The Journal of Antibiotics (2010) 63, 530–532

www.nature.com/ja

NOTE

Antimicrobial spectrum of the antitumor agent, cisplatin

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The Journal of Antibiotics (2010) **63,** 530–532; doi:10.1038/ja.2010.64; published online 23 June 2010

Keywords: antimicrobial; antitumor agent; cancer; cisplatin

Cisplatin (cisplatinum, *cis*-diamminedichloroplatinum [II]), is one of the most important anticancer agents used in medicine. Its structure is shown in Figure 1. Owing to its ability to bind to DNA, cause the cross-linking of adjacent intrastrand purines, and interfere with DNA repair, cisplatin is an effective DNA-damaging and anticancer agent. Although cisplatin is nearly curative for testicular cancer and active against ovarian, head and neck cancer, the potential of this drug as a cure for many other types of cancer is limited because of cellular resistance to cisplatin[1] and cisplatin's toxicity to humans; for example, renal toxicity, emesis, neurotoxicity, bone marrow suppression, anemia and hearing loss. Owing to the toxicity, cisplatin is administered intravenously in low dosage. The inhibition of *Escherichia coli* by cisplatin was discovered by Rosenberg *et al.*[2–4] before it was known to be an effective antitumor agent. They made this discovery while performing an experiment to analyze the effect of an electric field on the growth of bacteria, the experiment involving the use of platinum electrodes.

Although Rosenberg *et al.*[3] found that *E. coli* and other Gram-negative bacteria such as *Aerobacter aerogenes, Alcaligenes faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae and Serratia marcescens* were sensitive to cisplatin, it was unclear whether other bacteria were also inhibited. At a concentration 15-fold higher than that which inhibited cell elongation in *E. coli*, Gram-positive bacteria such as *Streptococcus lactis, Streptococcus faecalis, Staphylococcus aureus, Sarcina lutea* and *Neisseria catarrhalis* were not inhibited. Although other Gram-positive organisms were inhibited by this high concentration of cisplatin, they were much more resistant than *E. coli*. We felt it important to revisit this situation and determine the antimicrobial spectrum of cisplatin. Since those early days, three yeasts have been reported to be inhibited by cisplatin; that is, *Saccharomyces cerevisiae*,[5] *Schizosaccharomyces pombe*[6] and *Candida albicans*.[7] One mold, *Dictyostelium discoideum*, has been reported to be sensitive to cisplatin.[8] However, there has been very little screening effort focusing on the molds. Hence, we felt it important to expand our screening effort to include more filamentous fungi.

Inhibition of Gram-negative bacteria *E. coli, A. aerogenes, A. faecalis, P. mirabilis, P. aeruginosa, K. pneumoniae and S. marcescens* by cisplatin has been known for almost 45 years.[2] In our initial tests, we confirmed the sensitivity of *E. coli* and *S. marcescens* to cisplatin. Table 1 shows such a test with *E. coli* strains 153_z^γ, ZK 650 and C600 R1 and with *S. marcescens*. Our further experiments showed inhibition of *E. coli* strains ZY and ESS as well as *P. aeruginosa*. The effect of

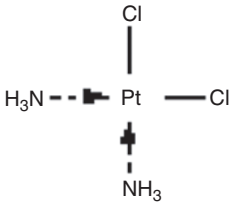

Figure 1 Molecular structure of cisplatin.

Table 1 Inhibition of bacteria by cisplatin[a]

Organism	*Agar medium*	*Day*	*Zone size (mm)*
Gram-positive			
Bacillus brevis 9999	LB	1	13 clear
Bacillus cereus 9139	LB	1	15 clear
Bacillus subtilis K	LB	1	15 clear
Streptomyces lividans B18	YEA	1	Trace (7 hazy)
Gram-negative			
Escherichia coli C600R1	LB	1	Trace (7 hazy)
Escherichia coli C600R46	LB	1	11 clear
Escherichia coli $J53_z^y$	LB	1	15 clear
Serratia marcescens	LB	1	15 clear

Abbreviation: YEA, yeast extract agar.
[a]16 μg per disc.

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Received 6 April 2010; revised 20 May 2010; accepted 26 May 2010; published online 23 June 2010

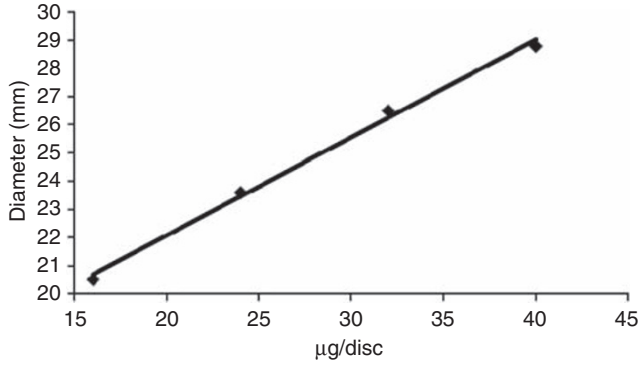

Figure 2 Inhibition of *Escherichia coli* C600 R1 by increasing doses of cisplatin. Inhibition was measured by the diameter of the clear zones in mm.

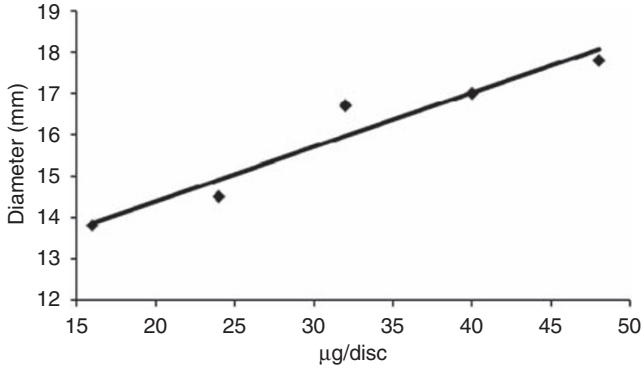

Figure 3 Inhibition of *Bacillus subtilis* K by increasing doses of cisplatin. Inhibition was measured by the diameter of the clear zones in mm.

Table 2 Cisplatin inhibition of *Escherichia coli* ZK650 in liquid culture in TSB medium

Experiment	*Contents of tube*	*Time*	*Growth (Klett units)*
1	No addition	4.5 h	141
	50 µl DMF	4.5 h	130
	50 µl cisplatin in DMF[a]	4.5 h	25
2	No addition	3.5 h	73
	50 µl DMF	3.5 h	71
	50 µl cisplatin in DMF[a]	3.5 h	19

Abbreviations: DMF, dimethylformamide; TSB, Tryptic Soy broth.
[a]Cisplatin concentration: 60 µg ml^{-1}.

Table 3 Cisplatin inhibition of *Bacillus subtilis* JH642 in liquid culture in TSB medium

Experiment	*Contents of tube*	*Time*	*Growth (Klett units)*
3	No addition	18.5 h	149
	225 µl DMF	18.5 h	155
	225 µl cisplatin in DMF*	18.5 h	74

Abbreviations: DMF, dimethylformamide; TSB, Tryptic Soy broth.
[a]Cisplatin concentration: 60 µg ml^{-1}.

Table 4 Inhibition of fungi by cisplatin[a]

Organism	*Agar medium*	*Day*	*Zone size (mm)*
Yeasts			
Saccharomyces cerevisiae YRH499	SM	1	18 clear
Schizosaccharomyces pombe 972	SM	2	Trace (7 hazy)
Schizosaccharomyces pombe NW158	YEA	3	22 clear
Schizosaccharomyces pombe NW214	YEA	3	24 clear
Schizosaccharomyces pombe NW240	YEA	4	41 clear
Schizosaccharomyces pombe sp6	YEA	4	22 clear
Molds			
Alternaria altenata	SM	1	16 clear
Aspergillus niger	SM	1	10 clear
Penicillium sp	SM	1	Trace (7 hazy)

Abbreviations: SM, Sabouraud maltose; YEA, yeast extract agar.
[a]16 µg per disc.

increasing dosages of cisplatin from 16 to 40 ug per disc against *E. coli* C600 R1 is shown in Figure 2. It can be observed that the increase in zone size is proportional to the cisplatin dosage. We also studied the effect of cisplatin on growth of *E. coli* ZK650 in liquid medium and found inhibition (Table 2).

Owing to the question in the literature regarding the sensitivity of Gram-positive bacteria to cisplatin, a number of such organisms were tested. Initial testing with *Bacillus brevis* 9999, *Bacillus cereus* 9139, *Bacillus subtilis* K and the actinomycete *Streptomyces lividans* B18 revealed inhibition by cisplatin at the lowest dose tested; that is, 16 ug per disc (Table 1). The results against *B. subtilis* K using different concentrations of cisplatin varying from 16 to 48 ug per disc, are shown in Figure 3. Later experiments showed inhibition of *B. subtilis* 168, *B. subtilis* JH642 and *Staphylococcus aureus*. The effect of cisplatin on growth of *B. subtilis* JH642 was also examined. Table 3 shows that cisplatin made up in dimethylformamide inhibited growth at levels of dimethylformamide that were not inhibitory (because of the disk evaporation technique used).

It is known that the yeasts *S. cerevisiae*,[5] *S. pombe*[6] and *C. albicans*[7] are inhibited by cisplatin. Our initial studies, shown in Table 4, confirmed cisplatin inhibition of *S. cerevisiae* and *S. pombe*. Five strains of *S. pombe*; that is, WT, 972, NW158, NW214, NW240 and sp6 were tested and all were found to be inhibited. A later experiment showed inhibition of an additional strain of *S. pombe*; that is, Rad3Δ.

The molds were difficult to test because their growth in liquid culture was filamentous rather than as an evenly distributed turbid suspension. They often grew as pellets, sometimes very large, with or without the apparent presence of sporulation. To solve this problem, small glass beads (65 beads per flask) plus either 1.5% of carboxymethylcellulose or 0.3% carboxypolymethylene[9] were added to the flasks. This resulted in a much more homogeneous type of growth. Our initial results showing cisplatin inhibition of *Penicillium* sp., *Alternaria alternata* and *Aspergillus niger* are shown in Table 4. Later experiments revealed cisplatin inhibition of additional molds such as *Aspergillus fumigatus, Fusarium oxysporum, Pythium ultimum* and *Geotrichum candidum*.

To summarize, the antitumor agent cisplatin has an extensive antimicrobial spectrum of activity. Growth of all 29 microbes, including seven Gram-negative bacterial strains, eight Gram-positive bacter-

ial strains, seven yeast strains and seven mold strains was found to be inhibited. This agrees with the point made by Newman and Shapiro[10] that most antitumor agents have antimicrobial activity. We are now using these cisplatin-inhibitable strains as prescreens to test the antimicrobial activity of novel cisplatin analogs chemically synthesized by Dr Baldwin King and his Drew University students. Those that are antibiotically active will be further tested for antitumor activity in the hope of identifying cisplatin analogs that do not have the toxicity problems associated with cisplatin.

EXPERIMENTAL PROCEDURE

Organisms were preserved by storage in 30% glycerol solution at –80 °C. The cultures were transferred to liquid medium (Tryptic Soy broth for the bacteria and Sabouraud maltose broth for the fungi) at 40 ml per 250 ml flask and grown on the rotary shaker at 28 °C and 220 r.p.m. until heavy growth was observed. This usually required 2 to 3 days for the bacteria and 4 to 6 days for the fungi. The liquid cultures were then used to seed melted agar media at 500 µl per 100 ml of agar medium. The agar media used were as follows: LB agar and Tryptic Soy agar for unicellular bacteria; yeast extract agar for the actinomycete; Sabaroud's maltose agar, Tryptic Soy agar and yeast extract agar for yeasts; and Sabaroud maltose agar as well as Tryptic Soy agar for the molds. The inoculated agar was poured into Petri dishes at 10 ml per plate and allowed to solidify. Cisplatin was dissolved in water up to 5 mM concentration or in dimethylformamide at higher concentrations (10 and 20 mM). Cisplatin was then added to paper discs of 6 mm diameter, which were allowed to dry on an aluminum foil surface. Those discs that received the high concentration of cisplatin were incubated in a vacuum oven at 42 °C for 2–3 h to remove the dimethylformamide before placing them on the inoculated agar surface. Those that received the low concentration of cisplatin could be placed on the agar directly. Plates were incubated at 35–37 °C for bacteria and 26–28 °C for fungi until growth was observed. Activity was shown by inhibition zones, the diameters of which were measured.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr Baldwin King for samples of cisplatin and Prakash Masurekar, Douglas Eveleigh and Charles Martin for *S. cerevisiae* strains. We acknowledge Drew undergraduate student Christian Maggio and high school students Anisha Mukherjee and Allison Dyevoich for laboratory assistance. The work was supported by a grant from AAAS/Merck and financial assistance from the Drew Summer Science Institute (DSSI) and RISE. We acknowledge the encouragement given by former RISE Director, Dr Ashley H Carter, the present Director, Dr Jon Kettenring and colleague Dr Vincent Gullo. Administrative assistance was provided by Miriam Donohue. All the authors of the paper except AL Demain were or are undergraduate students of Drew University.

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