Microbiological Study of Copiamycin

KANJI SEIGA AND KUNIHIKO YAMAJI

Obstetric and Gynecological Department of Health Insurance, Kobe Central Hospital, Ikuta-Ku, Kobe, Japan, and Kinki Mother's and Children's Infectious Diseases Center, Kobe, Japan

Received for publication 23 November 1970

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 per cent 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 aminitroazole and azolomycin F. From these results we conclude that copiamycin is an effective antifungal and antitrichomonal agent equivalent in activity to azolomycin 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 10⁶ 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 polypeptone, 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. In 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 × 10⁶/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
TABLE 1. Sensitivity of clinical isolates of yeasts to copiamicin

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MIC* (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td></td>
</tr>
<tr>
<td>M16</td>
<td>1.56</td>
</tr>
<tr>
<td>M21</td>
<td>1.56</td>
</tr>
<tr>
<td>M22</td>
<td>0.78</td>
</tr>
<tr>
<td>M23</td>
<td>3.13</td>
</tr>
<tr>
<td>M24</td>
<td>1.56</td>
</tr>
<tr>
<td>M25</td>
<td>1.56</td>
</tr>
<tr>
<td>M29</td>
<td>1.56</td>
</tr>
<tr>
<td>M30</td>
<td>3.13</td>
</tr>
<tr>
<td>Torulopsis glabrata</td>
<td></td>
</tr>
<tr>
<td>M12</td>
<td>3.13</td>
</tr>
<tr>
<td>M14</td>
<td>3.13</td>
</tr>
<tr>
<td>M15</td>
<td>0.78</td>
</tr>
<tr>
<td>M22</td>
<td>1.56</td>
</tr>
</tbody>
</table>

* Minimum inhibitory concentration.

TABLE 2. Sensitivity of clinical isolates of Trichomonas vaginalis to copiamicin

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MIC* (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M09</td>
<td>50</td>
</tr>
<tr>
<td>M10</td>
<td>25</td>
</tr>
<tr>
<td>M11</td>
<td>25</td>
</tr>
<tr>
<td>M13</td>
<td>100</td>
</tr>
<tr>
<td>M14</td>
<td>100</td>
</tr>
<tr>
<td>M15</td>
<td>50</td>
</tr>
<tr>
<td>M16</td>
<td>25</td>
</tr>
<tr>
<td>M17</td>
<td>25</td>
</tr>
<tr>
<td>M18</td>
<td>100</td>
</tr>
<tr>
<td>M22</td>
<td>50</td>
</tr>
<tr>
<td>M24</td>
<td>12.5</td>
</tr>
<tr>
<td>M27</td>
<td>50</td>
</tr>
<tr>
<td>M29</td>
<td>50</td>
</tr>
<tr>
<td>M31</td>
<td>25</td>
</tr>
</tbody>
</table>

* 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 copiamicin 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 copiamicin 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 copiamicin was put into the accessory chamber. The interior of the flask was filled with N2 gas. The effect of copiamicin 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.

RESULTS

The following results were obtained on the activity of copiamicin against yeasts and protozoa and on the effect of the drug on the sugar metabolism of protozoa.

In vitro activity. The MIC of copiamicin 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 copiamicin 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 3. Comparison of antitrichomonal activity of protozoacides

<table>
<thead>
<tr>
<th>Protozoacides</th>
<th>MIC* against standard strain (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomycin</td>
<td>1.0</td>
</tr>
<tr>
<td>Azalomycol F</td>
<td>12.5</td>
</tr>
<tr>
<td>Copiamicin</td>
<td>25</td>
</tr>
<tr>
<td>Carbarsone</td>
<td>250</td>
</tr>
<tr>
<td>Quinoform</td>
<td>50</td>
</tr>
<tr>
<td>Chlorhexidine diacetate</td>
<td>60</td>
</tr>
<tr>
<td>Aminitrozole</td>
<td>2.0</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>2.0</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Minimum inhibitory concentration.

TABLE 4. Sensitivity of clinical isolates of protozoa to copiamicin

<table>
<thead>
<tr>
<th>Dose of Copiamicin</th>
<th>5th day after inoculation</th>
<th>10th day after inoculation</th>
<th>No. of tested mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>0.01mg × 1</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>0.1mg × 1</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1.0mg × 1</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>10mg × 1</td>
<td></td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Groups given Copiamicin three times

FIG. 1. Experiment on prevention of infection by copiamicin in mice inoculated with Trichomonas vaginalis.
Metronidazole
Aminitrozole
Azalomycin

This
be
range
wide
Aimitrozole
Metronidazole
Azalomycin
T.
vaginalis.
T.
coipiamycin
to
inhibition
of
T.
vaginalis.
standard
a
obtained
(Fig. 1).

Results of
It
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.

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
the
protozoa
can
be
explained
by
the
following
formula:

\[ \text{O}_{\text{H}} = \frac{(A - C)}{(A - B)} \times 100\% \]

in
which
\( \text{O}_{\text{H}} \)

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
\( \text{O}_{\text{H}} \) is
5%
(i.e.,
oxidation
was
only
very
slightly
inhibited)
and
that
in
the
case
of
10
µg
\( \text{O}_{\text{H}} \) is
47%
, presenting
a
value
similar
to
that
of
azalomycin
F.
This
effect
copiamycin
on
anaerobic
glucose
metabolism
can
be
explained
by
the
following
formula:

\[ \text{L}_{\text{H}} = \frac{(D - E)}{D} \times 100\% \]

in
which
\( \text{L}_{\text{H}} \)

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
\( \text{L}_{\text{H}} \)
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).

DISCUSSION

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

Fig. 2. Comparative effects of protozoacides on inhibition of glucose oxidation of Trichomonas vaginalis.

Inhibition rate
Number of Trichomonas vaginalis
0 20 40 60 80 100%
Aminitrozole
20 µg
10 µg
5 µg
0
500 × 10⁶
500 × 10⁶
500 × 10⁶

Metronidazole
20 µg
10 µg
5 µg
0
610 × 10⁶
600 × 10⁶
600 × 10⁶

Trichomycin
10 µg
5 µg
0
750 × 10⁶
750 × 10⁶
750 × 10⁶

Azalomycin F
40 µg
20 µg
10 µg
5 µg
0
1500 × 10⁶
1500 × 10⁶
1500 × 10⁶

Copiamycin
40 µg
20 µg
10 µg
5 µg
0
720 × 10⁶
720 × 10⁶
720 × 10⁶

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-

with protozoa reduced the per cent 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 aminitrozole and azalomycin F.

For these results, we conclude that copiamycin is an effective antifungal and antitrichomonal agent equivalent in activity to azalomycin F.

LITERATURE CITED