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● *Original Contribution*

EVALUATION OF STATIN THERAPY ON ENDOTHELIAL FUNCTION IN HYPERCHOLESTEROLEMIC RABBITS BY AUTOMATIC MEASUREMENT OF ARTERIAL WALL MOVEMENT USING ULTRASOUND IMAGES

TAVOOS RAHMANI-CHERATI,* MANIJHE MOKHTARI-DIZAJI,* ALIREZA VAJHI,† and ABDORRAZZAGH ROSTAMI†

*Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; and †Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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Abstract—The aim of this study was to evaluate arterial endothelial function, assessed as acetylcholine-mediated dilation (AMD), in a hypercholesterolemic atherosclerotic rabbit model to investigate the effects of atorvastatin in the atherosclerotic process, using a new computerized analysis model and ultrasound images. Twenty-seven rabbits were fed a high-cholesterol (2%) diet for 6 wk and then divided into three groups for an additional 9 wk: Group A received regular chow food, group B received a 2% cholesterol-rich diet plus atorvastatin drug, and group C received regular chow food plus atorvastatin. Ultrasound examinations of endothelial function of the rabbit abdominal aorta artery were performed immediately after the 6 weeks (0 wk) and then 3, 6 and 9 wk after that. For off-line analysis, a computerized analysis method for evaluating instantaneous changes in the wall of the rabbit abdominal aorta was used. As parameters of improvement resulting from treatment, endothelium-dependent acetylcholine-induced dilation and endothelium-independent nitroglycerin-induced dilation were evaluated in treated rabbits. Differences among groups were tested using analysis of variance. On histopathology, intima-media thickness decreased after treatment in all groups. There were no significant differences in arterial diameter and blood velocity changes among treated rabbits at 0, 3, 6 and 9 wk of treatment in all groups, except in end-diastolic velocity, radial strain percentage, pulse index and resistance index in group C. In group A, AMD did not significantly improve after 3, 6 and 9 wk, as compared with 0 wk. Atorvastatin treatment significantly increased AMD (18%) at 3 wk in group B, compared with week 0. AMD significantly increased after 3 (26%), 6 (124%) and 9 (182%) wk in group C, compared with 0 wk. It is concluded that the new automatic method enables accurate and repeated evaluation of endothelial function during the progression and regression of atherosclerosis. Also, the results obtained in this study indicate that short-term administration of atorvastatin can improve endothelial function in cholesterol-fed rabbits. (E-mail: mokhtarm@modares.ac.ir)

Key Words: **Ultrasound images, Endothelial function, Endothelium-dependent vasodilation, Atorvastatin, Motion detection.**

INTRODUCTION

Since the beginning of the 1980s when endothelial function and structure were elucidated, it has been known that a relationship exists between the risk for cardiovascular disease and the vascular endothelium (Furchgott and Zawadski 1980). The most important role of the vascular endothelium is control of vascular tone through the production and release of contracting and relaxing agents (Vane et al. 1990). These agents include nitric oxide (NO) (Lusher and Vanhoutte 1990; Palmer et al. 1987), which is released after stimulation of endothelial cells by blood flow (Vanhoutte et al. 1986), and some agonists such as acetylcholine and serotonin (Lusher and Vanhoutte 1990). But risk factors, such as oxidative stress (Bao et al. 2010; John et al. 2007; Kubota et al. 2010; Reis et al. 2009), hypertension (Gilbert et al. 2008) and hypercholesterolemia (Jarauta et al. 2010), alter the physiologic processes of the endothelium system and thereby damage it. Endothelium-dependent relaxation of the arteries decreases in the early stage of atherosclerosis (Kishimoto et al. 2010; Rundek et al. 2006). Some *in vivo* studies have found endothelial dysfunction in the arteries without angiographically detectable lesions

Address correspondence to: Manijhe Mokhtari-Dizaji, Department of Medical Physics, Tarbiat Modares University, Tehran 14115-331, Iran. E-mail: mokhtarm@modares.ac.ir

in hypercholesterolemic patients (Mano et al. 1996; Weidinger et al. 2002). Endothelial dysfunction is associated with diminished activity of endothelium-dependent hyperpolarizing factor and decreased production of nitric oxide. Therefore, the clinical consequences include increased inflammation, vasoconstriction and development of atherosclerotic lesions (Griendling and Alexander 1997).

Many studies have reported that inhibitors of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase are used extensively to lower serum cholesterol levels and to improve cardiovascular endpoints and coronary stenosis (Masoura et al. 2011). Atorvastatin, a HMG-CoA reductase inhibitor, was very effective in reducing triglyceride and low-density lipoprotein (LDL) cholesterol levels and in raising high-density lipoprotein (HDL) cholesterol (Aydin et al. 2009).

Ultrasonic baseline parameters such as diameter and blood velocity changes play an important role in evaluation of the biomechanical behavior of arteries, but it seems these parameters have little sensitivity at follow-up treatment throughout statin therapy. Therefore, the effect of a reactive hyperemic stimulus on arterial vasodilation was suggested.

Simons et al. (1998) reported that through reactive hyperemia, atorvastatin increases forearm blood flow in hypercholesterolemics. Non-invasive evaluation of endothelial function is an accurate method that involves measuring acetylcholine-mediated changes in the vascular lumen. Under experimental conditions, assessment of NO production is the most useful method for evaluation of endothelial function. Non-invasive testing involves calculating with an ultrasound device the changes induced in the diameter of vessels by acetylcholine administration (Hiss et al. 2006).

In a previous study (Rahmani-Cherati et al. 2011), we described a new automatic analysis method for detection of instantaneous changes in the wall of the rabbit abdominal aorta in sequential B-mode ultrasound images. We reported that this automatic method is accurate and highly reproducible and also that the processing time can be efficiently reduced. Another study (Rahmani-Cherati et al. 2012) reported that this method enables investigation of endothelial function repeatedly and accurately during the progression of atherosclerosis.

Because of the lack of a standardized method for measurement of arterial vasodilation throughout statin therapy, we evaluated arterial vasodilation in response to endothelium-dependent dilation produced by acetylcholine, as well as endothelium-independent nitroglycerin-induced dilation, in the abdominal aortic artery of atherosclerotic rabbits, using ultrasound images and an automated image processing program to detect diameter changes from available ultrasonic frames. To evaluate endothelial function, acetylcholine was used as an endothelial-dependent vasodilator. In this study, the automatic method was used to evaluate the effects of atorvastatin therapy on endothelial function of the abdominal aorta in hypercholesterolemic rabbits. Thus, acetylcholine-mediated dilation (AMD) and nitroglycerin-mediated dilation (NMD) were measured in sequential ultrasound images during infusion of acetylcholine or nitroglycerin infusion into the abdominal aorta of healthy and atherosclerotic rabbits. The findings from the ultrasound study were comparable to the results of histologic evaluation.

METHODS

A total of 27 male New Zealand White rabbits (2.5–3.0 kg) were purchased from the Pasteur Institute of Iran (Tehran, Iran) and entered into this study after 2 wk of adaptation to housing facilities. All animals were handled in accordance with the guidelines of the Universities Federation for Animal Welfare (Teicher 2002). All animal experiments and protocols were evaluated and approved by the Animal and Ethics Review Committee of Tarbiat Modares University (Tehran, Iran). Rabbits were individually housed in metal cages in an air-conditioned room (22 ± 1°C). All rabbits were fed a high-cholesterol (2%) diet (Narayanaswamy et al. 2000; Rahmani-Cherati et al. 2012; Schaar et al. 2005; Wang et al. 2006) for 6 wk, at which point (0 wk) they were divided in three equal groups for an additional 9 wk: Group A received regular chow food, group B received a 2% cholesterol diet plus atorvastatin administered orally at 10 mg/d and group C received regular chow food plus 10 mg/d atorvastatin.

Ultrasound examination of endothelial function of the rabbit aorta was performed immediately after the 6 wk of a high-cholesterol diet (0 wk) and 3, 6 and 9 wk after that. To reduce movement and gas artifact in the intestines, all rabbits were maintained off feeds for 12 h. The rabbits were anesthetized with 50 mg/kg ketamine and 5 mg/kg xylazine (Alfasan, Woerden, The Netherlands) by intramuscular injection. The abdomen was shaved, and the animal was placed in dorsal decubitus. Marginal ear vein were cannulated for drug infusions. All animals underwent color Doppler ultrasonography (Voluson 730 Pro, GE Medical Systems Kretztechnik, Tiefenbach, Austria) with a 6- to 12-MHz linear array transducer. Instantaneous changes in the diameter of the abdominal aorta were obtained using sequential B-mode images. Peak systolic, end-diastolic and mean diameters of the abdominal aortic artery were extracted, and relative change in diameter (radial strain percentage) was calculated. The ultrasound probe was placed at the abdominal aorta, 1.5 cm below the renal artery. Flow