ACTION OF PULVOMYCIN AND KIRROMYCIN ON EUKARYOTIC CELLS

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1. Introduction

The antibiotic pulvomycin has been shown to be related to kirromycin with respect to its structure and function [1]. Pulvomycin resembles the 5′-substituent of the tetrahydrofuran moiety of kirromycin [1]. Both antibiotics inhibit prokaryotic protein synthesis by virtue of their specific interaction with elongation factor Tu [1,2]. Since pulvomycin is known to be cytotoxic against malignant cells in tissue culture and against cells of the ascitic form of Ehrlich carcinoma (ECA) in mice [3,4], studies were undertaken to determine the effect of both pulvomycin and kirromycin on macromolecular syntheses in eukaryotic systems.

2. Materials and methods

ECA cells were propagated in mice by weekly intraperitoneal transfer. These mice were a gift of H. Probst (Tübingen). The human cell line studied was HeLa, strain S3; the cultured cells were a gift of O. G. Issinger (Stuttgart). Radiochemicals were purchased from Amersham Buchler (Braunschweig).

Macromolecular syntheses in ECA and HeLa cells were performed as in [5]. Cells (3×10^6) in 3 ml phosphate-buffered saline [6] containing 0.6 mg heparin (Serva) were incubated with the antibiotics for 10 min at 37°C. The cell suspension was then transferred to a tube containing 0.1 μCi [^{14}C]-thymidine (61 Ci/mol), 0.1 μCi [^{14}C]uridine (53 Ci/mol), or 0.1 μCi [^{14}C]leucine (59 Ci/mol). After 20 min at 37°C the cells were centrifuged, suspended in trichloroacetic acid (5%), and collected on cellulose nitrate filters. The filters were dried and the radioactivity was measured.

Extracts of ECA and wheat embryo cells (Keimdiät GmbH, Augsburg) for polypeptide synthesis were prepared as in [7]. Material of low molecular weight was removed from the 30 000 × g supernatant by Sephadex G-25 filtration. Polyphenylalanine synthesis was performed as in [7].

Pulvomycin (mol. wt 438) was isolated from *Streptoverticillium mobaraense* Tü 1063 according to [8], and kirromycin (mol. wt 796) from *Streptomyces collinus* Tü 365 as in [9].

3. Results and discussion

The effect of either antibiotic on DNA, RNA, and protein synthesis was tested in intact ECA and HeLa cells. Figure 1 and fig.2 show that pulvomycin and kirromycin reduced uridine incorporation and, to a lesser degree, thymidine incorporation in ECA cells, whereas the rate of leucine incorporation remained unchanged during 30 min incubation; 50% inhibition of uridine incorporation into acid-insoluble material was observed at 3×10^{-6} M pulvomycin and 1×10^{-5} M kirromycin, respectively. In kinetic studies, a constant rate of inhibition of uridine incorporation was observed after 3 min incubation time.

Similar results were obtained with HeLa cells (data not shown). Thus, the pronounced effect of these antibiotics on eukaryotic cells might be due to an inhibition of RNA synthesis.

The effect of either antibiotic on poly(U)-directed polyphenylalanine synthesis in cell-free extracts of

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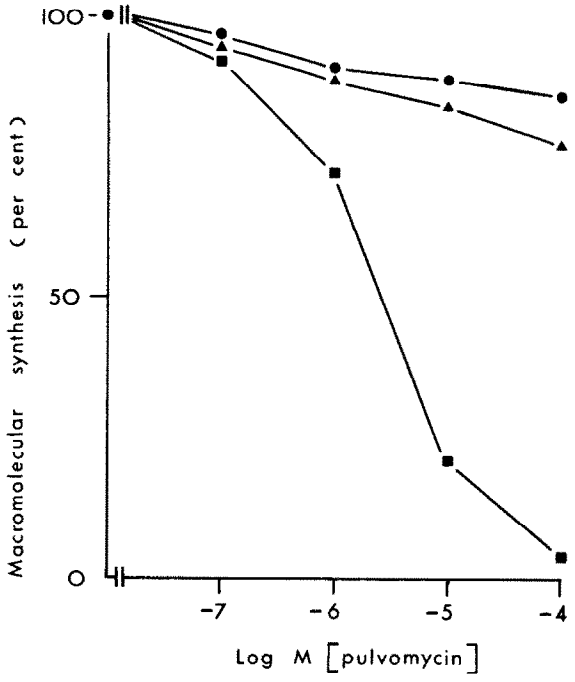

Fig.1. Effect of pulvomycin on macromolecular syntheses in Ehrlich carcinoma ascites cells (-●-) protein synthesis; (-▲-) DNA synthesis; (-■-) RNA synthesis.

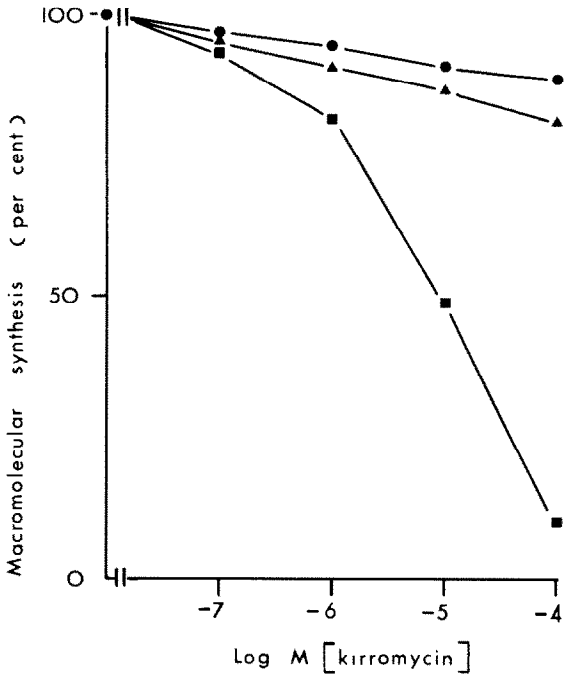

Fig.2. Effect of kirromycin on macromolecular syntheses in Ehrlich carcinoma ascites cells: (-●-) protein synthesis; (-▲-) DNA synthesis; (-■-) RNA synthesis.

Table 1
Effect of pulvomycin and kirromycin on polyphenylalanine synthesis in a cell-free system of ECA and wheat embryo cells

Additions	Conc. (M)	Phenylalanine incorporated (pmol)	
		ECA	Wheat embryo
Control		16.4	14.3
– Poly(U)		0.5	1.9
Chloramphenicol	6×10^{-4}	13.0	12.5
Cycloheximide	7×10^{-5}	4.1	7.6
Pulvomycin	2×10^{-6}	15.8	13.8
Pulvomycin	2×10^{-5}	15.2	13.6
Pulvomycin	2×10^{-4}	12.8	13.2
Kirromycin	2×10^{-6}	15.1	13.9
Kirromycin	2×10^{-5}	14.5	13.9
Kirromycin	2×10^{-4}	13.0	13.4

Reaction mixtures contained in 100 µl 50 mM Tris·HCL (pH 7.6), 80 mM KCl, 10 mM $MgCl_2$, 5 mM dithioerythritol, 1 mM ATP, 0.1 mM GTP, 1 mM creatine phosphate, 8 µg creatine kinase, 0.5 mg tRNA (yeast), 100 pmol [^{14}C]phenylalanine (0.2 µCi), 20 µg poly(U), 2 µl methanol containing pulvomycin or kirromycin as indicated, and 10 µl ECA extract or wheat embryo cells. After 15 min at 37°C, the radioactivity incorporated into hot 5% trichloroacetic acid-insoluble protein was determined

ECA and wheat embryo cells was examined. The data represented in table 1 support the findings of the in vivo experiments. Except for a slight inhibition probably due to a contamination of the cytoplasmic protein-synthesizing system by the mitochondrial system, at up to 1×10^{-4} M, both antibiotics did not interfere with this reaction.

Our results indicate that the action of pulvomycin and kirromycin on eukaryotic cells is different from that on prokaryotic cells. The antibiotics inhibit bacterial protein synthesis by acting on elongation factor Tu [1]. They do not interfere with RNA and DNA synthesis in bacteria in vivo [8,10] and in vitro (data not shown). In contrast, the cytotoxic effect of either drug on transformed eukaryotic cells might be due to an inhibition of RNA synthesis. Low toxicity of pulvomycin [11] and kirromycin [12] suggest a different sensitivity of normal and transformed cells.

Acknowledgement

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