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Age-related histochemical changes in coronary arteries and aorta of pigs (*Sus scrofa*)

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Abstract

Histochemically, tunica intima and the elastic fibres of tunica media and tunica adventitia of coronary arteries showed PAS positive reaction and the internal elastic lamina was highly PAS positive. The tunica intima and tunica adventitia showed moderate reaction, while the tunica media showed weak reaction for acid mucopolysaccharides in the coronary arteries. In aorta, the tunica intima including the internal elastic membrane was PAS positive. In the tunica media and tunica adventitia all the elastic fibres showed PAS positive reaction. In aorta weak reaction for acid mucopolysaccharides was observed in the tunica intima with moderate reaction in tunica media and tunica adventitia. Fat droplets were observed in the tunica adventitia and the surrounding connective tissue of coronary arteries and aorta of Group-II and Group-III animals.

Keywords: Coronary arteries, aorta, tunica intima, tunica media, tunica adventitia, internal elastic membrane

Introduction

The prospect of using pig organs for human xenotransplantation is becoming increasing due to advances in the transgenic technology. Furthermore, pigs share important characteristics with the anatomy of human cardiovascular system, making them useful models for the study of human diseases (Sahni *et al.*, 2008) [12]. In the cardiovascular system, the main age-related change structures are within the heart, heart valves, and the vascular system which lead to clinical disorder (Komutrattananont *et al.*, 2019) [9].

Materials and methods

The present study was conducted on right and left coronary arteries and thoracic and abdominal parts of aorta of SVVU-T-17 breed of pigs slaughtered at ICAR-AICRP, Tirupati. The collected samples were divided into three groups based on their age i.e., Group I (up to six months), Group II (seven months to one year) and Group III (above one year) and in each group six animals were studied. The fresh tissue samples were fixed in 10% Neutral Buffered Formalin. Later these samples were processed for paraffin sections (Singh and Sulochana, 1997) [13]. For the present study, some paraffin sections of 5 µm thickness and some sections of 10 µm from fresh tissue samples were subjected to the following histochemical staining methods i.e., Periodic Acid Schiff (PAS) for Mucopolysaccharides, Periodic Acid Schiff – Alcian Blue (PAS-AB) for Acid mucopolysaccharides, Von Kossa's method for Calcium (Singh and Sulochana, 1997) [13], Oil red-O method for Lipids and Schultz method for Cholesterol (Luna, 1968) [10]

Results and Discussion

In the present study the tunica intima and the elastic fibres of tunica media and tunica adventitia of coronary arteries showed PAS positive reaction. In all the three age groups the internal elastic lamina was strongly PAS positive (Fig.1). Similar findings were also observed by Srinivas (2013) [14] in the tunica intima and tunica adventitia of coronary arteries of goats. Contrary to this Divya (2015) [5] observed that the endothelial lining in the tunica intima showed PAS negative reaction, but in conformity with the present findings she noted PAS activity in the internal elastic membrane and elastic fibres including tunica externa.

In the present study moderate PAS activity was observed in the smooth muscles of tunica media of coronary arteries as reported by Waller *et al.* (1992) [16] in humans. But contrary to the present findings Cook *et al.* (1977) [3] reported weak PAS activity in the smooth muscle cells of coronary

arteries of mongrel dogs.

In both thoracic and abdominal aorta, the tunica intima including the internal elastic membrane was PAS positive. Further, in the tunica media and tunica adventitia all the elastic fibres showed PAS positive reaction in thoracic aorta (Fig.2) and abdominal aorta.

In the present study various intensities of acid mucopolysaccharides were observed in different layers of the coronary arteries of pigs. The tunica intima and tunica adventitia showed moderate reaction, while the tunica media showed weak reaction for acid mucopolysaccharides in all the three age groups of pigs (Fig.3). These observations are in line with the findings of Divya (2015)^[5] and Ratcliffe *et al.* (1970)^[11] in pigs. Srinivas (2013)^[14] in goats also noted similar findings but he reported no activity for acid mucopolysaccharides in tunica media.

In aorta weak activity of acid mucopolysaccharides was observed in the tunica intima but moderate activity in tunica media and tunica adventitia (Fig.4). Similar findings were observed by Jense and Bertelsen (1961)^[7], De Faria (1965)^[4], Fritze *et al.* (2012)^[6] in human and Arab *et al.* (2006)^[1] in rat. Divya (2015)^[5] in pig aorta reported that the tunica intima, the ground substance showed weak reaction for acid mucopolysaccharides but the tunica adventitia was negative for acid mucopolysaccharides. In contrary, Campbell (1965)^[2]

reported acid mucopolysaccharides in the tunica intima of the aortic arch and abdominal aorta in pig.

Lipid deposition was not evident in coronary arteries of pigs in group-I, but weak in group-II and moderate in group-III (Fig.5). The lipid deposits were observed in the tunica adventitia and the surrounding connective tissue in both coronary arteries and aorta. Further, the endothelial lining and subendothelial lining showed the presence of fine lipid granules in both the coronary arteries. It is in agreement with the findings of Jensen *et al.* (2010)^[8] who reported lipid infiltration into the endothelial cells of the intimal layer of aorta in pigs. Similarly, Srinivas (2013)^[14] observed lipid deposition in all tunics of the coronary artery in all age groups of goats as observed in the present study. The lipid droplets were less in thoracic aorta when compared to abdominal aorta (Fig.6).

In the present study calcification was not observed in any layer of the coronary arteries and aorta in all the three age groups. It is in conformity with the findings of Divya (2015)^[5] in pigs and Srinivas (2013)^[14] in all the age groups of goats and in aorta of humans except in certain types of arteriosclerosis.

In the present study cholesterol was not observed in all the three age groups of pigs in both coronary arteries and aorta. These findings were in line with the observations of Tuzcu and Weissman (2010)^[15] and they also reported absence of cholesterol in coronary arteries of swine.

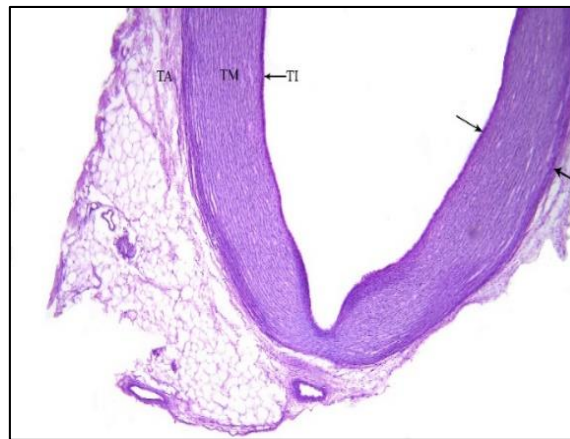


Fig 1: Photomicrograph of right coronary artery of pig of group-I showing PAS reaction (arrows) in tunica intima (TI) and elastic fibres of tunica adventitia (TA). TM-Tunica media (PAS X 40)

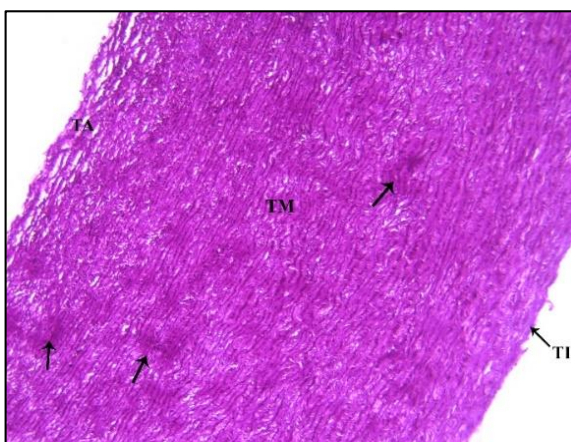


Fig 2: Photomicrograph of thoracic aorta of pig of group-I showing PAS activity (arrow) in tunica intima (TI), tunica media (TM) and tunica adventitia (TA). (PAS X 40)

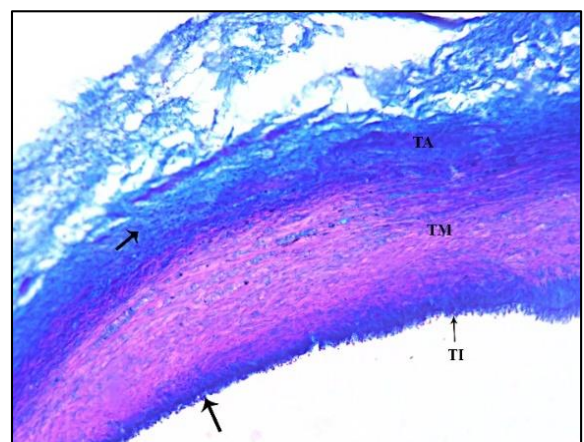


Fig 3: Photomicrograph of left coronary artery of pig of group-II showing PAS-AB reaction (arrow) in tunica intima (TI) and tunica adventitia (TA) (PAS-AB X 100)

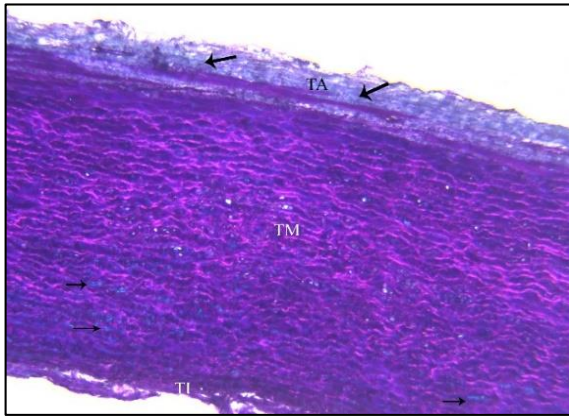


Fig 4: Photomicrograph of thoracic aorta of pig of group-II showing PAS-AB reaction (arrow) in tunica media (TM) and tunica adventitia (TA). TI-Tunica intima (PAS-AB X 100)

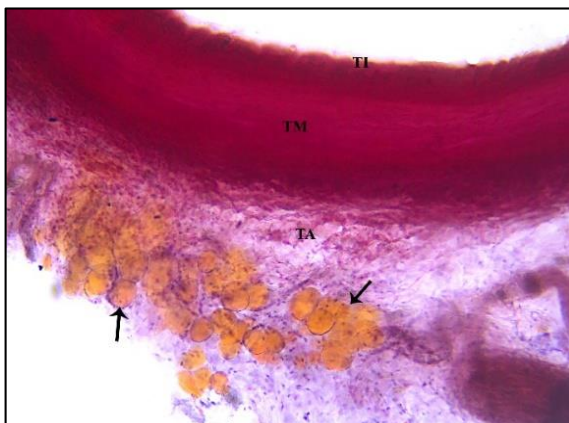


Fig 5: Photomicrograph of right coronary artery of pig of group-III showing lipid deposition (arrow) in tunica adventitia (TA). TI-Tunica intima TM-Tunica media (Oil red 'O' X 100)

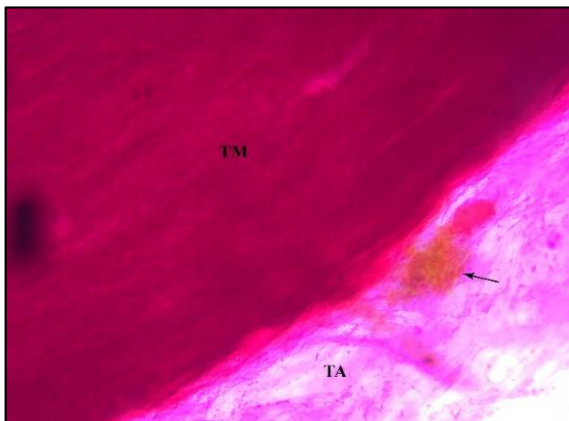


Fig 6: Photomicrograph of abdominal aorta of pig of group-III showing lipid deposits (arrow) in tunica adventitia (TA). TM-Tunica media (Oil red 'O' X 100)

Conclusion

Histochemically, tunica intima and the elastic fibres of tunica media and tunica adventitia of coronary arteries showed PAS positive reaction and in all the three age groups the internal elastic lamina was highly PAS positive. The tunica intima and tunica adventitia showed moderate reaction, while the tunica media showed weak reaction for acid mucopolysaccharides in the coronary arteries. In both thoracic and abdominal aorta, the tunica intima including the internal elastic membrane was PAS positive. All the elastic fibres of tunica media and tunica adventitia of aorta also showed PAS positive reaction. In aorta

weak reactions for acid mucopolysaccharides was observed in the tunica intima but moderate activity was observed in tunica media and tunica adventitia.

The presence of calcium and cholesterol was not observed in any layer of both coronary arteries and aorta in all the age groups of animals. Fat droplets were observed in the tunica adventitia and the surrounding connective tissue of coronary arteries and aorta of Group-II and Group-III animals.

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