Superiority of Methylprednisolone over Dexamethasone for Induction of Pneumocystis carinii Infection in Rats

A. SUKURA,* T. SOVERI, AND L.-A. LINDBERG

Department of Anatomy, College of Veterinary Medicine,
P.O. Box 6, 00581 Helsinki, Finland

Received 22 February 1991/Accepted 28 June 1991

Because of difficulties in in vitro cultivation, the basic Pneumocystis carinii studies have been carried out on animal models, mainly on rodents immunosuppressed by corticosteroids. Commonly used dexamethasone and methylprednisolone procedures were evaluated. The intensity of infection in rats was statistically significantly higher after 9 weeks' immunosuppression with methylprednisolone than with dexamethasone.

Experimental studies of Pneumocystis carinii have commonly been carried out on animal models. Rats immunosuppressed by different corticosteroids have been widely used in basic research and in testing anti-P. carinii drugs (6). The steroids of choice have been cortisone acetate (25 mg/kg of body weight twice a week) (6), 7, dexamethasone (2 or 1 mg/liter in drinking water) (2, 4, 5, 8), or methylprednisolone (depot injections of 16 mg/kg once a week) (9). In most of the experiments, antibiotic prophylaxis, mainly with tetracycline or a mixture of penicillin and streptomycin, has been used to control secondary infections. A combination of corticosteroid immunosuppression with a low-protein diet has also been used.

Eighteen Wistar rats from a colony known to be infected with P. carinii were used to evaluate differences between methylprednisolone and dexamethasone in an animal model. They were divided into three groups, six rats in each, with group 1 a control group and groups 2 and 3 immunosuppression groups. Three male and three female rats made up each group, except for the control group, which comprised five males and one female. The rats were kept in ordinary cages which were in close contact with one another. The animals were weighed once a week.

All rats received tetracycline (500 mg/liter) in their drinking water and consumed a normal commercial diet. Since group 1 was a control group, it did not receive any additional medication. Group 2 received subcutaneous injections of methylprednisolone (Depo-Medrol, 16 mg/kg once a week), and group 3 received dexamethasone (1 mg/liter) in its drinking water.

After 9 weeks, the rats were euthanized by means of ether anesthesia and then autopsied; on the same occasion, blood samples were collected by an open cardiac puncture method, and touch imprints were taken from the caudal part of the left lobe and then stained with toluidine blue O. The quantification was a modification of a previously published method (11), in which an approximately 0.1-g sample of the right cranial lobe was weighed and digested in a 10-fold volume of 0.1% collagenase at 40°C with magnetic stirring. After the pellet was centrifuged and washed, it was diluted to 1:10, 1:100, and 1:1,000. A 10-μl drop of each dilution was stained with toluidine blue O, and a sufficient dilution was chosen for quantification. Cysts per microscopy field were counted along the diameter of the sample spot, and the final concentration was calculated. All samples were quantified. Coding was performed to prevent the examiner from knowing the results of the imprint investigations. Student's t test was used to evaluate the differences in cyst counts between groups 2 and 3, variance analysis was used to evaluate the differences between blood cell values, and Tukey's test was used for comparisons between means.

All rats survived throughout the experiment. None of the animals in the control group showed any detectable P. carinii organisms. The intensity of the infection was, however, significantly different between the immunosuppression groups. The averages of the cyst counts were 19.9 × 10^7 ± 9.8 × 10^7 (mean ± standard deviation) cysts per g of lung tissue in group 2 and 5.7 × 10^7 ± 4.3 × 10^7 cysts per g of lung tissue in group 3 (P < 0.01). The different intensities were obviously due to the differing effects on the animals' immunostatus. Both groups which received corticosteroid showed lymphopenia (Table 1, P < 0.001), and group 2 showed neutrophilia (P < 0.01).

It has been reported that cellular immunity is important for protection against subclinical P. carinii infections (3). It is therefore logical that a medication having a more powerful influence on lymphocytes also yields a more severe infection. In their work on mice, Walzer et al. (10) found that the intensity of P. carinii infection in mice was independent of the dexamethasone dose, whereas Azab and Abdel-Mawla (1) found that intraperitoneal dexamethasone injections led to dose-dependent P. carinii infections in rats.

A severe catabolic effect of the corticosteroids on the animals' weight gain was also seen, the effect of methylprednisolone being stronger than that of dexamethasone. The relative body weight gain was 42% ± 44% (mean ± standard deviation) in the control group, but both corticosteroid-treated groups lost weight: the dexamethasone group lost 11% ± 23%, and the methylprednisolone group lost 43% ± 7%.

A logical explanation for the differences seen in this study could be merely the size of the dose. Higher doses of dexamethasone could result in the same or more intense P. carinii infections than with methylprednisolone. The methylprednisolone used in this study was a depot preparation and therefore able to be administered only once a week. This fact makes it more practical than cortisone acetate, which should be given twice a week. When parenterally administered steroids are used, the dose is not dependent on water consumption and exact doses can be used.

We conclude that with the doses used in our study

* Corresponding author.
TABLE 1. Differential cell counts after 9 weeks of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Differential cell countb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leukocytes</td>
</tr>
<tr>
<td>1</td>
<td>7,200 ± 3,122</td>
</tr>
<tr>
<td>2</td>
<td>5,600 ± 2,422</td>
</tr>
<tr>
<td>3</td>
<td>3,183 ± 2,153</td>
</tr>
</tbody>
</table>

a Group 1 (controls) received no corticosteroid, group 2 received subcutaneous injections of methylprednisolone (16 mg/kg/week), and group 3 received dexamethasone (1 mg/liter of drinking water).

b Mean number of cells per microliter ± standard deviation. Statistical differences were evaluated with variance analysis by Tukey’s test in comparison with the control group (***, P < 0.001; **, P < 0.01).

methylprednisolone elicits a more intense *P. carinii* infection than dexamethasone.

REFERENCES