Immunohistochemical evaluation of mast cells and mark activity tryptase and chymase in experimental fibrosarcoma

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Abstract

The aim of the study was an evaluation of the activity of mast cells and mark activity tryptase and chymase and of protein levels in experimental fibrosarcoma, induced in rat skin. The experiments were carried out on 50 male Wistar rats. The cancer was induced in rats by one subcutaneous injection of 0.2 mg 3-methylcholanthrene in 0.25 ml of olive oil. Tissue material was fixed in Bouin's fluid. Immunohistochemical tryptase detecting reactions were performed - using specific antibodies and the ABC complex. The activities of tryptase and chymase and protein levels were determined in supernatant of 10% homogenate. We found a very significant growth of mast cell quantity in the connective tissue of tumours. We observed slight differences in the activity of examined enzymes in tumours of different mass.

Key words: mast cells, tryptase, chymase, protein, fibrosarcoma, rat.

Introduction

The synthesis of tryptase and chymase occurs in mast cells. These cells, while homing the connective tissue, are ubiquitous in the whole organism. Especially numerous are they in tissues which are on the borderline of the external and internal environment. That is why their increased numbers are observed in the skin, under the epidermis, in the gastric mucosa, as well as in the mucosa of the respiratory system and the uro-genital system. The mast cells occur in the neighbourhood of blood vessels and lymphatic ducts, however, their presence has not been confirmed in circulation [1, 2].

Since the first identification of the mast cells, research has been carried out at many scientific centres to explain their role in homeostasis and pathology of the system. Till now, it has not been possible to determine either the role or the function of mastocytes in the maintenance of health condition, however, growing is the number of diseases in which the mast cells affect their pathophysiology. Experimental studies, performed on models of mastocyte functioning in these processes, are very significant for the explanation of the specific role of mastocytes in the course of various pathological processes. Neither biological nor clinical consequences of mastocyte occurrence in neoplasmic tumours have yet been unveiled [3, 4]. Preliminary own studies have demonstrated a distinct increase of the mast cells in tissues of an experimentally induced tumours in rats [5].

The aim of this study was to evaluate the activity of mast cells and neutral proteases, such as tryptase and chymase, and protein levels in the homogenate of methylcholanthrene (3-MC) fibrosarcoma of different masses, induced in rats.

Materials and methods

The study involved 50 male Wistar rats (100-120g b. w.). The animals were given standard rat chow and water. All the animals were divided into three groups: 1. Fibrosarcoma was induced in 34 rats by one subcutaneous injection of 0.2mg 3-MC dissolved in 0.25ml of olive oil in the dorsal skin area; 2. Controls (a) - 8 animals received subcutaneous injection of 0.25ml pure olive oil; and 3. Controls (b) - 8 rats with no treatment. The animals from the experimental group were killed soon after tumour growth was observed. The tumours were extracted on the day of detection, and after 1, 2 and 3 days from their initial observation. Dissected tumours were weighed and measured. Next, two wedge-shaped sections of each tumour were taken. In the control animals, only subcutaneous tissue sections were collected. Some tissue sections

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Figure 1. Subcutaneous tissue from the control rat. Spindle-shaped mast cells are distributed close to small blood vessels. The nuclei of mastocytes are small and obscured by granules. Toluidine blue. x300.

Figure 2. A fragment of a large fibrosarcoma periphery. Mast cells are very numerous. Most of them contain large, light nuclei and very little number of granules (they are strongly degranulated). Toluidine blue. x 300.

Figure 3. A fragment of a large fibrosarcoma centre. Immunostaining for tryptase is visible in the main, large and mature mastocytes. x 300.

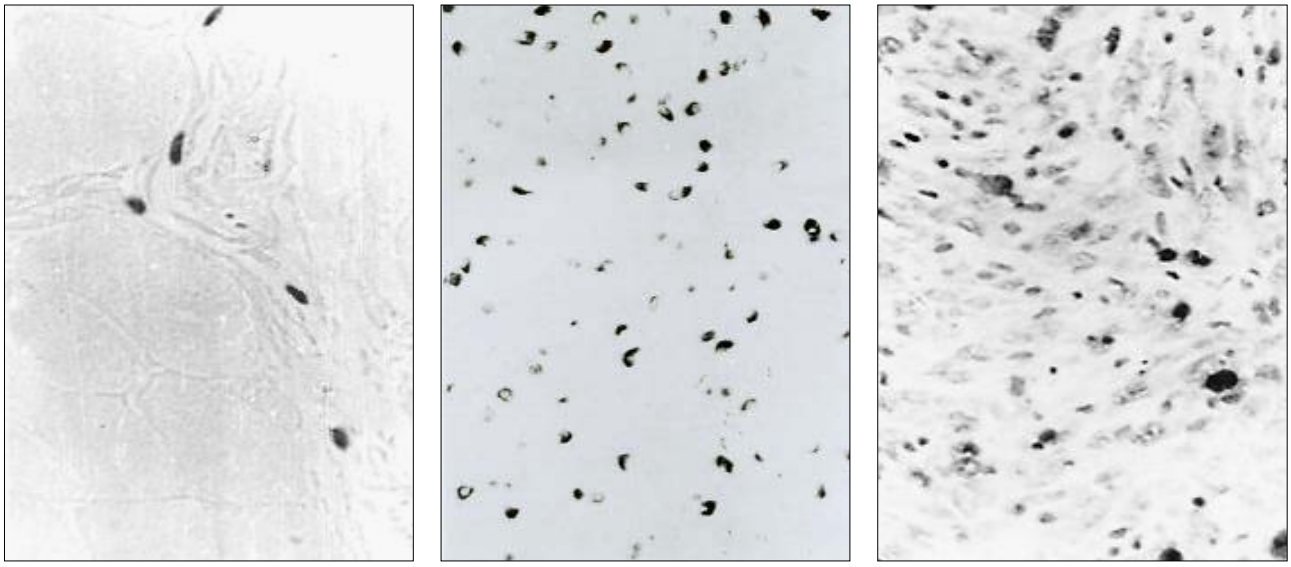

Table 1. Activity of tryptase and chymase and protein levels of methylocholanthrene fibrosarcoma.

Ingredient	Rats with tumour		
	small	intermediate	large
Tryptase	8.82 ± 1.0	9.58 ± 0.97	9.28 ± 0.82
Chymase	4.74 ± 0.43	5.06 ± 0.49	5.40 ± 0.5
Protein	30.39 ± 2.1	28.88 ± 1.9	28.24 ± 2.0

were fixed in Bouine's fluid and routinely embedded in paraffin. The obtained 5μm sections were stained with haematoxylin-eosin (H+E), as well as by the Azan method. Mast cells were stained with toluidine blue or alcjan blue+saphranine (Csaba methods) in pH 1.5. Immunohistochemical studies were performed by the ABC method (avidin-biotin peroxidase complex), using monoclonal specific mouse antisera against tryptase (DAKO Copenhagen). Control reactions always yielded negative results. Out of the second section, 10% homogenate was prepared in 0.15 M NaCl. The activity of tryptase was determined by means of Boc-Phe-Ser-Arg-AMC, and that of chymase by the use of Suc-Leu-Leu-Val-Tyr-AMC in pH of 7.5. The volume of released 7-amino-4-methylocoumarin was determined by measuring the absorbance at 560 nm, following the staining reaction with N-(1-naphthyl) in presence of sodium nitrite and ammonium sulphite. The results were read out of a calibration chart, made with the use of standard β-naphthylamine. The protein was determined by Bradford's method.

The obtained results were statistically evaluated by means of Student's 't' test.

Results

No significant differences were found between the control groups of rats, regarding the results of performed studies. For that reason, only the results of studies, performed on the animals injected with olive oil, were taken into account (Fig. 1.).

In the animals, in which the cancer was induced by 3-MC, tumours developed in the subcutaneous tissue after 3-4, 5-6 and 7-8 months and demonstrated the mass of 1-6, 20-55 and 80-176 g, respectively. All the tumours had a histological structure typical for fibrosarcoma, as mainly spindle-shaped cells constituted their structure. Many giant cells with numerous nuclei were found, as well as monstrous cells with giant nuclei, as well as other atypical cells. In the structure of the tumours, a tendency was observed towards an increase in the number of M cells during tumour growth (Fig. 2 and Fig. 3.).

However, significant individual differences were observed, as tumours of a similar size (especially the very large ones) sometimes differed in the concentration of M cell distribution.

Table 1 presents results of the measurements of tryptase and chymase activities and protein levels in the tumours with small, intermediate and large mass.

Discussion

So far, neither biological not clinical consequences of mast cell functioning in neoplastic tumours have unequivocally been determined.

In the performed own studies, a very distinct increase in the number of mastocytes was noted, mainly in the peripheral areas of fibrosarcoma. Other authors also observed an increased number of mast cells during carcinogenesis and their degranulation

related to tumour growth intensity [3, 6, 7]. However, in vitro experiments showed an anti-tumour activity of mast cells in culture conditions [2, 8]. The results of certain studies suggest that mastocytes may suppress the growth of neoplasms, e.g., via polysaccharide neutralisation [9] or by release of the tumour necrosis factor alpha (TNF-α), just as it has been reported from *in vitro* studies [4]. These views are contradicted by results of other studies, which demonstrate that TNF induces angiogenesis in normal tissues [10].

Fibrosarcomas of different mass, induced by 3-MC, showed slight differences in the activity of tryptase and chymase. No differences were found in the contents of protein in tumours of different mass, either. Immunopositive reaction for tryptase was seen only in some large M cells in the stroma of the fibrosarcoma. Toluidine blue staining always showed a few times higher number of mast cells in the same tumour fragments. It may probably have resulted from the occurrence of a large number of young and immature mast cells with scarce volumes of cytoplasm with a small number of secretory granulations [11]. Presumably, the parallelly growing number of mast cells in their stroma is of significant importance for quick tumour development. This problem, however, requires further investigations in order to explain the nature of mast cell influence on fibrosarcoma development.

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