PREVENTION OF BORRELLIA DUTTONII, TRYpanosoma Gambiense, Spirillum Minus AND Treponema Pallidum Infections Conveyable Through Transfusion*

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Spirochetemia of syphilitic patients was demonstrated by Uhlenhuth and Mulzer (1913). Since transfusion syphilis was first reported by Fordyce (1915) and importance of its prevention repeatedly emphasized by Levy and Ginsburg (1927), Feldman (1928), McCluskie (1939), and others, a number of experimental and clinical studies have been made.

Kast et al. (1939), Eichenlaub et al. (1941), Chin (1950), and Schmidt (1953) recommended the use of arsenic preparations for its prevention, while Nakao (1954) proposed such antibiotics as penicillin.

Employing the pathogenic agents of syphilis, relapsing fever, sleeping sickness, and rat-bite fever, the authors studied the prophylactic effect of two kinds of arsenic preparations and five of antibiotics against infections. The followings are the results obtained therefrom.

MATERIALS AND METHODS

A single strain of Borrelia duttonii (B.d.), Trypanosoma gambiense (T.g.), Spirillum minus (S.m.) and Treponema pallidum (T.p.) respectively was used. The T.p. strain was received from Prof. Harold J. Magnuson of the North Carolina University and the other strains were those maintained in the authors’ laboratory by mouse passage.

Mice weighing approximately 15 g were divided into groups of five. As for the inocula of B.d. and T.g., blood drawn from a mouse on the severest stage of the diseases was diluted 10-fold serially in B-N solution (One part of broth mixed with 4 parts of physiological saline) after counting the number of pathogenic microorganisms contained therein. The dilutions were placed in a water bath regulated at 23°C for 15 minutes. A group of 5 mice received a dilution intraperitoneally at a dose of 0.5 cc. The mice received B.d. were observed for 14 days and those received T.g. for 17 days, during which examinations were made with their blood.

In the case of S.m., blood and ascites of an infected mouse were mixed and treated similarly as described above. After inoculation, the mice were observed for 30 days, during which examinations were made with their blood as well as with ascites.

As for T.p., the testicles of a rabbit at the severest stage of orchitis were removed and

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minced, to which 2 volumes of physiological saline were added. The mixture was shaken for an hour and then centrifuged at 100 xg for 10 minutes. A serial 10-fold dilution of the supernatant was made in B-N solution. One half cc of each dilution was inoculated intracutaneously into 5 white rabbits, weighing approximately 2.5 kg, at the dorsal part which had been shaved. When T.p. was demonstrated in the induration formed at the site of inoculation, infection was judged as positive.

Experiments by intratesticular inoculation of T.p. diluted in citrated blood will be explained later.

Counting of pathogenic microorganisms was made microscopically using a Leitz microscope. With an ocular lens 3, objective lens 1/12, and a tube length 140 mm, the field was 0.02 mm². 0.005 cc of a microbial suspension was placed on a slide glass and covered with a cover glass of 20×30 mm. The number of pathogenic microorganisms in one cc of the suspension was calculated by the formula: Mean of the number of microorganisms per field x 30,000 x 200.

Descriptions on the drugs used will be made in the text.

EXPERIMENTAL RESULTS

I. Determination of Infectious Doses.

Results are summarized in Table 1. The minimal infectious dose for B.d., T.g., or T.p. was just a single microorganism, while that for S.m. was 1,000. The 50% infectious dose was 3.7 microorganisms for B.d., 1 for T.g., 464 for S.m., and 14.7 for T.p. The 100% infectious dose was 100 microorganisms for B.d., 10 for T.g., 10,000 for S.m., and 100 for T.p. Thomas and Morgan (1934) failed to infect rabbits with 1-6 spirochetes, whereas Magnuson et al. (1948) succeeded with a single spirochete.

Table 1. Infectious doses of Borrelia duttonii, Trypanosoma gambiense, Spirillum minus and Treponema pallidum

<table>
<thead>
<tr>
<th></th>
<th>B. duttonii</th>
<th>T. gambiense</th>
<th>S. minus</th>
<th>T. pallidum</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID₁₀₀</td>
<td>100</td>
<td>10</td>
<td>10,000</td>
<td>100</td>
</tr>
<tr>
<td>ID₅₀</td>
<td>3.7</td>
<td>1</td>
<td>464</td>
<td>14.7</td>
</tr>
<tr>
<td>MID</td>
<td>1</td>
<td>1</td>
<td>1,000</td>
<td>1</td>
</tr>
</tbody>
</table>

ID₁₀₀ = 100% infectious dose,
ID₅₀ = 50% infectious dose,
MID = minimum infectious dose.

II. Determination of Toxicity of Drugs.

Mapharsol (ML), Salvarsan-natrium (Sal), Penicillin G natrium crystal (P), Achromycin (AM), Terramycin (TM), Chloramphenicol (CM), and Dihydro-streptomycin (SM) were determined for their toxicity to mice. Serial 10-fold dilution of each drug was made in physiological saline. After placing in a water bath regulated at 23°C for 15 minutes, each dilution was injected into tail vein of 5-6 mice which were observed for 7 days. The results obtained are summarized in Table 2. The 50% lethal dose was the largest with P and Sal, CM, AM, TM, SM, and ML followed in the order.
III. Experiments for Prevention of Transmission.

Kast et al. (1939) stated that transfusion syphilis could be prevented by adding 1/10,000 volume of arsphenamine or neoarsphenamine to blood and by standing the mixture for 15 minutes before use. Eichenlaub et al. (1941), Chin (1950), and Schmidt (1953) reported that the use of 0.01 g of mapharsen or mapharsol per 500 cc of blood and standing the mixture for 15 minutes before use was satisfactory. While, Nakao (1954) reported 10,000 units or more of penicillin, 5 mg or more of aureomycin or terramycin, or 25 mg or more of chloramphenicol per cc of blood with standing the mixture for 20 minutes before use could prevent transfusion syphilis.

The number of pathogenic microorganisms presented in blood may have a great influence on the results of such experiments. Tani et al. (1931) had previously investigated the infectivity of blood of syphilitic rabbits and found that 1 cc of blood diluted 10,000-fold was still infective. In the present experiment, an inoculum of $10^8$ spirochetes (approximately ID$_{50} \times 7,000$) was used for T.p., $5 \times 10^6$ organisms for B.d. (approximately ID$_{50} \times 10^6$), $10^6$ organisms for T.g. (ID$_{50} \sim$...
10^5), and 12,500–50,000 organisms for S. m, approximately (ID_{50} × 20–100).

Each of dilutions of 7 drugs in physiological saline was mixed with an equal amount of a spirochete suspension to make a total volume of 0.5 cc, which was allowed to stand at 23°C for 15 minutes before inoculation. T. p. was inoculated intradermally at the dorsal portion of rabbits and the other pathogens were intraperitoneally into mice. The results obtained are shown in Table 3.

The 50% prophylactic dose against B. d. was the minimum with AM and TM and ML, Sal, SM and P followed in the order. CM had no effect within the range of the concentrations tested. When T.g. was used, prophylactic dose was the minimum with ML and TM, Sal and AM followed. P, CM, or SM had no effect. Against S. m, the dosage increased in the order of ML, Sal, SM, TM, AM, P and CM. Against T. p., the dosage increased in the order of ML, Sal, AM, TM, and P and CM had no effect. A close resemblance in the drug response was seen between S. m. and T. p.

IV. Coefficient of Chemotherapy.

Coefficient of chemotherapy was calculated for each of the chemotherapeutics against 4 kinds of pathogens from the ratio of LD_{50}, as determined by the intravenous injection of mice, to PD_{50}, either by intraperitoneal inoculation of mice or by intradermal inoculation at dorsa of rabbits.

### Table 4. Coefficients of chemotherapy (LD_{50}/PD_{50})

<table>
<thead>
<tr>
<th>Drug Microorg.</th>
<th>Mapharsol</th>
<th>Salvarsan-Natrium</th>
<th>Penicillin</th>
<th>Achromycin</th>
<th>Terramycin</th>
<th>Chloramphenicol</th>
<th>Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. duttonii</td>
<td>6.5</td>
<td>12.8</td>
<td>3.8</td>
<td>116.3</td>
<td>103.4</td>
<td>&lt;2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>T. gambiense</td>
<td>3,187.5</td>
<td>833.8</td>
<td>&lt;1.1</td>
<td>117.7</td>
<td>788.1</td>
<td>&lt;2.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>S. minus</td>
<td>3,187.5</td>
<td>395.6</td>
<td>169.6</td>
<td>157.0</td>
<td>154.0</td>
<td>&lt;2.1</td>
<td>144.1</td>
</tr>
<tr>
<td>T. pallidum</td>
<td>283,333.3</td>
<td>17,483.9</td>
<td>22.6</td>
<td>117.7</td>
<td>71.3</td>
<td>&lt;4.3</td>
<td>—</td>
</tr>
</tbody>
</table>

As shown in Table 4, ML was found to have the highest coefficient against T.g., S.m. or T.p., indicating the best prophylactic effect. Sal came next. The value for P was not so high as expected. Against B.d., AM and TM showed the highest coefficient and ML or Sal was much inferior. Coefficient of AM or TM was high against all 4 kinds of pathogens, which indicates the effectiveness against all of them. While, the results with P were different depending on pathogens tested. SM was especially effective against S.m. only. CM was entirely ineffective.

V. Prevention of Transfusion Syphilis by ML.

As ML was found to have the best prophylactic effect against infection of T.p. in rabbit dorsal skin, its effect on prevention of transfusion syphilis was studied.

To 9 parts of human blood containing 10^5 T.p./cc and 0.8% sodium citrate, 1 part of a ML dilution was added. After incubation at 23°C for 15 minutes, each 1 cc of a mixture was inoculated into testicles of a rabbit, which were then ob-
served for more than 80 days. The final concentration of T. p. was $9 \times 10^4$/cc. Three rabbits were used each dilution of ML. When testicles showed appreciable changes, Wassermann reaction became positive, and T.P. recovery was positive, infection was regarded as positive. The results obtained are shown in Table 5.

In the first experiment, concentrations of ML used in the blood were 0.01–10 $\mu$g/cc. All the rabbits received 1.0 $\mu$g or less ML developed orchitis. When concentration was raised to 10 $\mu$g/cc, T.p. was demonstrated from only one testicle of 1 out of 3 rabbits on the 60th day after inoculation. In another experiment using 10, 30, and 100 $\mu$g/cc of ML, no single case developed signs of infection. Namely, 10 $\mu$g/cc of ML inhibited the development of infection in most of the cases, yet it does not seem satisfactorily effective. While 30 $\mu$g ML/cc showed complete prevention of the infection. Therefore, it may be concluded that 3 mg of ML mixed with 100 cc of blood and standing the mixture at 23°C for 15 minutes before transfusion would guarantee the safety for T.p. The concentration of ML, 15 mg per 500 cc of blood, is one fourth of an adult therapeutic dose. It appears to be slightly higher than those previously reported, but it may probably have been due to the quantitative difference of T.p. used for experiment.

<table>
<thead>
<tr>
<th>Table 5. Findings of the preventive effect of mapharsol on transfusion syphilis</th>
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<tbody>
<tr>
<td><strong>Experiment I</strong></td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>0.01 $\mu$g</td>
</tr>
<tr>
<td>0.1 &quot;</td>
</tr>
<tr>
<td>1.0 &quot;</td>
</tr>
<tr>
<td>10.0 &quot;</td>
</tr>
<tr>
<td>30.0 &quot;</td>
</tr>
<tr>
<td>100.0 &quot;</td>
</tr>
</tbody>
</table>

*4/4: all the 4 testicles of 2 rabbits developed the infection.  
**20–39 days, 29 days: incubation period of 20–39 days, in average 29 days.  
***0/4: one of the 3 rabbits undergone the experiment died on the way.

**Conclusion**

The authors investigated the prophylactic effect of mapharsol, salvarsan-natrium, penicillin, achromycin, terramycin, chloramphenicol, and streptomycin upon Borrelia duttonii, Trypanosoma gambiense, Spirillum minus, and Treponema pallidum. First, the infectious dose was determined for each of the pathogens by intradermal inoculation of rabbit dorsum in case of Treponema pallidum and by intraperitoneal inoculation into mice in case of the other agents.

Then, 50% lethal dose (LD₅₀) was determined for each of the drugs tested by intravenous injection into mice. For determining prophylactic effect, an appropriate dose of each pathogen was mixed with serial dilutions of the drugs, which were
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inoculated into animals. The ratio of 50% prophylactic dose (PD₅₀) thus determined against LD₅₀ was calculated for comparison of prophylactic effect.

Finally, Treponema pallidum suspended in human blood was added with mapharsol dilutions. After standing at 23°C for 15 minutes, the mixtures were inoculated in testicles of rabbits for determining prophylactic dose. The results of these experiments may be summarized as follows:

1. The 50% infectious dose determined by intraperitoneal inoculation into mice was 3.7 spirochetes for Borrelia duttonii, 1 for Trypanosoma gambiense, and 464 for Spirillum minus, and 14.7 for Treponema pallidum as determined by intradermal inoculation into rabbit dorsal skin.

2. The 50% lethal dose as determined by intravenous injection into mice were as follows: mapharsol 0.51 mg, salvarsan-natrium 5.42 mg, penicillin G natrium crystal 54,600 u., achromycin 3.72 mg, terramycin 3.31 mg, chloramphenicol 4.28 mg and dihydro-streptomycin 2.58 mg per 15 g of body weight.

3. The 50% prophylactic doses against 4 kinds of pathogens as determined by inoculation of the mixtures of appropriate quantity of each pathogen (Borrelia duttonii approximately ID₅₀×10⁶, Trypanosoma gambiense ID₅₀×10⁶, Spirillum minus ID₅₀×20-100, Treponema pallidum ID₅₀×7×10⁴) with dilutions of drugs after standing at 23°C for 15 minutes were different as shown in Table 3. Achromycin and terramycin manifested a consistent prophylactic effect on all the pathogens tested.

4. Comparison of chemotherapeutic coefficients calculated by the ratio of 50% lethal dose and 50% prophylactic dose revealed that the figures of mapharsol and salvarsan-natrium were extremely high on Trypanosoma gambiense, Spirillum minus and Treponema pallidum, while their figures on Borrelia duttonii were very low. Achromycin and terramycin showed even chemotherapeutic coefficients on all the 4 kinds of pathogenic agents. While the coefficient of penicillin varied considerably depending on the pathogen tested. The figures of chloramphenicol and streptomycin were very poor.

5. Prophylactic effect of mapharsol was investigated by intratesticular inoculation into rabbits with human blood containing Treponema pallidum. The results suggest that transfusion syphilis could be prevented by addition of 3 mg of mapharsol per 100 cc of blood containing Treponema pallidum and standing the mixture at 23°C for 15 minutes before use.

REFERENCES


