Acta Veterinaria(Beograd), Vol. 48. No. 2-3, 191-196, 1998.

UDK 619:611.13:636.91

MORPHOMETRICAL AND STEREOLOGICAL ANALYSIS OF NONPREGNANT FEMALE GUINEA PIG AORTA

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(Received, 12. February 1998.)

Using light microscopy, the morphometrical and stereological characteristics of non-pregnant female guinea-pig aorta was studied. The animals were killed by cervical dislocation, the aorta was removed, fixed and embedded in paraffin. Resultant blocks were cut on a microtome and sections were stained with hematoxylin and eosin. Each section was examined under 10x magnification. To determine cross-sectional areas of the lumen, intima, media and adventitia, the standard Weibel M42 point-counting system was used. The cross sectional areas were calculated using the equation: area=Pi/PtxAg, where Pi is the number of points falling on an area, Pt is the total points of the test grid and Ag is the area of the whole test grid. The following values for crosssectional areas (expressed as 10^4 mm^2) of the different aortic layers were obtained: 1150.8± 108 for the lumen, 60.3± 5.3 for the endothelial layer, 454.9± 39 for the medial layer and 152.2± 13 for the adventitial layer. The cross-sectional area of guinea-pig aorta in total was 1818.3± 178. We concluded that the lumen occupies 63.3%, intima 3.3%, media 25.0% and adventitia 9.4% of the total cross-sectional area of guinea-pig aorta. The results obtained provide a standard that can be used in further investigations of remodeling of guinea-pig aorta under different conditions, including pregnancy.

Key words: Aorta/Stereology/Guinea-pig

Short Title: Stereology of aorta

INTRODUCTION

The guinea-pig is an experimental animal often used for medical research and represent a common model in anatomical, histological, physiological and other studies (Sisk, 1975). Numerous studies related to vascular physiology have been performed on different guinea-pig arteries, usually the aorta. So far, the elementary morphological and histological properties of guinea-pig aorta have been described (Sisk, 1975), but the details regarding histological structure of this artery have not been analyzed yet, while the morphometric and stereological

characteristics of guinea-pig aorta are still entirely unknown. For example, the basic stereological parameters such as cross-sectional area of the lumen, intima, media and adventitio of this blood vessel have not been determined yet.

Recently, ithas been recognized that changes in blood flow may be associated with alterations in morphology of the affected blood vessels (Baumbach et al., 1991; reviewed by Angus, 1994), and that the circulatory system is capable of being remodelled(reviewed by Langille, 1993). It this regard, it has been shown that changes in blood flow could lead to alterations in the morphology of the aorta (Karr-Dullin et al., 1981; Lias et al., 1982). However, in the majority of cases, the detection of vascular remodeling is possible only if morphological analysis is performed by morphometric and stereological methodology. Therefore, the purpose of this study was to examine, and to establish, the morphometric and stereological characteristics of guinea-pig aorta. In this regard,the values obtained may serve as control values in further investigations of vascular remodelling of the aorta during different conditions, including pregnancy.

MATERIALS AND METHODS

Preparation of specimens for light microscopy

Guinea-pigs (450-500 g) were used in this study (n=8).On the day of the experiment guinea-pigs were sacrificed, the thoracical cavity was opened and the descending part of the thoracic aorta was removed. The vascular segments (one segment per animal, 1 cm-long) were fixed in 10% buffered neutral formaldehyde (48 h), cut into 2 mm-long rings and subsequently the specimens were dehydrated through a graded series of ethanol solutions (70-100%) and embedded in paraffin wax. Resultant blocks were cut on a Sorval JB-4 microtome at 3 μm. Section were stained with hematoxylin and eosin.

Morphometric analysis

Each section was examined at 10x magnification (Olympus Vanox microscope). Section (3 μm thick) that included the entire circumference of each ring were cut from 5 different blocks of each animal and viewed by light microscopy with an ocular micrometer accurate to0.01 mm. For each block 5 sections were examined. Maximal and minimalinternal and external diameters were determined at 10x magnification, and these values, were used to determine the mean values of internal and external diameters. Wall thickness was calculated as the difference between external and internal diameters.

Stereological analysis

To determine cross-sectional areas of the different aortic layers including total cross sectional area, a standard pointcounting system (Weibel M42) was used. In brief, this frame-square test grid is composed of 21-line segments used to count intersects, and the 42-line endpoints. During point and interesect counting, each series of horizontal lines waned to count all the points and interesects falling on the various components of the vessels. The cross sectional areas were calculated using the following equation: Am = (Pi/Pt) x Ag; where Am cross-sectional area, Pi - number ofpoints falling on a specific layer, Pt - total

points of test grid and Ag - area of the whole test grid. Correction for eccentricity due to sectioning angle with reference to the long axis of the vessel was used for all calculations: = d1/d2 where dl - minimal radius of vessel, d2 - maximal radius of vessel. Thus, the definite form of the equation used was Am=(Pi/Pt) x Ag x (d1/d2).

Statistical analysis

For all vessel parameters, separate data measurements of diameters, wall thickness and cross-sectional profiles of vascular components, from each animal were respectively pooled and an average value recorded, so that in the analysis each animal contributed only one value for each parameter. The results are expressed as means æ S. E. M.; n refers to the number of animals.

In light icroscopy (see methods) the aorta from non-pregnant guinea-pigs displayed the following layers: 1) endothelial layer; 2) media layer and 3) adventitia layer.

The total cross-sectional area occupied by the lumen, endothelial, medial and adventitial layer were calculated as described by Lee et al. (1983). The values of the cross-sectional areas for guinea-pig aorta are depicted in Table 1. This procedure revealed that the cross-sectional area occupied by the lumen was $1150.8 \pm 93 \times 10^2$ mm^2 (n=8), which represented 63.3 ± 4.1% of the total aortic cross-sectional area. The same parameter for the intima was estimated to be $60.3 \pm 4.1 \times 10^2$ mm^2 (n=8). This value represented 3.3± 0.2% of the total aortic cross-sectional area. On the other hand, the same parameter for aortic media layer was calculated to be $454.9 \pm 36 \times 10^2 \pm 36 \times 10^2$ mm^2 (n=8), which is 25% of the toal cross-sectional area value (n=8). Finally, the cross-sectional of adventitia was $152.2 \pm 13 \times 10^3$ mm^2 (n=8), which is 8,4% of the total crosssectional value.

Table 1. Values of cross-sectional areas of lumen, intima, media and adventia for each animal. The values are expressed in 10^4 mm^2.

Animal	lumen	Intima	Media	Adventitia
1.	740.1	59.5	539.3	205.1
2.	980.4	50.4	361.1	135.2
3.	1000.4	44.5	371.3	179.1
4.	1323.6	77.8	549.2	123.2
5.	1301.4	50.3	346.4	121.4
6.	1561.6	70.3	549.2	181.2
7.	1321.4	69.4	361.3	105.4
8.	978.3	60.2	556.1	167.1

DISCUSSION

In the present study, we confirmed that guinea-pig aorta is composed of tunica intima, media and adventitia (Sisk, 1975), However, the morphometric and stereological parameters of these layers has not been previously determined yet. Until recently, the structure of the vascular system was generally viewed as being

very stable. However,it has now been proposed that the circulatory system is capable of remodeling it salf over a surprisingly short time frame (reviewed by Angus, 1994). For example, such structural reorganization is manifest whenever functional changes persist for more than a few days (reviewed by Langille, 1993). In this regard, it has been demonstrated that aortic blood flow in both ewes and humans is increased during pregnancy (Rosenfeld, 1977; Easterling et al., 1991), which may be due to pregnancy-associated decrease of aorta sensitivity to vasoconstrictors (Jansakul and King, 1990; St. Louis and Sicotte, 1992). Therefore, it seems likely that functional changes of the cardiovascular system during pregnancy could lead to remodeling the guinea-pig aorta. However, for detail analysis of pregnancy - associated remodeling of guinea-pig aorta the morphometrical and stereological characteristics of non-pregnant guinea-pig aorta need to be established. Thus, the results obtained provide a standard that can be used in further investigations invalving remodeling of guinea-pig aorta under different conditions, including pregnancy.

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MORFOMETRIJSKA I STEREOLOŠKA ANALIZA AORTE ŽENKE NEGRAVIDNOG ZAMORCA

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SADRŽAJ

Primenom svetlosne mikroskopije ispitivane su morfometrijske i stereološke karakteristike aorte negravidnih ženki zamorca. Životinje su žrtvovane cervikalnom dislokacijom, aorta je izvađena, fiksirana i ukalupljena u parafinu. Dobijeni kalupi su sečeni na mikrotomu i odsečci su bojeni hematoksilinom i eozinom. Svaki odsečak je ispitivan pod uveličanjem 10x. Za određivanje površina poprečnih preseka lumena, intime, medije i adventicije upotrebljen je standardni Weibel-ov M42 sistem za brojanje. Površine poprečnih preseka su izračunate primenom jednačine: Površina =Pi/PtxAg, gde je Pi broj tačaka testnog polja koji pada na ispitivanu površinu, Pt je ukupan broj tačaka testnog polja i Ag je ukupna površina testnog polja. Ustanovljene su vrednosti površina poprečnih preseka (izražene u 10^4 mm^2) za različite slojeve aorte: 1150.8 ± 108 za lumen, 60.3 ± 5.3 za endotelijalni sloj, 454.9 ± 39 za medijalni sloj i 152.2 ± 13 za adventicijalni sloj. Ukupna površina poprečnog preseka aorte zamorca je 1818.3 ± 178. Tako je ustanovljeno da lumen zauzima 63.3%, intima 3.3%, medija 25.0% i adventicija 8.4% od ukupne površine poprečnog preseka aorte zamorca. Dobijeni rezultati se mogu koristiti kao standard koji se može upotrebiti za buduća ispitivanja remodelovanja aorte zamorca pod različitim uslovima, uključujući graviditet.