A Phase Contrast Microscopic Study on the Induction of Cellular Deformation in Mast Cells

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ABSTRACT

The influence of colchicine, colcemid, ginseng-alcohol extract, saponin, garlic alcohol-ether extract, and X-irradiation on free floating peritoneal mast cells were studied in rats by means of a phase contrast microscope. After the intraperitoneal injection of colchicine or colcemid the mast cells of the albino rat peritoneal fluid show an altered morphology. Ginseng-alcohol extract, allicin extracted from garlic, and X-irradiation do not elicit this cytological response. Mast cells exposed to saponin exhibit a severe destruction and degranulation. The relation of the mast cell-damaging agents, metabolic poisons, and X-irradiation to the mast cell response is discussed with reference to the morphological changes. These are compared with the data of Padawer (1960, 1965).

INTRODUCTION

In the rat the connective tissue mast cells are large and polyhedral; in other mammals, including man, they are smaller, irregularly oval or flattened cells. Occasionally slow amoebism can be observed. The free floating peritoneal mast cells of the rat are spheroidal with a nucleus located centrally. The cytoplasm is filled with granules which are uniformly distributed and stain meta-

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chromatically. Padawer (1966) demonstrated that, after the administration of colchicine or its derivatives to albino rats, the morphology of the free floating cells was specifically altered. The literature on this subject includes a few reports made by Jacques Padawer.

This investigation is undertaken to determine the effect of ginseng-alcohol extract, saponin, garlic alcohol-ether extract, and X-irradiation on free floating peritoneal mast cells of the albino rat and also to compare the effect of colchicine and mast cell-damaging agents as well as metabolic poisons on the mast-cell system.

MATERIALS AND METHODS

Mature adult Sprague-Dawley strain albino rats, weighing about 200 gm. were used. Food and water were allowed ad libitum. The substances to be tested were dissolved in 10 ml of normal physiologic saline just before use and injected intraperitoneally into the left abdominal region. Three to twenty-four hours later, the rats were anesthetized with nembutal (5 mg./100 gm. body weight) and the abdominal cavity was opened. Peritoneal fluid was sampled with a clean dropper. Normal and abnormal mast cells were observed and photographed with a phase contrast microscope (AO Spencer).

The doses of the substances tested in this study
were as follows: colchicine, 0.2 mg./100 gm. body weight; colcemid, 0.02 mg./100 gm. body weight; ginseng-alcohol extract, 430 mg./100 gm. body weight; garlic alcohol-ether extract, 5 gm. Eq./100 gm. body weight; saponin, 50 mg./100 gm. body weight; X-irradiation, 25 roentgen of whole body irradiation/minute for 30 minutes. Ginseng-alcohol extracts were obtained by the following procedure. A water-ginseng mixture (water 500 ml and 30 gm. of dried ginseng) was boiled in the condenser-flask for 3 hours. The mixture was filtered and concentrated until 80 ml of the mixture remained. The concentrated extract was dissolved in 420 ml of 80% ethyl alcohol. Then the precipitates were removed by centrifuging; the filtrate was concentrated and evaporated. Finally, about 4.3 gm of a yellowish brown powder of dried ginseng was obtained.

Garlic alcohol-ether extracts were obtained by a procedure similar to that used in preparation of the ginseng-alcohol extract; An alcohol ether garlic mixture (alcohol 200 ml, ether 200 ml, and garlic (Allium sativum) 100 gm.) was ground with a crushing machine and filtrated. After complete evaporation of the filtrate the dried extracts were dissolved in 20 ml of physiologic saline and the final stock solution was kept in the refrigerator when not in use.

RESULTS

The data summarized in table 1 shows that neither the ginseng-alcohol extract nor the garlic alcohol-ether extract, nor X-irradiation effected any cellular deformation in the peritoneal mast cells. Whereas the intraperitoneal injection of the above substances did not affect the peritoneal mast cells, the intraperitoneal injection of colchicine and colcemid resulted in an extensive morphological change in all of the peritoneal mast cells, as was previously found by Padawer (1966).

1. The effect of colchicine was rapid, and was seen almost immediately following the intraperitoneal injection of the drug. Time-lapse microphotography (one frame per five minutes) of the treated mast cells in vitro showed nodal formation of the cytoplasm. (Fig. 6) Phase contrast microscopic observation of the colchicine-treated mast cells demonstrated not only the over-all cytomorphic deformations, but also nuclear displacement to the periphery and pronounced anisodiometry of the cytoplasm. (Fig. 1) Although all of the cellular components in the peritoneal fluid are similarly affected according to Padawer (1966), the changes were especially noticed in the mast cell. (Fig. 2) When mast cells from untreated rats were observed for prolonged periods, none of the changes seen before cell death and such as the autolysis accompanying vacuole formation were noted. Therefore, the cytoplasmic deformation of the colchicine-treated mast cells were not agonal.

Colcemid (deacetylomethyl colchicine), which is a tropolone of colchicine caused a cellular defor-

<table>
<thead>
<tr>
<th>Test Substances</th>
<th>Dosage/100 gm. body weight</th>
<th>Osmolarity mOsm/liter</th>
<th>pH</th>
<th>Sources</th>
<th>Deformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Physiologic saline sol.</td>
<td>5 ml</td>
<td>260</td>
<td>6.8</td>
<td>N.B.Co.</td>
<td>+</td>
</tr>
<tr>
<td>2. Colchicine</td>
<td>0.2 mg</td>
<td>223</td>
<td>7.1</td>
<td>Ciba Co.</td>
<td>+</td>
</tr>
<tr>
<td>3. Colcemid</td>
<td>0.02 mg</td>
<td>232</td>
<td>7.4</td>
<td>Panax ginseng</td>
<td>-</td>
</tr>
<tr>
<td>4. Ginseng-alcohol extract</td>
<td>430 mg</td>
<td>355</td>
<td>5.0</td>
<td>Coleman &amp; Bell</td>
<td>-</td>
</tr>
<tr>
<td>5. Saponin</td>
<td>50 mg</td>
<td>665</td>
<td>5.0</td>
<td>Allium Sativum</td>
<td>-</td>
</tr>
<tr>
<td>6. Garlic alcohol ether extract</td>
<td>5 gm. Eq.**</td>
<td>292</td>
<td>4.0</td>
<td>General Max.</td>
<td>-250</td>
</tr>
<tr>
<td>7. X-ray</td>
<td>375 r</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
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</table>

*Consistent dimorphism of the mast cells is expressed as positive (+).
**Dosage of garlic alcohol-ether extract being equivalent to 5 gm. of dried ginseng.
formation of the mast cells which was easily recognized even following a one tenth dilution of the colcemid. The effect of colcemid on mast cell deformation was greater than that of colchicine. (Fig. 3, 4, and 5)

2. Ginseng-alcohol extract and saponin caused only a slight difference from the normal in the number and the morphology of the mast cells. Fragments of the mast cells or clumped granules were floating in the peritoneal fluid. The number of intact mast cells was remarkably decreased. In all cases, many infiltrated lymphocytes were observed. These reactions were due to the disrupting and degranulating action of the ginseng-alcohol extract, which contained a saponin fraction as its main component. Actually following the saponin injection, there were more extensive hemolytic changes than found following the injection of ginseng-alcohol extract.

3. The garlic alcohol-ether extract (being equivalent to 10 gm. of dried ginseng) caused only minimal changes in the mast cells. The other cellular components of the peritoneal fluid maintained their typical round shape. Neither the stubby pseudopods of cytoplasm nor nodal constrictions were seen. In only a minority of the cells was there a slight dentation of the protoplasmic outline.

4. The effect of X-irradiation on peritoneal mast cells was similar to that seen following the garlic alcohol-ether extract injection. All of the cellular components of the peritoneal fluid remained intact.

Even 24 hours post-irradiation, at the time of maximal decrease (64.0% ± 5.3) of the intact mesenteric mast cells (An, 1964), no changes could be seen in the free peritoneal mast cells.

**DISCUSSION**

In normal albino rats, free floating peritoneal mast cells are spheroidal and the nucleus is centrally located. Normally the other cellular components of the peritoneal fluid cells of the rat (eosinophils, monocytes and lymphoid elements) are spheroidal. Padawer (1955, 1966) reported that the mast cells of rat peritoneal fluid are affected specifically and characteristically by the subcutaneous and intraperitoneal injection of colchicine or podophyllotoxin. Padawer (1960) studied the effects of colchicine, its congeners, and other pertinent drugs on the morphology of mast cells of the albino rat. After administration of colchicine to the albino rats, he found characteristic morphologic changes in the free cells. There was displacement of the nucleus to the periphery, pronounced anisodiametry of the cytoplasm, and elongation of the cytoplasmic mass with nodal constriction. Using the mast-cell morphologic response he compared the potency of these drugs with that of mast cell-damaging agents and metabolic poisons. He observed that compound 48/80, which is a histamine liberator, regardless of dose level had no effect on mast-cell morphology. Also he noted that allicin, which is known a metabolic poison and inhibit tissue respiration as well as arsenite, did not alter the morphology of the albino rat. Oh et al. (1962) reported that the administration of a large dose of dried-ginseng extract gave rise to degranulation and destruction of the mesenteric mast cells of the albino rat as well as a small dose of the extract. (Choi et al., 1964) As suggested by Lee et al. (1960) they postulated that these cytological changes were due to the existence of a histamine liberator in the dried ginseng. They described its effects on blood pressure and capillary permeability. Thereafter, Pak et al. (1963) confirmed the fact that the degranulation of mesenteric mast cells following the intraperitoneal or direct local injection of ginseng extract may be due to a saponin fraction of ginseng extract.

Induction of deformation of free floating peritoneal cells following colchicine or colcemid injections:

In response to the injection of colchicine, using
the same dose as given by Padawer (1960), the mast cells of peritoneal cavity became nodular. The cytoplasm appeared segmented, the nucleus was invariably located at one pole of the cytoplasmic mass and peripherally displaced to such an extent that most of its surface lacked cytoplasm. The effect of colcemid was more sensitive than that of colchicine. In all cases the effect on eosinophils and monocytes was easily observed. These cells were definitely elongated and their nuclei were at one pole. The doughnut-form of the nucleus of the eosinophil was not altered in its typical morphology.

**Induction of deformation of free floating peritoneal cells following injections of ginseng-alcohol extract or saponin:**

Following the injection of ginseng-alcohol extract, which may contain a histamine liberator, there was no change in the morphology of the peritoneal mast cells of the animals, nor any marked differences in the shape of eosinophils and monocytes as compared with those of the normal animals. Among the various effects of the chemical components extracted from ginseng, the hypotensive action has been studied by Fujidani (1905) and by others. Previously its effect as a histamine liberator was pharmacologically established by Lee et al. (1960). It is generally known that considerable destruction and degranulation of mast cells may be caused by treatment with several histamine liberators: Macintosh et al. (1949) with pepitone, Riley et al. (1953, 1955) with stilbamidine and others, and Fawcett (1955) with compound 48/80.

In response to the injection of a large dose of saponin, severe rupture and degranulation of the peritoneal cells appeared due to disruption of the granular membrane. This was clear evidence of the hemolytic effect of saponin.

**Induction of deformation of free floating peritoneal cells following garlic alcohol-ether extract injection or X-irradiation:**

Arsenite has been reported to have a toxic effect on the liver and other organs. Kim (1965) reported that allicin extracted from garlic also had an inhibiting action on tissue oxidation and on the TCA cycle. Lee (1967) demonstrated that vitamin C significantly prevented the toxic effect of allicin, but it did not prevent the effect of arsenite. Padawer (1960) reported that an administration of arsenite did not affect the mast-cell morphology regardless of the dose level. The authors observed that large doses of garlic alcohol-ether extract caused little change in the free peritoneal mast cells when they were compared with normal intact mast cells.

X-irradiation was stated to cause a decrease in the number, as well as degranulation and disruption of the tissue mast cells. (Smith et al. 1954, Maynard et al. 1955, Oh et al. 1964, and An 1964) In response to the same dose of irradiation as given by An (1964), no morphological disorder or changes could be seen in the mast cells.

With the exception of the ginseng-alcohol extract group, in all experimental cases the doses administered close to, or above, reported LD50 levels. The 24-hour survival of the animal was the only prerequisite for inclusion in the study.

The authors are in agreement with Padawer (1960) and found that, among thousands of living mast cells critically examined with the phase contrast microscope, not a single cell could unequivocally be referred to as being in mitosis.

The mast cell is fragile and easily cytolizes or degranulates following the mild trauma such as that due to chemical and physical agents. The osmotic pressures of the solutions tested in this study were within a 223 to 355 mOsm./liter with the exception of the saponin, 665 mOsm./liter. Making an exception of saponin it is not usual for free peritoneal mast cells seen in normal rats to undergo osmotic destruction or degranulation because of the above drugs. The hydrogen ion concentration of the solutions tested in this study were within a pH 4.0 to 5.0 with the exception of 0.8% normal physiologic saline, pH 6.8.
Padawer (1960) reported that the intraperitoneal injection of saline acidified to pH 4.5 with 0.2% acetic acid did not produce any effect on the mast cells.

The mechanism producing the morphological alterations after administration of colchicine, its congeners, and the other mast cell-damaging agents is not clear. Padawer (1960) suggested that there is a single process intimately linked to the sol-gel status of the cell. Therefore, derivatives of colchicine have been extensively studied in the hope of finding an antimitotic and interfering mechanism. However, Padawer et al. (1956) reported that mast cells are highly polymorphic when subjected to high hydrostatic pressure in the peritoneal fluid of young female rats which had been treated with colchicine. He attributed the polymorphism to the effect of colchicine on the cytoplasmic viscosity.

REFERENCES


Fig. 1. Free peritoneal cells treated with colchicine. An affected mast cell is visible. Phase contrast. \( \times 430 \).

Fig. 2. Free peritoneal cells treated with colchicine. Highly deformed mast cells and leucocytes are visible. Phase contrast. \( \times 430 \).

Fig. 3. Free peritoneal cells treated with colcemid. Several elongated mast cells are visible. Phase contrast. \( \times 430 \).

Fig. 4. Free peritoneal cells treated with colcemid. An affected mast cell with a displaced nucleus and a nodal constriction is visible. Phase contrast. \( \times 970 \).

Fig. 5. Free peritoneal cells treated with colcemid. An elongated mast cell with two nodal constrictions and an ectoplasmic extension is visible. Phase contrast. \( \times 970 \).

Fig. 6. Free peritoneal mast cells treated with colchicine. Several affected cells show highly polymorphic changes. Phase contrast. \( \times 970 \).