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ANTITUMOR ACTIVITY OF *l*-OHP IN MICE

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SUMMARY

The isomeric mixtures of platinum complexes of diaminocyclohexane (DACH) had been found active on several murine tumors. A recent separation of the oxalato-platinum complex of *trans-l*-DACH isomer allowed more precise screening studies and permitted the selection of one compound: *l*-OHP was submitted to our murine tumor screening system. The drug was given: (a) at doses of 1—12 mg/kg i.p. or i.v. on day 1, 5 and 9 compared to identical doses of *cis*-dichlorodiamine platinum II (CDDP) in L1210 bearing mice and (b) to AkR leukemia, LGC lymphoma, glioma 26, B16 melanoma, MA 16-C mammary carcinoma and Lewis lung carcinoma bearing mice at 2 dosages: 5 mg/kg (minimal effective dose on L1210), and 8 mg/kg (subtoxic dose in L1210). Acute LD_{10} and LD_{50} appeared similar to CDDP and *l*-OHP. *l*-OHP administered i.p. was more active on L1210 than CDDP. On L1210 grafted intracerebrally and on LGC lymphoma *l*-OHP increased significantly the lifespan while CDDP was inactive. On AkR leukemia, both drugs were active but *l*-OHP was less toxic. Both drugs were inactive on murine solid tumors. No renal toxicity was observed with *l*-OHP as compared to CDDP.

INTRODUCTION

CDDP, introduced by Rosenberg [1] in 1969, is a powerful cytostatic agent that is frequently and successfully used in clinical cancer chemotherapy [2]. CDDP, in the doses needed for a therapeutic effect, are sometimes imperfectly tolerated in the short term (nausea and vomiting), possibly

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later during treatment (especially by the kidney), and in the long term (by the oto-vestibular system) [2]. To try to avoid this toxicity, other Pt(II) complexes have been prepared, including the dichloro Pt(II) complex of DACH obtained as isomeric mixtures, and which is active on several murine tumors [3—6].

Kidani et al. [7] succeeded in separating DACH into geometric isomers, *cis* and *trans*, and then separated the *trans* into 2 optical isomers: *trans-d* and *trans-l* (Fig. 1A). Among the complexes they prepared, the oxalato Pt(II) complex of the *trans-l*-DACH (Fig. 1B) appeared to have the maximal *T/C* (treated/control) values on L1210 leukemia.

We have tested this *trans-l*-DACH complex, named *l*-OHP, on our murine tumor screening battery [8]. The object of this work was to test its cytostatic efficacy on the main leukemia and solid tumor types used in most screening systems. We studied the dose-effect relationship on L1210 leukemia [9]. In all experiments, *l*-OHP was compared to CDDP.

MATERIALS AND METHODS

The L1210 leukemia test for simultaneous detection of the oncostatic activity and the evaluation of acute LD_{50}

On day 0, B6D2F1 male mice, 3 months old, were inoculated i.p. with 10^5 L1210 cells. On day 1, the treated mice received various doses of *l*-OHP or CDDP: 1—22 mg/kg i.p. or i.v. in distilled water as solvent, 8 mice per dose. On days 5 and 9, drugs or solvent injections were repeated in the mice which did not show any sign of toxicity.

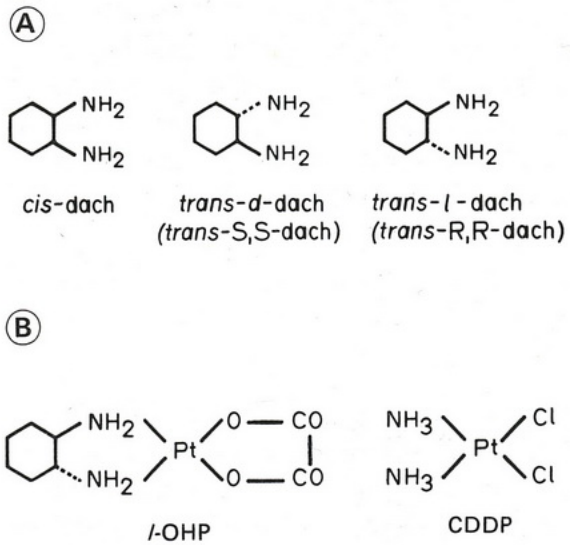

Fig. 1. (A) Structure of *trans S,S,*- and *trans R,R*-DACH. (B) The formula of *l*-OHP compared to CDDP.

The mortality was monitored daily and autopsies were performed to find out whether death was due to leukemia or to toxic action of the drugs. The acute LD_{50} was graphically determined.

The oncostatic effect of each dose was expressed as an index, *I*:

$$I = T/C \times 100$$

where *T* is the median survival time in the treated groups of mice and *C* that of the control group. The difference between median survival times of treated and control groups was statistically performed by the non-parametric Wilcoxon's test (ϵ) [11]. When *I* was greater than or equal to 125 and ϵ greater than or equal to 1.96, the compound was considered active.

For each route of administration (i.p. or i.v.) of *l*-OHP and CDDP, the 2 linear regressions were calculated and drawn: positive slope, the efficiency increased with the dose; negative slope, the toxicity appeared, increased with the dose and hid the activity of the compound. The intersection point of the 2 lines is considered as the optimally efficient dose (OED) (Fig. 2). The 2 doses determined by the intersection of the curves and the horizontal line at the level of 125% are called minimal and subtoxic doses of the maximally efficient dose range (MEDR).

Activity on other murine tumors

Besides intracerebrally (i.c.) grafted L1210 leukemia and CDDP-resistant L1210 line, 6 other murine tumors were used: L40 AkR leukemia, LGC lymphoma, glioma 26, B16 melanoma, MA16-C mammary adenocarcinoma and 3LL Lewis lung carcinoma [8]. The various tumors were grafted on day 0. The route of administration, the number of cells inoculated and the lines are shown in Table 1.

We compared the administration of *l*-OHP by the i.v. route at 2 doses, the OED and the minimum dose of MEDR (mMEDR) with that of CDDP.

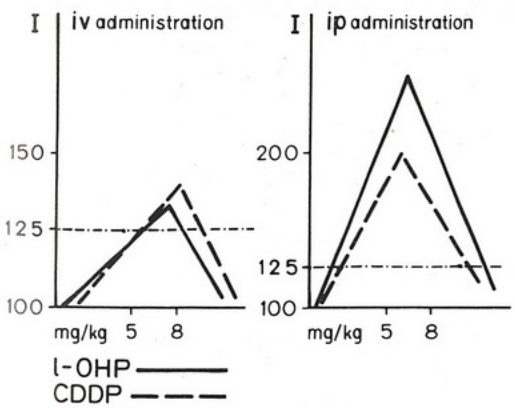

Fig. 2. Linear regression of *l*-OHP and CDDP efficacy after i.v. and i.p. administration.

TABLE 1

PANEL OF MURINE TUMORS USED FOR THE STUDY OF *l*-OHP

Tumor	Mouse strain	Graft route	Inoculum (no. of cells)
L1210 Leukemia { sensitive to CDDP / resistant to CDDP	B6D2F1	i.p.	10^5
L1210 Leukemia	B6D2F1	i.c.	10^4
L40 AkR Leukemia	AkR	i.p.	$\sim 10^6$
LGC Lymphoma	C57BL6	i.p.	$\sim 10^6$
Glioma 26	C57BL6	s.c.	$\sim 10^6$
B16 Melanoma	C57BL6	s.c.	$\sim 10^6$
MA16-C Mammary adenocarcinoma	C3H/He	s.c.	$\sim 10^6$
3LL Lewis lung carcinoma	C57BL6	s.c.	$\sim 10^6$

The conditions of administration of the compounds was the same as in the L1210 leukemia test; the solvent was distilled water, and the mice were injected on days 1, 5 and 9. Results were expressed according to the index $I = T/C \times 100$ and statistical analysis was performed according to Wilcoxon's test [10].

General and particular toxicities

These will be the subject of another study. We only examined in this investigation the renal toxicities of *l*-OHP and CDDP at their subtoxic dose of the MEDR.

RESULTS

Toxicity

Acute toxicity of *l*-OHP and CDDP is given in Table 2. LD_{50} and LD_{10} are similar for both compounds.

TABLE 2

ACUTE TOXICITY OF *l*-OHP AND CDDP

	LD_{10} (mg/kg)		LD_{50} (mg/kg)	
	i.v.	i.p.	i.v.	i.p.
l-OHP	10	15	17.5	20
CDDP	10	15	17.5	20

TABLE 3

COMPARED EFFECTS OF *l*-OHP AND CDDP ON L1210 LEUKEMIA: DOSE EFFECT CORRELATION FOR i.v. AND i.p. ROUTES

mg/kg	*l*-OHP				CDDP			
	i.v.		i.p.		i.v.		i.p.	
	I^a	P^b	*I*	*P*	*I*	*P*	*I*	*P*
12	100	—	Toxic		Toxic	—	Toxic	—
10	100	—	175	0.02	125	0.05	140	0.02
8	130	0.05	210	0.01	135	0.02	160	0.02
6	130	0.05	245	0.01	130	0.05	200	0.01
3	115	—	160	0.02	115	—	140	0.02
1.5	110	—	140	0.02	100	—	130	0.05
1	95	—	110	—	100	—	100	—

[a] I = (median survival in treated group/median survival in control group) × 100.
[b] Statistical significance for $\beta = 1.96$; $P = 0.05$.

Efficacy on L1210

Table 3 compares the effect of *l*-OHP to that of CDDP at different dose levels ranging from 1 to 12 mg/kg administered i.v. and i.p. Both compounds are active on L1210 leukemia and their efficacies are similar for each route of administration (Fig. 2). When injected i.p., *l*-OHP produces a stronger effect than CDDP in the same conditions. The linear regressions of *l*-OHP and CDP administered by the i.v. route are presented in Fig. 2. The OED is 7.5—8 mg/kg and the mMEDR is 5 mg/kg. These 2 doses were selected for further experiments on tumor panel.

When L1210 leukemia cells were grafted i.c. (Table 4), CDDP was found to be inactive while *l*-OHP significantly increased the lifespan of the treated mice. Both drugs are active against L40 AkR grafted leukemia. However, *l*-OHP is less toxic than CDDP. It is maximally efficient at 5 mg/kg on LGC lymphoma (Table 5).

TABLE 4

COMPARISON OF THE EFFECT OF *l*-OHP AND CDDP ON INTRACEREBRALLY GRAFTED L1210 LEUKEMIA. (10^4 CELLS)

	I^a	P^b
l-OHP	165	0.02
CDDP	100	

Dose 5 mg/kg i.p.
[a] $I = T/C \times 100$.
[b] P, statistical significance.

TABLE 5

EFFECTS OF *l*-OHP AND CDDP ON L40 AkR GRAFTED LEUKEMIA AND LGC LYMPHOMA

	mg/kg i.v.	I^a	
		l-OHP	CDDP
L40 AkR Leukemia	5	144	194
	7.5	177	Toxic
LGC Lymphoma	5	∞[c]	NA
	7.5	NA[b]	NA

Tumor graft (10^6 cells, i.p.) on day 0.
Treatment i.v. on days 1, 5 and 9.
[a] $I = T/C \times 100$.
[b] NA, not active.
[c] ∞, more than 50% of mice were cured.

TABLE 6

CROSS-RESISTANCE IN L1210 LEUKEMIA

Dose (mg/kg)	L1210/S[a]		L1210/R[a]	
	l-OHP	CDDP	*l*-OHP	CDDP
5	129[b]	133	NA	NA
7.5	133	NA[c]	NA	NA
10	Toxic	Toxic	Toxic	Toxic

[a] CDDP-sensitive line, L1210/S; CDDP-resistant line, L1210/R. 10^5 cells grafted i.p. on day 0. i.v. treatment on days, 1, 5 and 9.
[b] numbers are statistically significant with Wilcoxon's test ($P < 0.02$).
[c] NA = not active.

TABLE 7

RENAL TOXICITY IN B6D2F1 MICE (Subtoxic dose, 10 mg/kg i.p., day 4)

	No. of animals	Urea (mmol/l)	Creatinine (μmol/l)
l-OHP	10	10.4[a]	32
CDDP	10	98	3.80
Control	10	10	34

[a] These figures are obtained from the pool of the blood of 10 mice. Pooling was necessary because a large quantity of serum (5 ml) was needed for the analysis.

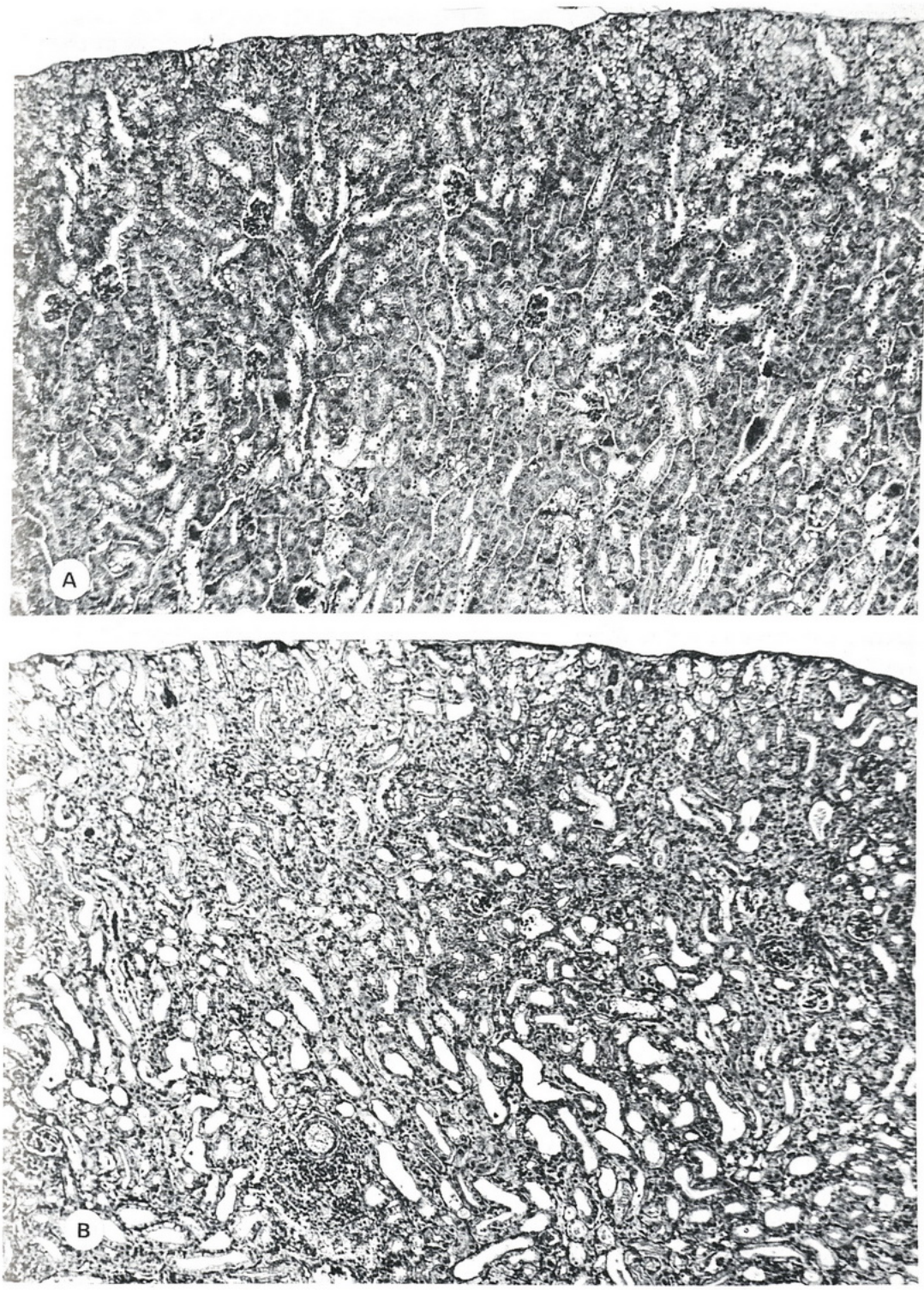

Fig. 3. (A) Kidney of mice treated with *l*-OHP: normal aspect. (B) Kidney of mice treated with CDDP: distal tubule dilatation, epithelial vacuolization, atrophy and necrosis. x 25.

Efficacy on other neoplasias

l-OHP and CDDP are inactive at both doses on the 4 solid tumors in the panel: glioma 26, B16 melanoma, MA16-C mammary adenocarcinoma and 3LL Lewis lung carcinoma. Table 6 shows that a cross-resistance was found between *l*-OHP and CDDP.

Toxicities

l-OHP and CDDP were compared for renal toxicity at the same dose, 10 mg/kg injected i.p. and Table 7 shows that, while CDDP appeared markedly nephrotoxic, while the urea and creatinine levels remained completely normal in *l*-OHP-treated mice. The histological examination confirms the remarkable tolerance of this platinum complex (Fig. 3).

DISCUSSION

Today there is a wide experience of the remarkable chemotherapeutic effects of the platinum complex, CDDP, in man [2]. CDDP is efficient in many more types of tumors in man than in the murine grafted neoplasias used in our screening test or have been published in the literature [2]. The efficiency of *l*-OHP can be expected to be as useful in human neoplasms as CDDP: we have not yet found any reason against this hypothesis in our preclinical examination in baboons [11] and early clinical trials (unpublished data) or pharmacokinetic study [10]. We also observed a tolerance superiority of *l*-OHP over CDDP in baboons [11] at the MEDR doses extrapolated from mice to monkeys according to their relative body surface area [12]. Finally, in our current *l*-OHP phase I trial employing the new method we have proposed of the intra-patient escalation [13], we can already confirm the remarkable tolerance of the product and have obtained the partial regression of a hepatoma and of heart carcinoma (unpublished data).

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