The Protective Role of Pentoxifylline on Diabetes-Induced Vasculopathy in Male Albino Rats

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Sarah Abdelmonem Arakib

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LIST OF ABBREVIATIONS

Abb	
AGER	Advanced Glycation End Product Receptors
AGEs	Advanced Glycation End Products
DM	Diabetes Mellitus
H&E	Hematoxylin And Eosin.
ICAM	Intercellular Adhesion Molecule
IDF	International Diabetes Federation
IL1	Interleukin 1
IL6	Interleukin 6
LDL	Low Density Lipoprotein
NF-κB	Nuclear Factor Kappa B
No	Nitric Oxide
$(OH)^-$	Hydroxyl Group
PDGF	Platelet Derived Growth Factor
PKC	Protein Kinase C
PTX	Pentoxifylline
ROS	Reactive Oxygen Species
SMCs	Smooth Muscle Cells
SO	Superoxide
STZ	Streptozotocin
TNF-α	Tumor Necrosis Factor Alpha
VCAM	Vascular Cellular Adhesion Molecule
α-SMA	Alpha Smooth Muscle Actin

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease that tends to spread throughout the world. Recent reviews showed that about 382 million people were found to be diabetic,with expected rise to 592 million by 2035 ***(Forouhi and Wareham, 2014).***

The disease leads to development of multisystem complications which contribute greatly to high rate of morbidity and mortality ***(Forouhi and Wareham, 2014).*** One of these serious complications is development of macro- and microvascular changes ***(Chen et al., 2012),*** including vascular retinopathy, nephropathy and neuropathy. Also macrovascular complications may have a role in ischemic heart disease, cerebrovascular disease and peripheral vascular disease ***(Cade, 2008).***

The pathophysiological effects of diabetes on blood vessels include impaired vasodilatation due to decrease nitric oxide (NO), smooth muscle cells (SMCs) proliferation, chronic inflammation, atherosclerosis, hemodynamic dysregulation, increased platelet aggregation and impaired fibrinolytic ability ***(Rahman et al., 2007 and Cade, 2008).***

The underlying mechanisms of these vascular complications are the oxidative stress and overproduction of reactive oxygen species (ROS) ***(Reza et al., 2015).***

Overproduction of ROS including O_2^- and H_2O_2, formation of advanced glycation end products (AGEs), and Lipid peroxidation have been implicated for vascular dysfunction in diabetes ***(Reza et al., 2015).***

Pentoxifylline (PTX) is a synthetic methylxanthine, prescribed for cerebrovascular and peripheral vascular diseases. PTX improves the effectiveness of microcirculation, decreases platelet aggregation and lowers plasma viscosity **(*Zhang et al., 2004).*** It also inhibits the phosphodiesterase and can cause vasodilatation of blood vessels by endothelium-dependent and independent mechanisms ***(Kabbesh et al., 2011).***

Pentoxifylline decreases the oxidative stress and inhibits lipid peroxidation ***(El-lakkany et al., 2011).*** It also reduces the adhesion of the neutrophils to endothelial cells and lowers the production of free radicals ***(Barbara et al., 2017)***.

Aim of the Work

The present study is designed to:

- Evaluate the possible protective effect of pentoxifylline on diabetic vasculopathy induced in rat model.
- Demonstrate the mechanism of action of pentoxifylline.

Review of literature

Normal Structure of Aorta and Renal Artery

I- Aorta:

A- Anatomy of Human Aorta:

The human aorta is the largest artery, which gives many branches carrying the oxygenated blood to different tissues for their nutrition. It arises from the left ventricle, ascends for a short distance, after that it arches backwards and to the left side, and then descends within the thorax and abdominal cavity. It ends by dividing into right and left common iliac arteries. Hence it is described in several positions; Ascending aorta, Arch of aorta, and Descending aorta which is divided into thoracic and abdominal parts ***(Buja and Butany, 2016*)**.

The ascending aorta, about 5cm long, begins at the upper part of the base of left ventricle, at the level of lower border of left third costal cartilage, then passes obliquely upward, forward and to the right to end at the level of the right second costal cartilage by becoming arch of aorta. It lies within the fibrous pericardium where it is enclosed within a tube of serosal sheath together with the pulmonary trunk. The branches of ascending aorta are the right and left coronary arteries which supply the heart ***(Anderson, 2000).***

Arch of aorta begins at the level of the sternal angle, arches backwards and to the left, over the left bronchus, to reach the body of fourth thoracic vertebra. The great arteries of head, neck and upper limbs arise from its upper convexity in order; the brachiocephalic trunk, the left common carotid and left subclavian arteries ***(Standring, 2016).***

Descending thoracic aorta begins at the lower border of fourth thoracic vertebra. At first, it is present to the left of the midline, then passes gradually to the midline and leaves the posterior mediastinum at the level of twelfth thoracic vertebra by passing behind median arcuate ligament. It gives nine pairs of posterior intercostal arteries, a pair of subcostal arteries, bronchial, esophageal, pericardial and phrenic branches ***(Chummy, 2011).***

The abdominal aorta begins at the aortic opening of the diaphragm, infront of the twelfth thoracic vertebra. It descends behind peritoneum on the anterior surface of the bodies of the lumbar vertebrae and ends at the level of fourth lumbar vertebra by dividing into two common iliac arteries ***(Standring, 2016).***

On the right, the abdominal aorta is related above to the cisterna chyli, thoracic duct, the azygos vein and the right crus of the diaphragm which separates it from the inferior vena cava. Below the second lumbar vertebra, it is related directly to the inferior vena cava ***(Standring, 2016).***

On the left, abdominal aorta is related above to the left crus of the diaphragm and left coeliac ganglion. At the level of second lumbar vertebra, it is related to the duodeno-jejunal flexure and left sympathetic trunk ***(Standring, 2016).***

There are four main groups of branches rise from abdominal aorta; single visceral arteries arise from its ventral aspect which supply the gut and its derivatives (celiac artery for foregut, superior mesenteric artery for midgut and inferior mesenteric artery for hindgut), paired lateral visceral branches (suprarenal, renal and gonadal arteries), paired lateral branches to the anterior abdominal wall (inferior phrenic arteries and lumbar arteries), and three terminal branches (common iliac arteries and median sacral artery) ***(Chummy, 2011).***

B- Histology of Human Aorta:

Regarding the histological structure of the human aorta, It is considered as a large elastic artery and it is composed of three layers: tunica intima (the innermost layer), tunica media (the middle layer), and tunica adventitia (the outer layer) ***(Jungueria et al., 2005).***

The tunica intima is made of an endothelial layer which is formed of single layer of squamous endothelial cells with flattened nuclei, subendothelial connective tissue layer, and internal elastic lamina which separates the tunica intima from the

tunica media. The role of the tunica intima is to provide a smooth non thrombogenic surface for blood flow ***(Hutchison, 2009).***

The tunica media is the thickest layer in the aortic wall. It is made of circularly arranged SMCs and collagen sheets seperated by multiple layers of circularly arranged elastic lamellae. The last elastic lammella is called external elastic lamina and it separates the tunica media from the tunica adventitia ***(Collins et al., 2014).***

The numerus elastic lamellae have a great role in keeping the blood flow more uniform and maintaining the normal arterial blood preasure. During systole, the pumped blood stretches the elastic lamellae and distends the arterial wall within limit, the present collagen fibers prevent the harmful over distension. During diastole, the arterial preasure is manitained by the rebound action of elastic fibers ***(Jungueria et al., 2005).***

The tunica adventitia of aorta is thin and consists of dense irregular connective tissue which is formed mainly by collagen fibers and fibroblasts. It provides support to the aortic wall and anchores it to adjacent tissues ***(Buja and Butany, 2016*)**.

The arterial wall takes its blood supply by diffusion from the lumen which supplies the inner part while the outer part is supplied by small blood vessels called vasa vasorum lie in the

tunica adventitia and outer part of tunica media ***(Buja and Butany, 2016*)**.

C- Anatomy and Histology of Rat Aorta:

The anatomy of cardiovascular systems in both human and rat are less or more similar ***(Suckow et al., 2006)***.

The histological structure of the rat aorta has the similar picture to that of human aorta and is composed also of the three characteristic layers: tunica intima, tunica media and tunica adventitia ***(Berry et al., 1972).***

The tunica intima is formed of endothelial layer seperated from the internal elastic lamina by subendothelial layer, which is very small and can be seen only by electron microscope ***(Berry et al., 1972).*** Intimal folds may be present in rat aorta but less prominent in the abdominal segment ***(Mello et al., 2004).***

The tunica media is formed of interrlated elastic lamellae, collagen fibers and smooth muscle cells. There are no blood vessels or nerves in the tunica media of rat aorta ***(Mello et al., 2004).***

The tunica adventitia consists of densely packed bundles of collagen irregularly arranged. Numerous fibroblasts, blood vessels, nerves, few elastic fibers and occasional SMCs are also found ***(Mary, 1978).***

II- Renal Artery:

A- Anatomy of Human Renal Artery:

Renal arteries are considered the largest lateral branches of abdominal aorta ***(Standring, 2008),*** which arise at the level of the intervertebral disc between the first and second lumbar vertebrae ***(Moore et al., 2014).*** Their origin lies one cm below the origin of the superior mesenteric artery ***(Koplay et al., 2010).*** They run laterally and slightly downwards almost at right angles to the aorta towards the hila of the kidneys crossing the corresponding crus of the diaphragm ***(Dăescu et al., 2012).***

The left renal artery usually arises a little higher than the right renal artery due to higher position of the left kidney. It lies behind the left renal vein, the body of the pancreas and the splenic vein ***(Standring, 2008).***

On the other hand, the right renal artery has a long downward course. It passes behind the inferior vena cava, right renal vein, head of the pancreas and the second part of the duodenum ***(Kumar et al., 2010).***

Near the hilum of the kidney each renal artery divides into anterior and posterior divisions in relation to the renal pelvis, each division also divides into a number of segmental branches, then lobar, interlobular and finally afferent and efferent glomerular arterioles ***(Budhiraja et al., 2011).***

B- Histology of Human Renal Artery:

Renal artery is a medium sized artery and is considered of muscular type ***(Nowrazani, 2016).***

The histological structure of the wall of the renal artery is formed of three layers as any blood vessel: tunica intima, tunica media and tunica adventitia. The intimal internal elastic lamina is prominent and the tunica media is formed of SMCs mainly (up to 40 layers). These cells are interlaced with various numbers of elastic lamellae. The tunica adventitia is thicker than the aorta and consists of dense connective tissue. It contains vasa vasorum, nerves and lymphatic capillaries. These structures may penetrate the outer part of the tunica media ***(Jungueria et al., 2005).***

The renal artery may control the blood flow to the kidney by contracting or relaxing the SMCs of the tunica media ***(Jungueria et al., 2005).***

C- Anatomy and Histology of Rat Renal Artery :

The rat renal arteries arise from abdominal aorta as human but they divide into primary branches (dorsal and ventral branches) before entering the hilum of kidney which in turn will give segmental arteries, then interlobar, arcuate and interlobular arteries ***(Yoldas and Dayan, 2014).***

The renal artery histology in rats shows gradual transposition from elastic type to muscular type. The intimal

structure is similar to that of mamalian arteries but the internal elastic lamina differ from aorta by becoming thinner as it passes towards the kidney. Also, the medial elastic lamellae become thinner as the vessel decrease in size. The medial SMCs are mainly spirally arranged as that of aorta ***(Mary, 1978).***