Ultrasound in Med. & Biol., Vol. 38, No. 8, pp. 1391–1403, 2012

0301-5629/$ - see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2012.03.008

● *Original Contribution*

ULTRASONOGRAPHIC ANALYSIS *VERSUS* HISTOPATHOLOGIC EVALUATION OF CAROTID ADVANCED ATHEROSCLEROTIC STENOSIS IN AN EXPERIMENTAL RABBIT MODEL

HOSSEIN MEHRAD,* MANIJHE MOKHTARI-DIZAJI,* HOSSEIN GHANAATI,†
AMIR-ALI SHAHBAZFAR,‡ and MOJDEH SALEHNIA§

*Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; †Department of Radiology, Imam Khomeini Hospital, University of Tehran Medical Sciences, Tehran, Iran; ‡Department of Pathobiology, Faculty of Veterinary, University of Tabriz, Tabriz, Iran; and §Department of Histology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

(*Received* 24 *June* 2011; *revised* 6 *March* 2012; *in final form* 8 *March* 2012)

Abstract—Advanced carotid atherosclerosis with severe stenosis (>70%) is a major clinical risk factor for ischemic stroke. Our ability to test new protocols for the treatment of atherosclerotic stenosis in humans is limited for obvious ethical reasons; therefore, a suitable animal model is required. The aim of this study was to generate an easily reproducible and inexpensive experimental rabbit carotid model of advanced atherosclerosis with morphological similarities to the human disease and the subsequent assessment of the reliability of B-mode ultrasound technology in the study of lumen area stenosis in this model. Briefly, New Zealand white rabbits underwent primary perivascular cold injury at the right common carotid artery followed by a 1.5% cholesterol-rich diet injury for eight weeks. All of the rabbits' arteries were imaged by B-mode ultrasound weekly, after which the rabbits were sacrificed, and their vessels were processed for histopathology. Ultrasound longitudinal view images from three cardiac cycles were processed by a new computerized analyzing method based on dynamic programming and maximum gradient algorithm for measurement of instantaneous changes in arterial wall thickness and lumen diameter in sequential ultrasound images. Histopathology results showed progressive changes, from the lipid-laden cells and fibrous connective tissue proliferation in neointimal layer, up to the fibro-lipid plaque formation, resulting in vessel wall thickening, remodeling and lumen stenosis. The B-mode ultrasound images and the histologic measurements showed an increase in the mean wall thickness and the lumen area stenosis within eight weeks. Quantitative and morphometric analysis of the mean wall thickness and the lumen area stenosis percentage showed a significant correlation between the B-mode ultrasound and the histological measurements at each time point ($R = 0.989$ and $R = 0.995$, $p < 0.05$, respectively). In conclusion, we successfully produced advanced atherosclerosis in the rabbit carotid artery that is similar to the condition seen in patients. This condition in rabbits can be properly assessed by B-mode ultrasound image processing. (E-mail: mokhtarm@modares.ac.ir) © **2012 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Histopathology, Stenosis, Carotid artery, Rabbit model, Cold injury.**

INTRODUCTION

Atherosclerosis is a general process that affects the entire arterial system. Atherosclerotic lesions of the common carotid artery have been described as a marker of the total atherosclerotic burden, and the degree of common carotid stenosis is associated with the severity of coronary and femoral artery stenosis and the clinical outcome of cardiovascular events (O'Leary et al. 1999; Molnár et al. 2009). Atherosclerotic lesions are considered to be advanced when the accumulation of lipid, cells and matrix is associated with the disorganization, repair, thickening and deformity of the arterial wall (Stary et al. 1995).

Advanced carotid atherosclerosis with severe stenosis is a major risk factor for ischemic stroke. The condition can be stable and asymptomatic or it can be a source of embolization. Clinically, the risk of stroke from carotid stenosis is evaluated by the presence or absence of symptoms and the degree of stenosis as seen with imaging techniques (Bakoyiannis et al. 2010; Knur 2009). Stenosis of the human internal carotid artery may be the cause of 10–20% of all ischemic strokes. In

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

symptomatic patients, carotid revascularization is indicated in 50% of stenoses. In asymptomatic patients, the indication for revascularization, based on randomized trials, is ≥60% stenosis, provided the estimated preoperative death or stroke risk is <3%. However, in clinical practice, asymptomatic stenosis is usually treated only if luminal narrowing exceeds 70–80%. The choice of revascularization strategies includes endarterectomy or balloon angioplasty, and the method used for stenting should be based on the surgical risk profile of the patient and on the locally available expertise. Carotid artery stenting is particularly beneficial for patients who are at high risk for surgery (Roffi et al. 2009).

Direct investigation of advanced atherosclerosis in human carotid arteries is by nature difficult to do. As a result, animal models offer an alternative that can replicate the physiopathology aspects of atherosclerotic stenosis while allowing for controllable variables and statistical data in a short period of time (Ma et al. 2008). Therefore, an appropriate animal model could prove critical to the research and development of new diagnostic and therapeutic modalities.

Various animal models of atherosclerosis have been developed for experimental investigations and testing of new therapeutic protocols and diagnostic devices. Several characteristics of the rabbit make it an excellent model for the assessment of atherosclerosis. Cholesterol has been known to cause atherosclerotic changes in rabbit arteries that are very similar to human atherosclerosis (Yanni 2004; Brousseau et al. 1999). In rabbits, lesion morphology is altered by the percentage of cholesterol added to the diet and the duration of the diet. Diets with a percentage of cholesterol of >1% cause hypercholesterolemia, and atherosclerotic lesions rich in foam cells originate from macrophages (Finking et al. 1997; Kolodgie et al. 1996).

Several models of arterial injury have been used to rapidly induce atherosclerosis in the arteries of rabbits fed high-cholesterol diets. These can be classified as models that use an intravascular approach such as balloon catheters (Phinikaridou et al. 2009; Shimizu et al. 2009), cold gas injection (Fang et al. 2009) or models that use a perivascular approach such as electric currents (Chiesa et al. 2001) and ligation (Zhang et al. 2010).

To investigate atherosclerosis in the rabbits, intravascular ultrasound (IVUS) and angiography (Chen et al. 2007; Chiesa et al. 2001, 2004), magnetic resonance imaging (MRI) (Ma et al. 2008; Chiesa et al. 2004; Helft et al. 2001), positron emisssion tomography/computed tomography (Hyafil et al. 2009) and optical coherence tomography (Zimarino et al. 2007) diagnostic systems can be used; however, histopathological evaluation remains the gold standard. The degree of carotid stenosis is often determined with the diameter method (lumen diameter reduction) by B-mode ultrasonography and angiography in longitudinal views of the artery (Schulte-Altedorneburg et al. 2001), but measurement of carotid stenosis with the area method (lumen area reduction) is often determined with 3-D ultrasound (Yao et al. 1998), IVUS and MRI technology (Chiesa et al. 2004; Ma et al. 2008; Yan et al. 2009) in cross-sectional views of the artery.

IVUS and angiography are invasive methods, thus the ability to use these techniques to make repeated measurements is limited, even in animal studies. MRI is very powerful but is very expensive and difficult to use in small animals. In human carotid studies, noninvasive and inexpensive B-mode ultrasound has been used to assess physical measurements of the arterial wall, and this technique can be used to perform repeated measurement of the arterial walls. However, most researchers have used the manual tracing method to evaluate arterial properties (Baldassarre et al. 1994; Bots et al. 1993), which is unreliable because it is based on subjective operator assessment and is time consuming. Using automated algorithms increases the reproducibility of the measurements and reduces the time needed for image processing (Rahmani-Cherati et al. 2011).

In this study, we generated a rabbit carotid model of advanced atherosclerosis with severe stenosis (>70%) and evaluated the reliability of noninvasive B-mode ultrasonography based on image processing in the extraction of mean wall thickness and lumen area stenosis parameters of carotid advanced atherosclerotic stenosis.

MATERIALS AND METHODS

Experimental design

Forty-two healthy male New Zealand white rabbits weighing 2.5–3.0 kg were used in this study. All animals were handled in accordance with the guidelines of the Universities Federation for Animal Welfare (UFAW) (Teicher 2002). All animal experiments and protocols were evaluated and approved by the Animal and Ethics Review Committee of the Tarbiat Modares University (Tehran, Iran). Rabbits were divided randomly into a healthy group (A) in which the rabbits consumed a standard chow diet (n = 14), and an injured group (B) in which the rabbits underwent perivascular cold injury to the right common carotid artery and then consumed an atherogenic diet (chow supplemented with 1.5% cholesterol) for eight weeks. For surgery, rabbits were anesthetized by an intramuscular injection of xylazine (5 mg/kg), ketamine (35 mg/kg) and acepromazine (0.75 mg/kg). The animals' necks were shaved, a midline skin incision was made and the right common carotid artery was surgically exposed and dissected from the surrounding tissue. After surgical exposure, approximately 1 cm of