Histomorphometry of marrow megakaryocytes in experimental uraemia in rats

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Abstract

Uraemic patients frequently demonstrate tendencies towards life-threatening bleeding. Reduced platelet counts and their functional immaturity seem to be caused, among other things, by disorders in the system of marrow megakaryocytes. The aim of the study was a histomorphometric evaluation of marrow megakaryocytes in the course of experimental uraemia in rats. Uraemia was induced by means of right nephrectomy and a partial removal of the left kidney cortex in rats. Morphometric analysis was conducted, using the Microlmage program set. The number of MK, MK area, N/C, CDMK, CDNMK and MK cluster formation were analysed. Uraemic rats showed a reduction in the MK count and their area and an increase in the N/C ratio, CDMK, CDNMK and in the incidence of MK clusters. The results indicate that platelet disorders, observed in uraemia, can also be conditioned by disturbed maturation of MK.

Key words: megakaryocyte, histomorphometry, uraemia.

Introduction

Patients with uraemia frequently demonstrate haemostatic disorders and platelet dysfunction. Different types of haematological alterations, such as bleeding tendency or thrombotic events, are observed. They lead to rapid progression of atherosclerosis, thrombosis and cardiovascular complications [1, 2]. Platelet count in uraemic patients is usually normal but the function of platelets is impaired and they tend to aggregate. Platelet dysfunction likely results from failure of bone marrow thrombopoiesis and peripheral platelet destruction-sequestration. There are no conclusive studies that would explain the thrombopoietic status in uraemic patients. The main factor, responsible for these disturbances is probably the circulating TPO - the major regulator of megakaryocyte production. Bleeding tendency in uraemic patients is associated with an excessive formation of nitric oxide (NO) [3, 4]. Kienast and Schmitz [5] suggest that an RNA-rich reticulated platelet count may be an exponent of thrombopoiesis in the marrow. Ando et al. [3] showed a reduction in MK production in the marrow, indirectly by evaluating the number of RNA-rich reticulated platelet counts. The majority of studies on thrombopoiesis have been based on morphological and functional analyses of platelets. Histological investigations of bone marrow MK in uraemia are still missing.

The aim of the study was a histomorphometric assessment of bone marrow MK in experimental uraemia in rats.

Material and methods

The study was carried out on 60 male Wistar rats (200-220 g b.w.). Experimental uraemia was produced by surgical resection of the right kidney and removal of approximately 50% of the left kidney cortex [6]. Control Group I (20 rats) consisted of animals without surgical manipulation and Control Group II (20 rats) contained animals with decapsulation and removal of fat adherent to the kidney. After decapitation, a fragment of bone marrow (0.1 x 1 cm) was fixed in the so-called "Oxford solution" and put into paraffin bars. Paraffin sections (5μm) were cut with a microtome. Routine haematoxylin and eosin, and immunohistochemistry stainings were used to identify immature MK (antibodies CD61 and VIII factor - DAKO). Morphometric analysis was conducted, using the standard Microlmage program set (Olympus) with DP 12 Analysis. The number of

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Table 1. Histomorphometric features (means ± standard deviations) of megakaryocytes in bone marrow in the study group.

	MK number	MK area	N/C ratio	CD MK	CD NMK	Cluster Forms (%)
Group I No surgical manipulation	38.9±6.2	189.9±46.2	32.7±6.7	0.84±0.08	0.57±0.07	0.0
Group II Sham operation	39.6±5,9	186.2±53.7	33.2±7.1	0.85±0.06	0.58±0.09	0.0
Group III Uraemic rats	35.2±6.9	164.5±41.7	36.9±5.4	0.89±0.09	0.61±0.08	15.0

MK/mm^2, MK area in μm^2, N/C ratio, circular deviation factor of MK (CD MK) and their nuclei (CD NMK), and the incidence of MK clusters were determined. The obtained results were analysed, using the Statistica Pl. computer program.

Results and discussion

We found statistically significant differences in the mean MK area ($p<0.05$) and in the number of cluster forms of MK between rats with uraemia and the animals in Control Groups I and II. Statistically insignificant differences in the total number of MK were observed between those groups. The highest, but not statistically significant was the N/C ratio, CD MK and CD NMK in uraemic rats Table 1.

Until now, the studies on thrombopoietic disorders in uraemic patients have concerned blood platelets [7]. Uraemic patients show platelet dysfunction, associated with multifunctional platelet defects. It has been demonstrated that blood platelets are characterized by, e.g., disorders in membrane glycoprotein expression, reduction in platelet phospholipids and Ib glycoproteins. A functional defect may cause a decrease in the expression of IIb - IIIa glycoproteins. Interesting is also the reduced mean platelet volume in uraemic patients. Neither cause of platelet fragmentation nor its functional dysfunction has been elucidated. The mechanism may be complex and related to lipid metabolic disorders [8, 9]. The most common platelet disorders include a low content of granule nucleotides [7, 10], an impairment of thromboxane formation and an increase in cholesterol-rich platelets [11]. The results of the present study may help explain these disturbances. Our experiments indicate changes in marrow MK. Experimental uraemia, induced in rats, causes a reduction in the total MK count and in the MK area, thus suggesting an accelerated production of platelets, which may develop abnormalities, as reported by other authors. Marrow MK are immature, and this is indicated by changes, observed in CD MK and their nuclei - they have a characteristic immature shape and form clusters. The mechanism of their formation is unknown. Immaturity of marrow MK in uraemia has also been confirmed by the analysis of their ploidy. Winkelmann et al. [12] have shown that high creatinine levels and low haemoglobin or creatinine clearance correlate with a low average ploidy of MK. Our results indicate that platelet disorders, observed in uraemia, can also be conditioned by MK maturation disturbances.

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