[Chem. Pharm. Bull. 33(12)5511—5514(1985)]

# Antitumor Effect of Oral Cisplatin on Certain Murine Tumors[1)]

YOSHINARI HASEGAWA* and MASUYUKI MORITA

*Bristol-Myers Research Institute, Ltd., 1 Futagoyama, Sakazaki, Kohta-cho, Nukata-gun, Aichi 444-01, Japan*

(Received April 19, 1985)

The comparative antitumor effects of cisplatin were investigated against several murine tumors after *i.p.*, *i.v.*, and oral administrations. A single administration of cisplatin could significantly inhibit the growth of both L1210 and P388 leukemias, and EL-LP-12 tumors under the conditions used. Oral cisplatin was tested against EL-LP-12 in combination with 6 other antitumor drugs. Cisplatin was inhibitory in binary combination with mitomycin C, vinblastine, 5-fluorouracil, carmustine (BCNU), and cyclophosphamide. In particular, the drug combined with either BCNU or cyclophosphamide showed marked therapeutic synergism.

**Keywords**——cisplatin; antitumor effect; oral administration; murine tumor

## Introduction

*cis*-Dichlorodiammineplatinum (II) (cisplatin) shows a broad spectrum of antitumor effect when administered *i.p.* in experimental animals, as mentioned by Rosenberg.[2)] The effects of the drug when administered orally, however, have not yet been investigated in detail. Handelsman *et al.* carried out primary screening and found that *i.p.* injection of cisplatin was effective, while oral administration was ineffective against L1210.[3)] Wolpert-DeFilippes also reported no activity on oral administration of the drug.[4)] We have found that cisplatin is effective against ascites and solid type of Ehrlich carcinoma and sarcoma 180 when administered orally as well as *i.p.*[5)] Since oral application of antitumor drugs is of importance in the clinical management of patients, an additional investigation on oral cisplatin was performed with L1210, P388 and EL-LP-12 tumors. Oral cisplatin was also tested against EL-LP-12 tumor in combination with other antitumor drugs. The results are presented here.

### Materials and Methods

The animals used in these experiments were $B6D2F_1$ female and ddY male mice weighing about 20 g (5 weeks old) and 21 g (4 weeks old), respectively. Animals were purchased from Shizuoka Laboratory Animal Center and kept on a standard solid diet with water *ad libitum*. L1210 and P388 leukemias were propagated in DBA/2 mice at weekly intervals by *i.p.* inoculation. The L1210 cells ($10^5$) and P388 cells ($10^6$) thus obtained were inoculated into the axillary region of the right hind leg or the peritoneal cavity of $B6D2F_1$ mice. EL-LP-12 (subline of Ehrlich carcinoma)[6,7)] tumor was similarly propagated in ddY mice at weekly intervals by *i.p.* inoculation. EL-LP-12 cells ($6 \times 10^6$) thus obtained were inoculated into the tail vein.

The cisplatin was supplied by Johnson Matthey Chemicals Ltd. of England. The survival periods of all tumor-bearing mice in each group were expressed as mean ± standard deviation (S.D.), and the significance of the differences between the dosed groups and the control group was evaluated by using Student's *t*-test. Animals surviving more than 30 d were calculated as 30-day survivors. Moreover, all results were expressed as percentage increase in lifespan (ILS) values of treated mice over untreated controls.

## Results and Discussion

In this approach, the tumor cells invade systemically and produce metastases when

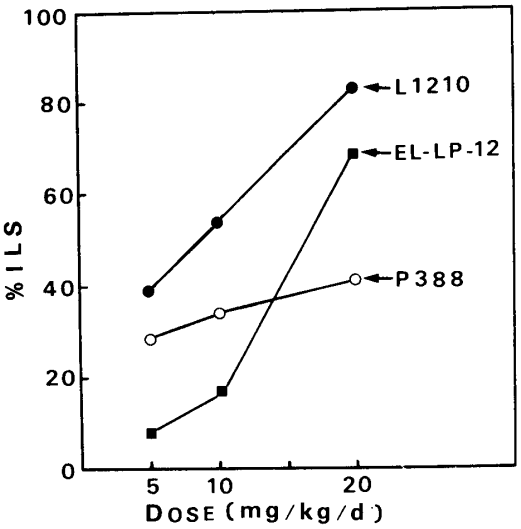


Fig. 1. Antitumor Effect of Cisplatin on L1210 and P388 Inoculated *s.c.* and EL-LP-12 Inoculated *i.v.*

The drug was administered *p.o.* on days 1 and 5 after tumor inoculation. One group consists of 10 mice.

TABLE I. Antitumor Effect of Cisplatin on L1210 (*i.p.* Inoculation)

| Cisplatin | | $\Delta G^{b)}$ (%) | $S/T^{c)}$ | Survival day (mean ± S.D.) | ILS (%) |
|---|---|---|---|---|---|
| Dose (mg/kg/d)[a)] | Route | | | | |
| Control | | 4 | 0/20 | 10.7 ± 2.0 | |
| 2 | *i.p.* | 1 | 0/10 | 16.4 ± 1.6[d)] | 53 |
| 4 | *i.p.* | −3 | 8/10 | 27.6 ± 5.1[d)] | 158 |
| 8 | *i.p.* | −16 | 10/10 | 30.0 | 180 |
| 2 | *i.v.* | 7 | 0/10 | 13.4 ± 1.3[d)] | 25 |
| 4 | *i.v.* | −5 | 1/10 | 17.7 ± 4.6[d)] | 65 |
| 8 | *i.v.* | −23 | 2/9 | 21.2 ± 6.3[d)] | 98 |
| 5 | *p.o.* | 10 | 0/10 | 12.1 ± 2.9 | 13 |
| 10 | *p.o.* | 1 | 0/10 | 13.5 ± 1.4[d)] | 26 |
| 20 | *p.o.* | −11 | 0/10 | 16.0 ± 1.8[d)] | 50 |

*a*) The drug was administered *p.o.* on days 1 and 5 after tumor inoculation. *b*) Body weight change: from 0 to 7 d. *c*) 30-Day survivors/number of mice tested. *d*) Significantly different from the control ($p < 0.001$).

TABLE II. Antitumor Effect of Cisplatin on L1210 (*s.c.* Inoculation)

| Cisplatin | | $\Delta G$ (%) | $S/T$ | Survival day (mean ± S.D.) | ILS (%) |
|---|---|---|---|---|---|
| Dose (mg/kg/d) | Route | | | | |
| Control | | 14 | 0/20 | 9.2 ± 0.4 | |
| 2 | *i.p.* | 3 | 0/9 | 13.7 ± 2.1[a)] | 49 |
| 4 | *i.p.* | −2 | 0/10 | 15.3 ± 2.5[a)] | 66 |
| 8 | *i.p.* | −15 | 1/10 | 20.1 ± 4.0[a)] | 118 |
| 2 | *i.v.* | 0 | 0/10 | 14.0 ± 1.2[a)] | 52 |
| 4 | *i.v.* | −4 | 0/10 | 18.3 ± 3.1[a)] | 99 |
| 8 | *i.v.* | −19 | 1/10 | 20.5 ± 5.5[a)] | 123 |
| 5 | *p.o.* | 5 | 0/10 | 13.7 ± 1.5[a)] | 49 |
| 10 | *p.o.* | −1 | 0/10 | 14.8 ± 1.0[a)] | 62 |
| 20 | *p.o.* | −14 | 0/10 | 17.1 ± 3.8[a)] | 86 |

For symbols, see Table I. *a*) Significantly different from the control ($p < 0.001$).

TABLE III. Antitumor Effect of Cisplatin on EL-LP-12 (*i.v.* Inoculation)

| Cisplatin Dose (mg/kg/d) | Route | $\Delta G$ (%) | $S/T$ | Survival day (mean ± S.D.) | ILS (%) |
|---|---|---|---|---|---|
| Control | | 12 | 0/20 | 9.7 ± 2.8 | |
| 2 | *i.p.* | 15 | 0/10 | 13.5 ± 2.8[a)] | 39 |
| 4 | *i.p.* | 13 | 0/10 | 16.8 ± 5.8[a)] | 73 |
| 8 | *i.p.* | −13 | 4/10 | 23.1 ± 8.3[a)] | 138 |
| 2 | *i.v.* | 18 | 0/10 | 14.3 ± 2.8[a)] | 47 |
| 4 | *i.v.* | 12 | 1/10 | 20.6 ± 4.1[a)] | 112 |
| 8 | *i.v.* | −11 | 1/10 | 18.9 ± 7.2[a)] | 95 |
| 5 | *p.o.* | 13 | 0/10 | 12.5 ± 3.4[b)] | 29 |
| 10 | *p.o.* | 15 | 0/10 | 13.0 ± 1.6[a)] | 34 |
| 20 | *p.o.* | 0 | 2/10 | 17.9 ± 7.6[a)] | 85 |

For symbols, see Table I. Significantly different from the control, *a*) $p < 0.001$; *b*) $p < 0.005$.

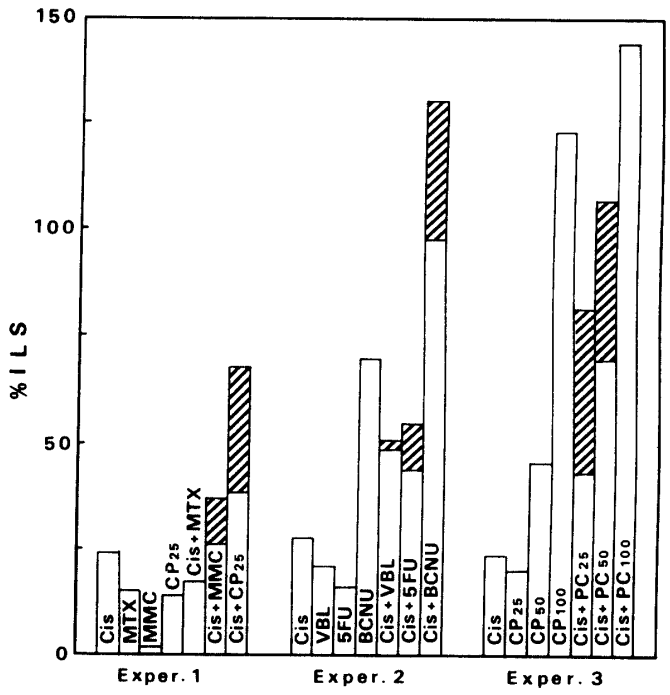


Fig. 2. Life-Prolonging Effect of Cisplatin on Mice with EL-LP-12 When Combined with Other Antitumor Drugs

The tumor was inoculated *i.v.* All drugs were administered simultaneously on days 1 and 5; cisplatin was given *p.o.* and the others *i.v.* One group consists of 9 or 10 mice.

▨, the % ILS observed in excess of a mere summation of individual % ILS (may be regarded as synergism).

Cis, cisplatin 10 mg/kg/d; MTX, methotrexate 3.1 mg/kg/d; MMC, mitomycin C 1 mg/kg/d; VBL, vinblastine 2 mg/kg/d; 5FU, 5-fluorouracil 50 mg/kg/d; BCNU, BCNU 5 mg/kg/d; $CP_{25}$, cyclophosphamide 25 mg/kg/d; $CP_{50}$, cyclophosphamide 50 mg/kg/d; $CP_{100}$, cyclophosphamide 100 mg/kg/d.

L1210 and P388 leukemias are inoculated *s.c.* into mice. The test system is regarded as a model for lymphogenous tumor metastases. A system of inoculation into the vein was adopted for the EL-LP-12 tumor in order to obtain pulmonary metastases. The antitumor effects of cisplatin on these test systems are summarized in Fig. 1; the effects were dose-dependent. Cisplatin showed a greater effect on L1210 than P388.

Subsequently, we compared the effects of *i.p.*, *i.v.*, and *p.o.* administrations of cisplatin on L1210 and EL-LP-12. Table I summarizes the effect of cisplatin on L1210 inoculated *i.p.* The drug, at its optimal dose of 8 mg/kg/d for *i.p.* injection, produced a 100% survival rate and showed an ILS of 180%, though body weight was decreased. The antitumor effect in each dose of *i.v.* injection was approximately half that of *i.p.* injection. Oral cisplatin was also significantly effective; the ILS was 50% at a dose of 20 mg/kg/d. This value exceeds those given in previous reports.[3,4)] Table II summarizes the effect of the drug on L1210 inoculated *s.c.* The results were similar in pattern to those in Table I. These responses to the drug appeared to be

dose-dependent. As shown in Table III, the results of comparative trials in EL-LP-12 tumor-bearing mice were similar to the results in the case of L1210. The drug, at its optimal dose of 20 mg/kg/d for *p.o.* administration, gave a 20% survival rate and 85% ILS. The mean survival period of the 40 mg/kg/d group was no longer than that of the control group as a result of drug toxicity.

Next, combination therapy was tested with cisplatin given *p.o.* and other antitumor drugs (methotrexate, mitomycin C, vinblastine, 5-fluorouracil, carmustine (BCNU), and cyclophosphamide) given *i.v.* simultaneously. These drugs were given at an empirically minimum effective dose level of approximately 1/10 $LD_{50}$. Figure 2 shows the effects on the survival of tumor-bearing mice when cisplatin was combined with each of the 6 conventional antitumor drugs. No clear therapeutic synergism of cisplatin plus methotrexate was seen, but the other 5 drugs used in the combination therapy showed increased % ILS values. Although the experimental conditions and model systems differ from those used in earlier work[8-10] on combination chemotherapy with cisplatin, our findings are essentially similar to the reported data.

Recently, Siddik *et al.*[11] reported antitumor, pharmacokinetic and toxicity studies with orally administered cisplatin and its congeners. They reported significant antitumor activities against ADJ/PC6A plastocytoma, and a peak plasma concentration of 4.3 μg Pt/ml was achieved within 30—60 min in mice receiving 50 mg/kg oral cisplatin. In our unpublished preliminary studies, blood concentrations of Pt following a single oral dose of 40 mg/kg of cisplatin in ddY mice were 1.7, 1.35, and 1.15 μg/ml at 30, 60, and 120 min, respectively. A confirmatory study clearly demonstrated that Pt did enter the blood after oral administration of cisplatin. Our data demonstrate that cisplatin is orally effective against several murine tumors. Thus, clinical trials involving oral administration of cisplatin and platinum compounds would appear to be justified.

**Acknowledgement** The authors are grateful to Prof. Yutaka Kawazoe of Nagoya City University for his valuable guidance and encouragement.

## References and Notes

1) A part of this work was presented at the 13th International Cancer Congress, Seattle, Washington U.S.A., Sept. 1982. Abstract of Papers, p. 549.
2) B. Rosenberg, *Cancer Chemother. Rep., Part 1*, **59**, 589 (1975).
3) M. Handelsman, M. Goldsmith, L. Broder, S. Carter, and M. Slarik, *Clinical Brochure, NCI*, **1**, 10 (1976).
4) M. K. Wolpert-DeFilippes, *Cancer Treat. Rep.*, **63**, 1453 (1979).
5) Y. Hasegawa, M. Morita, and N. Muto, *Gan To Kagakuryoho* (*Jpn. J. Cancer and Chem.*), **7**, 621 (1980).
6) Y. Hasegawa, T. Irikura, M. Ishidate, Jr., and D. Mizuno, *Gann*, **61**, 73 (1970).
7) Y. Hasegawa, T. Irikura, and D. Mizuno, *Chem. Pharm. Bull.*, **18**, 810 (1970).
8) G. R. Gale, L. M. Atkins, S. J. Meischen, A. B. Smith, and E. M. Walker, Jr., *J. Natl. Cancer Inst.*, **57**, 1363 (1976); *idem, Cancer Treat. Rep.*, **61**, 445 (1977).
9) F. M. Schabel, Jr., M. W. Trader, W. R. Laster, Jr., T. H. Corbett, and D. P. Griswold, Jr., *Cancer Treat. Rep.*, **63**, 1459 (1979).
10) A. Hoshino, T. Kojima, and S. Machida, Abstruct of Papers, the 40th Annual Meeting of the Japanese Cancer Association, Sapporo, October, 1981, p. 224.
11) A. H. Siddik, F. E. Boxall, P. M. Goddard, C. F. J. Barnard, and K. R. Harrap, Abstracts of Papers, 75th Annual Meeting of American Association for Cancer Research, Toronto, May 1984, p. 369.