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# Mast Cell Degranulation with Special Reference to the Effect of a Saponin Extract of Ginseng upon the Mesenteric Mast Cell of Albino Rats

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## *ABSTRACT*

Water extract of dried ginseng, which is known as a histamine liberator and induces degranulation and disruption of mesenteric mast cells, is thought to contain many different chemical factors. The essential component, a saponin extract of dried ginseng, was obtained and administered to albino rats. Even minute amounts (1 mg in 0.01 cc of normal saline solution) when locally injected into the mesentery of albino rats caused degranulation of mesenteric mast cells. Degranulation of mesenteric mast cells followed the intraperitoneal injection of a crude water extract, of an alcohol extract of dried ginseng, and a direct injection of both extracts into the connective tissue of mesentery. This degranulation is believed to be a saponin fraction of ginseng in the ginseng extracts.

## *INTRODUCTION*

Since tissue mast cells were first described by Ehrlich (1879), numerous experimental studies concerning the effects of hormones, chemicals and stresses on mast cells have been carried out in the fields of cytology, cytogenesis, and cytochemistry.

Experiments on the degranulation of mast cells have been presented by Riley and other workers (1953) using histamine liberators. Riley and West (1953) demonstrated a decrease in extractable histamine in rat mesenteries which was accompanied by a disappearance of mesenteric mast cells following the addition of chemical histamine liberators, namely, stilbamidine and d-tubocurarine.

Fawcett (1955) reported that rats, in which distilled water and a potent histamine liberator or compound 48/80 were injected intraperitoneally, showed degranulation and disruption in the mesenteric mast cells. The subsequent regeneration of mast cells was followed by disruption of the cell.

Smith (1962) using electron microscopy studied tissue mast cells in various animals and noted that histamine liberators cause dramatic vacuoles about the granules with a breakdown of the granular membrane and release of the intact granule from the edge of the cell.

Oh, et al. (1962) postulated that the disruption and degranulation of mesenteric mast cells in albino rats which had received the water extract of ginseng intraperitoneally which be due to a histamine liberator.

The present investigation was made to determine the active fraction of the extract of ginseng which causes the degranulation and disruption of mesenteric mast cells in rats.

## *MATERIALS AND METHODS*

Dried Panax Ginseng (so called Baik-Sam) which is officially guaranteed for quality was used for this study. A water extract of dried ginseng was prepared using the method described by Oh et al. (1962). The alcohol extract of dried ginseng was prepared by placing an alcohol mixture containing ground pieces of ginseng into a boiling water bath for 6 hours. The mixture was then filtered and concentrated so that 1 ml of the product contained 1 gm of dried ginseng. The saponin extract was

prepared as follows; 95% ethyl alcohol mixture containing 400 gm of ginseng particles was placed into a boilng water bath for 3 to 5 hours and then filtered and concentrated. This procedure was repeated again. The concentrated extract was dissolved in 95% ethyl alcohol, precipitated with ether and filtrated. The precipitate was dissolved in 95% ethyl alcohol and the above precipitation procedure was repeated 3 times. The precipitate in the cellophane bag was then dialysed for 14 days, dissolved in 95% ethyl alcohol, and again precipitated with ether. Finally, after evaporation of the ether, about 400 mg of a yellowish-white precipitate was obtained.

The water extract and alcohol extract of dried ginseng, and a pure carbohydrate fraction of the alcohol extract were diluted with normal saline solution. In each extract 1.0 cc of the solvent contained 1 gm of dried ginseng. The saponin fraction extracted from dried ginseng, was also diluted with normal saline solution. Two solutions of saponin extract were prepared: one, used for direct injection into mesentery, contained 1 mg of saponin in 0.01 cc of the solvent and the other, used for the intraperitoneal injection, contained 80 mg of saponin in 20 cc of Tyrode solution. The 80 mg saponin extract contained 8 gm of dried ginseng which caused severe reaction in the mesenteric mast cells of albino rats with disruption and degranulation after intraperitoneal injection of the water extract.

The animals used in this experiment were well-developed mature albino rats weighing approximately 200 gm 25 males and 15 females. Each experimental group contained 5 rats. Under light ether anesthesia the abdominal cavities of rats from groups one to five were opened using an anterior median incision followed by direct injection of 0.01 cc of water extract for the 1st group and the alcohol extract for the 2nd group into the mesentery near the ileocecal region. Using the same procedure we injected 0.01 cc of a pure carbohydrate fraction into the rats of the 3rd group. The rats of 4th group The rats of 4th group received 0.01 cc of saponin solution and the 5th group, the control, received 0.01 cc of normal saline solution.

In order to compare our results with others the 6th group of rats were given 20 cc of saponin solution intraperitoneally. Rats of the 7th group used as a control for group 6 received 20 cc of Tyrode solution intraperitoneally. Six hours later these animals were killed by air embolism.

Pieces of the injected site of the mesenteries from groups one through six were taken from the sacrified animals, carefully spread over clean slides. fixed with absolute methyl alcohol for 10 to 20 minutes, and then stained with toluidin blue, Wright's solution and Pugh solution used by Leblanc and Rosenberg (1957).

## *OBSERVATIONS*

1. The 1st group was given 0.01 cc of water extract directly into the ileocecal mesentery:

At or near the injected site of the ileocecal mesentery many mast cells showed the morphological changes of degranulation of metachromatic mast cell granules and disruption of the mast cells which in some cases was accompainied by a diffuse dispersion of the metachromatic granules of mast cells extracellularly around mast cells site. As the distance from the injected site increased, the damage to the mast cells decreased until no damage was noted in the intact mesentery.

Already 6 hours after the injection macrophages around the damaged mast cells showed an active phagocytic reaction against the exteracellular metachromatic granules. Extracellular metachromatic granules which had not been phagocytosed by the macophages were sporadically scattered through out the mesenteric connective tissue.

2. The 2nd group was given 0.01 cc of alcohol extract of it into directly the ileocecal mesentery:

In this experimental group the mast cells of injected site of mesentery showed less damage than was seen in the former group. However, degranulation and disruption of mast cells were found near the injected mesenteric sites and some extracellular metachromatic granules were phagocytosed by neighboring macrophages.

3. The 3rd group was given 0.01 cc of a pure carbohydrate fraction of alcohol extract directly into the ileocecal mesentery;

The animals of this group showed no morphological change in the mesenteric mast cells. This suggests that the pure carbohydrate fraction of alcohol extract does not contain any active agent producing degranulation and disruption of the mesenteric mast cells.

4. The 4th group was given 0.01 cc of saponin extract directly into the ileocecal mesentery:

The animals of this group were given a small amount of saponin extract, which is known to be one of the main active components of ginseng and showed the cytological change of degranulation of the mesenteric mast cells. 6 hours after the injection macrophages around the injected site showed an active phagocytic reaction against extracelluar metachromatic granules.

5. The 5th group, the control, was given 0.01 cc of normal saline solution directly into the ileocecal mesentery:

The animals showed no cytological change in the mesenteric mast cells of the injected site compared with the intact mesenteric mast cells except a very few extracellular metachromatic granules near the mesenteric mast cells.

6. The 6th group was given 20 cc of saponin extract intraperitoneally:

The animals of this group showed clear evidence of degranulation of the mesenteric mast cells after the injection. Some of the damaged mesenteric mast cells appeared to disruption.

However, the severity of the damage of the mast cells was decreased compared with the result obtained by Oh et al. (1962). Macrophages near the damaged mesenteric mast cells showed an active phagocytic reaction.

7. The 7th group, the control of the 6th group, was given 20 cc of Tyrode solution intraperitoneally:

The mesenteric mast cells of these animals yielded litle noticeable change in comparison with the mast cells of normal rats, except for sporadic extracellular localization of metachromatic granules around a few mast cells.

## *DISCUSSION*

The action of the water extract of ginseng as a histamine liberator was established by Lee et al. (1960). Lee (1936) found that this water extract of ginseng also accelerated the growth of the tadpole while inhibiting its metamorphosis.

Degranulation of mas cells by histamine liberators has been noted specifically by MacIntosch and Paton (1949) using peptone or a chemical histaimine liberator. Riley and West (1953) used with stilbamidine and d-tubocurarine, Paff and Margenthaler (1953) used protamine sulfate, and Fawcett (1955) used compound 48/80. On the other hand, the same phenomenon has been demonstrated following the use of many non-histamine liberators such as bacter ia by Fahr, (1905), egg albumin and carbon particles by Webb (1931), benzol by Sylven and Larsson (1948), snake venom by Zahl and Novak (1951), and cortisone by Cavallero and Bracchini (1951) etc. These nonhistamine liberators, while bearing no chemical similarity to each other, commonly cause degranulation of the mast cells.

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

Asboe-Hansen (1950, 1952) inferred that mast cells contribute to the elaboration of the hyaluronic acid of ground substance, after he demonstrated the disappearance of mast cells at the site of hyaluronidase injection. Fawcett (1955) maintained that in rat mesenteries treated with hypotonic solution, including distilled water, the mast cell degranulation was caused by osmotic disruption. However, he concluded that the same phenomenon appearing in rat mesenteries treated with the potent histamine liberator in compound 48/80 was due to the specific activity of the compound.

Oh et al. (1962) observed the degranulation of

mesenteric mast cells of rats injected intraperitoneally with 4 ml of water extract of ginseng mixed with 16 ml of Tyrode solution, thus proving that the degranulation of mesenteric mast cells was not due to hypotonicity of the injected solution.

Paff and Margenthaler (1955) noted that liver mast cells immersed in running water for 24 hours remained intact and thereafter were easily stained by methylene blue. Fawcett (1955) demonstrated that the mesenteric extracellular grannles of disrupted mast cells placed in distilled water remained undissolved for several hours, despite the general belief that mast cell granules are water-soluble.

The authors prepared a saponin extract of ginseng, which is a relatively essential component of ginseng, injected a minute amount into a rat's mesentery and observed degranulation and disruption of the mesenteric mast cells. Intraperitoneal injection of the extract produced similar changes. By this experiment the authors deduce that a water or alcohol extract of ginseng, contains the saponin fraction, induced degranulation and disruption of mesenteric mast cells of rats.

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## *REFERENCES*

Asboe-Hansen, G.: *Ann. of Rheumatic Disease 9:149, 1950.*

Asboe-Hansen, G: *Proc. Soc. Exp. Biol. and Med. 80: 677, 1952.*

Cavallero, C. and Bracchini, C.: *Proc. Soc. Exp. Biol. and Med. 78: 141, 1951.*

Ehrlich, P.: *Arch. u. Anat.u. Physiol., Physiol. Abt. 3: 571, 1879.*

Fahr: *Virchows Arch. 179: 450, 1905.*

Fawcett, D.: *Anat. Rec. 121: 29, 1955.*

Lee, P. H.: *Journal of Chosen Medical Association 6:4, 1936.*

Lee, W. C., Chang, W. S. and Lee, S. K.: *The New Medical Journal 3: 5, 1960.*

MacIntosch, F. C. and Paton, W. D. M.: *J. Physiol. 109: 190, 1949.*

Oh, Y. K., Pak, S. Y. and Choi, K. D.: *Yonsei Medical Journal 3: 5, 1962.*

Paff, G. H. and Margenthaler, D. D.: *Anat. Rec. 121: 579, 1955.*

LeBlanc, J. and Rosenberg, F.: *Proc. Soc. Exp. Biol. and Med. 96: 234, 1957.*

Riley, J. F.: *J. Path. Bact. 65: 471, 1953.*

Riley, J. F. and West, G. B.: *J. Physiol. 120: 528, 1953.*

Shelley, W. B.: *Arch. Dermat. 86, 2: 138, 1962.*

Sylven, B. and Larsson, L.G.: *Cancer Rec. 8: 449, 1948.*

Webb, R. L.: *Am. J. Anat. 49: 283, 1931.*

Zehl, P. A. and Novak, A.: *J. Morph. 89: 135, 1951.*