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EFFECT OF AN ANTI-TUMOR PLATINUM COMPLEX, Pt(II)DIAMINOTOLUENE, ON MITOCHONDRIAL MEMBRANE PROPERTIES

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SUMMARY

The effects of platinum complexes, selected for their potent anti-tumor activities, have been studied on rat liver mitochondria. Among the mitochondrial properties which have been studied, the most marked effects of platinum complexes were obtained on functions linked to the inner membrane.

cis-Pt(II)(3,4-diaminotoluene) dichloride is shown to stimulate state 4 respiration. It inhibits the phosphate transport into mitochondria, decreases the accumulation of Ca^{2+}, and induces a more rapid release of the accumulated Ca^{2+}. A release of Mg^{2+} from mitochondria incubated in the absence of added divalent cations, and an efflux of divalent cations from mitochondrial membranes are also observed.

All these results indicate a profound modification of the permeability of mitochondrial membrane.

INTRODUCTION

Biological effects of heavy transition metal coordination complexes have been studied during the past few years, and results have shown that these molecules have potent anti-tumor, bactericidal and immunosuppressive activities [1, 2].

The major part of the studies on the mechanism of action of these compounds had been done on the interaction between DNA and complexes [3, 4]. A direct interaction between platinum (II) complexes and DNA was shown to be responsible for the inhibition of DNA, RNA and protein synthesis, and hence for their anti-tumor activity.

There is little information on the effect of platinum complexes on membranes, although an interaction between cellular membrane and platinum complexes had been demonstrated in erythrocytes [5]. In this paper, we report results obtained by studying the effects of newly synthesized Pt (II) and Pd (II) complexes [6] on a typical intracellular membrane, the inner membrane of rat liver mitochondria.

MATERIALS AND METHODS

Rat liver mitochondria were obtained from Long Evans rats, according to the

method described elsewhere [7]. They were suspended in 250 mM sucrose, 10 mM Tris, pH 7.2, medium.

Respiratory assays were performed using a Gilson oxygraph, in 250 mM sucrose, 10 mM Tris, pH 7.2, 5 mM potassium phosphate, pH 7.2, 4 mM $MgCl_2$, 10 mM succinate and rotenone medium.

Swelling experiments were performed in an Eppendorf photometer according to Chappell's method [8]

The movements of divalent cations were measured both in intact mitochondria, and in the membrane phase. First, in whole mitochondria: aliquots were taken out from incubation medium, rapidly centrifuged in an Eppendorf microcentrifuge 3200. Mg^{2+} and Ca^{2+} were assayed by atomic absorption with a Perkin-Elmer 300, both in the diluted supernatant, and in the pellet dissolved in cholate and water. Both methods give identical results. Second, in membrane phase. the level of divalent cations in the membrane phase was followed using the fluorescence of a specific probe, chlorotetracycline, which is much more fluorescent in the presence of Ca^{2+} or Mg^{2+} in an hydrophobic medium [9]. Fluorescence was measured in an Eppendorf filter fluorimeter (excitation: 366 nm; emission: 530 nm $\rightarrow \infty$) [10]

The studied complexes have been synthesized and tested for their anti-tumor activities by Jordanov (Laboratoire de Chimie de Coordination, Strasbourg). They were dissolved in dimethylsulfoxide at 10^{-1} M for stock solutions. It was checked that dimethylsulfoxide in the concentrations used had no effects on mitochondrial properties.

RESULTS

We have reported in this paper some of the effects of a platinum complex on

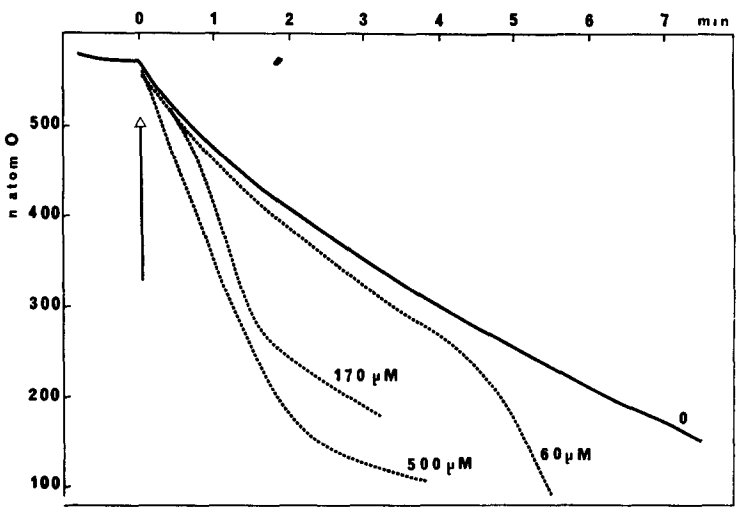

Fig. 1. Effects of Pt-dat on mitochondrial respiration. Rat liver mitochondria were incubated in the medium indicated in Materials and Methods, at 25 °C. Pt-dat was present at the concentrations indicated (dotted lines), before the addition of mitochondria at zero time (1 6 mg in 1 2 ml final).

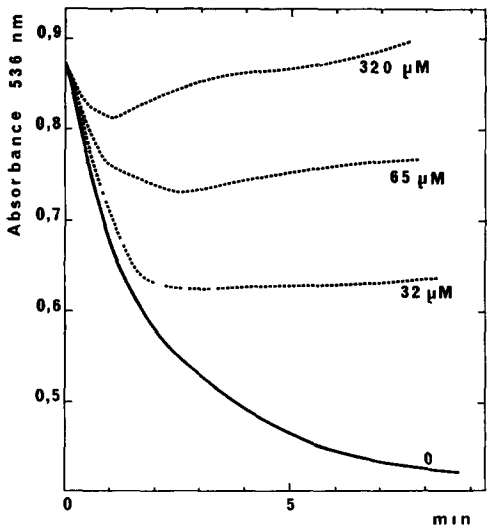

Fig 2 Effects of Pt-dat on phosphate transport Rat liver mitochondria (0.64 mg) were added in 100 mM ammonium phosphate, pH 7 0 (final volume 1 5 ml), and swelling was followed by measuring the absorbance of the suspension at 546 nm in an Eppendorf photometer at 20 °C. Mitochondria were added at zero time and Pt-dat, if present (dotted lines), was added at the indicated concentrations, before the mitochondria

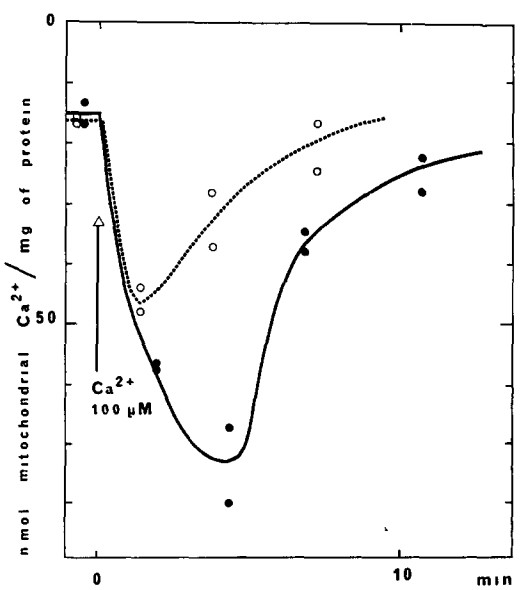

Fig 3 Effects of Pt-dat on mitochondrial Ca^{2+} transport Rat liver mitochondria (1 8 mg/ml) were incubated in 250 mM sucrose, 10 mM Tris, pH 7 2, 0 5 mM ADP, at 30 °C. 100 μM Ca^{2+} (55 nmol/mg of protein) was added 2 min after the mitochondria (zero time of the figure) Aliquots were taken at various times, immediately centrifuged and Ca^{2+} assayed both in the pellet and in the supernatant by atomic absorption (these two values are confused) ●–●, control, ○–○, +44 μM Pt-dat

mitochondria metabolism. The experiments shown are all related to *cis*-Pt(II)(3,4-diaminotoluene) dichloride (Pt-dat). Similar results have been observed with some other complexes, but the potencies observed were lower.

Effects on respiration. Fig. 1 shows the effect of Pt-dat on mitochondria respiring at state 4. The addition of this compound enhances the oxidation rate of succinate in the absence of phosphorylation substrates. A lag period is observed between the addition of complexes and the acceleration of respiration, which depends on the concentration used (4 min for 60 μM, 50 s for 170 μM).

No effect of the complexes was observed on the mitochondria in a state 3 respiration

Effects on phosphate transport. The transport of phosphate into mitochondria was followed by measuring the swelling of mitochondria incubated in 100 mM ammonium phosphate [8]. The results shown in Fig. 2 indicate a relatively strong inhibition of this transport (a complete suppression of the swelling had been obtained for 100 μM). This inhibition is not reversed by mercaptoethanol, as it does for SH reagent-induced inhibition of swelling [11].

A discrepancy between the lag times of inhibition by Pt-dat appears from Figs. 1 and 2. This can be explained by the experimental conditions, which were different in the two experiments. Moreover, the experimental temperatures were not the same.

Effects on the accumulation of Ca^{2+} When liver mitochondria are incubated in the presence of Ca^{2+}, in the range 50–150 μM, and in the absence of phosphate, a limited active accumulation of Ca^{2+} into the mitochondria is observed, followed by an efflux of this accumulated Ca^{2+} [7, 12]. A typical experiment of this phenomena is indicated in the control curve of Fig. 3. In this experiment, the addition of 44 μM Pt-dat, in the incubation medium, before the addition of mitochondria, is shown to

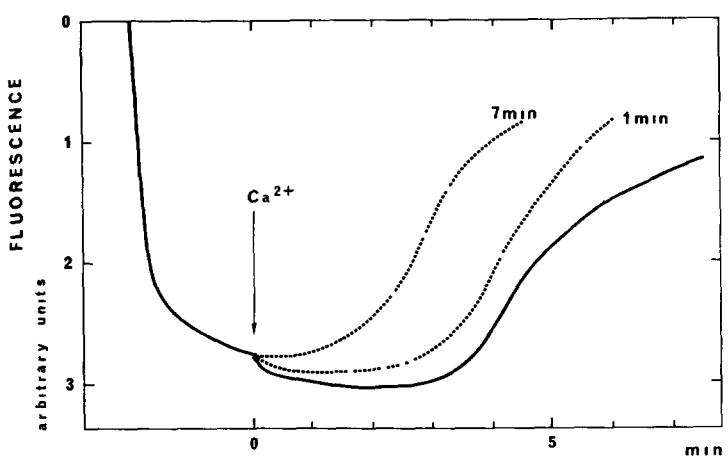

Fig. 4 Effects of the preincubation time of Pt-dat on Ca^{2+} movements Rat liver mitochondria (2 mg/ml) were added at zero time in 250 mM sucrose, 10 mM Tris, pH 7 2, 0 5 mM ADP, 3 μg/ml chlorotetracycline at 30 °C. 44 μM Pt-dat was added (—) before Ca^{2+} (dotted lines), at various indicated times. 100 μM Ca^{2+} (50 nmol/mg of protein) was added at the zero time of the figure. Chlorotetracycline fluorescence (366 → 530 nm) was followed in an Eppendorf fluorimeter (Corrections were made for the small fluorescence quenching induced by Pt-dat)

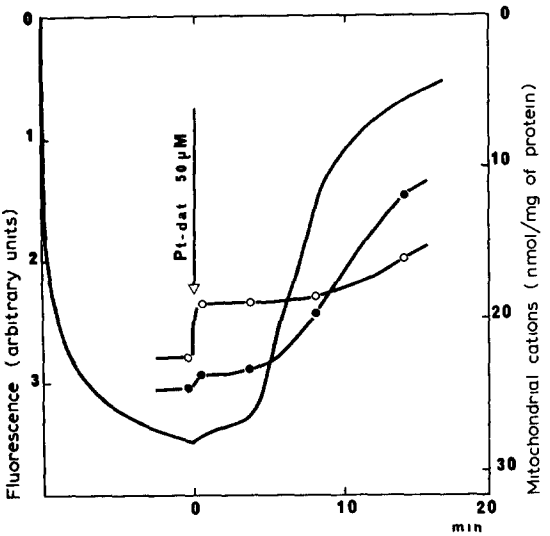

Fig 5. Effects of Pt-dat on endogenous divalent cations. Rat liver mitochondria were incubated in 250 mM sucrose, 10 mM Tris, pH 7.2, 0.5 mM ADP, 3μg/ml chlorotetracycline at 30 °C. Chlorotetracycline fluorescence and Ca^{2+} and Mg^{2+} content were simultanously followed as indicated in Figs 3 and 4 50 μM Pt-dat was added at the zero time of the figure. ——, fluorescence; ○–○, mitochondrial Ca^{2+}, ●–●, mitochondrial Mg^{2+}

induce a limitation of the Ca^{2+} accumulation, but especially an acceleration of the efflux of Ca^{2+} from mitochondria.

Comparing with the results of Fig. 1, we have studied the effect of the preincubation time of the Pt-dat before Ca^{2+} addition. This is shown in Fig. 4, where the Ca^{2+} movements are followed by the chlorotetracycline fluorescence method [9, 10, 13]. When Ca^{2+} is added 7 min after the Pt-dat, the inhibition of accumulation is complete, whereas the fluorescence decay, corresponding to the loss of endogenous Ca^{2+}, see ref. 13, begins earlier, than when Ca^{2+} is added just 1 min after the complex.

Effects of Pt-dat on endogenous divalent cations. As it has been shown that the efflux of Ca^{2+} from mitochondria loaded with Ca^{2+} in the absence of phosphate is due to a displacement of mitochondrial Mg^{2+} by Ca^{2+} [13], it was interesting to study whether the acceleration of the Ca^{2+} efflux by Pt-dat might be related to a movement of Mg^{2+}. Such an experiment is shown in Fig. 5. The addition of Pt-dat on mitochondria, incubated in the absence of added divalent cations, provokes, after a short rapid efflux of about 2 nmol Ca^{2+}/mg of protein, a release of mitochondrial Mg^{2+}, preceding the efflux of endogenous Ca^{2+}. A concomitant decay of chlorotetracycline fluorescence is observed, which corresponds to the efflux of membrane divalent cations.

DISCUSSION

All the effects reported in this paper, as well as those obtained with *cis*-Pt (II) (2-mercaptomethylimidazole)$_2$ dichloride, *cis*-Pt (II) (2-mercaptothiazoline)$_2$ dichloride and *cis*-Pd (II) (2-mercaptothiazoline)$_2$ dichloride complexes, which are not

described here, may be related to an action on the mitochondrial membrane. We have observed an apparent inhibition of the phosphate transport (no absorbance modifications were observed in potassium phosphate medium), a decrease of the accumulation of Ca^{2+} and increase of the passive permeability of Ca^{2+}. The stimulation of state 4 respiration, which is similar to an apparent uncoupling of oxidative phosphorylation, might also be related to modifications of membrane permeability.

However, it seems that these effects are not caused by the primary action of the platinum complexes. In fact, it is important to note the existence of a lag period between the addition of complexes and its subsequent action. This lag period is a function of the concentration of the complex used, higher concentrations corresponding to shorter lag periods. This observation is consistent with the idea that platinum complexes have another mechanism of action at the level of the mitochondrial membrane.

In line with the hypothesis of the basic role of Mg^{2+} in the maintenance of the permeability properties of the mitochondrial membrane [13–15], we have studied the influence of platinum complexes on the movements of Mg^{2+}. The results show that these compounds induce a release of the mitochondrial Mg^{2+}, occurring before Ca^{2+} release, which might be considered as the primary event of their action.

Thus, we have demonstrated in this paper the existence of an action of anti-tumor compounds at the membrane level. Further experiments would be necessary to ascertain if there is a direct relation between the anti-tumor properties and the interaction of these complexes on mitochondrial membrane described here. This work is now continued in our laboratory.

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