Effect of Doxycycline on Immune Response in Mice
AREZKI BELLAHSENE AND ARNE FORSGREN*
Department of Clinical Bacteriology, University of Lund, Malmö General Hospital, S-214 01 Malmö, Sweden
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The effect of doxycycline on immune response has been studied in mice, cell-mediated immunity being evaluated with the split heart allograft technique. Survival duration of heart transplants in animals treated with 2.5 mg of doxycycline per kg per day from the day of transplantation until rejection was slightly but significantly longer than in untreated animals, 18.8 days (P < 0.05) as compared with 14.5 days. In doxycycline-treated animals, both agglutinating and hemolytic antibody response to sheep erythrocytes was slightly but significantly decreased, though there was no inhibition of splenic production of antibodies to sheep erythrocytes (as measured by the number of plaques of hemolysis detected). The results show the immune response in mice to be only moderately inhibited by doxycycline. The relevance of experiments in mice is also discussed.

Doxycycline α-6-deoxytetracycline is an antibiotic that inhibits the bacterial synthesis of protein by blocking the fixation of amino acids to the ribosome (17). Recent studies in our laboratory have demonstrated that doxycycline also inhibits the chemotaxis and phagocytosis of human neutrophil leukocytes, both in vitro and in vivo (8, 9). Doxycycline also restrains the mitogen-induced lymphoproliferative response of human peripheral lymphocytes (7). The purpose of the present study was to investigate whether doxycycline inhibits the immune response in vivo. The interaction between doxycycline and the cellular and humoral immune response was studied in vivo in mice. The cellular immune response was investigated with the split heart allograft technique, and the humoral immune response was investigated with the plaque-forming cell assay and agglutinating and hemolytic antibody response to sheep erythrocytes.

MATERIALS AND METHODS

Animals. CBA/H mice (Anticimex AB, Evelund 19171 Sollentuna, Sweden) were used. They were 8 weeks old when first treated. Donor hearts were obtained from 12- to 36-h-old DBA/2J mice (Gl. Blomholtgård Ltd, 8680 Ry, Denmark).

Antibiotics. Every morning, the animals were given doxycycline (Vibramycin, Pfizer AB, Täby Sweden) intraperitoneally (i.p.) in doses of 2.5 mg/kg (human therapeutic dose [HTD]), or of 3 × HTD (7.5 mg/kg). Penicillin (Benzylenicillin, Kabi) was injected i.p. in a single dose of 200 mg/kg per day.

Antigens and immunization. Sheep erythrocytes (SRBC) were collected and stored in Alsever solution at 4°C for 1 week before use. The cells were washed 4 times in isotonic balanced salt solution (BSS) (pH 7.4), and 0.2 ml of a 25% (vol/vol) suspension was injected i.p. (16).

Agglutinating antibodies. Antisera were serially diluted in BSS, each dilution being mixed with an equal volume (25 μl) of 2% washed SRBC. The tests were performed in microtiter plates (Sterlin, Teddington, Middlesex, England), agglutination being read after 45 min at 37°C. Sera from treated but unimmunized mice were used as controls (16).

Hemolytic antibodies. Serial dilutions of antisera in BSS were incubated with equal volumes (25 μl) of 1.5% SRBC and guinea pig complement, and hemolysis was determined after 45 min at 37°C. Sera from treated but unimmunized mice were used as controls (16).

Antibody-producing cells. The splenic production of antibodies to SRBC was measured by a direct plaque-forming cell assay, using a modification of the Jerne-Nordin method (6, 13). From spleens removed 4 days after immunization with SRBC, lymphocytes were prepared by passage through a Millipore filter (Millipore Corp., Bedfor, Mass.) and isolation by density gradient centrifugation on lymphoprep (Lymphoprep, Nyegaard and Co A/S, Oslo, Norway). A solution of 0.5% agar (Bacto-Agar, Difco Laboratories, Detroit, Mich.) and 0.5% DEAE-dextran (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) in BSS at 46°C was mixed with 25 μl of SRBC diluted 1:5 in BSS, 25 μl of guinea pig serum (previously absorbed with SRBC for 20 min at 4°C) diluted 1:4 in phosphate-buffered saline, and 100 μl of the spleen lymphocytes in a concentration of 10⁶ lymphocytes per ml. Three separate 100-μl drops of the mixture were placed on a petri dish, and a glass cover slip (24 by 32 mm) was immediately placed on each drop. Plates were incubated for 3 h at 37°C, and plaques were counted under a stereo-microscope (Wild M5, Wild Heerburg, Inc., Heerburg, Switzerland). Representative plaques were checked for a central lymphocyte.

Transplantation. Heart grafts were transplanted by a modification of the Judd and Trentin method (14, 20). Hearts from newborn DBA mice were sectioned along the ventricular septum, each half then being inserted into a subcutaneous pouch in the dorsal part of the ear of the recipient CBA mouse. The survival of the transplant was monitored by measuring electric activity, using a Mingograf 61 equipped with an integrated amplifier. The activity of the host heart was also recorded. This activity differed from that of the transplanted hearts and did not confuse measurements.

Statistics. Student’s t test and Wilcoxon’s rank sum test were used to check the statistical significance of results.

RESULTS

Toxicity of doxycycline. Doxycycline was apparently well tolerated by the mice when given in HTDs or 3 × HTDs.

Survival duration of allografts. Untreated transplanted mice were used as controls. Figure 1 shows the effect of i.p. doxycycline on the survival duration of split-heart allografts from DBA mice in CBA mice. Both pretreated and non-pretreated mice, daily antibiotic treatment was given

* Corresponding author.
from the day of transplantation until rejection of the grafts was detected by electrocardiograph. Graft survival was slightly prolonged on the mice given doxycycline in a dose of 2.5 mg/kg per day (HTD) (Fig. 1).

The mean survival time (± standard deviation) of the allografts was 18.8 ± 4.7 days in the mice treated daily from the day of the transplantation until rejection of the grafts, as compared with 14.5 ± 3 days in the controls not given antibiotic treatment (P < 0.05). In mice also treated 10 days before the operation, the mean survival duration of allografts was 18.6 ± 3.8 days (P < 0.01). Penicillin given i.p. from the day of transplantation until graft rejection did not prolong the survival of the grafts (mean survival time = 15.9 ± 3.9 days) (Fig. 1).

**Antibody-producing cells.** The direct plaque-forming cells from spleens of mice immunized with SRBC and treated with either doxycycline or penicillin from the day of immunization (D0) until day 3 (D3), with or without pretreatment, were studied. The number of plaques detected in mice given 2.5 mg of doxycycline per kg per day was only slightly less (17%; not significant) than that in untreated, immunized mice (Fig. 2). Plaque formation was not inhibited in spleen cells from animals treated from day 20 before immunization (D – 20) until D3 or from D – 20 until D – 1. Mice treated with doxycycline in a dose of 7.5 mg/kg per day from the day of immunization had a normal primary antibody response to SRBC (Fig. 2). Administration of penicillin did not affect assay results.

**Agglutinating and hemolytic antibodies.** The titers of the agglutinating and hemolytic antibodies in mice immunized with SRBC and treated with doxycycline or penicillin were compared with those in untreated immunized mice (Table 1).

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**FIG. 1.** Percentage of surviving split-heart DBA allografts in CBA mice treated daily with an HTD of doxycycline (○) or with an HTD of benzylpenicillin (□) from the day of the transplantation until graft rejection. Mice treated for the same period with an HTD of doxycycline but also pretreated for 10 days with a HTD of doxycycline (■) are included. Untreated transplanted mice (○) were used as controls. The number of transplantation experiments is given at the top of each curve.

**FIG. 2.** Direct plaque-forming cells from spleens of mice immunized with SRBC and treated daily with either doxycycline (HTD, 3× HTD) or benzylpenicillin (HTD) from the day of immunization (D0) until day 3 (D3), with or without pretreatment (PT). Mice treated daily with doxycycline (HTD) from day 20 (D – 20) until day 1 (D – 1) before the day of immunization are included. The results (mean ± standard error) are expressed as percentages of those in the control group. Each group consisted of 10 mice.
TABLE 1. Antibody titers in SRBC-immunized mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Agglutinating antibodies</th>
<th>Hemolytic antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.7 ± 4.6</td>
<td>7.7 ± 5.6</td>
</tr>
<tr>
<td>Doxycycline HTD: D0 to D3 (PT = 0)</td>
<td>5.8 ± 3.3</td>
<td>6.8 ± 4.4</td>
</tr>
<tr>
<td>Doxycycline HTD: D0 to D3 (PT = 20 days)</td>
<td>4.2 ± 2.0</td>
<td>6.2 ± 4.5</td>
</tr>
<tr>
<td>Doxycycline HTD: D-20 to D-1</td>
<td>6.5 ± 4.4</td>
<td>7.4 ± 4.6</td>
</tr>
<tr>
<td>Doxycycline 3x HTD: D0 to D3 (PT = 0)</td>
<td>5.6 ± 3.3</td>
<td>6.5 ± 3.7</td>
</tr>
<tr>
<td>Doxycycline 3x HTD: D0 to D3 (PT = 20 days)</td>
<td>5.2 ± 3.6</td>
<td>6.6 ± 4.4</td>
</tr>
<tr>
<td>Doxycycline 3x HTD: D1 to D3</td>
<td>6.6 ± 4.8</td>
<td>7.0 ± 4.5</td>
</tr>
<tr>
<td>Benzylpenicillin HTD: D0 to D3</td>
<td>6.5 ± 3.3</td>
<td>7.4 ± 4.7</td>
</tr>
</tbody>
</table>

* Table shows titers of agglutinating and hemolytic antibodies from sera of mice immunized with SRBC and treated daily with either doxycycline (HTD, 3x HTD) or benzylpenicillin (HTD) from the day of immunization (DO), or 1 day after immunization (D1), until day 3 (D3) with or without pretreatment (PT). Results for mice treated daily with doxycycline (HTD) from day 20 (D-20) until day 1 (D-1) before the day of immunization are also included. Agglutinating and hemolytic antibody titers from untreated immunized mice served as control references. The results are expressed as the mean (log ± standard error) titers. Each group consisted of 10 mice.

DISCUSSION

The present study showed that doxycycline, in doses of either 2.5 or 7.5 mg/kg per day, only slightly, though significantly, prolonged allograft survival. The primary antibody response to SRBC, as estimated from serological agglutinating and hemolytic antibody titers, was also inhibited. No significant effect could be detected on the number of plaque-forming cells. In vitro investigations have demonstrated an inhibitory effect of doxycycline on the mitogen-induced lymphoproliferative response of human peripheral lymphocytes. Forsgren and Banck found no lymphocyte transformation in experiments using phytohemagglutinin as mitogen and doxycycline in a concentration of 50 μg/ml and an inhibition at lower concentrations (7). A marked lymphocyte inhibitory effect was also confirmed by Thong and Ferrante (21) and Potts et al. (18). Tetracycline depression of in vivo cell-mediated responses has also been found (19, 22, 23).

It has also been reported that neutrophils, incubated in vitro with doxycycline, showed a decreased capacity to phagocytose yeast and bacteria. The same was true for leukocytes harvested from healthy volunteers after ingestion of tetracycline (19). Doxycycline also effectively inhibits the chemotaxis of human neutrophils in agarose plates (1, 2, 8, 12, 23). Recently, Elewski et al. (3), using a skin chamber technique, demonstrated a remarkable inhibition of leukocyte chemotaxis after systemical or topical administration of tetracycline to healthy volunteers. However, Glette et al. (10, 11) have shown that doxycycline does not reduce in vivo neutrophil migration to skin chambers and have indicated that the impairment of neutrophil functions measured in vitro may be due to photo-induced production of toxic oxygen species or to the divalent cation chelating effect of tetracyclines, or both. The present investigation showed only a slight in vivo effect of doxycycline on cell-mediated and humoral immunity. The in vivo observations of this study suggest that the in vitro results reported earlier may have in vivo implications. However, it should be emphasized that the half-life of doxycycline in mice is ca. 4 h (4), compared with ca. 20 h in humans (5). When cytotoxic drugs are given on a body weight basis, as in this study, mice often tolerate much larger doses (10 to 20 times higher) than humans, implying that the effects of antibiotics might be more pronounced in humans than those reported here for mice.

LITERATURE CITED


