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INFLUENCE OF METRONIDAZOLE ON TREPONEMA PALLIDUM IN VIVO AND IN VITRO

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Abstract. The effect of metronidazole in different concentrations on *Treponema pallidum* (Nichol) was tested in vitro and in vivo in rabbits. Under conditions similar to the TPI test, an immobilization of the spirochetes was demonstrated at 10 μg/ml and higher. No effect was seen below 1 μg/ml. This immobilization was not complement dependent and therefore cannot be mistaken for a specific TPI antibody mediated reaction. Experiments in rabbits with syphilitic orchitis failed to disclose any effect of metronidazole on the spirochetes, even in concentrations considerably higher than the currently recommended dose in man.

Metronidazole has been used successfully for more than 10 years in infections caused by *Trichomonas vaginalis*. It is recommended as the therapy of choice for this frequently met disease (6). It is estimated that 10–20% of fertile women harbour the flagellate in the vagina and/or lower urinary tract (1). A simultaneous infection with other sexually transmitted diseases occurs frequently (1). The incubation period of trichomoniasis is usually shorter than that of syphilis. Thus, a patient starting on metronidazole may be incubating syphilis. He may also have a previously contracted latent syphilis. If the drug has an antitreponemal effect in vivo, as has been demonstrated in vitro (2, 7, 12), it could possibly transform the syphilitic infection into a chronic, "silent" phase or even cure it. On the other hand, serum from a non-syphilitic man sent for TPI test would falsely be declared "positive" due to the treponemal immobilizing activity of metronidazole (3, 12).

The aim of the present investigation was to study the effect of metronidazole in vivo on experimental syphilis in rabbits, and in vitro on *T. pallidum* (Nichol) under conditions similar to the TPI test.

MATERIAL AND METHODS

Metronidazole (1-/Beta-hydroxetyl/2-methyl-5-nitro-imidazole) (Flagyl®, Clont®) was kindly supplied by AB Leo (Helsingborg, Sweden). It was delivered as a 2% solution in 70% propylene glycol or PBS (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.2).

Test of effect on T.p. in vitro. Suspensions of *Treponema pallidum* (T.p.) strain Nichol were prepared by extraction of infected rabbit testes as described by Nelson & Mayer (8). The medium used for suspending the spirochetes was similar to that of Nelson & Diesendruck (9). To 0.25 ml of spirochete suspension (6–8 spirochetes per view field; × 500 magnification) was added 0.15 ml fresh and heat-inactivated (56°C, 30 min) guinea pig serum, respectively, and 0.1 ml of the metronidazole solution in 70% propylene glycol diluted to give a final concentration in the mixture of 1 000, 100, 10, 1, 0.1, 0.01 μg/ml, respectively. Each concentration was tested in duplicate and controls with 70% propylene glycol were included. The tubes were evacuated and filled with a mixture of nitrogen (95%) and CO_2 (5%). This procedure was repeated three times and the tubes were finally incubated at 35°C for 18 hours. The effect of metranidazole was estimated by calculating the number of mobile and immobile spirochetes among 25 counted spirochetes from each tube.

Test of effect on T.p. in vivo. A spirochete suspension containing approximately $20–30 \times 10^6$ treponemata (60–70 spirochetes per view field, × 500 magn.) was injected into each testis of 12 rabbits (2.5–3.0 kg). Starting 3 days later 4 rabbits were injected intraperitoneally twice a day with metronidazole. Two of them received 0.5 ml of 0.5% solution in PBS and 2 others 5 ml of the same solution in each injection. This treatment was repeated until the seventh day when the rabbits, including 2 controls injected with only PBS in corresponding volumes, were killed by exsanguination. The testicular tissue from each rabbit was extracted as described by Nelson & Mayer (8) and the

ratio of mobile to immobile treponemata calculated. In a similar way 4 other rabbits were treated with metronidazole in the same amount and concentrations as the 4 rabbits mentioned above, but these rabbits were not injected until the seventh day after inoculation of the treponema suspension. The treatment continued with two injections daily until the 10th day, when the rabbits, including 2 controls, were killed and the testicular tissue analysed as described above.

RESULTS

In vitro tests. Table 1 illustrates the mobility of T.p. after incubation in vitro at different concentrations of metronidazole (average of two analyses). At high concentrations of metronidazole the treponemes are immobilized to a considerable degree. Whether this immobilization was correlated to a simultaneous killing of the organisms could not be determined in the present investigation. Those treponemes that were registered as mobile at a concentration of 10 μg/ml or higher were less vivid than the controls and showed athetoid movements. At 1 μg/ml and below there seemed to be no effect upon the treponemes. Neither propylene glycol, nor fresh or inactivated guinea pig serum influenced the mobility of spirochetes.

In vivo tests. The experiments in rabbits infected with T.p. and treated with intraperitoneal injections of metronidazole for 4 days starting at the 3rd or 7th day of infection showed that the spirochetes were not affected by the treatment. The mobility and the pattern of movement of the organisms were both similar to those of the untreated controls. No differences in this respect were noted in the rabbits that were injected with 25 mg/kg/day of metronidazole compared with the rabbits that received a tenth of this amount or between the rabbits that were treated early (3 days after the infection) compared with those that were treated late (7 days after the infection).

Table 1. *Influence of metronidazole in different concentrations on the mobility of Treponema pallidum in vitro*

	Metronidazole concentrations (μg/ml)					
	1 000	100	10	1	0.1	0.01
Mobile/immobile	0/25	5/20	19/6	25/0	25/0	25/0

DISCUSSION

The present investigation demonstrated that metronidazole does not inhibit the mobility of T.p. in vitro at a concentration below 1 μg/ml. A slight immobilizing activity was seen at a concentration of 10 μg/ml. Furthermore, the pattern of movement was changed in an athetoid direction, indicating profound though not lethal damage to the spirochetes. At higher concentrations all spirochetes exposed to metronidazole were immobilized. The results are not affected by the presence or absence of fresh guinea pig serum as a source of complement. The vehicle, propylene glycol, was also indifferent in this respect.

Patients treated for trichomonas infestations usually receive a dose of 0.2 g metronidazole 3 times a day for 7 days. This corresponds to a serum concentration of 3.6–9.8 μg/ml according to Kane et al. (5). Davies et al. (2) demonstrated a minimum inhibitory concentration of 0.02 μg/ml for metronidazole on Reiter treponemata. Wilkinson et al. (12) established the 50% immobilization of T.p. (Nichol) by metronidazole at 5.2 μg/ml (mean value). Our own results correspond fairly well with those of the latter investigation. On the basis of these results Davies (3) postulated that metronidazole treatment might interfere in the performance of the TPI test, and Wilkinson et al. (12) proposed that sera from such patients should not be submitted for TPI tests.

An important and always included routine control of the TPI test, establishing the specificity of the reaction as a complement fixing antigen-antibody reaction, is the performance of the test in the presence as well as the absence of complement. Our results showed no dependence of complement as an antigen-antibody mediated TPI would do. Thus, it is hard to understand why false TPI results would be obtained in analysis of sera from patients treated with metronidazole. Besides, unspecific immobilization in the absence of complement is also found against icteric sera and sera of patients under antibiotic treatment. In these cases the serological laboratory can easily test these adverse reacting sera by FTA-abs test (employing killed T.p.), thereby providing the clinician with the adequate and desired information.

The present in vivo experiments failed to reveal any inactivation or immobilization of the spirochetes. The highest dose administered is about 2.5 times that usually given to patients.

However, the animals were treated parenterally, which should result in a very high serum concentration. Our results are in agreement with those of Yobs et al. (13). They found that metronidazole administered orally in a dose corresponding to almost 150 times of that currently recommended for man, or intramuscularly in a dose about 20 times higher than that for man, was without effect on T.p. inoculated intracutaneously in rabbits.

In man, metronidazole in ordinary doses is reported to be without effect in primary syphilis (11, 12). On the other hand, higher doses (>2 g/day) were reported by Davies (3) and Ramachander et al. (10) to cause disappearance of spirochetes and healing of the syphilitic lesions although healing was slower than that seen during penicillin therapy.

The discrepancy between the negative results of metronidazole in rabbits and the positive effect reported in man on healing of syphilis may partly be attributed to different susceptibility of the T.p. in man and T.p. (Nichol) in rabbits. Thus, an effect of metronidazole on syphilis in man cannot be excluded, especially not if higher doses are administered as in the treatment of amoebiasis and giardiasis (4).

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