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Arterial wall remodeling under sustained axial twisting in rats

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ABSTRACT

Blood vessels often experience torsion along their axes and it is essential to understand their biological responses and wall remodeling under torsion. To this end, a rat model was developed to investigate the arterial wall remodeling under sustained axial twisting *in vivo*. Rat carotid arteries were twisted at 180° along the longitudinal axis through a surgical procedure and maintained for different durations up to 4 weeks. The wall remodeling in these twisted arteries was examined using histology, immunohistochemistry and fluorescent microscopy. Our data showed that arteries remodeled under twisting in a time-dependent manner during the 4 weeks post-surgery. Cell proliferation, MMP-2 and MMP-9 expressions, medial wall thickness and lumen diameter increased while collagen to elastin ratio decreased. The size and number of internal elastic lamina fenestrae increased with elongated shapes, while the endothelial cells elongated and aligned towards the blood flow direction gradually. These results demonstrated that sustained axial twisting results in artery remodeling *in vivo*. The rat carotid artery twisting model is an effective *in vivo* model for studying arterial wall remodeling under long-term torsion. These results enrich our understanding of vascular biology and arterial wall remodeling under mechanical stresses.

1. Introduction

Arteries *in vivo* are subjected to complex mechanical loads due to lumen blood flow, pressure, surrounding tissue tethering, and body movement. It is well documented that alterations in lumen flow, pressure and axial stretch lead to arterial wall remodeling (Ku, 1997; Han et al., 2003; Lawrence and Gooch, 2009; Lee et al., 2010; Chiu and Chien, 2011; Hayman et al., 2012; Bell et al., 2016). The remodeling is characterized by structural and cellular changes such as increases in lumen size and wall thickness, extracellular matrix (ECM) deposition, cell proliferation, and matrix metalloproteinase (MMP) expressions, as well as the adaptation of endothelial cell (EC) shape and alignment (Langille, 1996; Ku, 1997; Nerem et al., 1998; Han et al., 2003; Gleason et al., 2004; Lee et al., 2008; Kim et al., 2009; Chiu and Chien, 2011).

Arteries also often experience axial twisting due to body movement or surgical procedures (Barton and Margolis, 1975; Pao et al., 1992; Han et al., 1998; Norris et al., 2000; Dobrin et al., 2001; Ding et al., 2002; Selvaggi et al., 2004). Mobile arteries within the torso and lower extremities such as the iliac, superficial femoral, and femoropopliteal are subjected to torsion with hip and knee flexion (Cheng et al., 2006; Choi et al., 2009; Klein et al., 2009). Sustained twisting occurs in arteries *in vivo* due to pathological changes or vascular surgery procedures (Salgarello et al., 2001; Kalish et al., 2003; Topalan et al., 2003; Wong et al., 2007). It also occurs in perforator-based propeller flap procedures for skin grafting in which a skin island, still connected to its' perforating artery and vein, is elevated and rotated like a helicopter propeller up to 180° using the perforating vessels as a pivot point (Jakubietz et al., 2007; Chang et al., 2009). However, little is known about arterial wall remodeling induced by axial twisting though it is known that torsion alters the arterial wall stress (Humphrey, 2002; Garcia et al., 2013).

Severe twisting of arteries and veins can affect their patency, impair endothelium function and delay wound healing in the anastomosis area, and lead to distal ischemia (Barton and Margolis, 1975; Endean et al., 1989; Izquierdo et al., 1998; Topalan et al., 2003; Selvaggi et al., 2004; Garcia et al., 2017). These changes can cause increased risks for thrombosis and organ dysfunction (Endean et al., 1989; Bilgin et al., 2003; Selvaggi et al., 2004;

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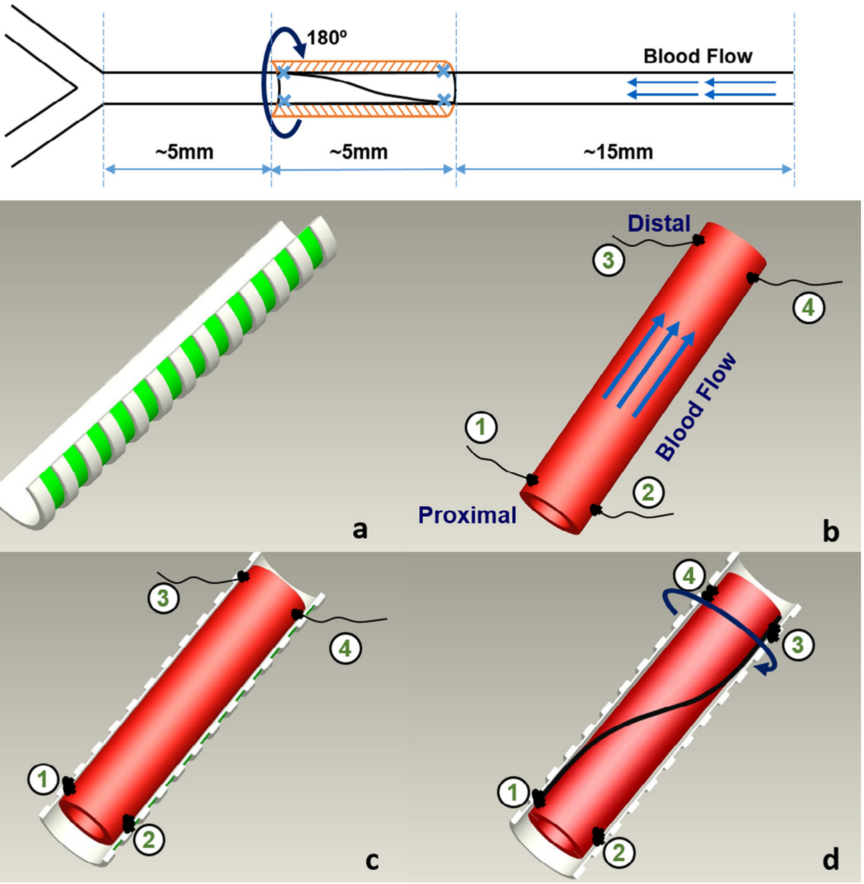

Fig. 1. Schematics illustrating the twisting of rat carotid artery *in vivo*. Top: location and dimension of the twisting segment. Bottom: surgical procedure: (a) rigid semi-circular tubular sheath used to support twisting; (b) artery segment selected with 4 sutures attached to adventitia (one at each side of the vessel at the proximal and distal ends, respectively; (c) Two sutures (① and ②) at the proximal end were sewn onto the sheath. (d) The two sutures (③ and ④) at the distal end were first pulled to swap position to rotate (twist) the vessel 180° along its axis, and then sewn onto the sheath to hold the twist in the artery segment.

Table 1
Experimental design: summary of measurements in 5 experiment sets and groups in each set for different time points with sample size in each group.

Set	Measurement	Group sample size[a]			
		0 Day	3 Days	1 Week	4 Weeks
I	H&E (Wall thickness) Verhoeff (Elastin) Van Gieson (Collagen)	–	6	6	6
II	BrdU (Cell proliferation)	–	6	6	6
III	Confocal (IEL fenestrae) Confocal (SMC nuclei)	–	5	5	5
IV	Silver Stain (EC morphology)	5	5	5	5
V	Western blotting (MMP)	–	7	7	7

[a] Sample size in each control group (sham operation) is the same as the corresponding experimental group.

Chesnutt and Han, 2011). It has been reported that cervical artery is extremely vulnerable to torsion injury that can lead to dissection and stroke (Norris et al., 2000). In order to better understand the artery functional change and augment vascular healing, it is essential to understand the mechanical behavior and biological responses as well as the adaptive remodeling of arteries under sustained axial twisting (Deng et al., 1994; Lu et al., 2003; Van Epps and Vorp, 2008; Garcia et al., 2013; Han et al., 2013).

Previously, we developed an *ex vivo* porcine carotid artery twisting model (Wang et al., 2015). It was shown that arterial wall remodels under axial twisting as demonstrated by elevated cell proliferation and MMP expression, changes in EC shape and orientation, as well as internal elastic lamina (IEL) fenestrae shape in 3 days under axial twisting. However, there has been no long-term study reported partially due to the limited duration of arteries in the organ culture model. Therefore, it is necessary to develop an animal model to investigate the long-term arterial remodeling under axial twisting.

Accordingly, the goal of this study was to investigate arterial wall remodeling under sustained axial twisting. A rat carotid artery twisting model was developed and the resulting arterial wall remodeling was investigated for up to 4 weeks.

2. Materials and methods

2.1. Animal

Male Sprague-Dawley rats, 9–10 weeks old, body weight 280–300 g, purchased from the SLAC Laboratory Animal Center were used in this study. The rats were randomly divided into experimental (twisting) and control (no-twisting) groups. The

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