were prepared by turning the rings gently several times on the distal portion of small forceps. Endothelial integrity was pharmacologically assessed by acetylcholine-induced vasodilatation (1 microM). Segments showing no relaxation were considered to be endothelium-denuded.

### 3.3. Experimental protocol

1. After the equilibration period, dose-response curves were obtained for aortic rings with endothelium that were previously contracted with 1 microM Phen, in order to determine whether the lactone of rosuvastatin (1 nM to 10 microM) had a relaxant effect. The results for the lactone were compared with those obtained for rosuvastatin in order to establish their relative potencies.

2. Participation of the endothelial layer in the relaxant effect was studied by comparing the presence and absence of this tissue in aortic rings that were precontracted with Phen, then relaxed with rosuvastatin or its methyl ester. The results of the latter two agents were compared in order to establish the relative contribution of the endothelial layer.

3. Experiments were performed in order to determine if NO, prostaglandins or $K^+$ channels are involved in the mechanism of the relaxant effect of rosuvastatin lactone. Aortic rings without endothelium were assayed in the presence or absence of the following inhibitors: 10 microM L-NAME (a non-selective NO synthase inhibitor), 10 microM indomethacin (a prostaglandin synthesis inhibitor), and 10 mM tetraethylammonium (TEA, a non-selective potassium channel blocker).

4. To establish whether NOS-2 activity and expression is involved in the relaxant effect of the rosuvastatina lactone, three strategies were employed. Firstly, the effect of this lactone on denuded aortic rings pretreated with 1400 W (a selective NO-2 inhibitor) was evaluated. Secondly, the effect of this rosuvastatin derivative on aortic rings pretreated with 10 microM cycloheximide (a general protein synthesis inhibitor) was determined. Thirdly, an immunoblot of iNOS in denuded aortic rings was obtained.

5. Finally, aortic rings were pretreated with 1 mM mevalonic acid before administering the lactone

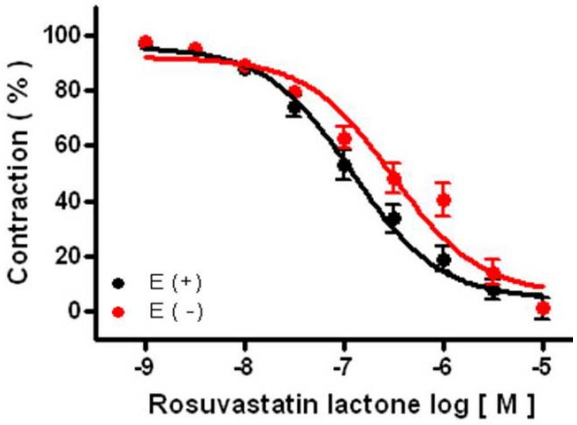


**Figure 1.** Rosuvastatin lactone-induced relaxation of rat aortic rings with or without endothelium precontracted with 1 μM phenylephrine. Measurements were made after 20 min of incubation. Data are reported as means ± SEM for N = 6 experiments. A two-way ANOVA was used.

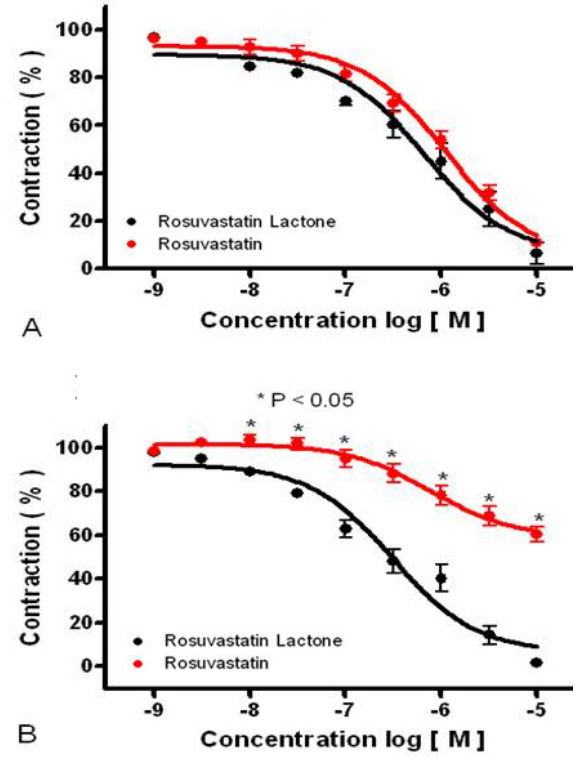


**Figure 2.** Comparative relaxant effects of lactone and rosuvastatin on rat aortic rings with (E (+) Figure A) or without (E (-), Figure B) endothelium precontracted with 1 μM phenylephrine. Data are reported as means ± SEM for N = 6 experiments. *P < 0.001 compared to E (+) (two-way ANOVA).

derivative of rosuvastatin, with the aim of evaluating the effects of the inhibition of HMG-CoA reductase on the effects of the test compound.

### 3.4. NOS immunoblot Experimental protocol

Briefly, samples were homogenized in Tris-HCl, pH 7.4, with a protease cocktail (MiniComplete-EDTA free, Roche, Mannheim, Germany), and the total protein was analyzed by Lowry's method. (18) Immunoblots were carried out in duplicate using 50 micro-grams of protein per lane on a 10 % SDS-polyacrylamide gel and transferred onto polyvinylidene fluoride membrane (PVDF; Hybond-P, Amersham Biosciences, UK). The PVDF membrane was then blocked with TBS containing 5% skim milk and 0.05% Tween for 2 hours at room temperature. The blot was incubated overnight at 4°C with a polyclonal antibody against iNOS (Santa Cruz Biotechnology, CA, USA), at a final dilution of 1:400. We previously tested that this antibody was NOS-2-specific and did not cross-react with NOS-1 or NOS-3. The membrane was then washed and incubated with the corresponding secondary (anti-rabbit) HRP-labeled antibody (Zymed), diluted 1:10,000 in blocking solution, for 2 hours at room temperature. Blots were washed and developed using an ECL detection system (Luminol, Santa Cruz Biotechnology, CA, USA). The blots were stripped and reprobed with a β-actin polyclonal antibody as a control. Images from films were digitally acquired and a densitometrical analysis was performed using the Quantity One Image Acquisition and Analysis Software (BioRad, Hercules, CA, USA). Data are expressed as normalized absorbance (A).

### 3.5. Statistical analysis

Data are presented as means ± SEM throughout the report. In all experiments, n equals the number of rats from which vessel segments were obtained. Statistical comparisons were performed by two way ANOVA to determine the statistical significance of differences in the data obtained, followed by a post hoc test. In all cases, a p value of less than 0.05 was considered statistically significant. The program used to perform the statistical analysis was Prism 4.0 (Graph Pad Software; San Diego, CA, USA)

## 4. RESULTS

### 4.1. Effect of rosuvastatin lactone on aortic rings precontracted with Phenypherine

As shown in Figure 1, rosuvastatin lactone ($10^{-9}$-$10^{-5}$ M) elicited a concentration-dependent relaxation of the same magnitude in aortic rings with and without endothelium ($CI_{50}$ values were -6.90 M vs. -6.50 M, and maximal relaxations were 98.63 ± 3.96 % vs. 98.48 ± 0.85 % in the presence and absence of endothelium, respectively).

### 4.2. The effect of rosuvastatin and its lactone on precontracted aortic rings

In the presence of endothelium tissue, the effects of rosuvastatin and its lactone derivative were not different ($E_{Max}$ values = 93 %), although the potency of the lactone (log $IC_{50}$ = -6.18 M) was higher than that of rosuvastatin (log $IC_{50}$ = -5.95 M. Figure 2A). On the other hand, in aortic rings without endothelium (Figure 2B) both the maximum effect and the potency were greater for the lactone derivative versus rosuvastatin ($E_{Max}$ values were

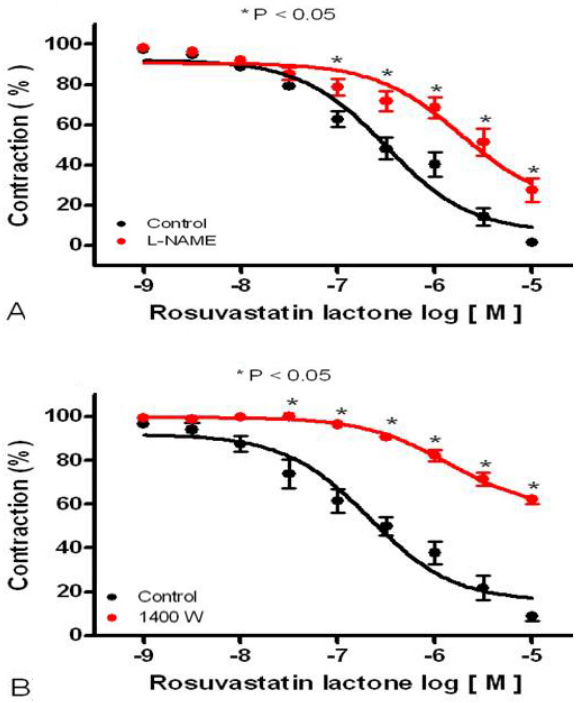


**Figure 3.** Effect of (A) L-NAME (10 µM) or (B) 1400 W (10 µM) on lactone-induced relaxation of rat aortic rings without endothelium precontracted with 1 µM phenylephrine. Data are reported as means ± SEM for N = 6 experiments. *P < 0.01 compared to control (two-way ANOVA).

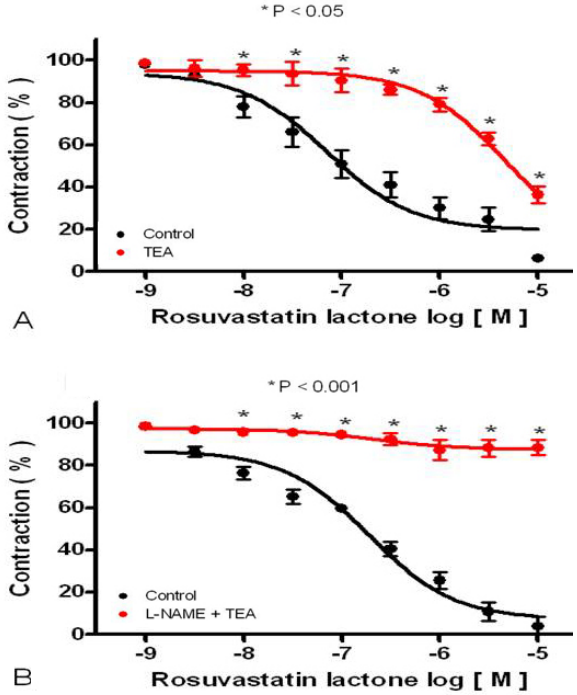


**Figure 4.** Effect of (A) Tetraethylammonium (TEA, 10 mM) or (B) 10 mM TEA plus 10 µM L-NAME on lactone-induced relaxation of rat aortic rings without endothelium precontracted with 1 µM phenylephrine. Data are reported as means ± SEM for N = 6 experiments. *P < 0.001 compared to control (two-way ANOVA).

39.42 ± 3.47 and 98.48 ± 0.85, whereas log $IC_{50}$ were -6.10 and -6.50 for rosuvastatin and its lactone derivative, respectively).

### 4.3. Effects of L-NAME, 1400 W and indomethacin on the relaxant effect of the lactone on denuded rings

As shown in Figure 3, pretreatment with L-NAME (a non-selective NO synthase inhibitor, A) or 1400 W (a selective NOS-2 inhibitor, B) significantly reduced the relaxant effect of the rosuvastatin lactone on aortic rings without endothelium. The maximal relaxant effect was reduced by 98.48 ± 0.84 *vs.* 72.34 ± 5.84 % and 91.20 ± 2.41 *vs.* 37.66 ± 2.17 % in the absence versus presence of L-NAME and 1400 W respectively. Furthermore, aortic rings precontracted with Phen and exposed to a high concentration of the rosuvastatin lactone (10 microM) showed immediate relaxation, which reached its maximum at 20 min and was inhibited in the presence of 1400 W (data not shown). Finally, pretreatment with indomethacin (a prostaglandin synthesis inhibitor) did not modify the relaxant effect of the lactone (data not shown).

### 4.4. Participation of $K^+$ channels in the relaxant effect of the lactone

The effect of the rosuvastatin lactone on denuded aortic rings precontracted with Phen was evaluated in the presence and absence of 10 mM TEA, a $K^+$ channel blocker. As shown in Figure 4A, pretreatment with TEA inhibited the relaxation induced by the statin. The maximal relaxant effect was 93.61 ± 1.25 % without TEA, and 63.54 ± 3.97 % with TEA. Furthermore the combination of L-NAME and TEA almost completely inhibited the relaxant effect of the methyl ester (96.17 ± 0.47 vs. 11.71 ± 3.56, in the absence versus presence of L-NAME+TEA; Figure 4B).

### 4.5. Participation of protein synthesis in the relaxant effect of the lactone

As shown in Figure 5, pretreatment with cycloheximide, a protein synthesis inhibitor, almost completely inhibited the relaxant effect of the lactone of rosuvastatin on denuded aortic rings without endothelium. $E_{Max}$ was 95.64 ± 3.96 % without cycloheximide and 15.99 ± 4.0 % with this inhibitor. On the other hand, cycloheximide did not alter sodium nitroprusside-induced relaxation in endothelium-intact and endothelium-denuded aortic rings (data not shown). Thus, the possibility that cycloheximide might inhibit relaxing agents through a mechanism unrelated to the inhibition of protein synthesis is ruled out.

### 4.6. Participation of HMG CoA reductase in the relaxant effect of the lactone

As shown in Figure 6 pretreatment with mevalonic acid, the product of enzymatic conversion of HMG-CoA, (18) did not modify the rosuvastatin lactone induced relaxation in arteries without endothelium (98.63 ± 0.37 vs. 97.98 ± 0.89, in the absence versus presence of mevalonate).

### 4.7. Immunoblot

NOS-2 protein expression was assayed using specific antibodies against the enzyme. This antibody was

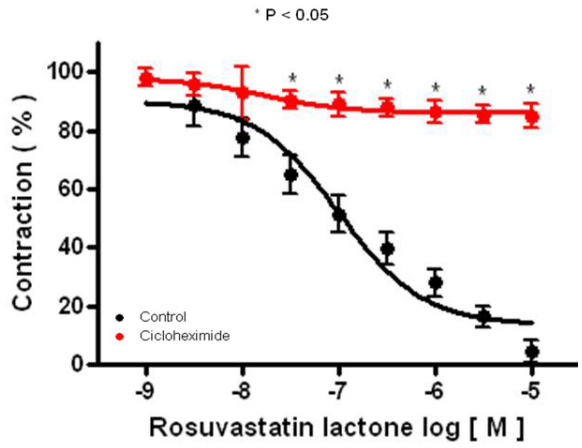


**Figure 5.** Effect of Cycloheximide (10 mM ) on lactone-induced relaxation of rat aortic rings without endothelium precontracted with 1 µM phenylephrine. Data are reported as means ± SEM for N = 6 experiments. *P < 0.001 compared to control (two-way ANOVA).

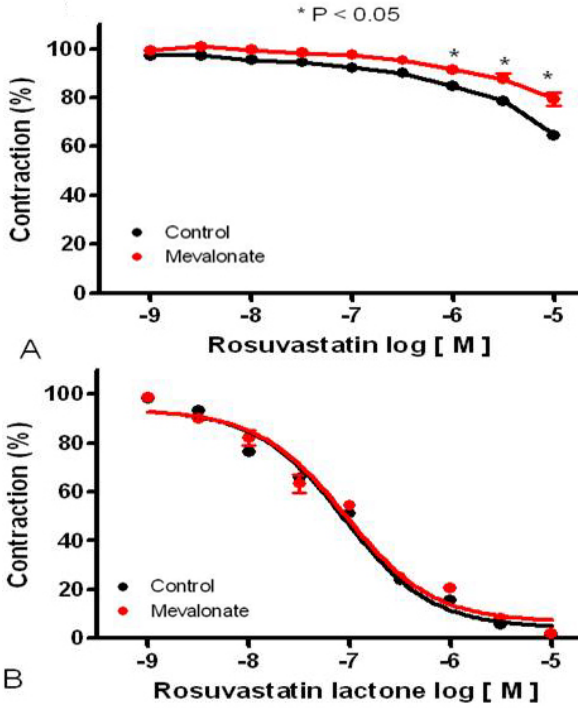


**Figure 6.** Effect of 1 mM mevalonate on (A) rosuvastatin- or lactone (B)-induced relaxation of rat aortic rings without endothelium precontracted with 1 µM phenylephrine. Data are reported as means ± SEM for N = 6 experiments. *P < 0.05 compared to control (two-way ANOVA).

tested to ensure that it did not cross-react with a different enzyme subtype. NOS-2 was expressed in thoracic aortic segments. When Phen was added to both tissues, the relative NOS-2 expression did not change significantly. When the lactone was added, however, NOS-2 expression significantly increased (Figure 7).

## 5. DISCUSSION

The results of the present study clearly show that the lactone of rosuvastatin has an acute concentration-dependent relaxant effect on vascular smooth muscle, in agreement with previous reports on rosuvastatin and several other statins (5-7). In rings with endothelium, the lactone derivative showed greater potency than the parent compound, but the maximum effects were similar (log $EC_{50}$ -6.17 vs. -5.95 and $E_{MAX}$ 93.59 ± 4.62 vs. 88.83 ± 1.99 for the lactone and rosuvastatin, respectively). In rings without endothelium, the lactone derivative showed both greater potency and a greater maximum effect compared to the parent compound (log $EC_{50}$ -6.50 vs. -6.10 and $E_{MAX}$ 98.48 ± vs. 39.42 ± 3.47 for the lactone and rosuvastatin, respectively). These results are related to the interesting finding that the relaxant effect of the lactone is independent of endothelium tissue, while that of rosuvastatin is partially dependent on the presence of this tissue (7,20). Therefore, this structural modification of rosuvastatin seems to be associated with a loss of its effect on the endothelium and an increase in the endothelium independent effect. Overall, the effect of the lactone is not significantly different from the parent compound.

We assessed the possible participation of NO and prostaglandins in the relaxant effect of the lactone derivative, in spite of its endothelium independent effect, as these mediators can be produced outside endothelium tissue (21-22), and that indeed, especially in the case of NO, rosuvastatin induces the production independently of the endothelium layer (7). In this sense, In this sense, we evaluated the participation of NO and prostaglandins by pretreating endothelium denuded rings with L-NAME and indomethacin, respectively. Whereas L-NAME partially but significantly inhibited the relaxant effect, indomethacin had no effect. These results strongly suggest that NO produced by vascular smooth muscle cells is involved in the relaxant effect of the lactone, while prostaglandins apparently play no role.

Because only partial inhibition was obtained with L-NAME, another mechanism in addition to NO production is likely to be involved in the relaxant effect of the lactone of rosuvastatin. Since a previous report by our group demonstrated that rosuvastatin can open $K^+$ channels, we suspected that this could be such a complementary mechanism (7). The results obtained with TEA in denuded aortic rings confirm that $K^+$ channels located in vascular smooth muscle cells are involved in the relaxant effect of the lactone. The complementary or additive role of NO and $K^+$ channels in the effect of this compound is demonstrated by the fact that simultaneous pretreatment with TEA and L-NAME almost completely inhibited the relaxant effect of the lactone derivative.

Because the NOS isoform present in smooth muscle cells is NOS-2 (23, 24), we hypothesized that the L-NAME-sensitive component of the relaxant effect of the lactone may result from the ability of this compound to induce this enzyme. Thus, endothelium-denuded aortic rings were pretreated with either 1400 W (a selective NOS

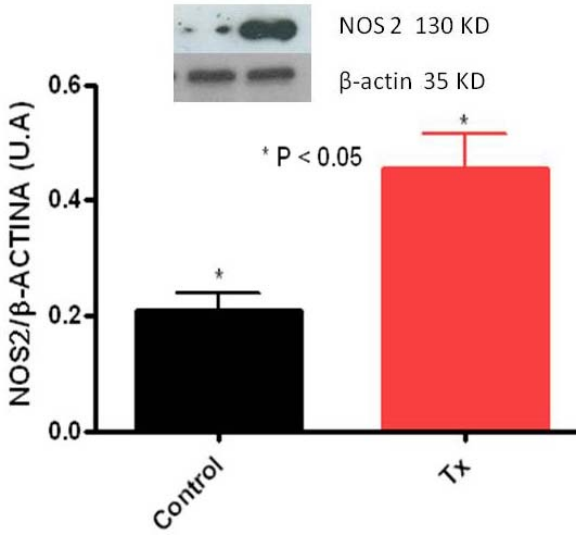


**Figure 7.** iNOS expression in the rat aorta. Data are reported as mean ± SEM for 3-4 rats. AU = arbitrary units; Ctrl = control (only phenylephrine); Tx = treatment (phenylephrine plus rosuvastatin lactone). Insert shows results from a typical experiment. *P < 0.05 compared to control (two-way ANOVA).

2 inhibitor) or cycloheximide (a protein synthesis inhibitor) before administration of the lactone, resulting in a partial inhibition in the former case, and an almost complete inhibition of the relaxant effect in the latter case. This suggests that NO produced by recently synthesized NOS-2 is involved in the effect of the lactone.

On the other hand, the fact that cycloheximide produced an inhibition similar to that obtained for L-NAME and TEA suggests that proteins of recent synthesis participate in the two complementary mechanisms of the relaxant effect of the lactone derivative: that mediated by NO and that involving $K^+$ channels. Providing further evidence for these two mechanisms was the fact that NOS-2 was detected by immunoblot only after lactone pretreatment of the denuded aortic artery. This result also suggests that NOS-2 is located in vascular smooth muscle.

The results of the present study suggest that the lactone induces NOS-2 expression within a shorter time than that proposed by various investigators, who have reported that several hours are required for the expression of iNOS to increase after exposure to an agent that prompts its induction (25, 26). Nevertheless, there is a report (27) of increased iNOS expression within a shorter time period.

The immunoblot results of the present study were obtained in cell culture 3 h after incubation with the first concentration of the lactone of rosuvastatin, enough time for NOS-2 expression according to the generally accepted concept. However, the fact that a relaxant effect was observed quickly and reached its maximum level within 20 min after exposure of aortic rings to a high concentration of the lactone of rosuvastatin, and that this effect was inhibited in the presence of 1400 W, supports the idea that this compound has a relatively rapid inducing effect on NOS-2.

Perhaps it is necessary to reconsider the role of iNOS in the regulation of vascular tone, taking into account that in some cases the participation of this enzyme does not need to be a product of a triggering stimulant related to inflammation.

Accordingly, it has been suggested that the original idea of constitutive synthases be modified: "The paradigm of constitutive NOS and iNOS isoforms has been modified from its original conception: although neuronal NOS (nNOS) and endothelial NOS are constitutively expressed, it is now clear that their activity can be regulated by various factors" (28). Then, iNOS may not always be a product of induction, nor always be associated with pathological conditions.

Results obtained in the presence of mevalonic acid are particularly important because they strongly suggest that the lactone of rosuvastatin has HMG-CoA reductase-independent pleiotropic effects. This contention is supported by the observation that pretreatment with mevalonic acid did not inhibit the endothelium-independent vasodilator effect of the lactone of rosuvastatin.

The present results show that structural modification of rosuvastatin elicited a qualitative but not quantitative change in its relaxant effect on vascular smooth muscle. Whereas this structural modification decreased the endothelium-dependent component, it increased the endothelium-independent component of this effect. Further experiments are needed to clarify the mechanisms involved in these changes, as well as the relationship between these changes and the structural modification of rosuvastatin. On the basis of these results, we suggest that some pleiotropic actions of statins are independent of HMG-CoA reductase.

We conclude that, compared to rosuvastatin, its lactone elicited a qualitative but not quantitative change in the relaxant effect on aortic rings. Whereas rosuvastatin is associated with an endothelium- and hydroxymethylglulatyl coenzyme A reductase-dependent relaxant effect in rat aorta, the lactone of rosuvastatin exhibited an endothelium- and hydroxymethylglutaryl coenzyme A reductase-independent relaxant effect. The relaxant effect of the lactone was mediated by the NO produced by iNOS located in vascular smooth muscle, as well as by $K^+$ channel activation.

## 6. ACKNOWLEDGMENT

This work is supported by grants from Instituto Politecnico Nacional and AstraZeneca SA de CV, Mexico

## 7. REFERENCES

1. Pasterkamp G, van Lammeren GW. Pleiotropic effects of statins in atherosclerotic disease. *Expert Rev Cardiovasc Ther* 9, 1235-7 (2010)

2. Zhou Q, Liao JK. Pleiotropic effects of statins. - Basic research and clinical perspectives. *Circ J,* 5, 818-26 (2010)

3. Kirmizis D, Chatzidimitriou D. Pleiotropic vasoprotective effects of statins: the chicken or the egg? *Drug Des Devel Ther*, 3, 191-204 (2009)

4. Kones R. Rosuvastatin, inflammation, C-reactive protein, JUPITER, and primary prevention of cardiovascular disease--a perspective. *Drug Des Devel Ther,* 4, 383-413 (2010)

5. Alvarez De Sotomayor M, Herrera MD, Marhuenda E, Andriantsitohaina R. Characterization of endothelial factors involved in the vasodilatory effect of simvastatin in aorta and small mesenteric artery of the rat. *Br J Pharmacol*, 131, 1179-1187 (2000)

6. Sonmez Uydes-Dogan B, Topal G, Takir S, Ilkay AF, Kaleli D, Ozdemir O. Relaxant effects of pravastatin, atorvastatin and cerivastatin on isolated rat aortic rings. *Life Sci*, 76, 1771-1786 (2005)

7. Lopez J, Mendoza R, Cleva VG, Martinez G, Castillo EF, Castillo C. Participation of K+ channels in the endothelium-dependent and endothelium-independent components of the relaxant effect of rosuvastatin in rat aortic rings. *J Cardiovasc Pharmacol Ther*, 13, 207-213 (2008)

8. Kosmidou I, Moore JP, Weber M, Searles CD. Statin treatment and 3' polyadenylation of eNOS mRNA. *Arterioscler Thromb Vasc Biol*, 12, 2642-9 (2007)

9. Hattori Y, Nakanishi N, Kasai K. Statin enhances cytokine-mediated induction of nitric oxide synthesis in vascular smooth muscle cells. *Cardiovasc Res, 3,* 649-58 (2002)

10. Vaughan CJ, Delanty N. Neuroprotective properties of statins in cerebral ischemia and stroke. *Stroke*, 30, 1969-1973 (1999)

11. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, *et al.* Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*, 359, 2195-2207 (2008)

12. Tannous M, Cheung R, Vignini A, Mutus B. Atorvastatin increases ecNOS levels in human platelets of hyperlipidemic subjects. *Thromb Haemost*, 82, 1390-1394 (1999)

13. Kostapanos MS, Milionis HJ, Elisaf MS. An overview of the extra-lipid effects of rosuvastatin. *J Cardiovasc Pharmacol Ther*, 13, 157-174 (2008)

14. Meuwese MC, Mooij HL, Nieuwdorp M, van Lith B, Marck R, Vink H, *et al.* Partial recovery of the endothelial glycocalyx upon rosuvastatin therapy in patients with heterozygous familial hypercholesterolemia. *J Lipid Res*, 50, 148-153 (2009)

15. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*, 292, 1160-1164 (2001)

16. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*, 101, 207-213 (2000)

17. Paul D. Martin, PhD, Mike J. Warwick, PhD, Aaron L. Dane, MSc, Steve J. Hill, BSc, Petrina B. Giles, Paul J. Phillips, MRSC, and Eva Lenz, PhD. Metabolism, Excretion, and Pharmacokinetics of Rosuvastatin in Healthy Adult Male Volunteers. *Clin Ther, 10,* 2194-203 (2007)

18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193, 265-275 (1951)

19. Alvarez De Sotomayor M, Herrera MD, Marhuenda E, Andriantsitohaina R. Characterization of endothelial factors involved in the vasodilatory effect of simvastatin in aorta and small mesenteric artery of the rat. *Br J Pharmacol*, 131, 1179-1187 (2000)

20. Schafer A, Fraccarollo D, Vogt C, Flierl U, Hemberger M, Tas P, *et al.* Improved endothelial function and reduced platelet activation by chronic HMG-CoA-reductase inhibition with rosuvastatin in rats with streptozotocin-induced diabetes mellitus. *Biochem Pharmacol*, 73, 1367-1375 (2007)

21. Jones RJ, Jourd'heuil D, Salerno JC, Smith SM, Singer HA. iNOS regulation by calcium/calmodulin-dependent protein kinase II in vascular smooth muscle. *Am J Physiol Heart Circ Physiol*, 292 (6), H2634-42 (2007)

22. Frias MA, Dubouloz F, Rebsamen MC, Lang U. Prostacyclin production in rat aortic smooth muscle cells: role of protein kinase C, phospholipase D and cyclooxygenase-2 expression. Cardiovasc Res, 60 (2), 438-46 (2003)

23. Luoma JS, Stralin P, Marklund SL, Hiltunen TP, Sarkioja T, Yla-Herttuala S. Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic lesions: colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins. *Arterioscler Thromb Vasc Biol*, 18, 157-167 (1998)

24. Muniyappa R, Xu R, Ram JL, Sowers JR. Inhibition of Rho protein stimulates iNOS expression in rat vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol*, 278, H1762-H1768 (2000)

25. Nakata S, Tsutsui M, Shimokawa H, Yamashita T, Tanimoto A, Tasaki H, *et al.* Statin treatment upregulates vascular neuronal nitric oxide synthase through Akt/NF-

kappaB pathway. *Arterioscler Thromb Vasc Biol*, 27, 92-98 (2007)

26. Kosmidou I, Moore JP, Weber M, Searles CD. Statin treatment and 3' polyadenylation of eNOS mRNA. *Arterioscler Thromb Vasc Biol*, 27, 2642-2649 (2007)

27. Chittezhath M, Deep G, Singh RP, Agarwal C, Agarwal R. Silibinin inhibits cytokine-induced signaling cascades and down-regulates inducible nitric oxide synthase in human lung carcinoma A549 cells. *Mol Cancer Ther*, 7, 1817-1826 (2008)

28. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* , 357, 593-615 (2001)

**Abbreviations:** eNOS: endothelial nitric oxide synthase, iNOS: inducible nitric oxide synthase, HMG-CoA: hydroxymethylglutaryl coenzyme A: NMR: nuclear magnetic resonance: Phen, phenylephrine

**Key Words:** Rosuvastatin lactone, Aorta, Endothelium- and HMG-CoA reductase-independent relaxation; Potassium channels; NOS-2

**Send correspondence to:** Carlos Castillo Henkel, Plan de san Luis y Diaz Miron s/n, 11340, Mexico, D.F., Tel: 01555-57-29-63 00 Ext. 62827, Fax: 0155-53-41-27-26, E-mail: drcarloscastillo@hotmail.com

http://www.bioscience.org/current/vol4E.htm