The Effect of Small Doses of Prednisolone on the Incidence of Subcutaneous Sarcomas Induced by 3-Methylcholanthrene in Virgin Female Swiss Mice

SHAFIQ A. QURESHI1, 2 AND HABIBUZ ZAMAN

Department of Pathology, Jinnah Postgraduate Medical Center, Karachi, Pakistan

Summary

The effect of small doses of prednisolone on the incidence of chemically induced subcutaneous sarcomas by 3-methylcholanthrene in virgin female Swiss mice was studied. Each animal received, in the middle of its back, a single s.c. injection of 2 mg of 3-methylcholanthrene dissolved in 0.3 ml of trioctanoin. Dosages of 0.083, 0.050, and 0.035 mg of prednisolone contained in 0.05 ml of normal saline were given by daily i.p. injections to all the test animals for 20 weeks. Each of the control animals received i.p. injections of 0.05 ml of normal saline daily for the same period. The animals were weighed at weekly intervals. Animals from the test group showed a 7% decrease in body weight from the 2nd to the 6th week. Later these animals gained in weight at the same rate as the control animals. This subsequent increase in weight in the prednisolone-treated animals was not related to any obvious evidence of edema. Three animals of the control group presented tumors at the end of the 6th week, whereas the 1st sarcoma in the test group was detected on palpation at the end of the 10th week. All tumors were allowed to grow for a further period of 2 weeks following their initial recognition. The respective animals were then sacrificed and sections of the masses examined under the microscope. The experiment was terminated at the end of 20 weeks. All of the 23 surviving control animals developed tumors by the end of the 16th week. Thus there was a 100% yield of tumors in the control group of animals. On the other hand, only 20 of the 35 surviving test animals developed tumors, so the yield of tumors in the test group of animals was only 57.1%. This highly significant inhibitory effect of prednisolone on tumorigenesis was apparently reflected in the size and histologic appearances of the tumors. The tumors in the prednisolone-treated group were smaller and appeared histologically less malignant.

Introduction

The action of cortisone on the growth of experimental tumors has been very widely studied. Heilman et al. (11) appear to be the only authors who have reported the complete inhibition of an established tumor. Sugiuira et al. (19), Stoerk (18), and Higgins et al. (12) report a temporary inhibition. Similar results were also obtained with a rhabdomyosarcoma (12), certain transplantable sarcomas (19), multiple malignant mastocytomas in a dog (6), and an ependymoma (8). Many investigators have reported that growth of normal as well as of neoplastic mesenchymal tissues is inhibited by large doses of either cortisone or hydrocortisone (3, 5, 15). The purpose of this study was to determine the effect of small doses of prednisolone (suspensions of 25 mg in 5 ml, supplied by Masing and Company, Ltd., Denmark) on the incidence of chemically induced subcutaneous sarcomas in mice and to note whether it inhibits, delays, or accelerates the growth of these tumors.

Material and Methods

ANIMALS. The experiments were performed on adult virgin female albino Swiss mice of the SW (Swiss-Webster) strain. The animals were kept in ordinary metallic cages in an air-conditioned room at a temperature of 25°–27°C, about 10–12 animals per cage. The age of the mice varied from 10–12 weeks at the beginning of the experiment. The animals initially weighed between 22 and 24 gm each. All the animals were fed on Rockland mouse diet and water ad libitum. Seventy-seven animals were used in all: 45 were treated with prednisolone and 32 served as controls.

PREDNISOLONE. The suspension of prednisolone (suspensions of 25 mg in 5 ml, supplied by Masing and Company, Ltd., Denmark) was diluted with physiologic saline so as to give 3 different dosages, viz., 0.083, 0.050, and 0.035 mg in 0.05 ml saline solution for daily i.p. injections into the animals of the test group for 20 weeks. Likewise, animals of the control group were given 0.05 ml of physiologic saline solution by daily i.p. injections until the termination of the experiment at the end of the 20th week. The 1st injection of prednisolone was given 24 hr prior to the injection of the carcinogenic agent.

CARCINOGEN. 3-Methylcholanthrene (manufactured by Eastman Kodak Company) was dissolved in trioctanoin so that 0.3 ml of the solution contained 2 mg of the carcinogen. This amount was given to each animal of both groups by a single s.c. injection at about the middorsal line just above the tail.

The animals were weighed once every week. From the 3rd week onward the animals were carefully examined twice a week by palpation of the back. The presence of any tumor was recorded. Whenever the tumor progressed moderately the animals were sacrificed and the tumors were removed, cut into thin...
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slices, and fixed in 10% formalin. The maximum dimensions and approximate volumes of the individual tumors were determined. The average volume of the tumors of each group was calculated to serve as a basis for a rough comparison. When an animal having no apparent tumor died accidentally, a piece of skin with subcutaneous tissue was taken from the area where the s.c. injection of 3-methylcholanthrene had been given. This was fixed and studied in the same manner as the sections of the tumors. At least 3 sections of each tumor were examined after staining with the hematoxylin-eosin and trichrome stains (Van de Grift's modification). The lesions were classified as fibrosarcomas or rhabdomyosarcomas.

Observations and Results

**EFFECT OF PREDNISOLONE ON BODY WEIGHT.** At the beginning of the 1st week, the average body weight of the animals in both groups was 23 gm. The prednisolone-treated group of animals showed an appreciable fall in their body weight from an average of 22.6 gm in the 2nd week to 21 gm in the 6th week (Chart 1). From the 7th week onward till the termination of the experiment a gradual increase in the body weight was noted. During this period, the gain in body weight of the test animals ran parallel to that of the animals in the control group. In fact, the over-all trend was towards a rise of the body weight in the test group of animals from the 3rd week, although this was not prominent until the 7th week (Chart 1). There was no obvious difference in the amount of food consumed by the animals of the 2 groups, which had been kept in separate cages. There was no evidence of edema in the test group of animals. The control group of animals gradually gained weight from the 1st to the 18th week (Chart 1). The average body weight at the end of the 18th week was 29.1 gm for the control and 27 gm for the test animals.

**INCIDENCE OF SARCOMAS.** Within a fortnight of the initiation of the experiments, 9 animals from the control group and 10 from the test group appeared chronically ill with hunched posture, diarrhea, and ruffled hair (Table 1). They died during the 2nd and 3rd weeks (Table 1). The cause of death could not be established with certainty as only E. coli were isolated from the intestinal contents. Pieces of the skin with subcutaneous tissue from the site of injection of the carcinogenic agent in these animals failed to reveal any evidence of tumor on microscopic study. At the end of the 6th week, 3 animals from the control group showed a palpable tumor at the site of injection (Table 2). Two weeks later, they were sacrificed and the tumors were removed and examined under the microscope. This procedure was followed for all the tumors which appeared in the 2 groups of animals until the termination of the experiment at the end of the 20th week (Table 2). Bleeding ulcers were present in most of the tumors of the control group of animals. The percentage of tumors at varying weekly intervals was also recorded (Table 2). The 1st sarcoma in the test group was recognized by palpation in the 10th week. After the growth had once started, no tumor apparently regressed in either of the 2 groups. All 23 surviving animals of the control group developed tumors by the end of the 16th week. Thus there was a 100% yield of tumors in the control group of animals. On the other hand, only 20 of the 35 surviving test animals developed tumors, so the yield of tumors

![Chart 1](chart1.png)

**TABLE 1**

INCIDENCE OF DEVELOPMENT OF SARCOMAS IN THE CONTROL AND TEST GROUPS OF ANIMALS

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TOTAL NO. OF MICE</th>
<th>NO. OF EARLY DEATHS</th>
<th>TOTAL EFFECTIVE NO. OF MICE</th>
<th>NO. OF MICE WITHOUT TUMOR</th>
<th>NO. OF MICE BEARING TUMOR</th>
<th>INCIDENCE OF SARCOMAS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32</td>
<td>9</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>Test</td>
<td>45</td>
<td>10</td>
<td>35</td>
<td>15</td>
<td>20</td>
<td>57.1</td>
</tr>
</tbody>
</table>

**TABLE 2**

INCIDENCE OF DEVELOPMENT OF FIBROSARCOMAS AND RHABDOMYOSARCOMAS AT WEEKLY INTERVALS IN THE TEST AND CONTROL GROUPS OF ANIMALS (AS DETERMINED ON PALPATION)

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>EFFECTIVE NO. OF MICE</th>
<th>TYPES OF SARCOMAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wk. of experiment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 7 8 9 10 11 12 13 14 15 16 17 18</td>
</tr>
<tr>
<td>Control</td>
<td>23</td>
<td>Fibrosarcomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhabdomyosarcomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Test</td>
<td>35</td>
<td>Fibrosarcomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhabdomyosarcomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>
in the test group of animals was only 57.1% (Table 1). As seen in Table 2, 14 of the 23 tumors in the control group were fibrosarcomas and only 9 were rhabdomyosarcomas. Thus 60.9% of the tumors in this group were fibrosarcomas and 39.1% rhabdomyosarcomas. On the other hand, 19 of the 20 test animals developed fibrosarcomas (95%), and only 1 animal developed rhabdomyosarcoma (5%). The average volumes of the tumors in the control and test groups were 0.510 ml and 0.247 ml, respectively, with a S.D. of ±0.046 in the test group and ±0.067 in the controls.

**APPEARANCES OF THE TUMORS.** The tumors were usually round and ranged in size from 0.6 x 0.5 x 0.5 cm to 1.2 x 1.2 x 1 cm. They varied from a soft to firm consistency. Their surfaces were often ulcerated and hemorrhagic. The growths presented the usual microscopic features of fibrosarcomas or rhabdomyosarcomas.

**Discussion**

It is well known that caloric restriction may reduce tumor formation in animals. In this study, the test animals did initially show a 7% decrease in their body weight. This, however, was not sustained and the general trend from the 3rd week was that of a gain in weight, which was quite appreciable from the 7th week onwards (Chart 1). In the following period of 14 weeks, the rate of gain in weight of these animals ran parallel to that of the control group. The amount of food used by the 2 groups of animals, which had been kept in separate cages, did not seem to be any different. At the time the animals were sacrificed, the prednisolone-treated animals did not show any evidence of edema. Thus the possibility of a diminished food intake or a period of sustained or pronounced loss of weight in the test animals may be excluded.

For a long time the effect of cortisone on the incidence of epidermal and subcutaneous tumors has been a puzzle. Different explanations have been offered from time to time, but nothing definitive has been reported. The most extensive work using subcutaneous tissue as the site of carcinogen injection has been done in rats and mice. Previous data on the effects of cortisone on various tumor-host systems suggested that this hormone has 2 different and contradictory actions (1, 13, 14). “When it inhibits primary tumor growth most probably through the tumor stroma, it also facilitates the implantation and growth of circulating tumor emboli that form metastases by depressing the immunologic mechanism of the host” (2, 10). Results reported from other studies, however, are not in accord with these findings.

In this study a marked inhibitory action of prednisolone on the induction of subcutaneous sarcoma by 3-methylcholanthrene was noted. This finding is in keeping with that of Takashi (20), though with a few major differences, but contrary to the results of other studies (4, 7), where no inhibitory action of cortisone on the growth rate of induced skin tumors or s.c.-induced sarcomas was observed. Compared with the results of Takashi (20) the inhibitory effect of prednisolone on tumorigenesis was considerably more marked in the present study. As seen in Table 2, 3 sarcomas appeared in the control group by the end of the 6th week, whereas only 1 tumor had appeared in the test group of animals by the end of the 10th week. During these 10 weeks, 11 sarcomas were noted in the control group. This difference is all the more striking when one takes into account the fact that there were as many as 35 effective animals in the test series and only 23 in the control group. Thus, percentage wise, by the end of this period only 2.8% of the test animals had developed sarcomas as compared with 47% of the control group. The most prominent difference between these 2 groups appeared at the ends of the 12th and 13th weeks when 86 and 91%, respectively, of the animals in the control group presented tumors as compared with 25 and 31% of those in the test group. This represented a percentage difference of 60. The over-all incidence of sarcomas in the control and test groups was 100% and 57.1%, respectively. This difference between the 2 groups is statistically significant to a high degree ($P = 0.00026$).

This inhibitory effect of prednisolone on tumorigenesis is probably also reflected in the size and histologic appearance of the tumors. The tumors in the prednisolone-treated group were smaller and appeared histologically less malignant. The average volume of the neoplasms in the control group was 0.510 ml while that in the test group was 0.247 ml. This variation also is statistically significant ($P < 0.01$). Each of these animals had been killed 2 weeks after the initial recognition of a mass. While it is possible that the sizes of these growths may have varied to some extent at the time of their 1st recognition by palpation, the variation could not have been too great since the site of injection in each animal had been carefully palpated twice each week. The 2-week period between the initial recognition of a tumor by palpation and the sacrifice of the animal when the growths were measured, was available for each of these neoplasms to grow. Therefore, the size of the masses at the termination of this period may reflect the rate of growth of the individual tumor. The tumors in the test group of animals appeared considerably less malignant.

It is of considerable interest that the prednisolone-treated group of animals gave a significantly smaller yield of tumors, which took a longer time to appear, were smaller in bulk, and appeared histologically less malignant. A number of possible explanations may be given for the inhibitory action of prednisolone on the induction of subcutaneous sarcomas in our study. The route of administration seems to be a very important consideration in corticoid studies with animals. It has been seen that hydrocortisone acetate, probably because of a larger degree of absorption without inactivation, is 3 times as effective as hydrocortisone acetate when administered by the i.p. route (17). When used by the s.c. route it was found to be only 0.2 as effective as hydrocortisone. This probably explains, to some extent, the tumor-promoting effect of cortisone noted by certain workers who used cortisone in smaller dosage by the s.c. route (16).

The inhibitory effect of stress (weight loss due to caloric restriction) on mitosis has been shown by Wolf and Nishimura (21) in the mouse epidermis. The initial weight loss in our animals receiving prednisolone may have been associated with some degree of biologic stress. Thus a state of depressed mitosis may have led to the inhibition of tumorigenesis. Autoradiographic studies with several tissues have shown that the fibroblast is the chief site for the cellular localization of hydrocarbons (9). It has been noted that the anti-inflammatory corticosteroids inhibit the growth and function of fibroblasts (3). It is a well-known fact that corticosteroids also exert an inhibitory action on malignant fibrous tissue tumors and keloids (3, 5, 15). Most of the
workers also agree that a marked increase in the activity of alanine-α-transaminase following the injections of prednisolone may be associated with the inhibition of tumor growth.

Acknowledgment

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References

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