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Basic Investigations

INTERACTION OF CISPLATIN WITH MEMBRANES OF RAT EHRLICH ASCITES TUMOR CELL*

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Quenching effects of cis-DDP on fluorescence of membranes of rat Ehrlich ascites tumor cell and binding of cis-DDP with the membranes were studied. The results indicate that cis-DDP can react with tumor cell membrane proteins. If the Pt binding sites were grouped into two types, the apparent stability canstants (K) and number of binding sites (n) are estimated.

Key words: Cisplatin, Tumor cell membrane, Fluorescence.

The anticancer mechanism of cisplatin has been interpreted on the basis of interaction of cisplatin with DNA, resulting in inhibition of the DNA replication. Until now, there are not detailed interpretation concerning the toxic side effect and resistance of platinum drugs. We consider that to cisplatin, as an extraneous substance, target other than DNA may exist in cells. To understand both parmacological and toxicological actions of cisplatin, we have suggested a multiple target model to interpret the metal-cell interaction and the responses of a cell toward certain attacking metal ion.[1] There is plenty of evidences indicating the presence of target biomolecules other than DNA in the cell. We suggested that membrane phospholipids, membrane proteins and cytoskeletal proteins (actin and tubulin) on cells might be the potential target molecules. The effects of cisplatin on cells, its pharmacological and toxicological activities, are the manifestation of the reactions between cisplatin and various targets. Based on this model, the interaction of cisplatin with erythrocyte membranes was studied. The results indicate that cisplatin and its hydrolytic product tend to bind with membrane proteins and phospholipids. However, the membrane proteins are likely the preferential binding site. The binding results in the alteration of membrane structure or cytoskeleton organization.[2, 3] In order to investigate the reactions between cisplatin and cancer cell membranes, in the present work the binding of platinum to membrane of rat Ehrlich ascites tumor cells and the effects of cisplatin on the intrinsic fluorenscence of membranes were studied.

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MATERIALS AND METHODS

Materials

Rat Ehrlich ascites tumor cells were supported by Department of Cell Biology, Beijing Medical University. Cisplatin (cis-DDP) was obtained from Jing Zhou Pharmacy. Trichloroacetic acid, Tris, $SnCl_2$, NH_4Cl and HCl purchased from Beijing Chemical Inc.

RF-540 fluorescence spectrophotometer (Shimadzu) and UV-120 spetrophotometer (Shimadzu) were used.

Separation of Plasma Membrane of Tumor Cell

Seven days after injection of the Ehrlich ascites tumor cells, the tumor cells were harvested from ascites of rat. The ascites were centrifugally washed with isotonic pH 7.4 buffer solution at 800r/min. The pellets of rat Ehrlich ascites tumor cell were suspended in Tris-HCl buffer solution (0.05 mol/L, pH 7.4). After freezing and melting repeatedly the suspension of tumor cells was sonicated to break the membranes. According to reference,[4] the plasma membranes were separated from suspension of broken cells by sucrose gradient centrifugationl. The content of membrane proteins was determined with Lowry method (mg/ml).

Fluorescence Measurement

0.2 ml aliquots of tumor membrane suspension (0.558 mg/ml) were mixed with cis-DDP solution and diluted with Tris-HCl buffer solution. After incubation in 37°C for 20 h, the fluorescence of plasma membranes was determined, the wavelength of excitation: 286 nm, the range of emission: 300 to 400 nm, the slit 10, 10 nm, respectively.

The Binding of cis-DDP to Tumor Cell Plasma Membranes

0.2 ml aliquots of tumor membrane suspension (0.558 mg/ml) with cis-DDP were diluted to 1 ml with Tris-HCl buffer solution. The mixtures were incubated for 20 h at 37°C, then cooled under 4°C. 0.2 ml of 6.3% trichloroacetic acid were added to mixtures. After standing at 4°C for 5 min the mixtures were centrifuged at 10,000 r/min for 10 min. 0.2 ml of supernatants were drawn and 0.28 ml of distilled water; 0.2 ml of HCl; 0.4 ml of NH_4Cl (20%) and 0.4 ml of $SnCl_2$ (2.2 g of $SnCl_2$ were dissolved in 3 ml HCl for 8 h, then diluted to 10 ml) were added subsequently. The optical absorptions of mixtures at 403 nm were deter-mined after 2 h. The concentration of free and binding platinum was calculated.

RESULTS AND DISCUSSION

The Influence of cis-DDP on Fluorescence of Tumor Cell Membranes

Fluorescence spectra of rat Ehrlich ascites tumor cell membranes were recorded at 335 nm, which were intrinsic fluorescence of membrane proteins. In presence of cis-DDP the fluorescence was quenched without shift of peak. This is consistent with its effect on erythrocyte membrane,[2,3] showing cis-DDP does not directly bind to fluorescence groups in membrnane proteins. Quenching effects of cis-DDP on fluorescence of tumor cell membranes and a dose- dependent relation were observed (Figure 1). The phenomenon is similar with the interaction between mercurous compound and erythrocyte membranes.[5] The results indicate cis-DDP reacts with tumor cell membrane proteins and quenching effects of cis-DDP on fluorescence of

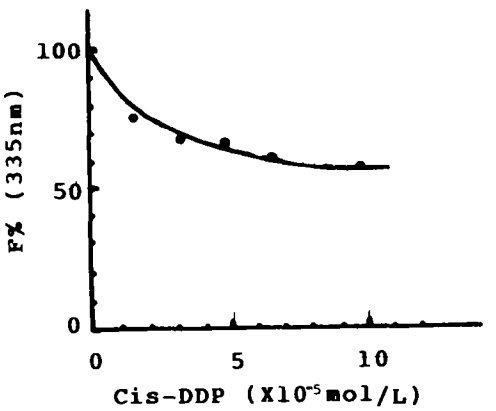

Fig 1. Quenching effect of cis-DDP on fluorescence of rat Ehrlich ascites tumor cell membranes (35.2 μg pr/mL). Excitation at 286 nm and emission at 335 nm

Table 1. Amount of cis-DDP binding to rat tumor cell membrane

Initial con. of cis-DDP$\times 10^{-6}$ mol/L	Free con. of cis-DDP$\times 10^{-6}$ mol/L	Bound con. of cis-DDP$\times 10^{-6}$ mol/L	$\bar{Y}(\times 10^{-3}$ mol/g)
139	64.4	74.6	0.76
209	131	78.0	0.80
278	184	94.0	0.96
418	307	111	1.13
557	435	122	1.24
696	536	160	1.63
974	789	185	1.89
1113	912	201	2.05

*Concentration of rat tumor cell membrane protein: 0.0980 g/L.

membranes could be used to measure the interaction between cis-DDP and tumor cell membranes.

Binding of cis-DDP to Tumor Cell Membranes

The amount of free and membrane-bound platinum were determined using a modified $SnCl_2$ method after reaction of cis-DDP with rat tumor cell membranes (Table 1). The binding isotherm of cis-DDP to membranes was obtained throug $\bar{Y}$, binding cis-DDP content per g membrane proteins, versus concentration of free cis-DDP (Figure 2). The result shows that the membrane-bound cis-DDP elevates with increasing of cis-DDP concentration.

To estimate binding parameter, we got Scatchard plot of cis-DDP binding to tumor cell membranes from the data of Figure 2.[6] If the platinum binding sites were grouped into two types, through the slope and into estimate binding parameter, we got Scatchard plot of cis-DDP binding to tumor cell membranes from the data of Figure 2.[6] If the platinum binding sites were grouped into two types, through the slope and intercept of curve in Figure 3 the apparent stability constant (K) and number of binding sites (n) were estimated as following:

$K_1 = 1..35\times 10^5$ L/mol $\quad n_1 = 6.80\times 10^{-4}$ mol/g protein

$K_2 = 2..50\times 10^3$ L/mol $\quad n_2 = 1.92\times 10^{-3}$ mol/g protein

Several experimental evidences indicate that cis-DDP can bind with blood plasma proteins[7] and inhibits enzyme activity.[8] Due to the affinity of platinum to S and N group, cis-DDP tends to attack membrane proteins, because SH and NH_2 groups are rich in proteins. The experimental results given here show that interaction of cis-DDP with membranes of rat Ehrlich ascites tumor cells induces quenching of fluorescence of membrane proteins, and there are two types of platinum binding sites in the cell membranes. These phenomena support our postulation of multiple target model. Su and co-workers[9] have found that cis-DDP causes the

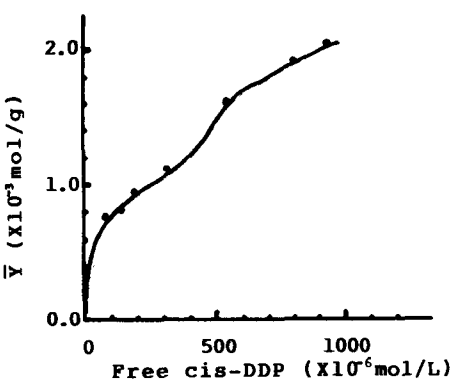

Fig. 2. The isothermo-binding between cis-DDP and rat Ehrlich ascites tumor cell membrane (37°C).

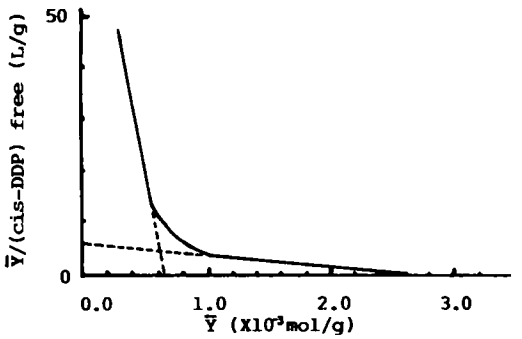

Fig. 3. Scatchard plot of cis-DDP binding to Ehrlich ascites tumor cell membrane.

disorganization of microfilament network of rat Ehrlich ascites tumor cells. This may be relevant to interaction between cis-DDP and skeleton proteins of membranes. Therefore, we suggest that the binding of cis-DDP to component of membrane, espe-cially membrane skeleton proteins may disturb cyto-skeleton structure. These effects perhaps attributed to anticancer activity of platinum drugs.

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