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A Comparative Analysis of Capillary Density in the Myocardium of Normotensive and Spontaneously Hypertensive Rats

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Changes in the vessels of the myocardium are of particular interest during its morphological development both under physiological conditions and conditions of hypertrophy. Measuring the morphometric parameter capillary density is a method for statistically significant assessment of capillary growth. The aim of the present study was to measure capillary density in the left and right ventricle of normotensive and spontaneously hypertensive rats and to conduct a comparative analysis of the received values between the two species of rats. A total of 15 male normotensive Wistar rats and 6 spontaneously hypertensive rats (SHR) were used for this study. Throughout the whole postnatal period capillary density decreased steadily and uniformly in both ventricles. The comparative analysis of corresponding age groups of Wistar rats and SHR revealed that changes occurred at a younger age in SHR. In conclusion, as age advanced, we established an inversely proportional correlation between cardiomyocytic hypertrophy and capillary density.

Key words: capillary density, myocardium, comparative analysis, rat

Introduction

Cardiac hypertrophy, regardless of the initiating event, is a structural consequence of one or more factors. These factors include the increase of the number and/or size of cardiomyocytes, the quantity of connective tissue elements and the blood vessel density, or a combination of some or all of the above-mentioned. Spontaneously hypertensive rats (SHR) represent an often analysed model of essential hypertension in humans, where the relation between hypertension and cardiac hypertrophy can be influenced and often interrupted [7, 22]. Changes in the vessels of the myocardium are of particular interest during its morphological development. These take place during the postnatal development of both normotensive and spontaneously hypertensive rats. Wearn [27], as well as Roberts and Wearn [21] were the first to show that increase in the weight of the myo-

cardium due to a higher workload leads to a significant decrease in capillary density. These and several later studies consider that capillary proliferation does not represent a primary response to hypertrophy by the myocardium [5, 9, 14, 19, 20]. Other studies state that the decrease in capillary density which takes place as hypertrophy develops is in fact reversed after the process of hypertrophy becomes more stable [24, 25].

There are three main morphometric markers for assessment of the capillary network, which are used to evaluate capillary proliferation under conditions of physiological and induced myocardial growth: the ratio between capillary and cardiomyocytic profiles [15, 16]; capillary density [1, 14, 26]; total length of the capillaries within the entire wall of the ventricle [1, 16]. Measuring capillary density is a method for statistically significant assessment of capillary growth. If this parameter increases together with myocardial growth, this is an unequivocal sign of capillary proliferation, since such change in the parameter can only result from addition of new capillary units per square millimetre of tissue. Cardiomyocytic hypertrophy leads to lateral displacement of the adjacent capillaries, which is also reflected in an increase in the surface area of the transverse section of the cardiac muscle cells [4]. Capillary proliferation in the myocardium of the left ventricle in SHR has been observed in a number of studies during different stages of the postnatal development both through quantitative and qualitative studies [12, 25].

The aim of the present study was to measure the morphometric parameter capillary density in the left and right ventricle of normotensive and spontaneously hypertensive rats and conduct a comparative analysis of the received values between the two species of rats.

Materials and Methods

In the present study, we used histological material from the hearts of male normotensive Wistar rats (WR) and SHR. The total number of WR was 15, divided into five age groups, each containing three animals: two-week-old; one-month-old; three-month-old; six-month-old; twelve-month-old. The SHR were distributed in two age groups, each containing three animals: one-month-old (young) and six-month-old (adult). The animals were anaesthetised with ether and cervical dislocation was performed. The hearts were removed from the chest cavity and were placed in physiological saline in order to rinse the blood in the cardiac cavities. After a short fixation in 10% neutral buffered formalin, a transverse incision was performed through the middle of the heart, a little below the level of the cardiac valves. The hearts were then placed again in 10% neutral buffered formalin for immersion fixation. Afterwards, they were fixed in paraffin blocks, from which we prepared 7 μm wide paraffin tissue slides, which were stained with haematoxylin and eosin.

The morphometric analysis was performed on five slides from the heart of each animal. Quantitative data were obtained with a computerised system for image analysis NIS-Elements Advanced Research (Ver. 2.30). The areas of interest in each slide were first found on low magnifications (×100, ×200), taking into account the respective age group. Afterwards, at magnification ×400 we marked a zone with surface area of 0.04 mm^2 (400 μm^2). The contours of the capillaries within the zone were outlined. In order to make a good comparison of the data, it was decided that the marked zone should include these capillaries which are adjacent, on the inside or outside, to two perpendicular contours and, respectively, should not include capillaries which are adjacent, on the inside or outside, to the other two borders. After outlining the borders, we automatically obtained the values of the morphometric parameter capillary density – calculated as number of capillaries per unit of surface area of the slide (number of capillaries/mm^2).

The obtained quantitative data were demonstrated with diagrams and were statistically evaluated through a Student's t test. Statistically significant differences were read in the case of $p < 0.05$. Microsoft Office Excel 2010 was used to process the data and to demonstrate the obtained results in an adequate way.

Results

The quantitative data, as well as the comparative analysis of the morphometric parameter capillary density in the free wall of the left and right ventricle during different periods of the postnatal development of normotensive Wistar rats and SHR were obtained through analyzing randomly selected areas of the myocardium without large or massive ruptures resulting from the processing of the material.

Throughout the whole postnatal period the values of the parameter capillary density decrease steadily and uniformly in both ventricles. Changes in the dynamic of this parameter were inversely proportional to changes in the transverse section of the cardiac muscle cells in all age groups. We established a directly proportional correlation between the morphometric index cardiomyocytic density in the left and right ventricle and capillary density. These comparative characteristics indicate that the development of the capillary network during the postnatal period of development in rats falls significantly behind the increase in the surface area of cardiac muscle cells both in left and right ventricle (**Fig. 1**; **Table 1**).

The comparative analysis of representative diagrams of capillary density demonstrated that in young (1-month-old) SHR the values of the parameter for both left and right ventricle are identical to those obtained for young (1-month-old) normotensive Wistar rats. A deviation from this correlation was observed both between adult (6-month-old) SHR and respective age groups of normotensive Wistar rats, as well as between adult (6-month-old) SHR and senescent (12-month-old) normotensive Wistar rats. The decrease in the values in adult (6-month-old) SHR constitutes approximately

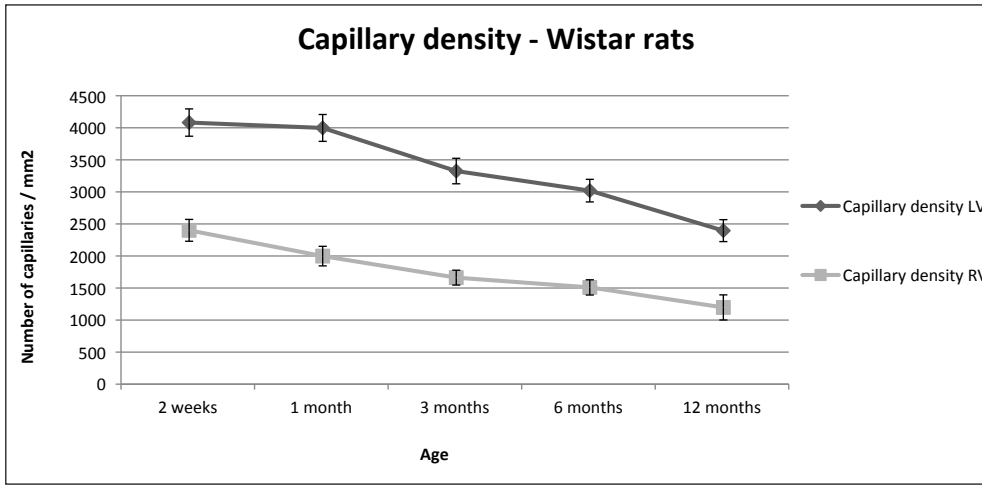

Fig. 1. Graphic representation of the changes in capillary density in the left and right ventricle. LV – left ventricle; RV – right ventricle

Table 1. Numerical representation of the changes in capillary density in the left and right ventricle

	Left ventricle		Right ventricle		
WR	**Number of capillaries/mm²**		**Number of capillaries/mm²**		
Age	**Mean value**	**SD**	**Mean value**	**SD**	**T-test**
2 weeks	4081.6	213.1	2400.1	170.3	p<0.001
1 month	3997.6	209.5	1998.1	152.6	p<0.001
3 months	3325.1	198.9	1662.3	115.9	p<0.001
6 months	3019.4	176.1	1509.8	118.8	p<0.001
12 months	2395.5	171.1	1197.3	195.9	p<0.001

Note: WR – Wistar rats.

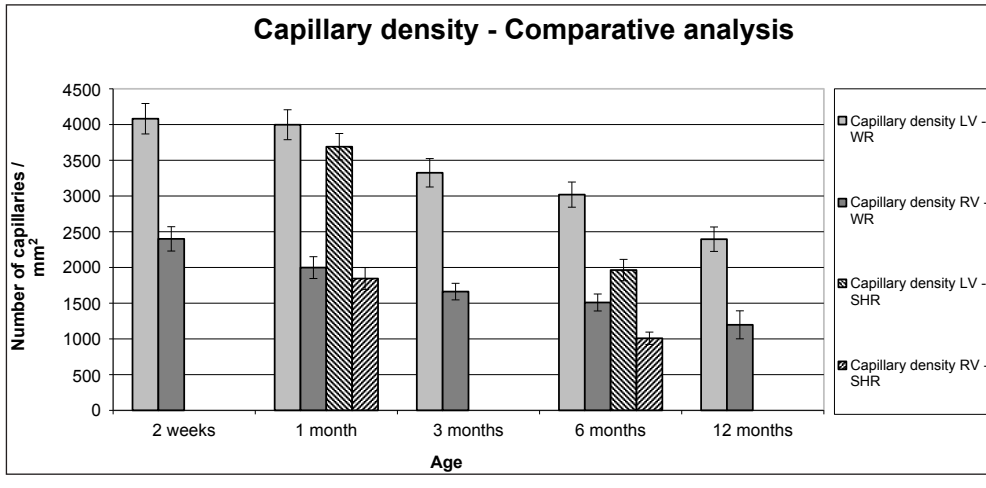

Fig. 2. Comparative analysis of the changes in capillary density in the left and right ventricle between Wistar rats and spontaneously hypertensive rats. LV – left ventricle; RV – right ventricle; WR – Wistar rats; SHR – spontaneously hypertensive rats

Table 2. Numerical representation of the changes in capillary density in the left and right ventricle and comparison between Wistar rats and spontaneously hypertensive rats

	Left ventricle			Left ventricle		
WR	**Number of capillaries/mm²**		**SHR**	**Number of capillaries/mm²**		
Age	**Mean value**	**SD**	**Age**	**Mean value**	**SD**	**T-test**
2 weeks	4081.6	213.1				
1 month	3997.6	209.5	1 month	3689.6	185.2	p<0.001
3 months	3325.1	198.9				
6 months	3019.4	176.1	6 months	1963.7	149.4	p<0.001
12 months	2395.5	171.1				

	Right ventricle			Right ventricle		
WR	**Number of capillaries/mm²**		**SHR**	**Number of capillaries/mm²**		
Age	**Mean value**	**SD**	**Age**	**Mean value**	**SD**	**T-test**
2 weeks	2400.1	170.3				
1 month	1998.1	152.6	1 month	1844.6	154.3	$p<0.05$
3 months	1662.3	115.9				
6 months	1509.8	118.8	6 months	1008.8	86.6	$p<0.001$
12 months	1197.3	195.9				

Note: WR – Wistar rats; SHR – spontaneously hypertensive rats.

one third of the values in adult (6-month-old) normotensive Wistar rats, and these values are lower even than the corresponding values for the two ventricles in senescent (12-month-old) normotensive Wistar rats (**Fig. 2**; **Table 2**).

Discussion

During the initial development of cardiac hypertrophy in SHR and normotensive rats (1- to 6-month-old), capillary density decreases significantly due to the increase in the transverse section of cardiomyocytes [6]. Our data show similar results. The decrease in capillary density demonstrates that compensatory neovascularisation does not take place during this period or, to say the least, is insignificant to sustain a normal vascular profile in SHR. Anversa et al. [4] describe various structural compensatory mechanisms in SHR aged under 2 months. During this period capillary proliferation in SHR exceeds the one in normotensive Wistar rats, despite capillary density remaining unchanged. This shows that the shortage of capillaries observed in 6-month-old SHR occurs after the second month, with Tomanek and Hovanec [25] reporting that decreased capillary density is observed in SHR aged 2-5- and 7-month-old. Together with the insufficient neovascularisation in SHR, the increasing size of cardiomyocytes leads to an increase in the mean diffusion distance. While at age 1 month the mean diffusion distance in both SHR and normotensive rats is similar, in 6-month-old SHR it increases to an extent that it does not differ substantially from the mean diffusion distance in 24-month-old normotensive Wistar rats. Insufficient revascularisation during ventricular remodeling in SHR as part of the development of cardiac hypertrophy can lead to a decrease in the cardiac output and ventricular necrosis, which has been observed in advanced age [17]. The data obtained in the present study for the period 1-6 months in normotensive Wistar rats and young SHR show a decrease in the capillary density which is more pronounced in the group of SHR.

During the full stage of development of hypertrophy (6-12 months) capillary density shows a similar degree of decrease in both ventricles [6]. The part of the left ventricle, occupied by cardiomyocytes in SHR remains significantly vast, with the amount of interstitial tissue being markedly decreased. Over the same period, the cardiac muscle cells in normotensive Wistar rats occupy a significantly smaller portion of the wall of the left ventricle, while the amount of interstitial tissue markedly increases [6]. This clearly demonstrates that during the full stage of development of hypertrophy, the increased surface area of the muscle fibres in SHR significantly exceeds the capacity of the neovascularisation and worsens the already advancing heart failure [6].

It is well known that the working potential of the muscle tissue depends on the blood supply which is a function of capillary density. The ratio between capillary and cardiomyocytic profiles in transverse sections of the myocardium increases 5 times in the left ventricle and 4 times in the right ventricle [18]. On the eleventh day after birth the capillary density in the myocardium is comparable to that observed in adults. These data are based on morphometric [1] and in vivo microscopic techniques [10, 11]. Together with the subsequent increase of the surface area of cardiomyocytes there is an almost directly proportional proliferation of capillaries [1, 13]. Similar data based on a comparative morphometric analysis between different age groups of normotensive Wistar rats and SHR were obtained in the present study. At a structural level, a decrease in capillary density is observed in the aging myocardium [23, 28]. The quantitative analysis of the structural features of capillaries shows a linear increase in capillary density as age advances, which helps maintain the number of capillaries per unit area of cardiomyocytes [2]. The rising afterload in senescent rats induces the development of concentric ventricular hypertrophy with an increase in the thickness of the free wall without an increase of the ventricular chamber [8]. The results obtained in the present study demonstrated a decrease in capillary density in rat ventricles as age advanced.

The changes observed during the comparative analysis of capillary density correlated with a more clearly pronounced hypertrophy, predominantly noted in the free wall of the left ventricle as age advanced in the group of SHR. Changes were more dynamic in the left ventricle due to the fact that the rising arterial pressure in the systemic circulation affects predominantly the afterload in the left ventricle. It should be noted that age-related hypertrophy was observed in normotensive Wistar rats as well. The development of hypertrophy had a profound effect on the parameter capillary density (the values of which showed a pattern of gradual decrease). The comparative analysis of corresponding age groups of Wistar rats and SHR revealed that changes occurred at a younger age in SHR.

Conclusion

Capillary proliferation observed in SHR exceeds the one in normotensive control animals, which indicates that myocardial hypertrophy induced by hypertension is related to an accelerated capillary growth in relatively young animals [4]. In that aspect, literature data correspond to a more dynamic rate of growth of the capillary network during postnatal development [16, 18, 20]. According to other data [3, 16, 18, 20] confirmed by the present study, the mechanism for increase of the capillary network under conditions of hypertrophy due to a rise in the afterload is inherent in young but not in senescent animals.

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