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Inhibition of adenylate cyclase by tetraplatin

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In the investigation of effects of platinum-containing compounds on dopamine (DA)-activated adenylate cyclase system, tetraplatin and cisplatin were found to suppress the increase of enzyme activity by various activators. However, tetraplatin was a much more potent inhibitor than cisplatin, with its I_{50} values being 1/25, 1/45, and 1/130 that of cisplatin in the presence of DA/Gpp(NH)p, NaF/$AlCl_3$, and forskolin/Gpp(NH)p respectively.

Key words: Adenylate cyclase, cisplatin, tetraplatin.

Introduction

Preclinical pharmacology of tetrachloro(*d,l-trans*)-1,2-diaminocyclohexane platinum (IV) (NSC-363812, tetraplatin) (Figure 1) shows antitumor activity similar to *cis*-diamminedichloroplatinum (II) (cisplatin). However, tetraplatin is effective at tolerated doses against leukemia sublines (L1210 and P388) with acquired resistance to cisplatin.[1] The two compounds have differences in their pharmacodynamic properties that may be due, in part, to differences in their valency states. We have previously reported that tetraplatin is a potent inhibitor of both choline acetyltransferase[2] and calcium-activated phospholipid-dependent protein kinase (protein kinase C),[3] whereas cisplatin exhibits little inhibitory activity against these two enzymes. We now report the *in vitro* effects of the two platinum compounds on dopamine (DA)-activated adenylate cyclase (AC) in rat brain striatum.

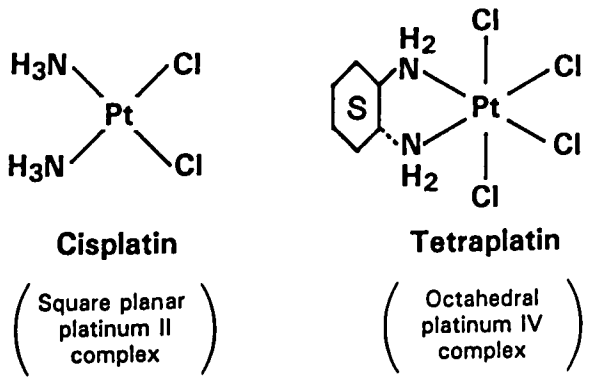

Figure 1. Structures of cisplatin and tetraplatin.

Materials and methods

Freshly dissected brain striatum from male Wistar rats (Charles River Laboratories Inc., Wilmington, MA), weighing between 240 and 280 g, were homogenized in 40 volumes (w/v) of an ice-cold buffer mixture (containing 2 mM Tris-maleate, pH 7.5, 2 mM ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA) and 2 mM $MgCl_2$), 0.32 M sucrose, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 48 000 × *g* for 20 min at 4°C. The pellet was resuspended in a volume equal to the original volume of the above buffer mixture. The suspension was centrifuged once more and the pellet was washed with the buffer, and then centrifuged again. The final pellet was resuspended in the buffer and stored at −70°C until use.

AC activity was measured by the conversion of [α-^{32}P]ATP to [^{32}P]c-AMP, using a modification of the method described by Salomon *et al.*[4] An incubation mixture (final volume 100 μl) was prepared by mixing (i) 50 μl of a solution containing (in final concentration) 40 mM HEPES, pH 7.7, 2 mM $MgCl_2$, 1 mM EDTA, 8 mM creatinine phosphate, 16 U/ml creatinine phosphokinase, 0.2 mM ATP, 10 μM GTP, 0.1 mM 3-isobutyl-1-methylxanthine, and 2 × 10^6 cpm[α-^{32}P]ATP; and (ii) 50 μl of a solution containing the enzyme (10 μg protein), appropriate amounts of activators [100 μM DA/50 μM Gpp(NH)p, 10 mM NaF/20 μM $AlCl_3$, or 50 μM forskolin/50 μM Gpp(NH)p], anti-cancer agent, and water. The mixture was incubated at 30°C for 10 min; and the reaction was terminated by adding 600 μl of solution made up of 25% (w/v)

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trichloroacetic acid (2 ml), 10 mM c-AMP (100 μl), [^{3}H]c-AMP (indicative of % recovery) (15 μl), and water up to 10 ml. The tubes were centrifuged at 300 × *g* for 5 min. The contents were then inverted onto pretreated columns packed with 1 ml of Dowex 50-X8 that retained (α-^{32}P]c-AMP. Three ml of water were added to each column to wash off the unreacted ATP. The Dowex columns were then placed over treated alumina columns packed with 0.6 g of neutral alumina. c-AMP was eluted from the Dowex columns onto the alumina columns, and the effluent was discarded. One ml of 0.1 N imidazole, pH 7.5, was then added to the alumina columns, and the effluent was again discarded. Finally, the c-AMP was eluted from the alumina columns into counting vials with 3 ml of imidazole. After addition of ScintiVerse I (Fisher Scientific Co.), ^{32}P and ^{3}H were assayed by liquid scintillation spectrometry.

The two anti-cancer agents cisplatin and tetraplatin were dissolved in water. AC activity was assayed with at least four different concentrations of one agent for each activator used (Table 1) to obtain an I_{50} (concentration of compound required to block 50% of an activator's effect on the enzyme) value. The effects of the two agents were then compared by their I_{50} values in the presence of the three activators.

Results

Tetraplatin was found to be a much more potent inhibitor of AC than cisplatin. When comparison was made between I_{50} values obtained in the presence of activators DA/Gpp(NH)p, NaF/$AlCl_3$, and forskolin/Gpp(NH)p, we found that 25-, 45- and 130-fold more of cisplatin respectively, was required to achieve the same degree of inhibition by tetraplatin (Table 1).

Table 1. Inhibition of DA-activated rat striatal adenylate cyclase by platinum-containing compounds

Activator	I_{50} (μM)[a]	
	Tetraplatin	Cisplatin
Dopamine/Gpp(NH)$_p$ (100 μM) (50 μM)	10	250
NaF/$AlCl_3$ (10 mM)/(20 μM)	10	450
Forskolin/Gpp(NH)$_p$ (50 μM) (50 μM)	1.5	200

[a] The concentration of compound required to block 50% of activator's effect on the enzyme. Each I_{50} value was obtained from the dose–effect curve of at least four concentrations of the platinum-containing compound in the presence of activator.

Discussion

Our results showed that tetraplatin and, to a lesser extent, cisplatin altered responsiveness of the DA-sensitive, striatal AC system. The inhibition of AC by the two platinum-containing compounds was demonstrated, with the aid of different activators, at the receptor-mediated (DA), nucleotide regulatory protein (NaF/$AlCl_3$), and catalytic subunit (forskolin) levels. Since the responsiveness of AC to forskolin stimulation was more sensitive to tetraplatin, it is suggested that tetraplatin exerted greatest action directly on the enzyme itself.

In our laboratory, data have consistently pointed out the differences between the biochemical properties of tetraplatin and cisplatin. Tetraplatin was previously shown to be a much better inhibitor than cisplatin of rat brain choline acetyltransferase[2] and protein kinase C.[3] The present study further revealed how their inhibition of rat striatal AC differs. However, what significance this difference in the effects of tetraplatin (bearing a tetravalent platinum, Figure 1) and cisplatin (bearing a divalent platinum) has in relation to neurotoxic side effects, remains to be explored.

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