Testicular Cultivation of *Treponema pallidum* (Nichols Strain) Facilitated by Sustained-Release Steroid Administration

BARRY D. BRAUSE,* SUSAN QUALLS, AND RICHARD B. ROBERTS

Division of Infectious Diseases, Department of Medicine, Cornell University Medical College, New York, New York 10021

Received for publication 15 September 1979

*Treponema pallidum* cultivation is facilitated by substitution of methylprednisolone acetate suspension for hydrocortisone administration during rabbit testicular infection. Methylprednisolone suspension reduces testicular mononuclear cell infiltration and should benefit future studies of virulent *T. pallidum*.

The fastidious agent of human syphilis, *Treponema pallidum*, can be grown only in vivo at present. *T. pallidum* is usually cultivated in rabbit dermal or testicular lesions (1, 2, 8). Simultaneous administration of cortisone to rabbits is associated with a 10- to 100-fold increase in the spirochete content of rabbit treponemal skin lesions presumably by suppressing the inflammatory response (7). Testicular lesions are also influenced by adenocorticosteroid administration, and rabbit testicular cultivation is the most established method for harvesting large numbers of treponemes (8). Daily or twice-daily intramuscular cortisone injections (6 mg/kg) have been routinely included in *T. pallidum* cultivation for over 20 years (8). This report describes the facilitation of treponeme cultivation by substituting methylprednisolone acetate suspension, a sustained-release corticosteroid, for hydrocortisone.

Virulent *T. pallidum* (Nichols strain) was cultivated in the testes of Venereal Disease Research Laboratories (VDRL)-negative Dutch Belt rabbits individually caged in air-conditioned quarters (60 to 63°F [15.6 to 17.2°C]). Each testis was inoculated with 10⁶ treponemes in 10% heat-inactivated normal rabbit serum. Two days after inoculation, four animals received intramuscular hydrocortisone sodium phosphate (Merck & Co., West Point, Pa.), 6 mg/kg daily and nine rabbits received intramuscular methylprednisolone acetate suspension (Depomedrol, Upjohn, Kalamazoo, Mich.), 4 mg/kg per week. Eight separate experiments were performed. Each experiment compared a testis from one hydrocortisone-treated rabbit with two or three testes from methylprednisolone suspension-treated rabbits. Animals studied in each experiment were from the same outbred shipment of rabbits, inoculated simultaneously from the same cryo-preserved treponeme inoculum, and followed at the same time in the same cooled animal room but in separate cages.

The initial weights (2 kg) and testicular dimensions of rabbits in both treatment groups were similar. Within 2 days of the development of fulminant orchitis, animals were sacrificed with 50 mg of sodium pentobarbital intravenously per kg and castrated. Each testis was sliced, and tissue sections (2.5 mm³) were shaken for 20 min in RPMI 1640 tissue culture medium (GIBCO, Grand Island, N.Y.). The extraction mixture was then centrifuged at 800 × g for 10 min to sediment gross tissue debris, testicular cells, and spermatozoa. The concentration of spirochetes in the supernatant was calculated by the method of Magnuson et al. (6). Extractions were performed with weight-dependent volumes of media (0.8 ml/g of testis). Small extraction volumes were employed in these studies because the treponemes were subsequently used in experiments requiring high densities of viable, virulent spirochetes. In other laboratories, 10- to 20-fold-larger volumes are used for extraction.

Methylprednisolone is fivefold more potent than hydrocortisone in anti-inflammatory activity (5). Doses of methylprednisolone (8 mg/kg per week) of equal potency to hydrocortisone were associated with a 25% reduction in rabbit weight over a 2-week period. To avoid this significant weight loss, doses of methylprednisolone were calculated to be one-half as potent as hydrocortisone. During steroid administration, methylprednisolone-treated rabbits (4 mg/kg per week) lost an average of 0.13 kg, whereas hydrocortisone-treated animals (6 mg/kg per day) gained a mean of 0.08 kg.

Comparative data from rabbits receiving hy-
drocortisone or methylprednisolone are summarized on Table 1. The average duration of incubation before mature orchitis developed was 16 days in the hydrocortisone group (range 14 to 20 days) and 17 days in those treated with methylprednisolone (range 14 to 21 days). The mean weight of testes from animals treated with methylprednisolone was 75% more than hydrocortisone-treated animals at the time of mature orchitis. Extractions performed with weight-dependent volumes of media (0.8 ml/g of testis) enhanced the yields associated with methylprednisolone administration by 350% per g and 660% per testis. T. pallidum harvests from both groups of rabbits were used as inocula in the rabbit skin syphiloma model and were equally virulent (1, 4, 9). In addition to the increased yield of treponemes, the amount of small-particle testicular debris accompanying extraction was markedly diminished in the methylprednisolone group as observed by dark-field and phase contrast microscopy.

The testicular inflammatory response was examined in both treatment groups by using hematoxylin and eosin-stained tissue sections after Formalin fixation. The cellular response in hydrocortisone-treated testes consisted of a paucity of leukocytes, but both lymphocytes and polymorphonuclear leukocytes were present with a mononuclear cell predominance. Testes from methylprednisolone-treated rabbits also had a mild leukocyte infiltration but consisted almost entirely of polymorphonuclear leukocytes.

Adrenocorticosteroid hormones have a profound effect on the evolution of experimental syphilitic lesions. Rabbit skin syphilomas do not become indurated and do not progress to an ulcerative stage if cortisone is administered during early infection (7, 8). The spirochete content of these skin lesions is 10- to 100-fold greater than lesions in untreated animals. The inflammatory response is less prominent and consists primarily of mononuclear cells in hydrocortisone-treated rabbits. Rabbit testicular infection with T. pallidum is influenced by cortisone in the same manner as is skin infection (8). In the absence of cortisone administration, an extensive, predominantly mononuclear cell infiltrate develops in infected testes. This inflammation is markedly diminished in magnitude by hydrocortisone treatment, but the infiltrate remains comprised primarily of mononuclear cells. In the present studies, a sustained-release steroid preparation was associated with suppression of the mononuclear cell response in testicular lesions.

The enhanced effects of methylprednisolone may be related to differences in distribution kinetics. In humans, the plasma half-life of methylprednisolone acetate suspension is approximately 40 h (personal communication, M. S. Cohon, Upjohn Co.), whereas the plasma half-life of hydrocortisone approximates 90 min (6). Therefore, methylprednisolone administration is associated with a sustained plasma level and perhaps a more sustained anti-inflammatory effect.

These results are consistent with observations of steroid effects on human leukocytes in vivo. The magnitude of human mononuclear cell accumulation at sites of injury is reduced to a far greater extent by daily prednisone than by alternate-day therapy (3). Moreover, mononuclear cells may be more sensitive to the effects of steroids than are granulocytes since accumulation of monocytes, but not neutrophils, is significantly reduced by prednisone.

Weekly intramuscular administration of methylprednisolone is far less tedious than daily injections of hydrocortisone currently employed.

### Table 1. Comparative data from rabbits receiving hydrocortisone or methylprednisolone acetate suspension

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean duration of orchitis incubation (days)</th>
<th>Mean testis wt (g) of mature orchitis</th>
<th>Extractable treponeme (Tp) yield&quot;</th>
<th>Tp/g of testis</th>
<th>Tp/testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone (N = 4)</td>
<td>16 (14-20)&quot;</td>
<td>2.4 (2.1-2.6)</td>
<td>8.6 x 10^7 (3.9 x 10^7 - 17.6 x 10^7)</td>
<td>2.1 x 10^9 (1.0 x 10^9 - 4.5 x 10^9)</td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone (N = 9)</td>
<td>17 (14-21)</td>
<td>4.2 (3.2-5.7)</td>
<td>3.9 x 10^6 (1.7 x 10^6 - 7.2 x 10^6)</td>
<td>1.6 x 10^9 (0.7 x 10^9 - 3.0 x 10^9)</td>
<td></td>
</tr>
<tr>
<td>Percentage increase in yield&quot;</td>
<td>350</td>
<td>660</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

" Testicular slices were extracted in RPMI 1640 tissue culture medium by using weight-dependent volumes (0.8 ml) of media per g of testis.
" Numbers in parentheses are ranges.
" Associated with methylprednisolone administration.
in many laboratories. The facilitation of *T. pallidum* cultivation in animals treated with methylprednisolone should be advantageous for future studies of treponemal biology, immunity, and metabolism.

This study was supported by Public Health Service grant AI-12932 from the National Institute of Allergy and Infectious Diseases.

**LITERATURE CITED**