Influence of thalidomide on megakaryocytes in multiple myeloma

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Abstract

The aim of the study was to assess the influence of thalidomide on megakaryocytes (MK) in patients with multiple myeloma (MM). The study was based on bone marrow trephine biopsies from 12 patients with MM before initiation of thalidomide administration and after three months of its duration. The morphometric examinations were done, using image analysis (DP 12). Quantitative assessment of MK and the analysis of the morphological parameters of MK were performed. MK with features of dysplasia were more frequently observed before the treatment. Additionally, a greater number of the so-called 'naked nuclei' was noticed then. Due to the effect of thalidomide, the mean number of MK increased and so did their area. During the treatment, a more frequent presence of emperipolesis was observed. The observations confirm the fact that thalidomide may cause changes in MK.

Key words: multiple myeloma, thalidomide, trephine biopsy, megakaryocyte.

Introduction

Multiple myeloma (MM) is a malignant neoplasm, characterised by an increase of clonal plasma cells in the bone marrow. In the biology of MM, the process of angiogenesis in bone marrow stroma plays an important role and this phenomenon correlates with the activity and the clinical stage of the disease. About half of the patients exhibit resistance to the first choice treatment. The patients, who initially responded to therapy, showed an increased resistance in the course of the disease [1]. In order to assess the stage of the disease and the effectiveness of the applied treatment, bone marrow trephine biopsy (BMT) is recommended [2]. So far, there have not been any effective methods of MM treatment. During recent years, reports of thalidomide use in the therapy of MM have become available [1, 3]. Thalidomide affects HIM by inhibiting angiogenesis and induces apoptosis of newly generated vessels. It has a specific quality of regulating the secretion of many cytokines [4]. The cytokine-like Vascular Endothelial Growth Factor (VEGF), with other cytokines secreted by HIM cells, can be a stimulator of angiogenesis in the bone marrow [5, 6]. VEGF expression is particularly high in MK in some haematological malignancies [7]. MK of the bone marrow are not only the precursor cells for the platelets but they comprise an essential element of the bone marrow-blood barrier which regulates the release of other cells of the haematopoietic system into blood [8]. The aim of the study was to assess the influence of thalidomide on bone marrow MK in patients with MM.

Material and Methods

The study was based on bone marrow samples, collected from the posterior superior iliac spine of 12 patients (8 women and 4 men, aged from 44 to 73 years) in the 2nd and the 3rd clinical stage of MM, according to the classification of Durie and Salmon. in whom thalidomide was applied. The specimens were obtained by BMT before the treatment and after 3 months of its duration. Bone marrow tissue was carried out in Oxford solution and paraffin embedding. Thin sections of 5 μm were cut by the use of a microtome. Routine techniques staining, such as a hematoxylin and eosin, were applied. Morphometric examinations were done, using an image analysis set (DP12). A quantitative analysis of MK (nuclear megakaryocyte, MK; naked nuclei, NN; anuclear

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Table 1. Histomorphometric features (means±standard deviations) of megakaryocytes in bone marrow of patients with MM before and after three months of thalidomide treatment.

Patient No	BMT	MK	NN	AMK	N/C	CDMK	CDNMK
		x ± SD	x ± SD	x ± SD	x ± SD	x ± SD	x ± SD
1.	before	*1.08 ± 0.86	0.67 ± 0.58	86.00 ± 21.63	0.43 ± 0.21	0.78 ± 0.08	0.72 ± 0.13
	after	6.00 ± 1.80	1.67 ± 1.53	177.00 ± 32.51	0.33 ± 0.08	0.70 ± 0.10	0.52 ± 0.13
2.	before	4.33 ± 3.21	1.67 ± 1.53	*74.00 ± 19.08	0.50 ± 0.10	0.77 ± 0.08	0.89 ± 0.04
	after	5.98 ± 1.01	1.31 ± 1.14	167.67 ± 41.06	0.40 ± 0.05	0.72 ± 0.10	0.46 ± 0.17
3.	before	1.33 ± 1.15	0.67 ± 0.58	88.00 ± 20.66	* 0.68 ± 0.08	0.84 ± 0.07	*0.79 ± 0.05
	after	4.00 ± 2.00	1.67 ± 0.58	155.00 ± 37.75	0.50 ± 0.10	*0.73 ± 0.10	0.45 ± 0.05
4.	before	*2.67 ± 1.15	2.33 ± 0.58	96.33 ± 18.50	0.80 ± 0.05	0.89 ± 0.04	0.70 ± 0.13
	after	7.00 ± 1.00	1.15 ± 0.76	123.67 ± 32.87	0.86 ± 0.06	0.65 ± 0.05	0.76 ± 0.09
5.	before	*6.33 ± 2.08	1.33 ± 0.58	112.00 ± 20.42	0.62 ± 0.10	0.78 ± 0.08	0.70 ± 0.10
	after	1.15 ± 0.77	0.67 ± 0.58	168.33 ± 53.46	0.62 ± 0.10	0.72 ± 0.10	0.62 ± 0.10
6.	before	*10.67 ± 3.06	0.67 ± 0.58	106.33 ± 29.26	0.68 ± 0.08	0.78 ± 0.08	0.82 ± 0.08
	after	2.33 ± 1.53	1.67 ± 0.58	171.67 ± 40.10	0.53 ± 0.10	0.82 ± 0.04	0.70 ± 0.05
7.	before	3.13 ± 1.06	1.07 ± 0.87	118.67 ± 22.03	0.43 ± 0.08	0.86 ± 0.11	0.80 ± 0.05
	after	4.67 ± 2.08	1.33 ± 0.58	165.00 ± 47.70	0.62 ± 0.10	0.65 ± 0.13	0.67 ± 0.08
8.	before	1.67 ± 0.58	0.67 ± 0.58	*113.00± 5.94	0.62 ± 0.08	0.82 ± 0.09	0.54 ± 0.13
	after	3.67 ±1.53	1.33 ± 0.58	137.33 ± 25.01	0.47 ± 0.07	0.79 ± 0.06	0.82 ± 0.07
9.	before	3.33 ± 1.53	0.67 ± 0.58	119.33 ± 23.80	*0.50 ± 0.05	0.68 ± 0.16	0.81 ± 0.10
	after	3.00 ± 2.65	0.67 ± 0.58	156.67 ± 45.37	0.73 ± 0.08	0.80 ± 0.04	0.74 ± 0.09
10.	before	4.67 ± 3.06	1.33 ± 0.58	151.33 ± 29.37	*0.46 ± 0.08	*0.80 ± 0.03	0.74 ± 0.08
	after	2.15 ± 1.76	0.67 ± 0.58	162.67 ± 53.54	0.65 ± 0.11	0.71 ± 0.03	0.44 ± 0.06
11.	before	2.00 ± 0.87	0.67 ± 0.58	124.00 ± 34.70	0.82 ± 0.08	0.72 ± 0.13	0.73 ± 0.08
	after	4.67 ± 1.53	1.67 ± 0.58	166.0 ± 37.32	0.55 ± 0.07	0.78 ± 0.06	0.53 ± 0.08
12.	before	1.33 ± 1.15	1.00 ± 1.00	121.33 ± 31.07	*0.83 ± 0.08	*0.85 ± 0.09	0.82 ± 0.10
	after	2.67 ± 2.08	1.67 ± 0.58	168.33 ± 48.00	0.54 ± 0.06	0.73 ± 0.08	0.67 ± 0.16

* statistically significant differences
MK- nuclear megakaryocyte, NN- naked nuclei, AMK- area MK. N/C- nuclear/cytoplasmic ratio,
CDMK- circular deviation of cells, CDNMK- circular deviation of nuclei.

cytoplasmic fragments, CF) per square millimetre of bone marrow was done, taking into account the presence of the so-called cluster forms. The analysis of MK morphological features, such as the MK area (AMK), the nuclear/cytoplasmic ratio (N/C), circular deviation of cells (CDMK) and their nuclei (CDNMK), the analysis of emperipolesis incidence were also performed. The identification of MK immature forms was done, using CD61 monoclonal antibodies and the factor VIII. LSAB+HRP and DAB (DAKO) detection kits were used as the chromogen. Statistical analysis of the results was done, using the Statistica PL computer program.

Results

It has been observed that MK, with the features of dysplasia, were present in the majority of the patients, especially in cases of myeloma cell infiltration. MK of a small area were more often found either around or inside the infiltration. Under the influence of thalidomide, a decrease in infiltration was noticed and the mean number of MK increased, their area being enlarged as well. MK were disseminated and could be found around the walls of the cavity vessels. Before the treatment, an increased mean number of NN was assessed, in comparison to the mean number of NN during the treatment. While assessing the mean CDNMK, significant differences were observed before and after the treatment with thalidomide. In the majority of cases, the values were higher before the treatment and MK were rounder. During the treatment, the presence of single cluster forms and the phenomenon of emperipolesis were noticed (Table 1).

Discussion

The process of angiogenesis in the stroma of bone marrow is important in the pathogenesis of MM. This process correlates with myeloma cell proliferation [5]. In the present study, we noted that the presence of plasma cell infiltration affected the morphological changes in MK. The presence of dysplastic megakaryocytes was also confirmed. Dysmegakaryocytosis, which is manifested by the presence of micromegakaryocytes, micromegakaryoblasts, promegakaryoblasts, naked nuclei and promegakaryocytes, may lead to production of functionally impaired platelets [9].

Haemostasis disturbances may result from those changes. The disturbances of MK may strengthen the action of proangiogenic cytokines by release of the factors, stimulating angiogenesis, such as VEGF [6]. These cytokines show an association with the production of myeloma cells. Thus, an increased expression of the proangiogenic factors may take place. Thalidomide is an inhibitor of angiogenesis, although its exact mode of action has not been

well recognised yet [4]. It regulates the secretion of many cytokines and affects HIM. In the present study, quantitative and qualitative changes of MK were observed, due to thalidomide treatment. During the therapy, there was a shift of MK into the localisation around the cavity vessels. MK constitute a vital element of the marrow-blood barrier which, in normal conditions, releases only mature forms of the haematopoietic system into circulation [8]. In case of its impairment, immature cells may occur. The presence of emperipolesis should also be emphasised. It may be suggestive of still existing unfavourable conditions in HIM, which persist during treatment [10]. Differences in the extent of the disturbances to the mean shape of MK nuclei, before and during thalidomide treatment, may be connected to an increased proliferation of MK and the resulting dissociation of the nuclear and cytoplasmic maturation. The observed changes confirm the fact that thalidomide is a multidirectional agent, causing alterations in the marrow MK. The interest in this subject may be important, especially in comparison to recent reports, concerning the biology of the myeloma cell, its interaction with HIM and new possibilities of MM multidirectional treatment.

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