Pravastatin Preserves Vasomotor Response in Atherosclerotic Arteries After Balloon Angioplasty

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Abstract: Background: Vasodilation response to pharmacological challenge is inhibited following balloon angioplasty. Pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, has been demonstrated to enhance endothelial cell production of nitric oxide and reduce low-density lipoprotein cholesterol. This study was conducted to evaluate the effect of pravastatin on vasodilation following balloon angioplasty in normal and atherosclerotic arteries. **Methods:** Three normal and three atherosclerotic New Zealand White rabbits were used. Atherosclerosis was induced by feeding a high cholesterol diet. Rabbits were sacrificed and carotid arteries were isolated and placed in a dual perfusion chamber. Both arteries from each rabbit were perfused with oxygenated physiologic buffered solution at 37^{o}C and 60 mmHg. One artery was exposed to pravastatin (100 μM) and the other served as control. Balloon angioplasty (BA) was performed in both arteries using a 2.5 × 15 mm balloon catheter inflated to 10 atm at 3 different sites for one minute each. Pharmacological challenge was given using acetylcholine (2×10^{-5} M) and sodium nitroprusside (2×10^{-5} M) in norepinephrine (2×10^{-6} M) preconstricted arteries. Vessel diameter was measured by a computer planimetry system. **Results:** After BA in normal rabbit arteries, acetylcholine did not demonstrate significant difference in percent lumen dilation between control and pravastatin (25.5±10.4 vs 16.6±7.5, p=ns) while atherosclerotic arteries had significantly preserved vasomotor response with pravastatin (16.9±7.2 vs 33.6±18.2, p<0.005). Similar results were noted with nitroprusside in normal arteries (29.0± 14.5 vs 18.0±10.5, p=ns) and atherosclerotic arteries (18.6±7.4 vs 38.4±19.8, p<0.003). **Conclusion and Discussion:** Pravastatin preserved vasomotor response in atherosclerotic arteries following BA when compared to normal arteries. This effect may be due to an enhanced production of nitric oxide in atherosclerotic arteries. However, pravastatin also appears to influence vasomotor response by either non-endothelial dependent or a combination of endothelial and non-endothelial dependent mechanism. [The Journal of American Science. 2008;4(1):83-89]. (ISSN: 1545-1003).

Keywords: artery; atherosclerosis; pravastatin; rabbit

Abbreviation: Ach: Acetylcholine; EDTA: Ethylenediamine-tetraacetic acid; G-6-Pase: Glucose 6-phosphatase; HDL: High-density lipoprotein; HMG-CoA: Inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A; LDL: Low-density lipoproteins; NE: Norepinephrine; PBS: Physiologic buffered saline solution; SN: Sodium nitroprusside

Introduction

Hypercholesterolemia is a recognized independent risk factor for coronary heart disease. Drug therapy is recommended for patients whose low-density lipoprotein (LDL) cholesterol concentrations are not adequately lowered by dietary modifications. Statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin and simvastatin) are the most effective agents currently available for lowering plasma levels of LDL cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the synthesis of cholesterol. HMG-CoA reductase is a rate-controlling microsomal enzyme that converts HMG-CoA to mevalonic acid, a precursor of cholesterol. HMG-CoA reductase inhibitors are the primary hypolipidemic drug treatment in most countries and statins are the mainstay of therapy for

hyperlipidemia (Illingworth, 1994). Pharmacological lowering of LDL cholesterol concentration has been shown in several primary and secondary intervention trials to decrease the occurrence of coronary heart disease and to prevent or delay coronary heart disease progression and the regression of atherosclerotic lesions has been demonstrated in some patients (Levy, 1984). Pravastatin is a new HMG-CoA reductase inhibitor for the treatment of hypercholesterolemia, which reduces LDL cholesterol and increases high-density lipoprotein (HDL) cholesterol (Jungnickel, 1992).

Pravastatin is formed by microbial transformation by the *microorganism Nocardia autotrophica*. The structural formula of pravastatin is shown in Figure 1 along with those of lovastatin and simvastatin. The dihydroxyheptanoic acid moiety is the substrate analogue that interacts with the active site of HMG-CoA reductase. The decalin ring interacts with the binding site. Pravastatin sodium is chemically designed as [1S-[1α (βS*, δS*), 2α, 6α, 8β(R*), 8α]]-1,2,6,7,8,8a-hexahydro-β,δ,6-trihydroxy-20methyl-8-(2-methyl-1-oxobutoxy)-1-naphthaleneheptanoic acid, monosodium salt. Its empirical formula is $C_{23}H_{35}NaO_7$ and its molecular weight is 446.52 (Arai, 1988). Pravastatin is a hygroscopic, crystalline powder that is freely soluble in water (>300 mg/ml) and methanol, slightly soluble in isopropanol, and practically insoluble in acetone, acetonitrile, chloroform and ether (Watanabe, 1988). Atherosclerotic plaque disruptions with subsequent arterial thrombosis are critical causes for acute coronary ischemic syndromes. Pharmacological protections of artery are under searching. We suppose that pravastatin will preserve the vasoactivity of the atherosclerotic artery by its affectation of lowering LDL cholesterol levels.

The effects of statin drug therapy on the cardiovascular system extend beyond their anti-hyperlipidemic properties. Many studies showed that statins have a pronounced antioxidant effect as well as well documented endothelial protective effect. Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, a key enzyme in the synthesis of cholesterol. They have been shown to decrease significantly the risk of cardiac events in the setting of primary prevention, secondary prevention or during the acute coronary syndrome (MIRACL trial). Since the effect of statin therapy is so diversified we elected to study the effect of pravastatinon the vasoreactivity of the endothelium following acute physical injury mimicking the current intravascular interventions.

Materials and Methods

Six, male, New Zealand White rabbits (Harlan-Sprague Dawley, Inc., Indianapolis, IN) weighing between 2.8 and 3.2 kg were used in this study. The control group consisted of three normal rabbits that were fed a regular diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN) for six months. To induce atherosclerosis, another three rabbits underwent balloon-induced arterial injury, then were maintained on a 1% cholesterol diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN) for one month followed by another month of alternated regular diet and the two months diet cycle was repeated for three times to keep the rabbits for total of six months. Balloon-induced arterial wall injury of the aorta was performed with a 4F Fogarty Arterial Embolectomy catheter (0.9 × 40 cm, Baxter Healthcare Corporation, Irvine, CA) introduced through the right femoral artery cutdown. The catheter was advanced in a retrograde fashion to the aortic valve and then withdrawn 3 cm. The balloon was inflated with 1.5 cm^3 of air, and the catheter was retracted down to the iliofemoral artery. This was repeated three times in each rabbit as described previously (Abela, 1985). Rabbits were anesthetized with ketamine (50 mg/kg, IM, Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine (20 mg/kg, IM, The Butler Company, Columbus, Ohio) in this surgery process (Abela, 1995).

After intravenous administration of heparin sulfate (1000 IU/rabbit) (Elkins-Sinn, Inc., Cherry Hill, NJ) to prevent postmortem clotting, rabbits were anesthetized by injecting nembutal sodium solution (pentobarbital 50 mg/ml, 1 ml/kg rabbit) (Abbot Laboratories, North Chicago, IL) through a marginal ear vein. Both carotid arteries from each rabbit were removed immediately after the rabbits were sacrificed and were immersed in oxygenated physiologic buffered saline solution (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 22.6 mM, glucose 5.5 mM and Na_2EDTA 0.03 mM) (PBS). Then the arteries were perfused in a dual organ chamber under 60 mmHg flow pressure and 2.5 ml/min flow rate at 37°C and the artery diameter vasodilation was measured instantly. Balloon angioplasty was performed in both arteries using a 2.5 × 15 mm balloon catheter inflated to 10 atm at 3 different sites for one minute each. One artery was served as non-pravastatin control and the other artery was exposed to

pravastatin (100 μM). After norepinephrine (NE, 2 × 10^{-6} M, Sigma Chemical Co., St. Louis, MO) preconstriction, pharmacological challenge was done with acetylcholine (Ach, 1 × 10^{-5} M, Sigma Chemical Co., St. Louis, MO) and sodium nitroprusside (SN, 1 × 10^{-5} M, Sigma Chemical Co., St. Louis, MO) (Table 1).

The data were calculated according to the formulas: (PBS-NE) (%)=(PBS-NE)/NE × 100, (Ach-NE) (%)=(Ach-NE)/NE × 100 and SN-NE (%)=(SN-NE)/NE × 100 separately, where Ach, NE, PBS and SN represented the average diameter (mm) of the arteries that were perfused by the PBS containing a corresponding chemical. Balloon angioplasty was performed in both arteries using a 2.5 × 15 mm balloon catheter inflated to 10 atm at 3 different sites for one minute each. Vessel diameter was measured by a computer planimetry system (Figure 1). Procedures were performed according to Michigan State University's Animal Care and Use Committee approved protocol.

Table 1. Artery Perfusion Step for Diameter Measurement

Treatments	Perfusion Steps	Perfusion Steps - Abbreviation in Figures	Perfusion Time (min)
Cycle 1: Before balloon injury Without pravastatin	Buffer 1	B1	10
	NE 1	N1	10
	Ach 1	A1	10
	SN 1	S1	10
	Buffer 2	B2	10
Cycle 2: Before balloon injury Half of arteries with pravastatin (100 μM)	Buffer 3	B3	10
	NE 2	N2	10
	Ach 2	A2	10
	SN 2	S2	10
	Buffer 4	B4	10
Cycle 3: After balloon injury Half* of arteries with pravastatin (100 μM)	Buffer 5	B5	10
	NE 3	N3	10
	Ach 3	A3	10
	SN 3	S3	10
	Buffer 6	B6	10

* Same arteries as in cycle 2

Statistical analysis: With Jandel Scientific program, SigmaStat (Sigma Chemical Co., St. Louis, MO) was used for data statistical analysis. $P<0.05$ was considered statistically significant difference. Measured data were reported as mean±SD. The student t-test was used for different studies.

Results

From the observation of aorta arteries, all the rabbits were atherosclerosis with the balloon-induced injury and maintained on a 1% cholesterol diet for one month alternatively up to 6 month feeding.

Myocardial infarction in human cases a triggering activity such as physical exertion precipitates the acute onset of the disorder (Mittleman, 1993; Muller, 1989; Tofler, 1990), but it is difficult to be studied in human. Therefore, a suitable animal model is important for the research in this field. This study demonstrated that atherosclerotic rabbit can be induced with balloon induced arterial injury surgery combined with 6 months of alternative 1% cholesterol diet (Abela, 1995). The rabbits which were balloon induced arterial injury and then were maintained in an alternative 1% cholesterol diet for a total of 6 months clearly caught atherosclerosis. This model is a useful method to get atherosclerotic animal for the related scientific research purpose.

In this experiment, the vasoactivity of both normal and atherosclerotic rabbit carotid arteries was

measured using NE preconstriction and pharmacological challenge with Ach and SN (Figures 2, 3, 4). The measurements were performed with the steps of a perfusion cycle as showed in Table 1.

For normal rabbit arteries under PBS perfusion and pharmacological challenged by Ach and SN to NE, there was no significant difference in percent vasodilation between control and pravastatin in both before balloon injury and after balloon injury cycle (25.5±10.4 vs 16.6±7.5 for Ach, p=ns; 4.5 vs 18.0±10.5 for SN, p = ns). However, compared to control, pravastatin demonstrated a significantly greater percent vasodilation on atherosclerotic arteries after balloon angioplasty (16.9±7.2 vs 33.6±18.2 for Ach, p<0.005; 18.6±7.4 vs 38.4±19.8 for SN, p<0.003) (Figure 2, 3, 4).

The vasodilatation range of ratio to NE of PBS and pharmacological challenge with Ach and SN was 30-50% for before balloon injury and 20-40% for after balloon injury. Pravastatin enhances the vasodilation response in atherosclerotic arteries following balloon angioplasty. Pravastatin may influence vasodilation by a combination of endothelial and non-endothelial dependent mechanism.

Pravastatin enhances both endothelium dependant and independent vasoreactivity of carotid arteries in the setting of acute balloon injury. This was true in the presence of atherosclerosis. The mechanism of this effect is unlikely to be due to the lipid lowering property of pravachol. This might be due to its antioxidant effect or to some other unidentified process through a direct endothelial process, or the activation of some receptors or the induction of some signal that ultimately relates to the genomic makeup of the endothelium. Its effect on the NO system is being established and might be part of the puzzle.

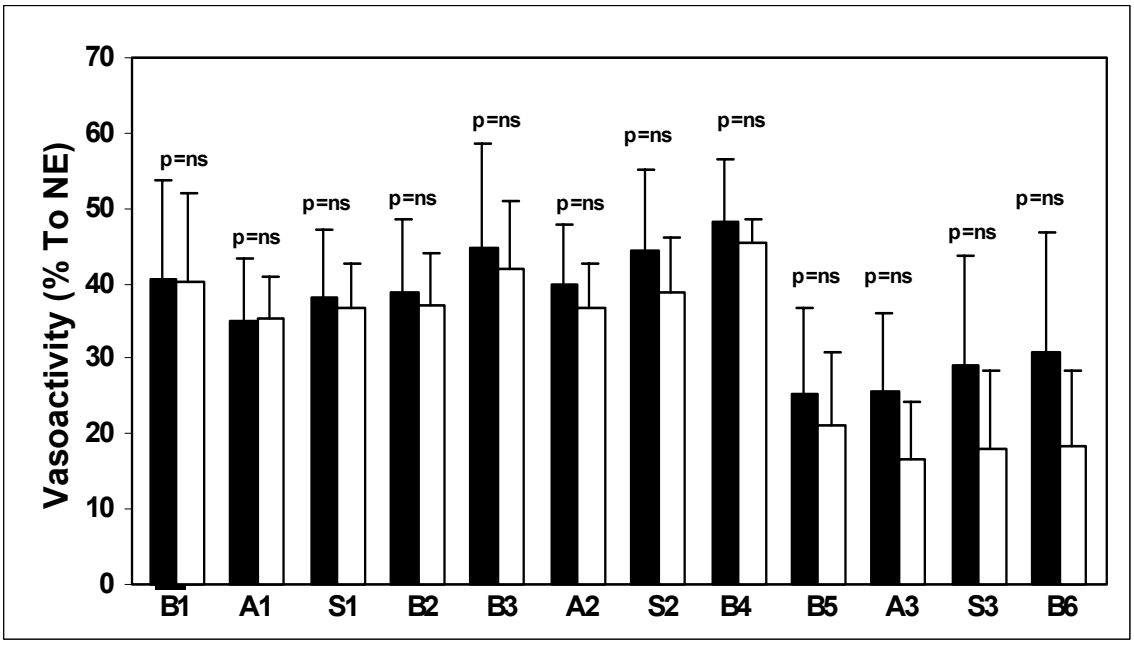

Figure 2. Vasoactivity (% to norepinephrine) of control rabbit.
: Control; : Pravastatin. Letter meanings are shown in Table 1.

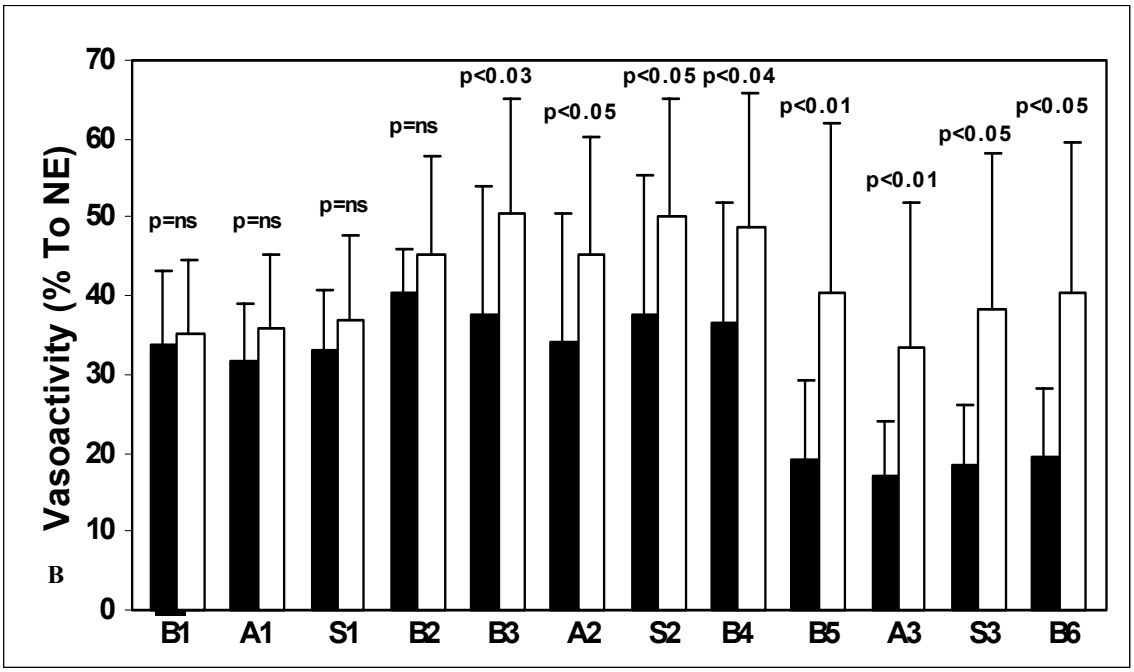

Figure 3. Vasoactivity (% to norepinephrine) of atherosclerotic rabbit.

: Control; : Pravastatin. Letter meanings are shown in Table 1.

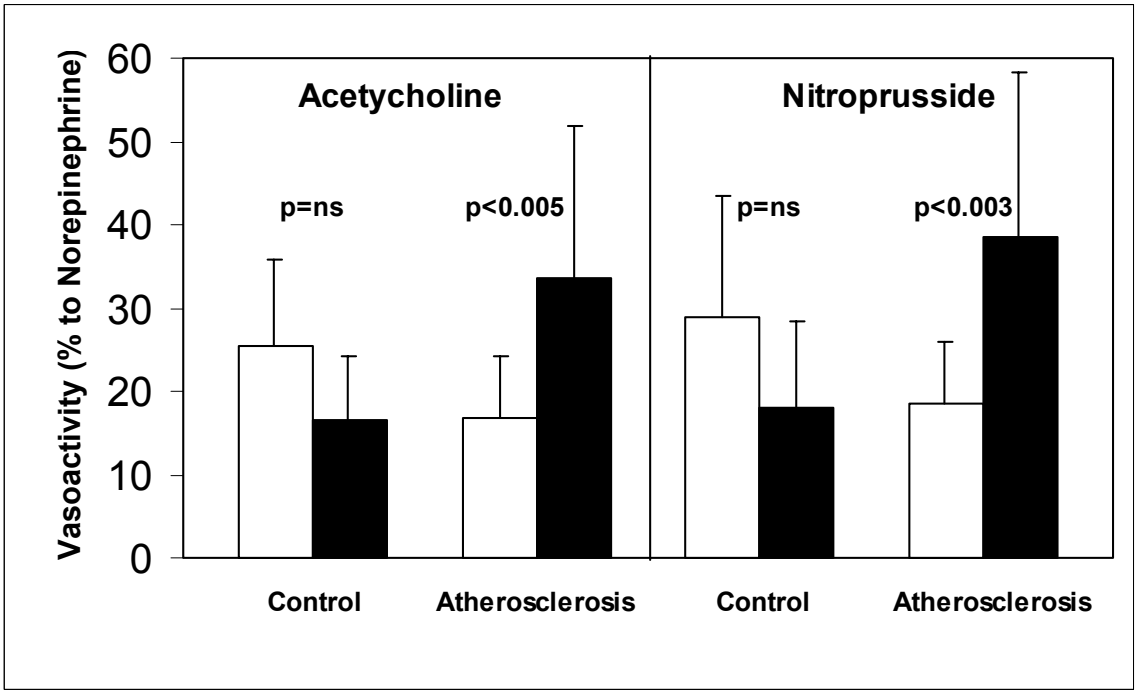

Figure 4. Vasoactivity (% to Norepinephrine) of rabbit carotid balloon injured. Perfused by buffer; Perfused by buffer plus pravastatin.

Discussions

Atherosclerosis, or "hardening of the arteries", is the process that causes heart attacks and most strokes. It is currently believed that cholesterol, especially the LDL, must be modified or oxidized before they can be taken up to cause foam cells. When cells use oxygen for energy, they produce by-products called free radicals. Free radicals damage cells and tissues during a process called oxidation - a factor in many chronic illnesses, including some forms of cancer, cataracts, arthritis and cardiovascular disease. LDL, known as the "bad cholesterol", is actually a protein that carries cholesterol throughout the body. The cholesterol carried by LDL deserves its bad reputation, however. It often ends up in our arteries, causing clots that can lead to heart attacks. Oxidation of LDL-cholesterol contributes to the plaque build-up in arteries, a process called atherosclerosis that can cause blockages and reduced blood flow. The process also plays a role in the loss of elasticity in arteries.

Antioxidants help neutralize free radicals and prevent them from causing cellular damage. Once oxidized, the cholesterol is less apt to be expelled by the body's cleaning mechanisms and more likely to be stored in arteries.

One of the problems for atherosclerotic rabbits is that their free radical and oxidation conditions are changed under the disease. Free radical modification of serum that is not the solely increased level of lipoprotein oxidation products in blood lipoproteins is an important cause for cholesterol accumulation in cells, and apparently for their transformation into foam cells during atherosclerosis.

Once altered by free radical oxidation, plasma lipoproteins undergo dramatic change, both in the manner in which they can interact with cells and in the ways in which they influence cell functions (Chisolm, 1991). Pravastatin preserved vasomotor response in atherosclerotic arteries following BA when compared to normal arteries. This effect may be due to an enhanced production of nitric oxide in atherosclerotic arteries. However, pravastatin also appears to influence vasomotor response by either non-endothelial dependent or a combination of endothelial and non-endothelial dependent mechanism. Pravastatin play protection function on vascular activity may through anti-oxidation.

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