GRISEORUBINS, A NEW FAMILY OF ANTIBIOTICS WITH ANTIMICROBIAL AND ANTITUMOR ACTIVITY

II. BIOLOGICAL PROPERTIES AND ANTITUMOR ACTIVITY OF THE ANTIBIOTIC COMPLEX GRISEORUBIN

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The antibiotic complex griseorubin has antimicrobial activity against Gram-positive as well as -negative bacteria, mycobacteria, mycoplasma and protozoa *in vitro* but it is not active against yeast and fungi. Tests with transplantable rodent tumors indicate that griseorubin is inhibitory to the growth of lymphatic leukemia L1210 in mice and ZAJDELA ascites hepatoma in rats. The acute LD_{50} of griseorubin in mice is 50 mg/kg of body weight when given intraperitoneally. Attempts to potentiate the antitumor activity by complexing with DNA proved to be unsuccessful.

As reported in the preceding paper griseorubin has been characterized as a complex of components belonging to the polycyclic C-glycosyl antibiotics of the "kidamycin" type. The present paper describes the antimicrobial properties of griseorubin and its chemotherapeutic activity against several transplantable rodent tumors.

Material and Methods

The sample of the griseorubin complex hydrochloride used in this report was prepared according to the procedure described in the preceding paper. Calf thymus DNA was a gift from Mrs. E. SARFERT (Zentralinstitut für Mikrobiologie und experimentelle Therapie Jena). This preparation was described as having an average molecular weight of about 25 millions[1].

Assay of Antimicrobial Activity

Both the agar diffusion test and the serial dilution test method were used for the determination of MIC in this work.

Anticancer Tests

Griseorubin was tested against leukemia L1210 on female $NMDF_1$ (NMRI × DBA2) or $ABD2F_1$ mice (body weight 18 ~ 24 g) and against WALKER 256 carcinosarcoma and ZAJDELA hepatoma on young Wistar rats (body weight about 70 g at mean time of experiments). Leukemia L1210 was implanted intraperitoneally by 0.2 ml saline containing 5×10^6 leukemic cells per mouse. Treatment was initiated one day after implantation and continued once daily for nine days with a stop of two days following the fourth injection. WALKER 256 carcinosarcoma was propagated subcutaneously by trocar with small fragments of about 3 ~ 4 mm diameters and treated intraperitoneally once daily for 4 consecutive days. ZAJDELA hepatoma was implanted intraperitoneally by 0.5 ml ascitic fluid. The schedule of treatment was the same as in L1210 system.

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Griseorubin-DNA Complex

Calf thymus DNA was dissolved in sterile 0.15 M NaCl to a concentration of 1.22 mg/ml, autoclaved for 15 minutes at 120°C, and cooled rapidly. Griseorubin was dissolved in this DNA solution to a final concentration of 0.3 mg/ml. The solution of griseorubin and DNA resulted in an immediate reduction of the polarographic step of griseorubin, indicative of complex formation.

The griseorubin-DNA complex was tested against L1210 on $ABD2F_1$ mice as described above. For the study of therapeutic effects, the antibiotic: DNA ratio was 1: 4 on the weight basis.

Test for Cytotoxic Effect in Tissue Culture Cells

The cytotoxic effect of griseorubin and the griseorubin-DNA complex was examined against chicken embryo cells grown in monolayers. The samples were dissolved in 0.9% NaCl solution in serial twofold dilutions and tested for cytotoxicity on 2-day-old cultures.

Acute Toxicity of Griseorubin

Water dissolved griseorubin was used for acute toxicity in male mice (AB Jena) weighing 18~23 g. Griseorubin was administered intravenously, intraperitoneally or subcutaneously in increasing doses. The observation period lasted 4 weeks. Approximate LD_{50} values were estimated according to the mortality of the mice.

Results and Discussion

Antimicrobial Activities of Griseorubin

As seen in Table 1 griseorubin exhibited antibacterial activity against a variety of Gram-positive and -negative bacteria. However, no activity was observed against *Saccharomyces, Kloeckera, Fusarium*, and *Penicillium*, respectively. The test spectrum differs from that of kidamycin in activity against some Gram-negative bacteria. Unlike kidamycin[2] griseorubin was effective against *Escherichia coli*. Significant activity against mycoplasma was noticed as illustrated in Table 2. Griseorubin had inhibitory activity only against a few species of protozoa. The results of the protozoan test are given in Table 3.

Antitumor Activities of Griseorubin

The results of *in vivo* experiments on leukemia L1210, WALKER 256 carcinosarcoma, and ZAJDELA hepatoma are illustrated in Tables 4~5. Griseorubin showed a significant increase of survival time in mice bearing L1210 and in rats implanted with ZAJDELA hepatoma. The antibiotic did not exhibit a growth inhibition of WALKER 256 carcinosarcoma. It is further noteworthy that the toxicity of griseorubin is much lower in $ABD2F_1$ mice than in $NMDF_1$ mice.

Griseorubin can be distinguished from kidamycin by a stronger antitumor activity against L1210. Kidamycin shows only a slight life-prolongation of about 10% at total doses of 18, 13.5, and 9 mg/kg when it was administered daily intraperitoneally for 8 days[3].

In attempts to enhance the antitumor activity of griseorubin without concomitant increase of host toxicity a griseorubin-DNA complex was prepared and administered to mice bearing L1210. Complexes formed with DNA as carrier behave like lysosomotropic drugs[4]. They are endocytized and the drugs are released intracellularly in a free form after intralysosomal digestion of the carrier. Under *in vivo* conditions a potentiation of the antitumor activity of adriamycin, daunomycin, and actinomycin D has been described[5~9].

Griseorubin forms a complex with DNA *in vitro*. The amount of calf thymus DNA required to bind griseorubin completely was determined by observing the change in the polarographic step of griseorubin as increasing amounts of DNA were added. Specially the anthracyclines exhibit pro-

Table 1. *In vitro* antimicrobial activity of griseorubin as determined by the agar diffusion test method.

Test organisms	MIC (mcg/ml)
Bacillus subtilis ATCC 6633	15
Escherichia coli C 600	15
Escherichia coli SG 458	15
Serratia marcescens SG 621	62
Aerobacter aerogenes SG 117	15
Pseudomonas sp. B 7	31
Pseudomonas aeruginosa SG 137	125
Bacillus globifer OH 11	15
Bacillus globifer EH 11	31
Mycobacterium phlei SG 346	15
Mycobacterium smegmatis SG 987	15
Corynebacterium equi SG 1144	15
Corynebacterium mediolanum SG 1145	15
Corynebacterium diphtheriae Typ gravis	31
Corynebacterium diphtheriae Typ mitis	31
Corynebacterium diphtheriae Typ intermed.	15

Table 2. *In vitro* susceptibility of *Mycoplasma* species to griseorubin as determined by the serial dilution test method.

Test organisms	MIC (mcg/ml)	
	Bacteriostatic	Bactericidal
Mycoplasma gallisepticum S 6	0.16	4
Mycoplasma hyorhinis	0.16	4

Table 3. *In vitro* susceptibility of protozoa species to griseorubin as determined by the serial dilution test method.

Test organisms	MIC (mcg/ml)	
	Protozoastatic	Protozoacidal
Trypanosoma equiperdum K 48	0.1	10
Trypanosoma gambiense K 51	0.25	10

Table 4. Effectiveness of griseorubin on leukemia L1210.

Doses (mg/kg mouse)	MIT	
	$NMDF_1$ mice	$ABD2F_1$ mice
30	tox**	34.5**
15	tox***	16.1**
7.5	2.2***	19.4**
4	18.5***	16.1
2	51.9***	8.7
1	38.4	10.5
0.5	17.6	

*** mean values of 3 experiments
** mean values of 2 experiments
remainder 1 experiment
MIT: mean increase of survival time in % of untreated controls
Day of L1210 injection is first day of experiment.

Table 5. Effectiveness of griseorubin on WALKER 256 carcinosarcoma and ZAJDELA hepatoma.

Doses (mg/kg rat)	WALKER 256 MIG	ZAJDELA hepatoma MIT
8.6	tox*	33.3
4.3	20.1*	32.2
2.15	15.7*	35.2
1.1	11.1*	21.6
0.6	6.2*	19.9
0.3	4.3	24.4

* Mean values of 2 experiments
remainder 1 experiment
MIG: mean inhibition of tumor growth in % of untreated controls
MIT: mean increase of survival time in % of untreated controls
Day of tumor injection is first day of experiment.

nounced signals by all polarographic techniques suitable for analytical measurements and binding studies[10], see Fig. 1.

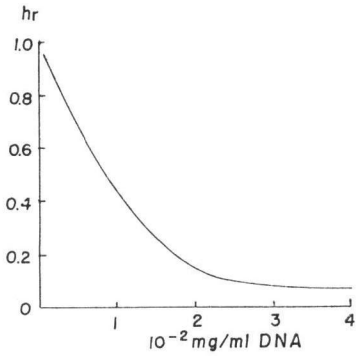
However, under conditions used it was found that the griseorubin-DNA complex was not more effective than the free griseorubin in prolonging survival of $ABD2F_1$ mice bearing L1210.

On the other hand, the griseorubin-DNA complex exhibited a reduced cytotoxic effect on the

growth of cultured chicken embryo cells *in vitro* in comparison with the free griseorubin. The maximum tolerate dose of griseorubin was 6.25 mcg/ml unlike the griseorubin-DNA complex which displayed a maximum tolerate level of >100 mcg/ml referring to griseorubin.

The LD_{50} values of griseorubin in mice were determined to be 10 mg/kg of body weight by intravenous, 50 mg/kg by intraperitoneal, and 100 mg/kg by subcutaneous injection, respectively. When the intoxication was lethal the animals died during the sixth and twelfth day after administration independent of the application route. The intraperitoneal injection of griseorubin led to a serious chronic fibroblastic peritonitis, the subcutaneous injection to skin necrosis.

Fig. 1. Titration curve of griseorubin (6×10^{-3} mg/ml in 0.9% NaCl solution)
hr: Relative polarographic step height of griseorubin



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