Microbiological Study of Copiamycin

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Copiamycin, an antibiotic agent, has shown in vitro activity against Candida albicans, Torulopsis glabrata, and Trichomonas vaginalis. Local administration of copiamycin to mice inoculated intraperitoneally with protozoa reduced the percent of infection as measured by decreased abscess formation. This antibiotic has had little effect on the glucose oxidation by protozoa. Its action on anaerobic glucose metabolism in these organisms was equal to that of amitrole and azalomycin F. From these results we conclude that copiamycin is an effective antifungal and antitrichomonal agent equivalent in activity to azalomycin F.

Copiamycin is an antibiotic produced by Streptomyces hygroscopicus var. crystallogenes ATCC 19040 as reported by Arai et al. (2) in 1965. This antibiotic is assumed to have a guanidyl group, COOH group, double bond, and lactone ring with a molecular weight of 1,100 to 1,200. It is highly insoluble in water. Copiamycin is effective in vitro against yeasts, fungi, and protozoa, having shown growth-inhibitory activity against Candida, Mucor, Cryptococcus, Trichophyton, and Trichomonas vaginalis (1). It has exhibited no activity against Bacillus subtilis, B. cereus, Staphylococcus aureus, Mycobacterium, Escherichia coli, Klebsiella pneumoniae, and certain other bacteria. The acute toxicity (LD50) of copiamycin in mice is 24.8 mg/kg by intraperitoneal administration and 61.5 mg/kg by subcutaneous injection. This study pertains to the in vitro and in vivo effectiveness of copiamycin and its action on the sugar metabolism of protozoa.

MATERIALS AND METHODS

In vitro activity. The activity of copiamycin against fungi and protozoa isolated from clinical patients was determined by the following dilution method. (i) For in vitro antifungal activity, dilutions of copiamycin were made in accordance with the standard dilution method of Japan. Fungi that had been incubated for 2 weeks in Sabouraud agar containing 1% glucose were suspended in physiological saline to give a 10⁵ cells/ml suspension and were streaked on Sabouraud' agar media by the usual agar-streak method. Plates were incubated at 27°C for 72 hr and were then examined for growth. The minimum inhibitory concentration (MIC) was determined.

(ii) For in vitro antitrichomonal activity, dilutions of copiamycin were made in simplified yeast serum (SYS) medium, and a solution of T. vaginalis cells that had been incubated at 37°C for 72 hr in this same medium was prepared to give a 10⁶ cells/ml suspension. After incubation in SYS medium with 10% serum added, the MIC was determined microscopically. The composition of SYS medium is as follows: yeast extract, 2.0 g; powdered polypropylene, 2.0 g; cystine hydrochloride, 0.2 g; glucose, 1.0 g; and distilled water, 100 ml. After adjusting this mixture to pH 5.6, 5 ml was poured into each test tube.

In vivo antitrichomonal activity. The in vivo antitrichomonal activity of copiamycin was evaluated on the basis of inhibition of the abscess formation caused when T. vaginalis is injected intraperitoneally in mice. Eight to 10 days after injection of T. vaginalis (500 × 10⁴/ml), several abscesses are usually formed in the liver and other abdominal organs of these animals.

The animals were divided into two control groups and four dose groups, each with two subgroups; 0.01, 0.1, 1.0, or 10 mg of copiamycin was administered locally either as a single dose or once daily for 3 days immediately after inoculation. There were 10 mice in each dosage subgroup and 20 mice in both control groups. The antibiotic was suspended in dimethyl sulfoxide-methanol-distilled water (3:4:3) and rubbed into the skin.

Laparotomy was carried out 5 or 10 days after inoculation, and abscess formation was evaluated microscopically.

Determination of the sugar metabolism of protozoa. The effect of copiamycin in protozoa on the oxidation of glucose and on the production of lactic acid was studied with a Warburg manometer. A buffered suspension of protozoa was prepared as follows. T. vaginalis that had been incubated in SYS medium for 74 hr was isolated centrifugally, and the precipitate was washed three times with Krebs-Ringer-phosphate (KRP) buffer (pH 6.4). Cells were then resuspended in buffer.

For studies on the oxidation of glucose, 1 ml of the buffered solution of T. vaginalis cells was transferred to the main chamber of the Warburg manometer and 0.5 ml of 0.01 m glucose was added to the solution to
The following results were obtained on the activity of copiامycin against yeasts and protozoa and on the effect of the drug on the sugar metabolism of protozoa.

**In vitro activity.** The MIC of copiамycin against eight clinical isolates of *Candida albicans* was in the range of 0.78 to 3.13 µg/ml, and five isolates were sensitive to 1.56 µg of copiамycin per ml. The MIC against four strains of *Torulopsis glabrata* was also 0.78 to 3.13 µg/ml. Against 14 clinical isolates of *T. vaginalis*, the MIC was in

| TABLE 1. Sensitivity of clinical isolates of yeasts to copiамycin |
|-------------------------|-----------------|
| Isolates            | MIC (µg/ml)    |
| --------------------|----------------|
| *Candida albicans*   |                |
| M16                 | 1.56           |
| M21                 | 1.56           |
| M22                 | 0.78           |
| M23                 | 3.13           |
| M24                 | 1.56           |
| M25                 | 1.56           |
| M29                 | 1.56           |
| M30                 | 3.13           |
| *Torulopsis glabrata* |              |
| M12                 | 3.13           |
| M14                 | 3.13           |
| M15                 | 0.78           |
| M22                 | 1.56           |

* Minimum inhibitory concentration.

| TABLE 2. Sensitivity of clinical isolates of *Trichomonas vaginalis* to copiамycin |
|-------------------------|-----------------|
| Isolates            | MIC (µg/ml)    |
| --------------------|----------------|
| M09                 | 50             |
| M10                 | 25             |
| M11                 | 25             |
| M13                 | 100            |
| M14                 | 100            |
| M15                 | 50             |
| M16                 | 25             |
| M17                 | 25             |
| M18                 | 100            |
| M22                 | 50             |
| M24                 | 12.5           |
| M27                 | 50             |
| M29                 | 50             |
| M31                 | 25             |

* Minimum inhibitory concentration.

give the same glucose concentration as the SYS medium. In addition, 0.2 ml of 20% KOH was transferred to the accessory chamber, and the copiамycin solution was transferred to the side chamber. The effect of the antibiotic on the uptake of O2 was observed for 74 hr at 37 C.

To study the effect of copiамycin on the production of lactic acid in protozoa, 1 ml of the buffered solution of *T. vaginalis* cells was transferred to the main chamber of the Warburg manometer, 0.5 ml of 0.5% glucose solution was added as a base medium, and an appropriate amount of copiамycin was put into the accessory chamber. The interior of the flask was filled with N2 gas. The effect of copiамycin on the anaerobic metabolism of glucose by protozoa was then measured by the decrease in the amount of lactic acid produced after incubation at 37 C for 1 hr. Determinations of the lactic acid concentrations were carried out by the Barker-Summerson method.

FIG. 1. Experiment on prevention of infection by copiамycin in mice inoculated with *Trichomonas vaginalis*.
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Aminitrozole
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FIG. 2. Comparative effects of protozoacides on inhibition of glucose oxidation of Trichomonas vaginalis.

Effect on sugar metabolism. When the effect of copiamycin on the oxidation of glucose by T. vaginalis was investigated, the rate clearly changed when 10 μg of copiamycin per ml was added. This effect of copiamycin on glucose oxidation by protozoa can be explained by the following formula:

\[ O_H = \frac{(A - C)}{(A - B)} \times 100\% \]

in which \( O_H \) is the oxidation-inhibitive rate of base medium, \( A \) is the consumption of oxygen when base medium was added, \( B \) is the consumption of oxygen when base medium was not added, and \( C \) is the consumption of oxygen when base medium as well as protozoacide was added.

Figure 2 gives a comparison of the oxidation-inhibitive rate in \( T. \) vaginalis by various kinds of protozoacides. This shows that in the case of 5 μg of copiamycin \( O_H \) is 5% (i.e., oxidation was only very slightly inhibited) and that in the case of 10 μg \( O_H \) is 47%, presenting a value similar to that of azalomycin F. This effect of copiamycin on anaerobic glucose metabolism can be explained by the following formula:

\[ L_H = \frac{(D - E)}{D} \times 100\% \]

in which \( L_H \) is the production-inhibitive rate of lactic acid, \( D \) is the amount of lactic acid produced by glucose, and \( E \) is the amount of lactic acid produced at the time of addition of protozoacide.

Figure 3 shows the effect \( L_H \) of various protozoacides on the inhibition of lactic acid production by \( T. \) vaginalis. The inhibition rate of copiamycin was 7 and 17%, respectively, when 20 and 40 μg were used. These values are similar to the lactic acid production-inhibitive rate of aminitrozole and azalomycin F (3).

FIG. 3. Comparative effects of protozoacides on inhibition of lactic acid production by Trichomonas vaginalis.

The range of 12.5 to >100 μg/ml, presenting a fairly wide range of sensitivity peaking at 25 or 50 μg/ml (Tables 1 and 2).

Results of the comparison of the sensitivities of a standard strain of \( T. \) vaginalis to copiamycin and to other protozoacides are shown in the Table 3. This table does not indicate copiamycin to be superior to the other drugs tested against \( T. \) vaginalis. It can be seen that the sensitivity of this protozoa to copiamycin is comparable to azalomycin F (reference 3; Table 3).

In vivo activity. After the administration of copiamycin to mice inoculated intraperitoneally with \( T. \) vaginalis, the following results were obtained (Fig. 1). The group given a single, 0.01-

mg dose of copiamycin did not differ from the control group either at 5 or 10 days after inoculation. The group that received a single, 10-mg dose of copiamycin showed the antibiotic to be approximately 60% effective.

On the other hand, in the groups given copiamycin daily for 3 days, the drug was effective even in the group given the lowest dose (0.01 mg × 3), both at 5 and 10 days after inoculation. After 10 days, the antibiotic was effective in 8% of the group given a dose of 1.0 mg × 3 and in 100% of the group given a dose of 10 mg × 3.

Discussion

Copiamycin, an antibiotic agent, has shown in vitro activity against \( C. \) albicans, \( T. \) glabrata, and \( Trichomonas vaginalis \). Local administration of copiamycin to mice inoculated intraperitoneally...
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