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KINETICS OF THE REACTION OF CIS-PLATINUM COMPOUNDS WITH DNA IN VITRO

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Abstract. The kinetics of the reaction of a series of cis-platinum(II) compounds with DNA in vitro has been studied using their ability to disturb the secondary structure of the macromolecule. The complexation modifies the stacking of the base pairs and causes an inhibition of the intercalation of ethidium bromide which is correlated with the number of platinum atoms bound per nucleotide. The compounds fall into three groups which react in a few minutes, in a few hours or in several days. The inhibition of the complexation by chloride and carboxylato ions indicates that the interaction occurs through hydrolysed species and that hydrolysis is the rate limiting step. In addition the results indicate that the carboxylato entities are able to react with DNA in vitro without enzymatic activation and that there is no correlation between the antitumoral activity of these compounds against L1210 Leukemia cells and their in vitro reactivity towards DNA.

Since the discovery of the antitumoral properties of the cis-$Pt(NH_3)_2Cl_2$ (cis-PDD) several authors have tried to establish relationships between the physio-chemical properties of the platinum compounds and their pharmacological effects. Cleare and Hoeschle in 1973 (1) established a series of "empirical rules" which are essential for the observation of antitumor activity against Sarcoma 180 in Swiss mice. In particular the compounds should contain a pair of cis-leaving groups whose lability, i.e. reactivity, was similar to that of the chlorides in the cis-PDD. However they mentionned that compounds having relatively non-labile ligands in vitro, such as carboxylato groups, could be active

Abbreviations: r_i = molar ratio Pt introduced/DNA nucleotide; r_b = number of Pt covalently bound/DNA nucleotide; DAC = 1,2-diaminocyclohexane = $C_6N_2H_{14}$; malonato = $C_3O_4H_2$; oxalato = C_2O_4; en = ethylenediamine = $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$; EtdBr = ethidium bromide = 3,8-diamino-6-phenyl-5-ethylphenanthridinium bromide.

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and suggested a possible biological (enzymatic) activation or a non specific oxidative attack in vivo.

Several biochemical and biological effects such as inhibition of DNA synthesis, mutagenicity, prophage induction, increased sensitivity of deficient cells in DNA repair systems suggest that the DNA is the privileged target of the platinum compounds (2). The interaction of cis-PDD with DNA in vitro has been particularly studied and it has been shown that the first step concerns the replacement of a chloride ion by a water molecule which permits the compound to bind in a monodentate manner to DNA (3-5). Then the second chloride is hydrolysed and followed by the formation of a bidentate complex between the platinum and the DNA bases. This complexation induces perturbations of the DNA secondary structure which have been revealed by different physico-chemical techniques (6,7). Among these techniques, the use of a fluorescent intercalating agent, ethidium bromide, showed that perturbations induced by bidentate platinum compounds inhibit the intercalation leading to a decrease of the fluorescence of the DNA-EtdBr complexes (8,9). A relationship between this fluorescence decrease and r_b was found and used to follow directly the kinetics of interaction without purification of the complexes in order to eliminate free platinum.

In this paper are reported the kinetics of interaction of a series of cis-platinum(II) compounds with DNA in vitro and the effects of different ions (chloride and carboxylato) upon the complexation.

MATERIALS AND METHODS

Platinum compounds : $K_2[PtCl_4]$, potassium tetrachloroplatinate(II), was bought from the Comptoir Lyon-Alemand (Paris, France) and was the starting salt for the other platinum compounds used is this study. The chemical denomination, formula and properties are summarized in Table I. For methods of synthesis see ref. 10.

Kinetics of DNA-platinum interaction : Salmon sperm DNA was bought from Worthington Biochemical Corporation (Freehold, N.J.). Stock solutions of DNA were prepared by dropping a 10. mM solution of $NaClO_4$ (Fluka, Buchs, Switzerland) on the fibers then gently stirred at 4°C for 48 h. This solution was centrifugated at 8000 g for 10 mn. and the concentration determined spectrophotometrically according to an

TABLE I

CHEMICAL NAME	FORMULA	ANTITUMORAL ACTIVITY TOWARDS L1210[a]	t 1/2 (HOURS)
HIGH REACTIVE COMPOUNDS			
cis-Diaquoethylenediamineplatinum(II) nitrate	cis-$[Pt(en)(H_2O)_2](NO_3)_2$ [b]	-	<0.1
cis-Diaquodiammineplatinum(II) nitrate	cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$	-	<0.1
cis-Sulfatomonoaquodiammineplatinum(II)	cis-$Pt(NH_3)_2(SO_4.H_2O)$	-	0.1
cis-Sulfatomonoaquo-1,2-diaminocyclohexaneplatinum(II)	cis-$Pt(DAC)(SO_4.H_2O)$ [e]	+	<0.1
cis-Diaquo-1,2-diaminocyclohexaneplatinum(II)nitrate	cis-$[Pt(DAC)(H_2O)_2](NO_3)_2$ [e]	+	<0.1
MODERATE REACTIVE COMPOUNDS			
cis-Dichloroethylenediamineplatinum(II)	cis-$Pt(en)Cl_2$ [b]	+	1.5
cis-Dichlorodiamminoplatinum(II)	cis-$Pt(NH_3)_2Cl_2$	+	3.9
cis-Dichloro-1,2-diaminocyclohexaneplatinum(II)	cis-$Pt(DAC)Cl_2$ [e]	+	2.3
LOW REACTIVE COMPOUNDS			
cis-Malonatodiammineplatinum(II)	cis-$Pt(NH_3)_2(mal)$ [c]	+	240
cis-Oxalatodiammineplatinum(II)	cis-$Pt(NH_3)_2(ox)$ [d]	+	160
cis-Malonato-1,2-diaminocyclohexaneplatinum(II)	cis-$Pt(DAC)(mal)$ [c][e]	+	110
cis-Oxalato-1,2-diaminocyclohexaneplatinum(II)	cis-$Pt(DAC)(ox)$ [d][e]	+	90

a : see Ref. (10) ; b : en = $NH_2-CH_2-CH_2-NH_2$; c = mal = $C_3O_4H_2$; d : ox = C_2O_4 ; e : DAC = $C_6N_2H_{14}$.

absorption coefficient of 6360 M^{-1} cm^{-1} at 258 nm. The DNA-Platinum complexation was carried out by stirring in the dark, at 37°C, in 10^{-2}M $NaClO_4$ and in the presence of chloro or carboxylato (malonato or oxalato) ions. The pH was 5.8 to 6.2 and those of malonic and oxalic acid solutions was brought to 6.0 by addition of NaOH. At zero time, freshly dissolved platinum compounds in 10^{-2}M $NaClO_4$ were added to DNA (0.25 mg/ml) and corresponded to a final r_b of 0.20. At different times, aliquots (0.10 ml) were taken and added to 2.4 ml of EtdBr (0.04 mg/ml) in 0.4 M KNO_3. This EtdBr concentration corresponds to the saturation of all the intercalation sites in DNA (8).

Spectrophotometric methods have been already been reported (8,9). They were expressed by the ratio $(I_1/I_0) \times 100$ where I_1 = fluorescence intensity of DNA-Pt-EtdBr complex minus fluorescence intensity of pure EtdBr and I_0 = fluorescence intensity of DNA-EtdBr complex minus fluorescence intensity of pure EtdBr. In the experimental conditions, the fixation of one platinum atom per five nucleotides induced a fluorescence decrease of 35 ± 5 %. Since a linear relationship was found between the fluorescence decrease and r_b up to 0.20 (8), this allows the determination, for each compound, of the time required to complex half of the platinum introduced, ($t_{1/2}$) i.e. the time corresponding to an r_b of 0.10 (an example is given in Figure 1). Control of platinum content in the DNA-Pt complexes was determined by atomic absorption spectrophotometry (7) after elimination of free platinum by dialysis of filtration through Sepharose 6B (Pharmacia, Uppsala Sweden) gel column.

RESULTS AND DISCUSSION

Various cis-platinum(II) compounds were reacted in 10^{-2}M $NaClO_4$ with salmon sperm DNA at an initial ratio of 0.20 i.e. five nucleotides

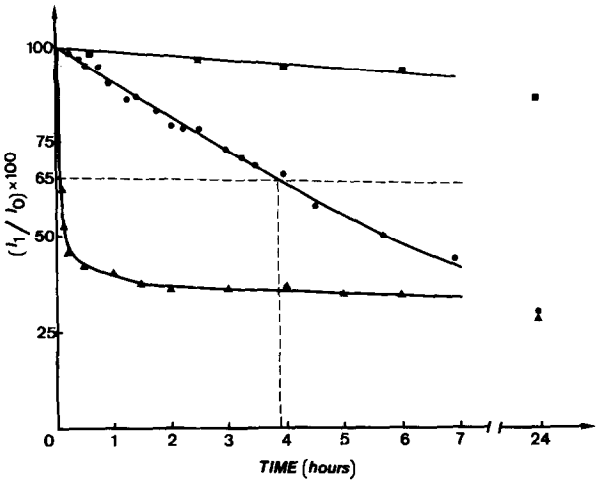

Fig. 1: Kinetics of the interaction between salmon sperm DNA and a series of cis-platinum compounds : (▲) cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, (●) cis-$Pt(NH_3)_2Cl_2$ and (■) cis-Pt(DAC) (malonato). These DNA-Pt complexes correspond to r_i = 0.20.

per platinum atom. Figure 1 shows characteristic profiles of the DNA-platinum complexation and it can be seen that, depending on the nature of the platinum compound, three different profiles are obtained. From these curves we can determine for each compound the time ($t_{1/2}$) necessary to bind one platinum atom per ten nucleotides. The results are presented in Table II which shows that the compounds can be classified in three classes :

TABLE II

PLATINUM COMPOUNDS	$[NaClO_4]$ x M	[Cl] x M			[malonato] x M			[oxalato] x M		
	10^{-2}	10^{-3}	10^{-2}	10^{-1}	10^{-3}	10^{-2}	10^{-1}	10^{-3}	10^{-2}	10^{-1}
cis-$[Pt(en)(H_2O)_2](NO_3)_2$	<0.1	<0.1	1	65						
cis-$Pt(DAC)(SO_4)(H_2O)$	<0.1	<0.1	1	70						
cis-$Pt(NH_3)_2(SO_4)(H_2O)$	0.1	<0.1	0.7	96						
cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$	<0.1	<0.1	0.25	96						
cis-$Pt(NH_3)_2Cl_2$	3.9	4.5	5.5	100	5	8	>150	6	16	>150
cis-$Pt(DAC)Cl_2$	2.3	2.5	3.5	95	2.5	3	>150			
cis-Pt(DAC)(malonato)	110	120	130	140	100	180	>240	110	120	>240
cis-Pt(DAC)(oxalato)	90	115	105	120				130	150	>240

HALF-TIME (IN HOURS) OF THE KINETICS OF THE DNA-Pt INTERACTION FOR A SERIES OF CIS-Pt COMPOUNDS AS A FUNCTION OF THE ANION CONCENTRATION.

- highly reactive compounds ($t_{1/2}$ <0.1 h)
- moderate reactive compounds ($t_{1/2}$ ~ few hours)
- less reactive compounds ($t_{1/2}$ ~ several days)

Within these three classes of compounds it can be observed that the nature of the ligands influences their reactivity. So the reactivity depends on the nature of the labile ligands and the order of decreasing rate is H_2O ~ SO_4 >Cl >oxalato >malonato.On the other hand, in each class, the (en)compounds are more reactive than the (DAC) and the (NH_3) species which shows the importance of the chelating effect of the inert ligands. This effect is correlated with the angle N-Pt-N assigned by the ligands which equals 77.59° for the cis-Pt(en)Cl_2 (11), 82.9° for the cis-Pt(DAC)Cl_2 (12) and 87° for the cis-Pt$(NH_3)_2Cl_2$ (13).

In Figure 2 are presented the profiles of the kinetics of DNA-platinum interaction for a series of cis-Pt compounds as a function of the anion concentration. Chloride ions inhibit the reaction of chloro, aquo and sulfato Pt compounds but do not seem to have any significant effect on the reaction of the carboxylato compounds. On the other hand carboxylato ions inhibit the reaction of both chloro and carboxylato Pt compounds. These observations are in agreement with the results of Teggins et al. who have studied the substitution of the chloride ligands of cis-Pt$(NH_3)_2Cl_2$ by oxylate (14). The fact that the reactions of all Pt species are inhibited by high concentration of anions indicates that they must be hydrolysed to be able to react with DNA in vitro. Our results are also in agreement with those obtained by Cleare et al. who have followed the course of the aquation reaction of platinum compounds using conductivity measurements (15,16).

From Table I it can be noticed that there are active compounds against the L1210 Leukemia cells in the three classes of reactivity indicating that there is no relationship between reactivity an antitumoral activity. However we have demonstrated a relationship between the reactivity of the platinum compounds and their toxicity

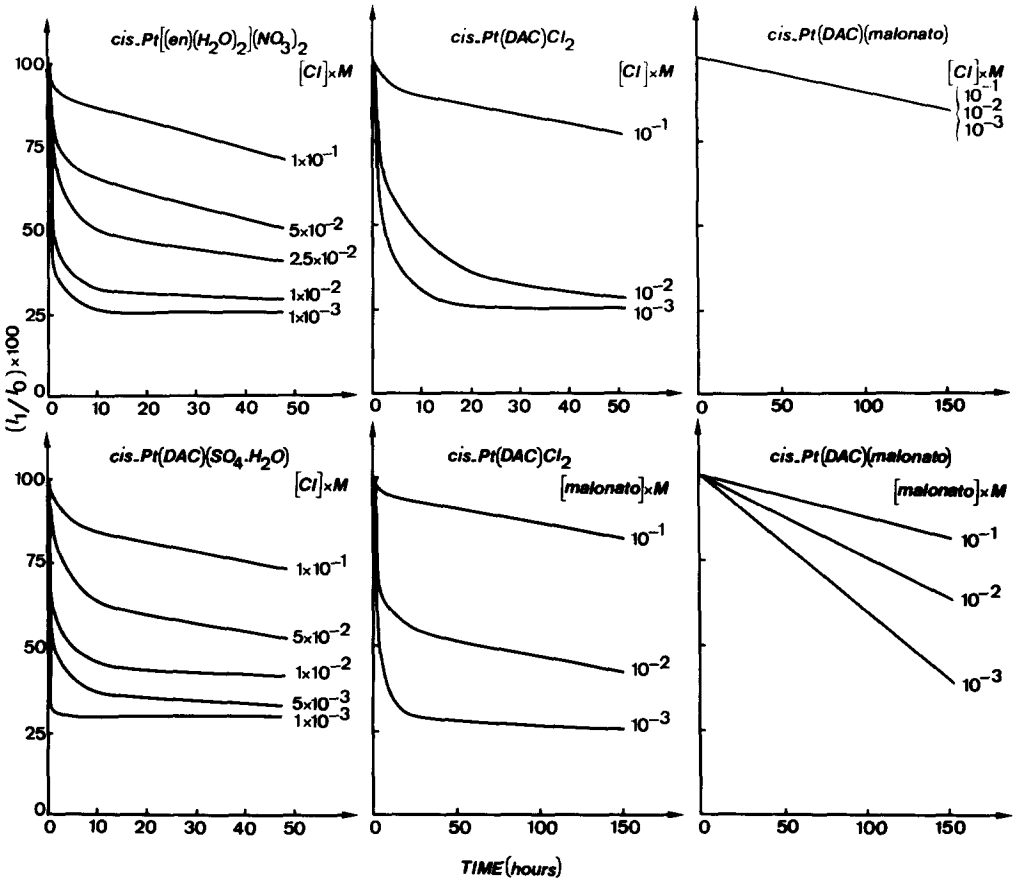

Fig. 2: Kinetics of the interaction between salmon sperm DNA and a series of cis-Platinum compounds as a function of the anion concentration. These DNA-Pt complexes correspond to r_i = 0.20.

towards Swiss mice (10). On the other hand, the carboxylato compounds (oxalato or malonato) are able to react directly *in vitro* with DNA in absence of any enzymatic activation as suggested by Cleare and Hoeschele (1), although such a system can not be excluded *in vivo*. Nevertheless it is not impossible that *in vivo* the quantities of drug which can reach the DNA will be very different depending on the reactivity of the compounds and on the concentrations of anions inside and outside the cells. The aquo, sulfato and chloro compounds can react rapidily with numerous nucleophilic sites present in the cells which decreases the concentration of the drug. On the contrary the weak hydrolysis rate of

carboxylato compounds may be compensated by a greater concentration of free drug available in the nucleus.

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