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Crospovidone: position in granulate and disintegration

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Three methods are generally employed for incorporating disintegrants into tablets, i.e. by positioning them intra-, extra- or intra/extragranularly [1]. Although it may theoretically be expected that this could lead to different effects the practical results are frequently marginal, probably due to encasement of the disintegrant in the binder and/or active. However, disintegrant positioning should be considered in tablet design as shown in the following example using a water soluble, high dose drug formulation with crospovidone, an excipient with powerful disintegrating properties [2, 3].

The drug used is soluble in water but very slightly soluble in ethanol. Granulations were carried out in an intensive mixer and tablet compression in a rotary tabletting machine.

Two 6 kg granulations, A and B, were prepared followed by drying, sizing, mixing with extragranular components (batch B), glidant and lubricant. The blends were compressed to a target tablet mass of 750 mg using 8×18 mm capsule shaped punches and similar compaction pressures. The compositions of the two batches are shown in Table 1.

The tablets were analyzed for hardness ($n = 20$), disintegration ($n = 6$, water, no disks) and dissolution ($n = 6$, water, 900 ml, 37 °C, paddle, 50 rpm). The results are enumerated in Table 2.

Table 1: Composition of batches A and B

Component	A (intragr.) mg/tablet	B (intra/extragr.) mg/tablet
Drug	583	583
Microcrystalline cellulose (intragranular)	97	61
Crospovidone (intragranular)	60 (8%)	30 (4%)
Ethanol	q.s.	q.s.
Microcrystalline cellulose (extragranular)	–	36
Crospovidone (extragranular)	–	30 (4%)
Colloidal silicon dioxide	5	5
Magnesium stearate	5	5

Table 2: Tablet properties

	A (intragranular)	B (intra/extragr.)
Hardness (kg)	27.8 (c.v. 4.1%)	27.3 (c.v. 3.9%)
Disintegration (min)	14.6	1.0
Time to 50% drug dissolved (min)	25	1.7

It is evident that positioning the disintegrant intra- and extragranularly makes a vital difference in this case, transforming the tablets from an unacceptable to a high quality product with almost flash disintegration.

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Pt(II) and Pd(II) complexes of 3-aminoflavone: *In vitro* and *in vivo* evaluation

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Since the discovery by Rosenberg et al. that *cis*-diamminedichloroplatinum(II) (cisplatin) exhibits antitumor activity [1], extensive studies of platinum amine analogues have been performed. Cisplatin was the first to be approved for clinical use in the treatment of genitourinary, head and neck tumors in humans. Unfortunately, cisplatin caused a number of negative side effects like nephrotoxicity, neurotoxicity and the development of resistance. Extensive effort has been devoted to the development of platinum compounds with a broader activity spectrum and lower toxicity [2]. Platinum complexes exhibiting antitumor activity should incorporate two *cis* nitrogen ligands each bearing at least one hydrogen (primary or secondary amines) together with two less strongly bound ligands (leaving groups) such as chlorides. The NH_2 moiety is believed to be essential because of the possibility of hydrogen bond formation between the amine and DNA fragments [3]. It is possible that amine release could play a role in the toxic side-effects of platinum anticancer agents. Our approach to design more effective anticancer drugs with less toxicity is based on the biological activity of flavanoids [4–8]. The observation that the flavanoide ligands themselves have anticancer activity [9, 10] prompted us to investigate novel platinum complexes, structurally related to cisplatin, containing a flavone ligand instead of amine with two *cis* bound labile chloride ligands. To minimize toxicity, 3-aminoflavone which possesses the desired NH_2 groups has been used as non leaving ligand. In the present preliminary study, we report on the antitumor activity of the complexes of the structure *cis*-$[Pt(AF)_2Cl_2]$ [11] and *trans*-$[Pd(AF)_2Cl_2]$, where AF = 3-aminoflavone. The alkylating ability of *cis*-platinum(II) and *trans*-palladium(II) complexes, containing 3-aminoflavone as ligands, has been also evaluated. The investigation is based on an *in vitro* test with 4-(4′-nitrobenzyl)pyridine (NBP) (Preussmann Test) [12].

The result of the NBP test show significant alkylating activities for the complexes (Table 1). The date are relative to those for cisplatin. The data revealed the markedly greater alkylating activity of *cis*-$[Pt(AF)_2Cl_2]$ than of *trans*-$[Pd(AF)_2Cl_2]$. In a previous study [12], a correlation between the alkylating activity *in vitro* and the *in vivo* cytostatic activity against a mice Sa180 sarcoma solid tumor was found for platinum(II) complexes. The antineoplastic activity of the *cis*-platinum(II) and *trans*-palladium(II) complexes of 3-aminoflavone were examined against the development leukemia L1210. In our experiments we implanted 3×10^5 L1210 cells intraperitoneally

Table 1: NBP test

Compd.	Concentration (M/l)	Absorbance* $\lambda_{max} = 545$ nm	ε	Relative alkylating activity (%)
cis-$[Pt(AF)_2Cl_2]$	1.25×10^{-4}	0.630	5040	2100
trans-$[Pd(AF)_2Cl_2]$	1.25×10^{-4}	0.100	800	333
Cisplatin	1.25×10^{-3}	0.300	240	100

* Means of 3 determination

Table 2: Activity of *cis*-$[Pt(AF)_2Cl_2]$ and *trans*-$[Pd(AF)_2Cl_2]$ in L1210 leukemia

Compd.	ALD_{50}* (g/kg) i.p.	Dose i.p. (ratio of ALD_{50}*)	Dose (g/kg) of body weight	T/C (%)	Antineoplastic effect according NCI–USA**
cis-$[Pt(AF)_2Cl_2]$	0.6	1.0	0.6	123	− [b]
		0.5	0.3	130	+ [a]
		0.1	0.06	130	+ [a]
trans-$[Pd(AF)_2Cl_2]$	0.6	1.0	0.6	123	− [b]
		0.5	0.3	123	− [b]
		0.1	0.06	123	− [b]
Control				100	

* The approximate LD_{50} (ALD_{50}) was calculated according to [12]
** a: T/C(%) > 125 (+); b: 100 < T/C(%) < 125 (−)

into mice. The approximate lethal dose 50 (ALD_{50}) of the investigated complexes was determinated as 0.6 g/kg. *Cis*-$[Pt(AF)_2Cl_2]$ prevented the development of L1210 leukemia after doses of 0.5 and 0.1 g/kg body weight of mice: T/C(%) = 130 (Table 2). *Trans*-$[Pd(AF)_2Cl_2]$ did not inhibit the survival time of treated mice versus control animals: T/C(%) = 123. The results of these experiments clearly demonstrate that the new analog of cisplatin, *cis*-$[Pt(AF)_2Cl_2]$ exhibit significant antitumor activity in the development of leukemia L1210 and may be regarded as a potential antitumor drug. More detailed pharmacological and biochemical experiments will be performed in the near future.

Experimental

1. Chemistry

To the solution of 3-aminoflavone (AF) (0.474 g, 2 mM) in ethanol (80 ml) the water solution (10 ml) of K_2PtCl_4 (0.415 g, 1 mM) or K_2PdCl_4 (0.326 g, 1 mM) was added dropwise. The reaction mixture was initiated by heating to 60 °C (for the Pt-complex) and stirred for 6 h in room temperature. The precipitate was filtered, washed with ethanol and dried in vacuo. Obtained: *cis*-$[Pt(AF)_2Cl_2]$ (740.4) (yellow), m.p., 206–209 °C, yield 62%, *trans*-$[Pd(AF)_2Cl_2]$ (651.7) (orange), m.p. 250-255 °C (dec.), yield 75%. The complexes gave satisfactory elemental analyses and the purity was verified by IR, ^1H NMR and ^{195}Pt NMR spectroscopy.

2. Animals

For the experiments hybrid male, F_1 (BALB/c × DBA/2) mice, weighing 23–29 g, 8–10 weeks old, purchased from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław) were used. They were given standard laboratory food and water ad libitum.

3. Leukemia

Leukemia cells L1210 was purchased from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław) and was maintained by serial passages *in vivo*. Leukemia cells from the fluid were resuspended in 0.9% NaCl, so that 3×10^5 L1210 cells were injected intraperitoneally (i.p.) into mice.

4. Therapeutics

The complexes were administered in a volume of 0.01 ml/g mouse weight in 1% methylcellulose solution. Control mice received equivalent volumes of 1% methylcellulose solution.

5. Toxicity determination

The approximate lethal dose was determined by the method described by Deichmann and Le Blance [13].

6. Antileukemic assay

On a day 0.3×10^5 of L1210 leukemic cells was implanted i.p. into F1 mice. Five mice were used per groups. Beginning on day 1, the mice received a solution of the investigated complexes (1× i.p.) after leukemia implanted. The mice of the control group received 1% methylcellulose solution on the treatment day. The mice were observed daily for survival. The median survival time (MST) according to Geran's method [14] is: MST = $(x = y)/2$, where x denotes the earlier day when the number of dead animals is $> N/2$, y denotes the number of animals in the group. The antileukemic effect of the drugs was assessed as percentage ratio of MST of the treated group (T) to that of the control group (C):

$$T/C(\%) = (MST_T/MST_C) \times 100\%$$

7. Statistical analysis

The results were evaluated using student's test for differences between means. Differences were considered significant when $p \leq 0.05$.

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