Histological Studies on the Degranulation of Mesenteric Mast Cells of the Rat by Water Extracts of Ginseng[*]

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ABSTRACT

Ever since Fujidani made his report in 1905, many workers have studied the chemical components of Panax Ginseng and their effects on depression of blood pressure. Riley (1952) and other workers have demonstrated the degranulation of mast cells in experimental animals treated with some histamine liberators, and the existence of a histamine liberator in the water extract of ginseng has been demonstrated by pharmacological assay by Lee et al (1960).

This present study was intended to observe the disruption and degranulation of mesenteric mast cells of rats administered the water extract of ginseng, which might contain the histamine liberator. Variable doses of the water extract were injected intraperitoneally, and the degranulation of mesenteric mast cells was histologically demonstrated by means of toluidin blue, Giemsa, May-Grünwald and Wright's stains.

Degranulation began in the experimental group given 4 ml of the extract mixed with 16 cc. of Tyrode solution; the severity of degranulation increased probably with the dose of the extract, and extreme degranulation took place in the groups injected with doses of 6 or 8 ml of the extract.

Several reports have reported on the chemical components and especially on the hypotensive effects of ginseng extract. Fujidani (1905) demonstrated depression of blood pressure in dogs and cats injected intravenously with ginseng alcohol extract. The inhibitory effect of ginseng ether extracts on the cardiovascular system was noted by Sakai (1915) in rabbits. Yonekawa (1926) reported on the transient and the peristent hypotensive action of a glycoside of ginseng extract. The ether extract and the saponin preparation of ginseng were observed by Kim (1931) to depress blood pressure and to increase the respiratory rate in rabbits, which is probably due to a central paralytic action.

Lee (1939) reported that in tadpoles the water extract of ginseng stimulated their growth and retarded their metamorphosis.

Lee et al. (1960) reported the existence of a histamine liberator in the water extract of ginseng and described its effects on blood pressure and capillary permeability.

Experiments on the degranulation of mast cells have been studied by Riley (1953, b) and by other workers with histamine liberators other than that in ginseng extract. Riley and West(1953)demonstrated a decrease in extractable histamine and in the contents of mast cells in rat mesenteries treated with chemical histamine liberators, namely, by stilbamidine and d-tubocurarine.

Fawcett (1955) reported that, in rats which were injected with distilled water and with a potent histamine liberator,compound 48/80, intraperitoneally,

the occurrence of degranulation in the mesenteric mast cells and the subsequent regeneration of the mast cell were followed by the disruption of the cell.

Based upon the above-mentioned research work, this study has been carried out to demonstrate the disruption and degranulation of mesenteric mast cells in rats by means of water extracts of ginseng containing a histamine liberator.

MATERIALS AND METHODS

Used for this study were 350 gm of dried Panax Ginseng (so called Baik-sam), officially guaranteed as to for quality. The animals used in this experiment were eighty well-developed mature albino rats weighing approximately 200 gm, 40 males and 40 females. Water extracts of ginseng were obtained by the following procedure. Water mixtures with ground pieces of ginseng were placed in a 95° C water bath for 24 hours and then filtered and concentrated so that 1 ml of the product would contain 1 gm of dried ginseng. The solution to be injected was prepared by adding varying doses of the water extract of ginseng i. e., 0.5 ml, 1 ml, 2 ml, 4 ml, 6 ml, and 8 ml added to Tyrode solution, the total volume of each mixture being 20 ml.

The solution obtained by the above procedure was injected intraperitoneally under ether anesthesia, and six hours later, the animals were killed by means of air embolism. Pieces of mesentery taken from the sacrificed animals were carefully spread over clean slides and fixed with absolute methyl alcohol for 10-20 minutes and then stained with Giemsa, Wright's, May-Grunwald and toluidin blue stains.

For the control group, Tyrode solution and distilled water were used instead, injecting 20 ml respectively.

The method of Miles and Miles (1952) was adopted to evaluate the potency of the water extract of ginseng as to the histamine liberator. Thus, to rats with an area on their back cleanly shaved off on the previous day, 10 mg of trypan blue was given intravenously. Then physiological saline solution, histamine phosphate and the water extract of ginseng, 0.1 ml each, were injected intradermally

in the previously prepared sites ten minutes before sacrifice. On dissection of the animals, it was noted that the severity and size of the blue spots appearing on the inner surface of the skin sites produced by the water extract of ginseng were about equal in degree to that produced by histamine phosphate.

OBSERVATIONS

1. The control group given 20 ml of Tyrode solution:

The peritoneum and mesentery of these animals yielded little noticeable change in comparison with normal intact rats, except for sporadic extracellular localization of metachromatic granules around a few mast cells. This degranulation is presumably attributed to senile degeneration of the cells.

2. The group given hypotonic solution, i.e., distilled water:

As a whole, the osmotic disruption of mast cells and a mild degree of change in the other mesenteric connective tissue cells were observed. An extensive dispersion of extracellular metachromatic granules was visible around the cells as a result of osmotic disruption.

The red-staining zone around disrupted mast cells with May-Grunwald and Giemsa stains, as noted by Fawcett (1955) in a similar experiment, was not always seen.

Fibroblasts appeared to be somewhat swollen in a lowered osmotic atmosphere, but no withdrawal (retraction) of cytoplasmic processes or cytoplasmic rounding up was visible.

Destruction due to osmosis in a hypotonic atmosphere was encountered in many of the tissue eosinophils of the mesentery, so that their definite recognition was difficult.

It was evident that the membrane of mast cells was more fragile than that of other mesenteric connective tissue cells. While it is generally reported that the mast cell granules are water soluble, extracellular granules were not dissolved during the experimental period of six hours, an a characteristic metachromatism of extracellular granule appeared almost the same as intracellular ones. Some of the granules were seen to be phagocytosed by neighboring macrophages. There was no change

in the staining qualities or in the shape of these phagocytosed granules seen in this experimental period of six hours.

Some mast cells, excepting the disrupted ones, remained intact in the perivascular areas of the large intestisnl arcades and also in the vicinity of the deep core of mesenteric fatty tissue.

3. The group given 0.5 ml of the water extract of ginseng mixed with 19.5 ml of Tyrode solution, and the group given 1 ml of the water extract of ginseng mixed with 19.0 ml of Tyrode solution:

These groups were little different from the ones given 20 ml of Tyrode solution, and the majority of mast cells remained intact. There was no change in the relative variability of numbers and cytology of tissue eosinophils, nor was there any marked difference in the shapes of fibroblasts and macrophages from that seen in the previous experimental groups.

4. The group given 2 ml of the water extract of ginseng mixed with 18 ml of Tyrode solution, and the group given 4 ml of the water extract of ginseng mixed with 16 ml of Tyrode solution:

In the first of these two groups there was very little sporadic appearance of mast cell disruption and degranulation and no clear difference was recognizable from the preceding group in paragraph 3. In the second of these two groups, however, the disruption and degranulation of mast cells occurred to some extent, and a tendency toward degranulation was clearly observable. To demonstrate a "red-staining zone" appearing around the disrupted mast cells was extremely difficult, and thus the shape, size and staining qualities of extracellular granules were little different from those of intracellular ones.

Little difference from the former was found in the numbers and the morphology of the tissue eosinpohils and no change in the staining qualities and the shapes of the eosinophils in the vicinity of disrupted mast cells was seen.

No swelling occurred in fibroblasts. Maerophages found near the disrupted mast cells were seen with phagocytosed mast cell granules. The phagocytosed granules were of almost the same shape and staining qualities as the intracellular granules of mast cells·

5. The group given 6 ml of the water extract of ginseng mixed with 14 ml of Tyrode solution, and the group given 8 ml of the water extract of ginseng mixed with 12 ml of Tyrode solution:

In both of these groups, diffuse degranulation appeared due to severe disruption of mast cells, which was clear evidence of the degranulating effect of the water, extract of ginseng on mast cells. A similarity to the group treated with 4 ml of the water extract of ginseng mixed with 16 ml of Tyrode solution was found in that very few mast cells remained intact alongside the disrupted ones. The intact mast cells remaining in the deep core of the fatty tissue and in the areas adjacent to the deep intestinal arcades gave no different a picture from the group treated with hypotonic solution. The "red-staining zone" described to appear around the disrupted mast cells in the group given hypotonic solution was hardly recognizable in these groups. As was the case with the preceding group, no change was found in the numbers, shape and staining qualities of tissue eosinophils. One recognizable difference from the group given hypotonic solution was the appearance of many intact tissue eosinophils close to the disrupted mast cells.

Fibroblasts appeared normal, and the macrophages nearby phagocytosed the extracellular granules extruded from the disrupted mast cells. The phagocytosed granules appeared to undergo little change from the intracellular granules, probably because there was not enough time to give rise to any appreciable degeneration or intracellular digestion.

DISCUSSION

Of the various actions of the chemical components extracted from ginseng, the hypotensive action has been studied by Fujidani (1905), Sakai (1915), Kim (1931), Lee et al. (1960) and by others, particularly, the action of the water extract of sinseng as a histamine liberator was established by Lee et al. (1960). Lee (1936) found that the water extract of ginseng accelerated the growth of the tadpole while inhibiting its metamorphosis.

Degranulation of mast cells by histamine liberators

has been noted specifically by MacIntosch and Paton (1949) with peptone or a chemical histamine liberator, by Riley and West (1953) with stilbamidine and d-tubocurarine, by Paff and Margenthaler (1953) with protamine sulfate and by Fawcett (1955) with compound 48/80. On the other hand, the same phenomenon has been demonstrated with many non-histamine liberators Such as bacteria by Fahr (1905), egg albumine and carbon particles by Webb (1931), benzol by Sylvén and Larsson (1948), snake venom by Zahl and Nowak (1951), and cortisone by Cavallero and Braccini (1951) etc. These non-histamine liberators, while bearing no chemical similarity to each other, are commonly known to give rise to degranulation of mast cells.

It can be readily assumed that the mast cell membrane is affected by hypotonic solution, by aqueous fixatives, and by the various steps in embedding and sectioning to a far greater degree than other connective tissue cell membranes, so that the mast cell membrane is more easily disrupted.

Devitt et al. (1954) claimed that the physiological secretion or function was not solely responsible for mast cell degranulation, and degranulation might be attributed to degenerative phenomena, as claimed by Fawcett (1955). Asboe-Hansen (1950, 1952) inferred that mast cells contribute to the elaboration of the hyaluronic acid of ground substance, when he demonstratetd disappearance of mast cells at the site of hyaluronidase injection. Fawcett (1955) maintained that mast cell degranulation was caused by osmotic disruption in rat mesenteries treated with hypotonic solutions, including distilled water. But he concluded that the same phenomenon appearing in rat mesenteries treated with the potent histamine liberator in compound 48/80 was attributable to the specific activity of the compound.

We observed a tendency toward degranulation of mast cells in the group injected intraperitoneally with 4 ml of the water extract of ginseng mixed with 16 ml of Tyrode solution, and we observed diffuse degranulation of mast cells in the group given 6 ml of the water extract of ginseng mixed with 14 ml of Tyrode solution and in the group given 8 ml of the water extract of ginseng mixed

with 12 ml of Tyrode solution.

The osmotic pressures of the solutions used in this experiment were: Tyrode solution was 295 osmols, compared with the 300 osmols of physiological saline solution; 0.5 ml of the water extract of ginseng mixed with 19.5 ml of Tyrode solution gave 313 osmols; and 8 ml of the water extract of ginseng mixed with 12 ml of Tyrode solution, gave 355 osmols. The osmotic pressures of the above solutions are not known to give rise to osmotic disruption of mast cells.

Therefore, it is deduced that there exists a component in the water extracts of ginseng which can induce mast cell degranulation. Concerning the extracellular mast cell granules, some of them were phagocytosed by neighbouring macrophages in this short experimental period of six hours, and no distinct changes in staining gualities and shape of the phagocytosed granule were observed. However, Fawcett (1955) noted, in a similar experiment with distilled water and a potent histamine liberator, compound 48/80 that the phagocytosed granules showed a characteristics staining quality and shape, then were fused into large acidophilic granules or droplets 24 hours later; intracellular digestion was followed by disappearance in 72 hours.

Paff and Margenthaler(1955) noted that liver mast cells immersed in running water for 24 hours remained intact and were easily stained by methylene blue thereafter. Fawcett (1955) demonstrated that extracellular granules of disrupted mast cells caused by distilled water remained unresolved for several hours in spite of the general relief that mast cell granules are water-soluble.

We reached a similar conclusion in that extracellular mast cell granules could withstand circumstances that disrupted mast cells, such as hypotonic solution or distilled water. The "red-staining zone" appearing around disrupted mast cells with Giemsa and May-Grunwald stains, as described by Fawcett (1955) and others, was visible only in the group treated with distilled water. This "red-staining zone" was probably due to local changes in staining qualities of the ground substance by substances secreted from disrupted mast cells by

distilled water.

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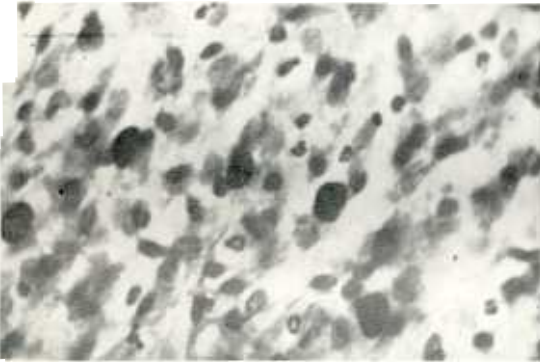
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Fig. 1. Mesentery of the rat 6 hours after intraperitoneal injection of 20 ml of Tyrode solution. Mast Cells are intact. Giemsa stain (× 400).
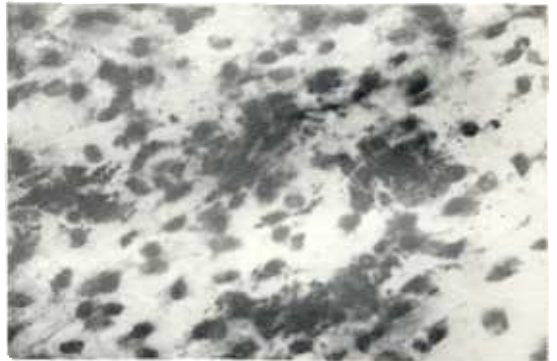

Fig. 2. Mesentery 6 hours after intraperitoneal injection of 20 ml of distilled water. Mast cells are ruptured releasing their granules. Giemsa stain (× 400).
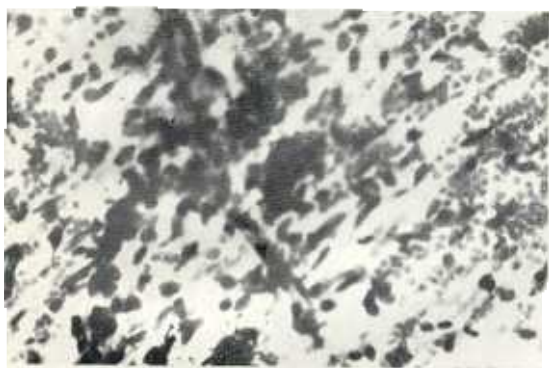
Fig. 3. Mesentery 6 hours after intraperitoneal injection of 4 ml of the water extract of ginseng mixed with 16 ml of Tyrode solution. 3 mast cells are degranulated. Giemsa stain (× 400).
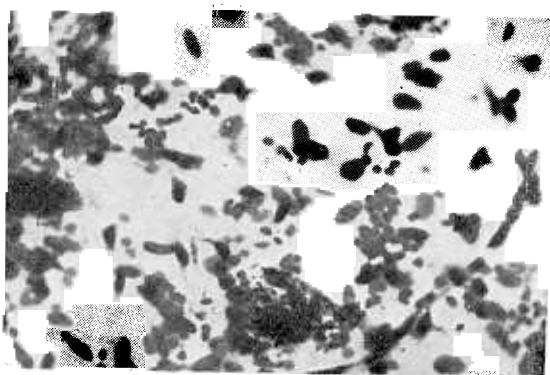

Fig. 4. Mesentery 6 hours after intraperitoneal injection of 6 ml of the water extract of ginseng mixed with 14 ml of Tyrode solution. Degranulated mast cells are visible. Giemsa stain (× 400).

Fig. 5. Mesentery 6 hours after intraperitoneal injection of 8 ml of the water extract of ginseng mixed with 12 ml of Tyrode solution. Several mast cells are disrupted and degranulated. Giemsa stain (× 400).