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RESPONSE OF THE MAST CELL POPULATION TO INTRATRACHEAL INJECTION OF HEPARIN AND FUCOIDAN

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Heparin, a sulfated polysaccharide produced by warm-blooded animals and with a regulatory action on activity of many components of hemostasis, is stored in the cytoplasmic granules of mast cells. To understand the pharmacokinetics of the therapeutic analogs of this biopolymer, the ingestion of substances of polysaccharide nature by mast cells is of great interest. The ability of mast cells to ingest polyanions, introduced into the body, has been demonstrated by light microscopy relative to changes in the number and saturation of the metachromatic staining of the granules by the use of basic dyes [1, 5, 6, 11-13]. It has shown by methods of light microscopy that mast cells can ingest and incorporate granules not only of heparin, but also of other polysaccharides, such as glycogen, inulin, dextran, and carboxymethylcellulose [9, 12]. Depending on structural characteristics, unequal relations would be expected between the internal structures of mast cells and of the polymers entering them. However, visual qualitative assessment of changes in metachromatic staining of the cells was unable to reveal any differences. Mistakes connected with the distribution of granules inside the cell and also with non-specificity of the cationic dyes traditionally used, were unavoidable.

Unlike other basic dyes used in light microscopy, berberine sulfate exhibits a linear relationship between the intensity of its fluorescence and the heparin concentration [10]. The selectivity of binding of berberine sulfate with sulfo groups, under appropriate conditions, enables changes in the content of sulfated polysaccharides in the mast cells to be estimated quantitatively. This has made possible an examination of the known qualitative results of cytochemical analysis at the quantitative level and new information to be obtained on activity of the mast-cell population following administration of various polysaccharides in vivo.

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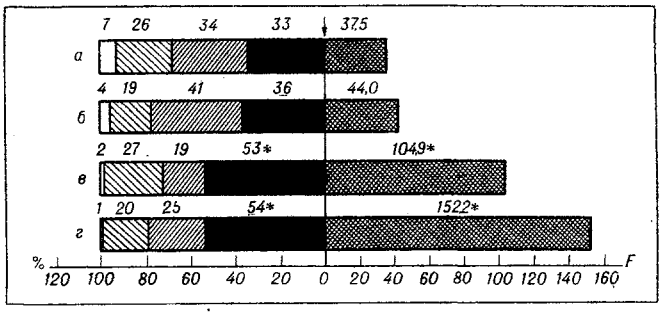

Fig. 1. Cytochemical and fluorometric analysis of reaction of mast-cell population of intact animals (a) and 5 h after intratracheal injection of physiological saline (b), heparin (c), and fucoidan (d). Abscissa: from O to the right – units of intensity of fluorescence, from O to the left – percentage of cells by intensity of metachromasia. Part shaded black) very dark cells, close oblique shading) dark cells with a distinguishable nucleus, wide oblique shading – pale cells with distinguishable granules, unshaded part — very pale, empty cells with single granules; cross-hatching – intensity of fluorescence.

The aim of this investigation was to compare the mast-cell population of the peritoneal cavity of rats after a single intratracheal injection of heparin and of the sulfated polysaccharide fucoidan, using cytofluorometry and cytochemical analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male albino rats weighing 180-230 g. Aqueous solutions of heparin ("Richter") and of fucoidan, obtained from brown marine algae *Laminaria cichoriodes* [8], were injected intratracheally once only into animals while anesthetized with ether, in a dose of 50 mg/kg. The volume of fluid injected did not exceed 0.6 ml. The total number of animals was divided into four groups with six rats in each group: group 1) intact rats, group 2) rats receiving an injection of isotonic sodium chloride solution under the same conditions, group 3) rats receiving heparin, and 4) rats receiving fucoidan. A suspension of mast cells was obtained from peritoneal washings by the method in [14] 5 h after injection of the substances. The procedure for preparing the suspension and staining with toluidine blue was described previously [6]; cytochemical analysis was carried out by the method in [5], with evaluation of the mast-cell population into four categories depending on the type of staining properties. Another series of preparations was obtained and stained with a 0.02% aqueous solution of berberine sulfate by the method in [10]. Fluorescence of the berberine was estimated quantitatively on a "Univar" microfluorometer ("Reichert," Austria), coupled with a "Conttron" computer (West Germany); a standard set of narrow-band interference filters was used [4]. The numerical data were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

The study of the mast-cell population of intact rats and of rats 5 h after intratracheal injection of physiological saline revealed no difference in the intensity of metachromatic staining or in the intensity of fluorescence between these two groups of animals (Fig. 1).

The similarity observed both in staining and in the intensity of fluorescence of the mast cells of intact rats and rats receiving physiological saline indicates that the procedure of instillation of the substance into the trachea and the general anesthesia to which the rats were subjected had no effect on the state of the peritoneal mast cells for 5 h after these procedures. The increase in degranulation index of the mast cells noted in the literature in response to ether anesthesia is of a short-term character [5].

The percentage of darkly stained cells (type 1) was significantly increased and the percentage of cells with a distinguishable nucleus (type 3) was reduced by more than half in rats 5 h after receiving an intratracheal injection of heparin, compared with the fluorescence of cells in rats receiving physiological saline, and the mean intensity of fluorescence in the experimental animals also was increased (Fig. 1).

When the mast cells of the rats were studied cytochemically at the same times after intratracheal injection of fucoidan, no significant differences could be found from the cell population of the rats receiving heparin.

The cytofluorometric study showed that the intensity of fluorescence of the mast cells after injection of fucoidan was significantly (by 1.5 times) higher than after injection of heparin.

The increase in the intensity of fluorescence of the mast cells after intratracheal injection of heparin confirmed the existing hypothesis concerning the phagocytosis of heparin, introduced into the body, by mast cells and its storage [5, 6], for many investigations have shown that the intensity of fluorescence of an individual mast cell is proportional to the concentration of heparin in it. Fucoidan, which is a heparinlike polysaccharide, has metachromasia similar to that of heparin, and also has a degree of sulfatation [7] which allows its selective interaction with berberine sulfate [10].

Thus the use of a more sensitive fluorometric method revealed the significantly higher content of the plant polysaccharide in the mast cells after intratracheal injection than after injection of heparin, despite the equal doses of the polyanions.

The greater intensity of fluorescence of the mast cells after injection of fucoidan can be explained by the greater number of sulfate groups contained in the branched macrochain of this polyelectrolyte. Another important fact is that negatively charged polymers, with a molecular weight 100-200 times greater than heparin, can accumulate in mast cells and be kept there for a long time. This conclusion stems from the stronger (by 1.5 times) fluorescence of the mast cells which we observed after injection of fucoidan than in the cells of animals receiving heparin.

Accumulation of fucoidane may be accompanied by displacement of a certain quantity of endogenous heparin from the granules into the blood stream, which will prolong for a short time the hypocoagulation effect that we described previously [2, 3].

We demonstrated cytofluorometrically that both heparin and fucoidan, with great differences in their molecular weight, can accumulate in the mast cells from the rat peritoneal cavity. The results of experiments by the cytofluorometric method not only confirm the conclusions of cytochemical analysis, but also enable differences in the response of the mast-cell population to injection of polysaccharides of different structure to be estimated quantitatively.

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