In vitro activity of ribostamycin against Prototheca sp.

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Abstract

The in vitro activity of ribostamycin against algae of the genus Prototheca was evaluated by minimum inhibitory concentration (MIC) studies on solid media. Concentrations of 4 mcg/ml were required to inhibit 100% of the P. zopfii strains; 16 mcg/ml inhibited 100% of the P. stagnora strains and 95% of the P. wickerhamii strains. These values are inferior to plasma concentrations obtained after injection of ribostamycin. It is likely that this antibiotic could be effective in the treatment of protothecosis in man.

Introduction

The unicellular algae of the genus Prototheca, although known since described by Kruger in 1894 (7), were not conclusively related with human pathology until 1964 when Davies and colleagues communicated the first case in man (2). Actually, their pathogenicity is accepted and the human disease they produce called protothecosis (1).

Ribostamycin is an aminoglycoside antibiotic produced by Streptomyces ribosidoficus, with a molecular formula C_{17}.H_{34}.N_{4}.O_{10} and a chemical structure that includes D-ribose in the C5 of neamine. It is water soluble and stable in powder form and in aqueous solution.

Ribostamycin inhibits the growth of: Gram positive microorganisms such as Staphylococcus aureus, Staph. epidermidis, Streptococcus hemolyticus, Strep. viridans, Diplococcus pneumoniae, Enterococcus; and Gram negative organisms like Neisseria gonorrhoea, Salmonella, Vibrio parahaemolyticus, Shigella, Escherichia coli, Klebsiella, Enterobacter, Citrobacter, Proteus, Pseudomonas aeruginosa. It does not inhibit yeasts or fungi.

In preliminary in vitro sensitivity studies using the disc-plate method, we observed that ribostamycin inhibited algae of the genus Prototheca (3, 4). This finding motivated us to make a more rigorous study of the minimal inhibitory concentrations (MIC) required for different Prototheca species.

Material and methods

Seventy-eight strains were assayed: 44 Prototheca wickerhamii, 24 P. zopfii and 10 P. stagnora. The strains came from our department's collection and international collections belonging to the following researchers: Ajello, L., Mycology Division, CDC Public Health Service, Atlanta, Georgia 30333, USA; Ball, E. H, Medical Mycology Section, University of Glasgow, G 11, Scotland; Feo, M., Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela; Hocquet, P., Service des Maladies Parasitaires et Exotiques, Centre Hospitalier Regional, 49036 Angers Cedex, France; Koenig, H., Institut de Parasitologie et Pathologie Tropical, Faculté de Médecine, Université L. Pasteur, Strasbourg, France; Kurtzman, C. P., Zymologist, ARS Culture, Department of Agriculture, Peoria, IL 61604, U.S.A; Pore, R. S., Department of Microbiology, Medical Center West, Virginia University, Morgantown, WV 26506, U.S.A. Wild strains isolated in our laboratory from residu-
al waters (2, 5) were also assayed.

All the strains were conserved in Sabouraud’s glucose agar and in Sabouraud’s glucose agar with chloramphenical at 25 °C until use. Sabouraud’s glucose agar (Difco 0109) was used for the MIC study.

Laboratorios Morrith, S.A. of Spain, provided the powdered ribostamycin.

The MIC was determined using a method of dilution in a solid medium.

To obtain the inoculum, a 5-day culture of the *Prototheca* under study was made in Sabouraud’s glucose agar. From this, a 10 mcg loop of the germ was taken and emulsified in 10 ml of sterile distilled water. The emulsion was corrected until its turbidity equalled 1 McFarland. From this seed suspension a 1/100 dilution was made (3).

Antibiotic concentrations ranging from 0.25 mcg/ml to 128 mcg/ml were tested in solid Sabouraud’s glucose agar.

The media were incubated in a Ster’s inoculator with 36 recipients.

Results were read after 3 days incubation and confirmed at 5 days incubation at 28 °C for *P. wickerhamii* and *P. zopfii* and at 25 °C for *P. stagnora*. The MIC was considered to be the lowest antibiotic concentration that totally inhibited algae growth.

Results

Results are presented in Table 1. *P. zopfii* required the least MIC; 100% of the strains were inhibited with 4 mcg/ml. The next lowest MIC was for *P. stagnora*, 16 mcg/ml to inhibit 100% of the strains, although 70% were inhibited with 4 mcg/ml. *P. wickerhamii* was the most resistant species; 75% of the strains were inhibited with 4 mcg/ml and 100% with 32 mcg/ml.

Discussion

The possible activity of antibiotics against algae of the genus *Prototheca* has been little investigated to date. The aminoglycoside antibiotics inhibit these algae *in vitro* and ribostamycin presents the largest inhibitory halos with the disc-plate method (3, 4, 5).

Ribostamycin reaches plasma concentrations of approximately 25 mcg/ml 30–60 minutes after intramuscular administration of 500 mg (8).

Summarizing the results of the MIC studies for the genus *Prototheca*, 100% of the *P. zopfii* strains were inhibited at 4 mcg/ml and 100% of the *P. stagnora* strains and 95% of the *P. wickerhamii* strains were inhibited at 16 mcg/ml. These values are inferior to the concentrations reached in plasma, therefore an *in vivo* trial in laboratory animals or in humans for the treatment of protothecosis is plausible. As of yet, no treatment has been determined for this disease.

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| Table 1. Susceptibility accumulative of Prototheca genus to Ribostamycin. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species         | No. of strains | Minimal inhibitory concentration in mcg/ml |
|                 |                 | 0.25 | 0.5 | 1    | 2    | 4    | 8    | 16   | 32   | 64   | 128  |
| *P. wickerhamii*| 44              | 15   | 23  | 32   | 33   | 40   | 42   | 44   |        |      |      |
|                 |                 | (34%)| (52%)| (73%)| (75%)| (91%)| (95%)| (100%)|        |      |      |
| *P. zopfii*     | 24              | 12   | 16  | 21   | 24   |      |      |      |        |      |      |
|                 |                 | (50%)| (67%)| (88%)| (100%)|      |      |      |        |      |      |
| *P. stagnora*   | 10              | 3    | 7   |      |      |      |      |      | 10    |      |      |
|                 |                 | (30%)| (70%)|    |      |      |      |      | (100%)|      |      |

The numbers in brackets correspond to the percentage.