Effect of Cortisone and X-ray Irradiation on Mast Cells of the Adrenalectomized Albino Rats

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ABSTRACT

The effects of cortisone administration and whole body irradiation by X-ray upon mesenteric mast cells of intact and adrenalectomized albino rats were studied.

In intact rats, the administration of cortisone (50mg./kg) and whole body irradiation by X-ray (800r) caused severe degranulation and disruption of mesenteric mast cells within 24 hours. However their degranulation and disrupting effects upon mesenteric mast cells were markedly inhibited after the removal of the adrenal gland. Although the adrenalectomy alone hardly caused these morphological changes of mesenteric mast cells of the albino rats.

According to this experiment it is fairly clear that the effects of cortisone and whole body X-ray irradiation inducing degranulation and disruption of mesenteric mast cells of the albino rats, were not direct phenomena but they indirectly affected the mesenteric mast cells through some special mechanism mediated by the adrenal gland.

INTRODUCTION

The effects of cortisone on the mast cells of experimental animals has been recently studied by many authors.

Asboe-Hansen (1950) demonstrated a decrease in number of mast cells in connective tissue accompanied with degranulation and disruption of mast cells in hamsters, rabbits, mice and guinea pig treated with cortisone. Cavallero and Braccini (1951) demonstrated a decrease in number of mast cells of the skin and the heart tissue, and a decrease of hyaluronic acid level in the connective tissue treated with cortisone in the rat. Fulton and Maynard (1953) observed that a decrease in number of the tissue mast cells, discharge of the stainable metachromatic granules and a reduction in blood clotting time had occurred after cortisone injection in the hamster. Stuart and Kligerman (1951), Smith and Lewis (1954), Lee (1959), Kim (1960), Oh (1961) and An (1964) reported that in experimental animals, cortisone injection had induced the disruption and degranulation of mast cells. Foley and Glick (1962) reported that histamine liberation and morphological changes of the mast cells had occurred after administration of histamine-liberator, compound 48/80 and cortisone.

The effect of X-ray irradiation on the mast cells of experimental animals had been studied by many authors. Murray (1948), Bloom (1954), Crabb and Kelsall (1957) and An (1964) observed that whole body irradiation of X-ray on experimental animals caused severe disruption and degranulation of the mast cells. Maynard (1955) reported that the more severe destruction had occurred on the 4th day after whole body irradiation of X-ray in the hamster. According to Smith and Lewis’s report (1953), the most severe destruction and degranulation had occurred on the 10th day after X-ray irradiation in the hamster.

While Devitt et al. (1953) reported that some significant numerical changes of mast cells in the
mesentery were observed but the heart and the skeletal muscle had not been affected in the rats treated with cortisone or thyroxin. Baker et al. (1951) also demonstrated that the injection of ACTH did not cause any morphological changes or numerical fluctuation of mast cells in the thymus.

Murray (1948) reported that the number of splenic mast cells increased after infusion of strontium 89 in the rat. Kelsall and Crabb (1952) also reported that the number of mast cells in the thyroid and parathyroid increased after X-ray irradiation in the hamster.

As mentioned above it was learned that under the treatment of cortisone and whole body X-ray irradiation on experimental animals, the cytological and the numerical changes of mast cells were still controversial.

The effect of adrenalectomy upon mast cells of experimental animals has been studied by many authors. Padawer (1954) reported that the adrenalectomy caused the decrease of the diameter of the floating mast cells in peritoneal fluid and hypophysectomy effected the increase of it in length and the alteration of its shape. Speirs (1955) reported that adrenalectomy caused reduction in number of the floating mast cells in peritoneal fluid.

Raesaenen (1961) reported that the administration of ACTH in intact rats caused almost complete degranulation of the mucosal mast cells and its degranulating effect was inhibited after an adrenalectomy.

The present investigation was made to determine the effects of cortisone and X-irradiation to induce degranulation and disruption of mesenteric mast cells in the albino rats, directly or indirectly through an adrenal gland or an unknown special mechanism of the adrenal gland.

**MATERIALS AND METHODS**

140 mature albino rats, allocating 5 for each group, were used for this study. These animals were divided into the control groups treated with cortisone and X-irradiation and the experimental groups received the operation of adrenalectomy prior to treatments of cortisone and X-irradiation.

The rat is lightly anesthetized with ether, the back skin, just below the 12th rib, is shaved, prepared and draped for a dorsal approach of the retroperitoneal space. A transverse incision is made just below the 12th rib and the erector spinae muscle is vertically split, the retroperitoneal adrenal glands exposed and both glands were excised one by one completely without leaving any tissue. When the experimental rats were sacrificed, a search was made for any remaining adrenal tissue.

Adrenalectomized rats were available for use in this experiment two weeks after the operation. In the post-operative period, 1% saline solution instead of tap water, ad libitum, was supplied to the adrenalectomized rats in order to compensate for the loss of sodium.

In one of the control groups, 50 mg./kg of cortisone (cortisone acetate, saline suspension 25mg./cc, 11-dehydro-17-hydroxy-corticosterone-21-acetate) was given intramuscularly under the light ether anesthesia into the thigh muscles of the rat. In the others of the control groups, with 250 kvp General Electric Therapeutic Units (technical factors: 220 kvp, 15 Ma, Focus-Object Distance 50 cm, using Thoraex filter II(0.4 mm Sn., 0.25 mm Cu. and 1 mm Al.) r/min., measured on the air 25 r) an 800 r whole body irradiation (50% of the lethal dose in the albino rat) was administered to the rats.

In the experimental groups, the rats were slaughtered by means of air embolism or decapitation and divided into the followings: the immediate group, the 3 hour group, the 6 hour group, the 12 hour group, the 24 hour group, the 2 day group and the 3 day group after treatment. In the immediate group, the mesenteries of the rats were obtained 10 minutes after treatment as mentioned above.

In the control and experimental groups, pieces of mesenteries taken from the sacrificed animals were carefully spread over clean slides and fixed with absolute methyl alcohol for 10 to 20 minutes and then stained with toluidine blue or Giemsa staining solution for examining the metachromatic
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granules. A count of 100 mesenteric mast cells selected at random were made with the high power magnification of 400 diameters.

The degrees of degranulation and disruption of mast cells were arbitrarily divided into 4 types; Intact (normal, Fig. 2), Grade I (slight degranulation, Fig. 3), Grade II (moderate degranulation, Fig. 4) and Grade III (marked or severe degranulation and disruption, Fig. 5) according to the classification adopted by An (1964).

In an adrenalectomized rat, the incidence of intact mesenteric mast cells did not differ from the normal rats that did not receive any experimental treatment or operation at all.

OBSERVATION

1. The control group given 50 mg./kg of cortisone; The effect of intramuscular injection of cortisone in about 40 experimental animals was shown in the Table I and Fig. I. In the immediate group, an acute decrease to 87.6±8.9(%) in incidence of intact mast cells was noted and the rest showed various degrees of degranulation and disruption of mast cells. In the 3 hour group, the incidence of normal mast cells decreased to 70.0±14.0(%), in the 6 hour group to 66.6±14.2(%), and in the 12 hour group, it reached the lowest value, 63.8±14.5(%). In the 2 day group after cortisone administration, such numerical and morphological fluctuations of mesenteric mast cells began to recover to the original status and in the 5 day group, intact mast cells increased to 90±9.0(%) and by 15 days after cortisone treatment, returned to an almost normal status, 95±6.5(%).

These findings indicated that cortisone injections have a disrupting or degranulating effect on mast cells.

Table 1. Effect of cortisone (50 mg./kg) on the mesenteric mast cell counts of the intact rat

<table>
<thead>
<tr>
<th>Time after Cortisone injection</th>
<th>No. of Degranulated &amp; Disrupted Mast Cells/100 of Mast Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Grade I</td>
</tr>
<tr>
<td>0 hour</td>
<td>87.6±8.9</td>
</tr>
<tr>
<td>3 hours</td>
<td>70.0±14.0</td>
</tr>
<tr>
<td>6 hours</td>
<td>66.6±14.2</td>
</tr>
<tr>
<td>12 hours</td>
<td>63.3±14.5</td>
</tr>
<tr>
<td>24 hours</td>
<td>69.5±13.5</td>
</tr>
<tr>
<td>2 days</td>
<td>86.6±10.2</td>
</tr>
<tr>
<td>3 days</td>
<td>85.0±10.4</td>
</tr>
</tbody>
</table>

m(%)±probable error(%).

Fig. 1. Incidences of normal mesenteric mast cells treated with cortisone injection and X-irradiation in the intact and the adrenalectomized rat.
cells associated with an increase in the number of atypical or irregular mast cells in cellular contour.

2. The control group treated with 800r of X-irradiation:

The effect of whole body X-irradiation on the mast cells of the albino rat is shown in Table II, Figure I. This group showed a little different pattern from the one given cortisone.

In this control group, there was, in the immediate group, only a slight decrease of normal mast cells to 94.0±7.1(%) accompanied with a slight degranulation.

Table II. Effect of X-irradiation (800r) on the mesenteric mast cell count of the intact rat

<table>
<thead>
<tr>
<th>Time after Cortisone inj.</th>
<th>No. of Degranulated &amp; Disrupted Mast Cells/100 of Mast Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>0 hour</td>
<td>94.0±7.1</td>
</tr>
<tr>
<td>3 hours</td>
<td>92.0±8.1</td>
</tr>
<tr>
<td>6 hours</td>
<td>83.3±11.2</td>
</tr>
<tr>
<td>12 hours</td>
<td>68.0±14.1</td>
</tr>
<tr>
<td>24 hours</td>
<td>64.0±14.4</td>
</tr>
<tr>
<td>2 days</td>
<td>90.0±9.0</td>
</tr>
<tr>
<td>3 days</td>
<td>91.2±8.5</td>
</tr>
</tbody>
</table>

(%)± probable error (%).

In the 3 hour group, the change of mast cells was not evident compared to former group. In the 6 hour group, the significant degranulation and disruption of mast cells was recognized and reached the value 83.3±11.2(%). This meant an increase of degranulation and disruption of mast cells. In the 12 hour and 24 hour groups, the incidence of intact mast cells was decreased to the values of 68.0±14.1(%) and 64.0±14.4(%) respectively and the severity of degranulation and disruption of the cells reached a maximal point. In the 2 day group, there was a tendency of recovery to a normal level showing an incidence of intact mast cells rising from 90.0±9.0(%) to the value of 97.0±5.2(%). Fifteen days after X-ray treatment, the incidence of intact mast cells had fairly well recovered back to a normal level.

3. The experimental group having an adrenalectomy prior to cortisone injection (50 mg./kg):

The effect of cortisone injection on the mesenteric mast cells of the adrenalectomized rat is shown in the Table III and the Figure I. Considerable difference from the control groups was found in the incidence of the various types of mast cells. However, no change in the staining ability of mast cells were observed. Hardly marked changes in the mast cells of the rat's mesentery were found even up to 12 hours after cortisone injection.

Table III. Effect of cortisone(50 mg./kg) on the mesenteric mast cell counts of the adrenalectomized rat

<table>
<thead>
<tr>
<th>Time after Cortisone inj.</th>
<th>No. of Degranulated &amp; Disrupted Mast Cells/100 of Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>0 hour</td>
<td>97.0±5.1</td>
</tr>
<tr>
<td>3 hours</td>
<td>95.6±6.2</td>
</tr>
<tr>
<td>6 hours</td>
<td>94.2±6.9</td>
</tr>
<tr>
<td>12 hours</td>
<td>92.6±7.8</td>
</tr>
<tr>
<td>24 hours</td>
<td>88.2±9.7</td>
</tr>
<tr>
<td>2 days</td>
<td>91.6±8.3</td>
</tr>
<tr>
<td>3 days</td>
<td>93.0±7.7</td>
</tr>
</tbody>
</table>

(%)± probable error (%).

In 24 hours, a slight degranulation began to occur in mesenteric mast cells and the incidence of intact mast cells was 88.2±9.7(%).

Comparing with the former control group given cortisone only, the significant difference was in the 12 hour and 24 hour groups as recognized in the incidence of intact mast cells or the grade of degranulation and disruption of mast cells.

In the 2 day and 3 day groups having had an adrenalectomy, the incidence of intact mast cells increased to 91.6±8.3(%) and 93.0±7.7(%) respectively and the tendency of recovery was recognized in these stages.

4. Experimental group having an adrenalectomy prior to X-irradiation:

The effect of X-irradiation on the mesenteric mast cells of the adrenalectomized rat is shown in Table IV, Figure I. No marked decrease of normal mast cells or increase of degranulated mast cells was recognized in the rat's mesentery of the group after X-irradiation except the 24 hour group after treatment which was associated with a slight decrease in the incidence of intact
mast cells to $87.2\pm10.0(\%)$. In the 2 day and 3 day groups, the incidence of intact mast cells increased to $97.2\pm4.9(\%)$ and $92.0\pm8.1(\%)$ respectively and the tendencies for recovery were recognized in these stages.

Table II: Effect of X-irradiation (800r) on the mesenteric mast cell count of the adrenalectomized rat

<table>
<thead>
<tr>
<th>Time after X-irradiation</th>
<th>No. of Degranulated &amp; Disrupted Mast Cells/100 of Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>0 hour</td>
<td>91.6±8.3</td>
</tr>
<tr>
<td>3 hours</td>
<td>--</td>
</tr>
<tr>
<td>6 hours</td>
<td>96.2±5.7</td>
</tr>
<tr>
<td>12 hours</td>
<td>96.8±5.3</td>
</tr>
<tr>
<td>24 hours</td>
<td>87.2±10.0</td>
</tr>
<tr>
<td>2 days</td>
<td>97.2±4.9</td>
</tr>
<tr>
<td>3 days</td>
<td>92.0±8.1</td>
</tr>
</tbody>
</table>

These findings indicate that the treatment of adrenalectomy prior to cortisone injection and X-irradiation acted as an inhibitory or protective factor on the degranulation and disruption of mesenteric mast cells of the rat.

**DISCUSSION**

The effect of cortisone administration on various organs or tissues, especially the numerical and morphological changes, have been discussed by many authors. Asboe-Hansen (1950), Cavallero and Braccini (1953), Stuart et al. (1951), Smith and Lewis (1954), Foley and Glick (1962), Lee (1959), Kim (1960), Oh (1961) and An (1964) reported that cortisone treatment altered the number or shape of mast cells in experimental animals. While Devitt et al. (1953) reported that no degenerative change of mast cells was demonstrable when the animals were treated with cortisone.

The effect of X-irradiation on mast cells of experimental animals has also been discussed by many authors. Smith and Lewis (1953) and Maynard et al. (1955) demonstrated that X-irradiation caused disruption and degranulation of mast cells. While Kelsall and Crabb (1952) have contended that no remarkable morphological changes in mast cells of the rat were seen after X-irradiation.

It is apparent that such appropriate doses of cortisone or X-irradiation are capable of causing the qualitative and quantitative fluctuation of the mesenteric mast cells of the albino rat. All these controversial reports and uncertainties regarding the relationship of cortisone and X-irradiation on them, has provided the impetus for this investigation.

Furthermore, as mentioned in the observations for the control groups, there was a significant or severe decrease of normal mesenteric mast cells, $63.3\pm14.5(\%)$ in the 12 hour group after a single injection of cortisone acetate (50 mg./kg), and also some sort of normal mesenteric mast cells in the 24 hour group after whole body X-irradiation.

While in the experimental group having an adrenalectomy prior to the cortisone injection and whole body X-irradiation, in both cortisone administered group and X-irradiated group, the degranulation and disruption of mesenteric mast cells was reduced or markedly unaffected in the absence of the adrenals.

Oh et al. (1962) and Pak et al. (1963) of this laboratory have studied the problem of degranulation and disruption of mesenteric mast cells in the albino rats with the water extract of ginseng and saponin itself which was probably known as a histamine liberator, and a mast cell degranulating or disrupting factor obtained from the water extract of ginseng and saponin itself.

An (1964) studied cortisone injection and X-irradiation in regards to the same problem in albino rats and a mast cell degranulating or disrupting factor was observed after the administration of cortisone and X-irradiation and also noted the chronological changes of mesenteric mast cells after these treatments in albino rats.

Concerning histamine and heparin liberation associated with mast cell disruption, Jorpes (1937) and Wilander (1939), and Riley and West (1933) reported that the degranulation and disruption of the rat’s mast cells induced experimentally was accompanied by the simultaneous secretion of heparin and histamine.
The mechanism of degranulation and disruption of mast cells due to compound 48/80 and antigen (horse serum, serum albumin etc.) was suggested by Hoegerberg and Uvanes (1952, 1957, 1958) and Uvanes (1955, 1969) to be due to the activation of an enzymatic process producing lysis of the mast cell membrane. The hypothetical enzyme was not isolated, but indirect evidence pointed to an enzyme identical with or related to phospholipase.

Two mast cell disrupting or degranulating factors of cortisone injection and X-irradiation used in this study did not, at least, take part in the mechanism of it directly. Therefore the possibility that the role of the adrenals as an intermediate activator to induce mast cell disruption has been emphasized. Although the authors found that the inhibitive action due to adrenalectomy in albino rats upon mast cell disruption was obtained, it showed considerable similarity to the results presented by Raasenenn (1961) who experimented using ACTH as a mast cell disrupting factor in albino rats after an adrenalectomy.

Acknowledgement: This investigation was supported by the research fund from the Graduate Training and Research Committee of Yonsei University College of Medicine.

REFERENCES

Fig. 2. Intact mesenteric mast cells (Normal) of the albino rat treated with adrenalectomy and X-irradiation, 400×, Giemsa stain.

Fig. 3. Slight degranulated mesenteric mast cells (Grade I) of the albino rat treated with cortisone, 400×, Giemsa stain.

Fig. 4. Moderate degranulated mesenteric mast cells (Grade II) of the albino rat treated with cortisone, 400×, Giemsa stain.

Fig. 5. Severe degranulated and disrupted mesenteric mast cells (Grade-III) of the albino rat treated with X-irradiation, 400×, Giemsa stain.