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Megakaryocytes and platelets in experimentally induced renovascular hypertension (2K1C) in rats

Megakariocyty i płytki w doświadczalnym nadciśnieniu naczyniowo-nerkowym (2K1C) u szczurów

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: Hypertension is one of the most frequently occurring diseases worldwide. Approximately 10% of the population with hypertension reveals the secondary type of the disease. The aim of the study was to evaluate the megakaryocyte-platelet system in the course of renovascular hypertension.

Material/Methods: An experimental model of hypertension in rats according to Goldblatt was used in the study. The experimental material (blood, bone marrow) was collected in the 4th, 8th, and 16th weeks of the study. Bone marrow megakaryocytes (MKs) were evaluated using immunohistochemical and morphometric methods. Blood platelets were analyzed based on their count (PLT) and mean volume (MPV). Plasma thrombopoietin (TPO) concentration was also assessed.

Results: The investigation showed increased numbers of MKs 16 weeks after partial unilateral ligation of the renal artery. Statistically significant increase in platelet count, platelet mass, and the number of MK naked nuclei (NKs) as well as elevation of the circular deviation of the nuclei (CDN) of MKs accompanied the changes. MPV and TPO concentration did not change during the experiment. There was significant positive correlation between the increase in blood pressure and the numbers of MKs and NKs. The number of MKs correlated positively with PLT and CDN. Although TPO plasma level did not change significantly, there was marked negative correlation between plasma TPO concentration and PLT.

Conclusions: Although features of intensified platelet turnover were not observed, on the basis of the study it can be assumed that the megakaryocytic system undergoes changes in the course of renovascular hypertension. This can contribute to blood platelet production and the development of possible hypertension complications.

Key words: megakarycytes • blood platelets • renovascular hypertension • rats

Streszczenie

Wstęp: Nadciśnienie tętnicze należy do najczęściej występujących chorób na świecie. U około 10% populacji osób z nadciśnieniem tętniczym ma ono charakter wtórny. Celem badań była ocena układu megakariocytarno-płytkowego w przebiegu nadciśnienia naczyniowo-nerkowego.

Materiał/metody: Wykorzystano model doświadczalny nadciśnienia u szczurów wg Goldblatta (2-kidney, 1-clip). Materiał do badań (krew, szpik kostny) pobierano w 4, 8 i 16 tygodniu eksperymentu. Za pomocą metod immunohistochemicznych i morfometrycznych oceniono megakariocyty szpikowe (MKs).

Analizy płytek krwi dokonano w oparciu o ich liczbę (PLT) i średnią objętość (MPV). Oceniono osoczowe stężenie trombopoetyny (TPO).

Wyniki: Przeprowadzone badania wykazały wzrost liczby megakariocytów po 16 tygodniach od zabiegu częściowego podwiązania tętnicy nerkowej. Zmianom towarzyszyły: istotny statystycznie wzrost PLT, masy płytkowej, liczby nagich jąder MKs (NK) oraz zwiększenie wartości współczynnika kształtu jądra (CDN). MPV i osoczowe stężenie TPO nie uległy zmianie podczas eksperymentu.

Wykazano istotną dodatnią korelację między wzrostem ciśnienia tętniczego a liczbą MKs i NK. Liczba MKs korelowała dodatnio z PLT i CDN. Mimo że stężenie TPO w osoczu nie uległo znaczącej zmianie, wykazano istotną ujemną korelację między osoczowym stężeniem TPO a PLT.

Dyskusja: Na podstawie przeprowadzonych badań, mimo że nie zaobserwowano cech wzmożonego obrotu płytkowego, można przypuszczać, iż w przebiegu nadciśnienia naczyniowo-nerkowego dochodzi do zmian w układzie megakariocytarnym. Może to rzutować na wytwarzanie płytek krwi i przyczyniać się do rozwoju ewentualnych powikłań nadciśnienia.

Słowa kluczowe: **megakariocyty • płytki krwi • nadciśnienie naczyniowo-nerkowe • szczury**

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Introduction

Hypertension is one of the most common diseases worldwide. Along with diabetes mellitus, lipid balance disturbances, smoking, and obesity, it is a significant risk factor of atherosclerosis, cardiovascular diseases such as coronary heart disease, brain stroke, peripheral artery disease, and their direct complications (heart failure, renal failure, encephalopathy). Renovascular hypertension is the most frequent cause of secondary hypertension with potentially removable cause [9]. The complications of hypertension are related to, among others, a hypercoagulative state and a tendency for the formation of thrombi [24,33]. Blood platelets play an important role in these events, in addition to their role in coagulation and fibrinolysis [4,33].

Blood platelets are un-nucleated structures of discoid shape produced as a result of megakaryocyte (MK) cytoplasm fragmentation [17]. Platelets' morphological and functional properties are conditioned during MK development [54]. The influence of various factors on megakaryocytopoiesis is reflected in the function and amount of circulating platelets [54]. Platelets and megakaryocytes create the system of a regulative circle, described as the megakaryocyte-platelet hemostatic axis (MPHA) [37,38,54]. The system is regulated mainly by thrombopoietin (TPO) [11,42]. TPO is a key cytokine which controls the stages leading eventually to the formation of fully mature MKs and enables the formation of the suitable number of platelets [10,11]. The influence of TPO on megakaryocytopoiesis is reflected by the elevated number of MKs and their progenitors in the bone marrow and spleen, MK size, nuclear ploidy (chromosomal DNA content), cytoplasm maturation, intensification of the expression of cellular differentiation markers, and the increase in pro-platelet formation by MKs [11,16,48]. MKs' size and their ploidy correlate directly with the circulating platelet mass, which is the product of the platelet number and mean platelet volume (MPV) [2,12,39]. After thrombocytopenia is induced, the first detectable change is an increase in ploidy and MK size [22,60]. MK number is elevated in a later period [60] and depends on the inflow of their progenitor cells [1,59].

Platelets are a heterogenic blood corpuscle population as far as their size and reactivity are concerned [14,54]. An inverse relationship was observed between platelet number and size [2,60]. It was shown that large platelets were hyper-reactive [2,14,60]. Such changes in platelet volume and function could be related to the release of young platelets or the effects of humoral factors that influence megakaryocytopoiesis regulation and platelet production and function [53,54]. It is assumed that MKs with high ploidy are the source of large hyper-reactive platelets and the increase in platelet volume is the effect of changes in MK cytoplasm fragmentation [54]. The size of platelets and their functional potential are determined in MKs during thrombopoiesis. Platelet size in the circulation does not undergo significant changes [2,35,55]. The physiological mechanisms that regulate platelet volume are not yet known. However, it is known that disturbances concerning both MK and platelet ionic canals are the background of these mechanisms [2]. Changes in platelet mass, involving an imbalance between the number of platelets and their volume, are observed under certain pathological conditions. Thus it seems that platelet number and volume undergo independent regulation [2,55].

In cases of atherosclerotic renal artery narrowing [2,3], diabetes mellitus [52], unstable angina, and myocardial infarction [46,60], normal or increased numbers of large platelets were observed. Patients after myocardial infarction showed increased MPV, which is a predictor of a subsequent ischemic incident. MPV did not correlate with other known vascular diseases risk factors. This suggests that MPV is an independent risk factor of a next acute coronary syndrome [2,54]. On the other hand, in the course of acute ischemic brain stroke, MPV increase was accompanied by a drop in blood platelet number [2,35]. MPV elevation was also observed in patients with hypertension, but the platelet number was normal or lowered [33,38]. It is now known that large hyper-reactive platelets contribute to the development of atherosclerosis [54]. The occurrence of such platelets in prothrombotic conditions is explained as a consequence of the elevated use of corpuscles and their accelerated production [54]. In atherosclerosis-induced diseases, both large and hyper-reactive platelet numbers as well as MK ploidy increase were observed [5,54,55,60]. Similar changes were also presented in diabetes [5] and primary hypertension [38].

So far, the evaluation of the megakaryocyte-platelet system in the course of renovascular hypertension, one of the secondary types of hypertension, has not been performed. In the present study a morphometric evaluation of bone marrow megakaryocytes (count, cell and nuclear size, nuclear-cytoplasmic ratio, and the circular deviation of the cells and their nuclei) was performed. We also analyzed blood platelets based on their count and mean volume. Moreover, the assessment of plasma TPO concentration was taken into consideration. In order to analyze the dynamics of possible changes in the megakaryocyte-platelet system, the study material was collected 4, 8, and 16 weeks after surgical narrowing of the renal artery. Blood pressure measurements were also conducted at the same intervals.

Materials and Methods

Animals and induction of renovascular hypertension

Male Wistar rats (180–220 g) were used in the experiment. A two-kidney one-clip model of hypertension was induced by partial standardized clipping of the left renal artery under pentobarbital anesthesia (40 mg/kg, i.p.). The animals were divided into six groups (Table 1). The rats were then left untouched for the next 4, 8, or 16 weeks. A sham-operated groups of rats (animals that received the same surgical intervention except for the clipping of the artery) served as the controls. During the operation all the animals obtained lincomycin to prevent infection.

Blood pressure measurement

Systolic and mean blood pressure were measured in conscious rats by a tail-cuff method (Harvard Rat Tail Pressure Monitor System) according to the method described by R. Zatz [58].

Hematological and biochemical analyses

The blood samples for these analyses were obtained from the living animals, from the study and control groups at 4, 8, and 16 weeks of the experiment. Venous blood was taken without stasis from the right ventricle of the beating heart into the vacutainers containing EDTA for platelet count (PLT) and sodium citrate (4:1) for MPV. Samples of the blood for estimation of TPO plasma levels were also obtained. During this procedure the animals were under pentobarbital anesthesia (40 mg/kg, i.p.) and the thoracic cavity was opened. PLT and MPV were measured by an autocounter. Moreover, platelet mass was calculated according to the formula PLT x MPV [2] for each sample. The blood samples for TPO level estimation were centrifuged immediately and the plasma was stored in several aliquots at -85°C until assayed. Thrombopoietin was assayed by sandwich-type ELISA (Santa Cruz Biotechnology, Inc.; TPO (N-19): sc-1298).

Table 1. Division of rats into groups in relation to the time of material collection after the surgical procedure

4th week	E 1 (n=9)	C 1 (n=6)
8th week	E 2 (n=10)	C 2 (n=7)
16th week	E 3 (n=10)	C 3 (n=7)

E – study groups (rats with narrowed renal artery): E1 – rats sacrificed after 4 weeks, E2 – rats sacrificed after 8 weeks, E3 – rats sacrificed after 16 weeks; C – control groups (rats operated on without ligation of the renal artery, so-called sham-operated rats): C1 – rats sacrificed after 4 weeks, C2 – rats sacrificed after 8 weeks, C3 – rats sacrificed after 16 weeks; n – number of rats in a group.

Analysis of bone marrow megakaryocytes

Experimental material

After the rats were sacrificed, the femur and sternum were removed, fixed in 10% buffered formalin, decalcified, and embedded in paraffin.

Staining procedure

Two 5-µm-thick longitudinally oriented bone marrow sections were removed from each sample of both the femur and sternum. One section each from the femur and sternum were stained with hematoxylin-eosin (HE) for complex morphometric analysis of MKs. Other sections (one from the femur and one from the sternum) were prepared for immunohistochemistry. The monoclonal antibody CD61 (clone Y2/51, catalog no. M 0753, DAKO) was used to identify glycoprotein IIIa (GPIIIa). The aim of this examination was to identify, for numerical analysis not only large mature MKs, but also the small young MKs that may be mistaken for other cells.

Morphometry

Following HE staining and immunostaining, morphometric evaluation was performed using an Olympus BX41 microscope with a digital camera connected to a computer in which a standard morphometric program (Micro Image IncD UDF Packed Writing Software for Windows, OLYMPUS) was installed. To estimate the number of MKs and naked nuclei (NKs), i.e. megakaryocytes after the loss

of cytoplasm transformed to thrombocytes, the immunohistochemical sections were analyzed. Three randomly selected fields in each section were chosen at a magnification 240× (only areas containing well-preserved hematopoietic tissue were accepted). Only nuclear MKs were included (NKs formed a separate group) [50].

For morphological analysis, HE-stained sections were used. In each section, MKs were identified as cells with diameters ≥20 μm and visible nuclei [23]. The following parameters were evaluated in the study: the area of the cell (AC), the area of the nucleus (AN), the nuclear-cytoplasmic ratio (N/C), and the circular deviation (CD) of both MKs (CDC) and their nuclei (CDN). The circular deviation is defined as $CD=4\pi A/C^2$ (C – circumference, A – surface area), giving the value of 1.0 for a circular shape and a lower factor for irregular outline [50]. The ratio of nuclear material to cytoplasm (N/C) is high in MKs without granulation (less mature MKs) and tends to decrease as granulation increases (more mature MKs) [41]. The N/C ratio was calculated from the quotient of AN and AC. Three randomly selected fields in each section were chosen at a magnification of 480× (only areas containing well-preserved hematopoietic tissue were accepted) and suitable MKs and their nuclei were measured. Then the median values of the parameters were calculated.

We obtained permission from the local ethics committee to perform the investigations on the animals (no. 1004/36).

Statistical analysis

The Statistica PL program was used for the statistical analysis of the results. Minimum, maximum, mean, and standard deviation (*SD*) were determined for the particular parameters. The evaluation of distribution normality was performed using the Shapiro-Wilk test. The results underwent analysis of variance ANOVA. The significance of differences between groups were estimated with the Tukey-Kramer test and correlation assessment was conducted using the linear correlation test according to Pearson.

Results

Systolic blood pressure (SBP), mean blood pressure (MBP)

SBP values in all the study groups (E1: 135.8, E2: 150.5, E3: 157.0 mmHg) were statistically significantly higher than in the control groups (C1: 116.2, C2: 124.7, C3: 127.3 mmHg, $p<0.01$). The same was true for MBP (E1: 100.4, E2: 117.0, E3: 119.8 mmHg) compared with the controls (C1: 81.3, C2: 101.7, C3: 102.1 mmHg). SBP values correlated positively with both MK number ($p<0.05$, $r=0.337$) and the number of NKs ($p<0.05$, $r=0.371$).

Megakaryocytes and blood platelets

The first changes in the megakaryocyte-platelet system were observed in the 8th week after partial renal artery ligation; however, they were statistically insignificant. They concerned decreases in MK and NK counts in the bone marrow and PLT in the blood compared with the control group as well as with the study group evaluated in the 4th week. The megakaryocyte-platelet system showed significant changes in the 16th week of the study. At that time, E3 presented elevated MK and NK numbers in the bone marrow with accompanying increased PLT, platelet mass, and CDN compared with the control group and the study groups assessed earlier (E1, E2; Table 2, Figure 1).

MK number correlated positively with both PLT ($p<0.05$, $r=0.477$) and platelet mass ($p<0.01$, $r=0.557$). Moreover, positive correlation was found between the number of MKs and CDN ($p<0.05$, $r=0.467$). There was also positive correlation between PLT and platelet mass ($p<0.001$, $r=0.922$) and PLT and NK ($p<0.05$, $r=0.434$). On the other hand, we did not find changes in MKs size (AC) and MPV, MK nuclear size (AN), or the values of the nuclear-cytoplasmic ratio (N/C) and circular deviation of MKs (CDC) at the particular stages of the study.

TPO plasma level

In the study rats, plasma TPO concentration, although it did not show any significant changes, oscillated in accordance with the regulating mechanism determined by platelet mass, i.e. in the 8th week an increasing tendency of TPO concentration was found in comparison with the values obtained in the controls and the first study group (E1), while in the 16th week a decreasing tendency of TPO level was observed (Table 2). Moreover, negative correlation was observed between plasma TPO concentration and PLT ($p<0.05$, $r=-0.4305$) and platelet mass ($p<0.01$, $r=-0.5306$).

Discussion

Hypertension is a disease in the course of which morphological and functional changes of various organs occur. This happens due to both the unfavorable influence of increased blood pressure on vascular walls and pathogenic factors. Hypertensive complications are connected, among others, with hypercoagulability and a tendency for thrombus formation. Besides the coagulative system and fibrinolysis, blood platelets play an important role in these events. Both experimental and clinical studies on hypertension have paid much attention to the evaluation of blood platelets. Only a few of them concerned MKs [1,38]. However, the cells were evaluated under conditions of primary hypertension and not renovascular hypertension, which is a secondary hypertension. As opposed to primary hypertension, the causes of which have not yet been fully determined, the etiology of secondary hypertension is well established.

The pathogenesis of renovascular hypertension is complex. The hemodynamically significant narrowing of a renal artery or arteries leads to kidney hypoperfusion and an increase in plasma renin-angiotensin system (RAS) activity. Afterwards, the activation of tissue RAS, overstimulation of the sympathetic nervous system (SNS), and increased aldosteron (ALDO) and vasopressin (VSP) synthesis and release take place. These factors contribute to the maintenance of increased blood pressure, mainly in the chronic phase of renovascular hypertension. The duration of the

Table 2. Megakaryocyte and platelet parameters and plasma thrombopoietin concentration in the study and control groups of rats

	Group Parameter	C 1 $\bar{x}\pm SD$	E 1 $\bar{x}\pm SD$	C 2 $\bar{x}\pm SD$	E 2 $\bar{x}\pm SD$	C 3 $\bar{x}\pm SD$	E 3 $\bar{x}\pm SD$
1	MKs count in field of vision (mag. 240×)	6.890±1.58	6.980±1.8	8.190±2.01	6.950±2.34	6.990±0.81	10.030±2.04^{k1,2}
2	NK number in field of vision (mag. 240×)	1.540±0.25	1.820±0.72	2.290±0.99	1.710±1.01	1.570±1.02	3.050±0.960^{k1,2}
3	Area of MKs (AC) [μm^2]	319.0±27	335.8±30.1	303.0±25.9	323.3±28	327.0±41.1	321.3±23.3
4	Area of nucleus (AN) [μm^2]	101.3±8.2	105.6±8.7	95.4±6.6	96.0±11.7	103.5±12.8	102.8±10
5	Nuclear-cytoplasmic ratio (N/C)	0.328±0.051	0.321±0.027	0.319±0.025	0.305±0.039	0.320±0.028	0.322±0.024
6	Circular deviation of MKs (CDC)	0.711±0.029	0.746±0.035	0.739±0.04	0.760±0.03	0.756±0.039	0.752±0.024
7	Circular deviation of MKs nuclei (CDN)	0.455±0.062	0.446±0.032	0.440±0.046	0.436±0.063	0.437±0.05	0.511±0.026^{k1,2}
8	Platelet count (PLT) [$10^3/\mu l$]	881±119	819±45	803±136	799±140	787±114	988±39^{k1,2}
9	Mean platelet volume (MPV) [fl]	5.43±0.32	5.30±0.25	5.50±0.09	5.38±0.21	5.56±0.45	5.59±0.55
10	Platelet mass [$10^3/\mu L$ x fl]	4814±860	4338±317	4421±768	4308±825	4374±730	5529±693^{k1,2}
11	Thrombopoietin plasma level [pg/ml]	20.60±1.67	20.71±1.7	21.50±2.81	22.25±2.6	19.50±1.38	18.75±2.87

Symbols of the groups were described in the Table 1. MKs – megakaryocytes; NK – naked nuclei; N/C – nuclear-cytoplasmic ratio.
Data presented as mean. Statistic significance level $p<0.05$ was determined:
^{k} - in comparison with the control group;
^{1} - in comparison with the 1st examined group;
^{2} - in comparison with the 2nd examined group.

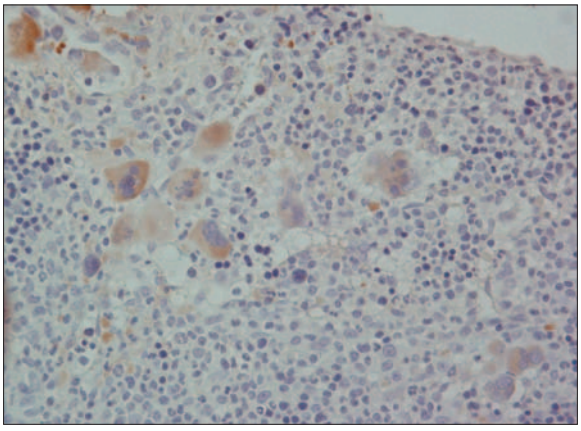

Figure 1. Rat bone marrow section. Loosely oriented megakaryocytes immunostained for CD61 (GP IIIa) to identify cells (group E3) (magnification: 480×)

particular phases of renovascular hypertension is variable. In rat studies it was observed that the acute phase developed from 2 to 4 weeks and the chronic phase, 9 weeks after partial unilateral ligation of the renal artery [26,32].

In the present study, the first changes in the megakaryocyte-platelet system were observed only 16 weeks after renal artery ligation, i.e. during the time of the chronic phase of renovascular hypertension in rats. The significant elevation of MK number in the bone marrow was found with a simultaneous, slightly decreased concentration of the main factor stimulating megakaryocytopoiesis, i.e. TPO. It is possible that ALDO and/or VSP, factors included in the pathogenesis of renovascular hypertension, had their effect on bone marrow. The results of some studies pointed to the potentially stimulating influence of these factors on megakaryocytic cell-line growth [7,19,27,28,47]. Some studies revealed a stimulating influence of SNS on hematopoietic progenitor cells, including a megakaryotic cell line [20,25,34]. Increased RAS activation could possibly occur in bone marrow, which contributed to the effect. In some studies concerning the issue, Haznedaroglu's hypothesis on tissue RAS functioning in bone marrow was confirmed [18]. It was shown that angiotensin II, through AT1 receptors, stimulated the proliferation of the progenitor cells of various hemopoietic cell lines, including megakaryocytic cells [44].

It should be stressed that hypertension is a factor contributing to the development of atherosclerosis. In previous studies it was observed that in the course of diseases with

atherosclerotic etiology, such as coronary disease (especially unstable angina), myocardial infarction, or ischemic brain stroke, large hyper-reactive platelets are produced, which can be accompanied by an increase in platelet mass [2,35,46]. This is a consequence of significantly elevated platelet use and, as a result, their increased production. Thus elevated platelet turnover occurs. In such a situation, the occurrence of large platelets with accompanying changes in MKs (ploidy increase and MK size in the bone marrow) were observed [13,54].

There is an opinion that MKs with abundant cytoplasm are a source of platelets with increased MPV [6,13,54]. However, the mechanism of large platelet formation has not yet been established. It could be the result of changes in MK cytoplasm fragmentation and the effects of cytokines on the process [13,54]. Therefore, suspecting possible changes of MPV, we analyzed not only MK size, but also nuclear-cytoplasmic ratios and the circular deviation of these cells. Other studies on essential thrombocytemia observed, besides the elevation of platelet count and increased mean volume, also the occurrence of MKs of very irregular shape [40,45]. However, CDC did not change during the experiment, which can indirectly explain the lack of MPV changes. It can be assumed that the study rats did not reveal intensive peripheral platelet turnover, which occurs in acute vascular incidents where a thrombus is formed in sites of atheromatous plaque rupture and ulceration [56]. This could be the effect of a lack of advanced atherosclerotic lesions in the blood vessels of experimental animals. Bath et al. observed positive correlation between MPV and the degree of atherosclerosis-induced renal artery narrowing [3].

According to the inverse relationship between MPV and PLT occurring in physiological conditions expressed by the constant product of the values [55], we expected platelet number to be constant provided that MPV was at a constant level. However, PLT unexpectedly increased significantly at the end of the experiment, i.e. after 16 weeks. A positive correlation between MK number and PLT was found, which shows that the PLT changes in rats with experimental renovascular hypertension corresponded with the bone marrow MK numbers. Senaran et al. observed, besides an MPV increase, the co-occurrence of PLT elevation and plasma TPO concentration. The relationship concerned patients with myocardial infarction and unstable angina, which are diseases with an atherosclerotic background with increased platelet turnover [46]. In our study, however, we did not evaluate the morphology of arteries with regard to atherosclerotic lesions, although it seems that such an examination could provide more information. We cannot exclude that TPO could be one of the factors that contributed to the effect observed by Senaran and revealed by our study. In the study rats, plasma TPO concentration did not undergo significant changes but varied in accordance with the regulating mechanism by platelet mass. Moreover, negative correlation was observed between plasma TPO concentration and PLT and platelet mass. The results confirmed the assumption that rats with renovascular hypertension do not show intensified peripheral platelet turnover.

In the 16th week of the experiment, besides the increase in PLT, platelet mass, and MK number, an elevation of NK number in the bone marrow was observed. The increase in NK number (remnants of MKs which released pro-platelets and platelets into the circulation) showed that the main site of platelet production could be the bone marrow vascular sinuses. Positive correlation between NK count and PLT was shown. Of the evaluated morphological parameters of MKs in rats with renovascular hypertension, CDN was significantly elevated. This shows that the MK nuclei had a more regular circumference, which is a feature of less mature MKs compared with MKs of more advanced maturation with multilobular nuclei of irregular circumference [23,41]. Young MKs are also characterized by a high value of N/C. However, mean N/C and MK size did not change in the course of the experiment. It should be considered that, according to Levine [23], the nuclear circumference of MKs that are in the last stage of maturation can become more regular than those in intermediate stages of maturation. The positive correlation between MK number and CDN found in the study can indicate that a marked part of the MK pool consisted of both young cells and MKs in the last stage of maturation, in which the formation and release of cells take place. The increased MK count and PLT in the last stage of the experiment could be a compensative reaction to earlier changes in the megakaryocyte-platelet system, the decreasing tendency of PLT observed in the 8th week of the study. Humoral and neurogenic factors included in the pathogenesis of the chronic phase of renovascular hypertension could participate in the condition.

On the other hand, it should be remembered that the changes in the megakaryocyte-platelet system observed in the 16th week (the last stage of the experiment) could be connected with the longer duration of hypertension and its higher values. This could contribute to the induction of atherosclerotic changes in vessels. Morshita et al., examining the abdominal part of the aorta of rats with hypertension induced by the Goldblatt method (2K1C), observed the presence of hypertrophy and thickening of the vessel wall and an elevation of wall-to-lumen ratio [31]. It can be assumed that in animals with experimental renovascular hypertension examined to evaluate the megakaryocyte-platelet system, the increase in platelet release was the reaction to so-called corpuscle use. Such factors as the elevation of shear stress, endothelial dysfunction/damage, RAS activation, and the influence of vasopressin could contribute as well [15,21,29,36,43,49,51,57]. In the study rats the process was not intensified, as MPV increase and MK size elevation (which reflects their ploidy) were not observed in the study.

On the basis of this study, although the features of intensified platelet turnover could not be observed it can be assumed that in the course of renovascular hypertension, the megakaryocyte-platelet system undergoes changes. This can contribute to blood platelet production and the development of possible hypertension complications. Previous studies on a similar experimental model showed intensified platelet aggregation induced using ADP compared with rats of a control group [8,30]. The evaluation of advancement stage of atherosclerotic changes and humoral factors related to their occurrence in rats with experimental renovascular hypertension need to be explained.

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