In Vitro Effect of Menfegol on Neisseria gonorrhoeae

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Summary

Bacteriostatic and bactericidal effect of Menfegol, which has been used as a spermicide, on Neisseria gonorrhoeae was investigated in vitro. The distribution of the MICs of N. gonorrhoeae to Menfegol consisted of 2 groups. Resistant strains showed the MICs of more than 3200 µg/ml while the MICs of sensitive strains were less than or equal to 200 µg/ml. When the resistant strains were suspended in several concentrations of Menfegol and were incubated at 35°C, no concentrations inactivated gonococci completely. However, the number of organisms was remarkably decreased within 30 minutes.

Introduction

Protective effect of chemical contraceptives on viral and bacterial agents of sexually transmitted diseases (STDs) has been reported1-5. These studies revealed the efficacy of contraceptive use to reduce the risks of not only pregnancy, but also STDs. Recently, the incidence of STDs is said to be rising. The variety of sexual activities and the increasing occurrence of drug resistant pathogens may play an important role on it. The need for control measures to prevent the spread of STDs in public health exists while administration of therapeutic agents to patients is fundamental.

Menfegol (p-menthanylphenyl polyoxyethylene (8.8) ether. M.W.: 620.03) has been developed and used as a spermicide since 1969. It is a nonionic surfactant derived from turpentine oil. This safe chemical has also the virucidal effect which has been reported on HIV6). In this study, we investigated the in vitro effect of Menfegol on N. gonorrhoeae.

Materials and Methods

The reference strains used were Bowden kindly given by Dr. Finkelstein at University of Texas, WHO VII and WHO 6988 by Dr. Reyn at the Statens Serum Institute, 905 W/NRL by Dr. Knapp at Washington University, and F62, F93 and 76-073389 from the Center for Disease Control. Clinical strains were isolated from patients who attended clinics in Tokyo, Osaka, Kanagawa or other areas in Japan. Clinical strains were confirmed with physiological characteristics and were stored by the gelatin-disc method at -80°C. Organisms were grown on GC medium base (Difco) with defined supplement7) in a candle jar at 35°C. Twenty per cent. w/v stock solution of Menfegol in saline or PBS, pH 7.0 was prepared and diluted as needed. The pH of Menfegol dilutions was adjusted to pH 7.0 with 0.1 N NaOH.

For the purpose of investigating the bacteriostatic effect, the minimum inhibitory concentrations (MICs) of 220 strains to Menfegol were determined. The procedure for the MICs according to CDC was employed except for using GC medium base with defined supplement. The inoculums were prepared by suspending 18-hour-old colonies in nutrient broth to give a concentration of approximately 10^6 CFU/ml. The suspensions were inoculated with a micro-inoculater on GC medium agar containing Menfegol in
serial two-fold dilutions. Plates were incubated in a candle jar at 35°C for 24 hours. After incubation, plates were read to determine the MICs.

To examine the bactericidal effect of Menfegol, the following study was made. Eighteen-hour-old cultures were suspended in PBS, pH 7.0. Suspensions were filtrated through 1.2 μm pore-sized filter (German Sciences) to remove bacterial clumps. Additional buffer solution was added to bring suspensions to a concentration of approximately 10⁶ CFU/ml. One ml of each suspension was added to 9 ml of PBS, pH 7.0 without Menfegol and serial ten-fold dilutions of Menfegol (0.001% to 1%) in PBS, pH 7.0, and were mixed. Test samples were incubated in a water bath at 35°C. After intervals, the total numbers of organisms in the mixture were determined by the standard plate dilution method on GC medium base with defined supplement.

**Results**

Bacteriostatic effect: The distribution of the MICs of *N. gonorrhoeae* contained 2 groups (Table 1). Sensitive strains were involved in the range between 1.563 μg/ml and 200 μg/ml. On the other hand, resistant strains had the MICs of more than 3200 μg/ml. About 18 per cent. of clinical isolates studied were resistant to Menfegol.

For a preliminary study to investigate the viability of *N. gonorrhoeae* suspended in buffer solution, an experiment carried out was as follows: after incubation in a candle jar at 35°C for 18 hours, colonies of *N. gonorrhoeae* were collected and suspended in 10 ml of PBS, pH 7.0. The numbers of organisms were adjusted to a concentration of approximately 10⁷ CFU/ml by adding additional buffer. The suspensions were incubated in a water bath at 35°C. After intervals, the suspensions were inoculated by streaking on GC medium base with defined supplement, using 3 mm wire loop. Plates were incubated in a candle jar at 35°C for 24 hours and were read. The results are shown in Table 2. The organisms suspended in PBS, pH 7.0 survived for 4 hours. These results enabled us to undertake the experiments for evaluating the

| Table 1 Distribution of MICs of *Neisseria gonorrhoeae* to Menfegol |
|--------------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         | Total No. | μg/ml %     | 0.0001 | 0.0003 | 0.0006 | 0.0010 | 0.0030 | 0.0050 | 0.010 | 0.020 | 0.032 |
| Reference strains       | 7          | 1.563 %     | 3      | 3      | 42.9   | 85.7   | 100    |        |       |       |       |
| Clinical strains        | 213        | 3.3 %       | 6      | 14     | 14     | 78     | 50     | 7      | 4     | 39    |       |
|                         |            |             |        |        |        |        |        |        |       |       |       |

*: Cumulative percentage.

| Table 2 Viability of *N. gonorrhoeae* in PBS (pH 7.0) |
|--------------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         | No. strains    | Time (min)    |                |                |                |                |                |                |                |
|                         | 0 30 60 120 180 | 240           |                |                |                |                |                |                |                |
| Reference strains       | 7 7* 7 7 NT NT NT |                |                |                |                |                |                |                |                |
| Clinical strains        | 7 7 7 7 7 7 |                |                |                |                |                |                |                |                |

*: Number of strains that showed colony growth.
**: Not tested.

![Fig. 1 Concentration and exposure time effects of Menfegol on Neisseria gonorrhoeae.](image)
bactericidal effect of Menfegol in which the viability of organisms in several concentrations of Menfegol was compared.

Bactericidal effect: The bactericidal effect of Menfegol on *N. gonorrhoeae* was examined with 3 resistant strains which were randomly selected from the resistant group (Fig. 1). Organisms in PBS without Menfegol survived for several hours without remarkable decrease in number. The total number of organisms in the Menfegol solutions was considerably reduced within 30 minutes. No concentrations of Menfegol, however, gave complete loss of organisms. The effect of concentration more than 0.001% was almost comparable.

Discussion

The inactivation of HIV by Menfegol was reported by Miyamoto et al. The virus was exposed to 0.063 mM (0.004%) and 0.25 mM (0.016%) of Mefegol. The tissue culture infectious dose (TCID<sub>50</sub>/ml) of recovered virus was decreased from $2 \times 10^{4.2}$ TCID<sub>50</sub>/ml to $2 \times 10^{1.8}$ TCID<sub>50</sub>/ml and less than 10 TCID<sub>50</sub>/ml.

Our results showed that some of clinical strains of *N. gonorrhoeae* were resistant to Menfegol according to the MIC values. However, even these resistant strains were inactivated, and the number of viable organisms was remarkably diminished in more than 0.001% Menfegol solution within 30 minutes. The concentration of 0.001% is less than an estimated level produced by the spermicidal tablet containing Menfegol. The tablet is formulated so that the final concentration is approximately 0.6% when administered and diluted with vaginal fluid and ejaculate.

The mechanisms of inactivating *N. gonorrhoeae* by Menfegol have not been revealed. Furthermore, studies of influence on attachment of gonococci to cells in low concentrations of Menfegol are needed. These further projects are in progress.

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References

リン菌に対するメンフェゴールのin vitroでの効果

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殺精子剤として使用されているメンフェゴールのリン菌に対する効果をin vitroで調べた。リン菌のメンフェゴールに対する最小発育阻止濃度（MIC）は二峰性を示し、耐性株は3,200μg/mlを示したが、感受性株では200μg/ml以下であっ

た。各種濃度のメンフェゴール溶液にリン菌を浮遊させ、35℃で静置したところ、どの濃度でもリン菌が完全に消失するには至らなかった。しかし、菌数は30分以内に急激に減少した。