NOTE

A SIMILAR ACTION TO UV-IRRADIATION AND A PREFERENTIAL INHIBITION OF DNA SYNTHESIS IN *E. COLI* BY ANTITUMOR PLATINUM COMPOUNDS

MIKIO SHIMIZU* and BARNETT ROSENBERG

Biophysics Department, Michigan State University, E. Lansing, Michigan 48823, U.S.A.

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Cis-dichlorodiammineplatinum (II) [*cis*-Pt (II) $(NH_3)_2Cl_2$], and related compounds were first reported by ROSENBERG *et al.*[1,2] to have potent antitumor activities against sarcoma 180 and leukemia 1210 in mice. Subsequently, these compounds were reported effective against DUNNING ascites leukemia and WALKER 256 carcinosarcoma in rats[3] and in other systems.[4~6]

Cis-Pt (II) $(NH_3)_2Cl_2$ also causes inhibition of cell division and elongation of bacterial cells,[7,8] prophage induction in bacteria,[9] inhibition of DNA synthesis in mammalian cells,[4] cross-linking of DNA *in vivo*[10] and *in vitro*,[11] and immunosuppression in mice.[12,13] These activities of *cis*-Pt (II) $(NH_3)_2Cl_2$ are very similar to those of difunctional alkylating agents. Bleomycin was reported to differ in mode of action from radiation even though it caused bacterial elongation and inhibition of DNA synthesis.[14,15] This study was performed to determine the susceptibilities of four isogenic sub-strains of *Escherichia coli*-Bs-1, Bs-2, B and B/r[16] to UV irradiation, *cis* and *tran*-Pt (II) $(NH_3)_2Cl_2$, mitomycin C, bleomycin, rifamycin and chloramphenicol. *In vitro* minimum inhibitory concentrations were determined by the dilution method. Overnight bacterial cultures in nutrient broth (Difco) were added by small capillaries to nutrient broth agar plates which contained two-fold dilutions of drug. After incubation for 16 hours at 35°C, plates were examined to determine the minimum concentration of drug which prevented visible growth of bacteria.

The effects of UV and drugs on the viability of *E. coli* strains were determined by the following procedures: Each strain, grown logarithmically in a medium ($(NH_4)_2HPO_4$ 2.5 g, KH_2PO_4 1.5 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, sodium glutamate 3.0 g, glucose 3.0 g per liter), was harvested, washed and resuspended in a saline or in this medium without glucose. To determine UV-sensitivity of strains, each cell suspension (5×10^8 cells/ml in saline, 10 ml/petri dish of 10 cm diameter) was exposed for 30 seconds to a 10-w UV germicidal lamp at a distance of 30 cm. Aliquots of the suspensions were plated out and incubated. Surviving cells were expressed relative to numbers in controls not irradiated. To determine lethality of drugs to these strains, various concentrations of drugs were added to each cell suspension (10^8 cells/ml in the medium without glucose). The mixtures were incubated at 37°C for 30 minutes. Dilutions of these sus-

Table 1. The sensitivity of the *E. coli* strains to drug.

	Minimum inhibitory concentration (μM)			
	E. coli Bs-1	*E. coli* Bs-2	*E. coli* B	*E. coli* B/r
UV*	3×10^{-10}	3×10^{-7}	0.2	1.0
Cis-Pt (II) $(NH_3)_2Cl_2$	38	38	150	300
Trans-Pt (II) $(NH_3)_2Cl_2$	>300	>300	>300	>300
Mitomycin C	0.1	0.02	0.4	3.3
Bleomycin	2.2	0.6	1.1	9.0
Rifamycin	6.3	3.2	6.3	12.5
Chloramphenicol	2.0	2.0	2.0	2.0

* Cells were exposed for 30 seconds to a 10-w UV lamp at a distance of 30 cm and figures indicate the survivors relative to unirradiated controls.

* Present address: Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan.

Fig. 1. Bacteriocidal effects of *cis*-Pt (II) $(NH_3)_2Cl_2$ and mitomycin C on UV-sensitive strains and UV-resistant strains.

Each strain, grown logarithmically in a medium ($(NH_4)_2HPO_4$ 2.5 g, KH_2PO_4 1.5 g, NaCl 5.0 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, sodium gultamate 3.0 g, glucose 3.0 g per liter), was harvested, washed and resuspended in the medium without glucose to a concentration of 10^9 cells/ml. Each concentration of drug was added to each cell suspension. The mixture was incubated at 37°C for 30 minutes without shaking. After appropriate dilutions, the suspension was spread on nutrient agar plates. The colonies were counted after an overnight incubation of the plates.

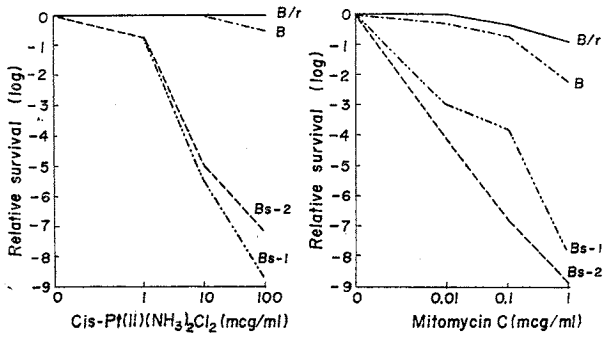
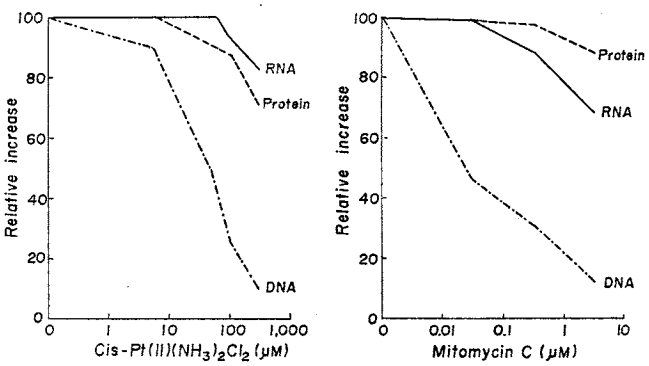


Fig. 2. Effects of *cis*-Pt (II) $(NH_3)_2Cl_2$ and mitomycin C on nucleic acids and protein synthesis by *E. coli* Bs-1.

The experiment was performed by the method described by SEKIGUCHI *et al.*[17] Briefly, bacterial cultures containing various levels of drug were incubated with shaking at 37°C for 30 minutes. After collecting cells by centrifuge, nucleic acids were extracted by heating cells in 0.5 N perchloric acid at 90°C for 15 minutes. DNA and RNA in the extracts were determined by diphenylamine reaction and by orcinol reaction, respectively. The residue after extraction was dissolved in 1 N NaOH, and protein was determined by the method of LOWRY *et al.*



pensions were spread on nutrient broth agar plates, incubated overnight and surviving cells were tabulated.

Table 1 shows that UV-sensitive mutants, *E. coli* Bs-1 and *E. coli* Bs-2, were much more sensitive to UV irradiation than the parent *E. coli* B strain. Table 1 and Fig. 1 show that *cis*-Pt (II) $(NH_3)_2Cl_2$ and mitomycin C were more effective on *E. coli* Bs-1 and *E. coli* Bs-2 than on *E. coli* B and the UV-resistant mutant *E. coli* B/r. Bleomycin, as reported by ENDO,[14] showed no significant difference in its antibacterial activity on UV-sensitive strains and the parent strain, but it was less active against the UV-resistant mutant *E. coli* B/r (Table 1). Rifamycin and chloramphenicol exhibited no significant differences in minimal inhibitory concentrations against UV-sensitive and UV-resistant strains (Table 1).

The effects of *cis*-Pt (II) $(NH_3)_2Cl_2$ and mitomycin C on nucleic acids and protein synthesis by *E. coli* Bs-1, were determined essentially by the method of SEKIGUCHI *et al.*[17] Bacterial cultures containing various concentrations of drug were incubated with shaking at 37°C for 30 minutes. Cells were separated by centrifugation and nucleic acids in the cells were extracted by heating in 0.5 M perchloric acid at 90°C for 15 minutes. DNA and RNA in the extracts were determined by diphenylamine and by orcinol reactions, respectively. The residues after perchloric acid extraction were dissolved in 1 N NaOH, and proteins were determined by the method of LOWRY *et al.* Fig. 2 shows that *cis*-Pt (II) $(NH_3)_2Cl_2$ and mitomycin C caused a preferential inhibition of DNA synthesis in bacterial cells as was observed with mammalian cells.[4]

These results suggest that *cis*-Pt (II) $(NH_3)_2Cl_2$ is very similar in mode of action to difunctional alkylating agents.

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