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INHIBITION OF METHIONINE UPTAKE BY *CIS*-DIAMMINEDICHLOROPLATINUM (II) IN EXPERIMENTAL BRAIN TUMORS

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cis-diamminedichloroplatinum (II) (CDDP) has been used both alone and in combination with other chemotherapeutics for cancer chemotherapy. Although CDDP acts primarily on DNA, it can also act at the tumor-cell membrane to inhibit methionine transport. The latter mechanism of CDDP is reported to have an important role as a chemical modulator in enhancing chemotherapeutic effects of 5-fluorouracil in tumor cells. We report here the effects of CDDP on methionine uptake in an *in vivo* brain-tumor model. C6 brain-tumor cells were stereotactically inoculated in the right basal ganglia of 6-week-old male Sprague-Dawley rats. Ten days after the inoculation, autoradiographic images were obtained using (14C-methyl)-L-methionine. The tracer uptake, represented as differential absorption ratio (DAR) and an acid-insoluble fraction (AIF), was measured in both brain tumors and normal brain with or without an intravenous injection of CDDP. The tumor/non-tumor DAR and AIF decreased significantly ($P < 0.01$, as determined by the Mann-Whitney U-test) after CDDP treatment, whereas the non-tumor DAR and AIF remained almost unchanged. These findings indicate that CDDP inhibits methionine uptake selectively in brain-tumor tissue and may therefore be a potent chemical modulator in the chemotherapy of brain tumors.

A large number of chemotherapeutic agents have been used to enhance the effectiveness of therapy in malignant brain tumors, although optimal therapy schedules have not yet been established. Chloroethylnitrosoureas (CENUs) are widely used; however, the results from patients receiving CENUs have been limited in randomized cooperative studies (Mahaley, 1991). Recently, *cis*-diamminedichloroplatinum (II) (CDDP) has been used increasingly in the chemotherapy of head-and-neck malignancies (Forastiere, 1993) and gastrointestinal cancers (Buswell *et al.,* 1994), also in recurrent brain tumors after CENU failure (Spence *et al.,* 1992).

High-dose CDDP, administered either i.v. or i.a., exhibits notorious effects, such as nephrotoxicity or peripheral nephropathy (Shani and Wolf, 1989; Spence *et al.,* 1992; Buswell *et al.,* 1994). Combinations of CDDP with other therapeutic agents may help to reduce the toxic effects. CDDP and 5-fluorouracil synergistically enhance cytotoxicity in tumor cells, including ovarian tumor cells, *in vitro* (Scanlon *et al.,* 1986, 1988), and have synergistic anti-tumor effects in gastrointestinal malignancies (Goseki *et al.,* 1995). CDDP is considered to be a chemical enhancer of the chemotherapeutic effects of 5-fluorouracil in tumor cells (Scanlon *et al.,* 1986, 1988).

CDDP inhibits methionine uptake *in vitro* in these tumor cells. Such exogenous methionine depletion in tumor cells results in increased intracellular methionine synthesis, *i.e.,* folate co-factors that consume thymidylate (dTMP) synthase are induced. The resulting accumulation of 5-fluorodeoxyuridylate (FdUMP) forms an FdUMP-dTMP synthase complex and enhances cytotoxic effects in tumor cells (Scanlon *et al.,* 1986, 1988). To determine the *in vivo* effect of CDDP on methionine uptake, we employed a stereotactically inoculated brain-tumor model and autoradiographic technique using (14C-methyl)-L-methionine.

MATERIAL AND METHODS

Cells and culture

C6 cells derived from rat glial tumors (Benda *et al.,* 1968) were grown in Eagle's minimal essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum, penicillin G (50 units/ml), streptomycin (50 μg/ml), and fungizone (2.5 μg/ml; all from Flow, McLean, VA). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

Rat brain-tumor model and CDDP treatment

C6 cells (5×10^6/10 ml) were stereotactically inoculated in the right basal ganglia of 6-week-old male Sprague-Dawley rats weighing 130 to 160 g (Mineura *et al.,* 1993). Ten days after inoculation, the rats received an i.v. injection of CDDP (5 mg/kg, kindly provided by Nippon Kayaku, Tokyo), and autoradiographic images of brain tumors and normal brain were obtained using (14C-methyl)-L-methionine 30 min after CDDP treatment.

Autoradiography of brain tumors

A total of 1.11 MBq of (14C-methyl)-L-methionine (1.9 GBq/mmol, NEN, Boston, MA) were administered i.v. to rats that were anesthetized with 1.5 to 2.0% halothane.

First, we measured the kinetics of the (14C-methyl)-L-methionine uptake in inoculated brain tumors and non-tumor regions. Rat brains were dissected and immediately frozen 15 to 90 min after the tracer injection. A cryostat was used to cut the tumor-bearing brains into 20-μm-thick sections. Dried sections were exposed to an X-ray film (Kodak NMC-1) together with an autoradiographic [14C] micro-scale (Amersham, Aylesbury, UK) for one month. The tissue uptake of (14C-methyl)-L-methionine was represented as the differential absorption ratio (DAR) in the following equation: tissue radioactive counts/tissue weight (g) × body weight (g)/total injected radioactive counts (Marrian and Maxwell, 1956). Alternatively, we washed sections containing tumor cells in 0.5 M perchloric acid solution for 60 min to measure (14C-methyl)-L-methionine uptake and to obtain images of the acid-insoluble fraction (AIF), identical to protein-bound fraction. The washed sections were subject to autoradiographic procedures as described above.

Optical density on autoradiographs was obtained with a densitometer, and converted into regional radioactivity on the basis of calibration standard curves plotted by concentration of the [14C] micro-scale. A 2-×-2-mm square was focused on the tumor regions and the non-tumor region (contralateral gray matter). Autoradiographic measurement was made once per rat.

RESULTS

The DAR or AIF in tumors and non-tumor regions was less different between 15 and 90 min after injection of (14C-methyl)-

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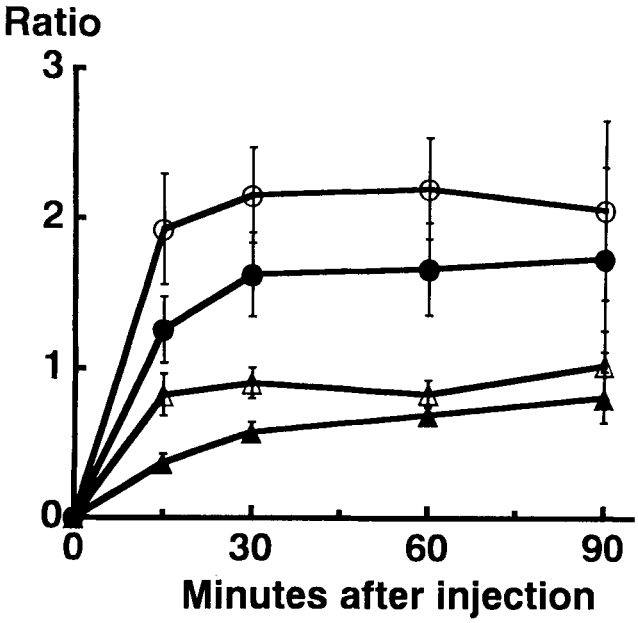

FIGURE 1 – Time course of DAR and AIF in the tumor and the non-tumor gray-matter region. The kinetics of DAR and AIF in the tumor and in the non-tumor region indicate fewer differences through 15 to 90 minutes after injection of (^{14}C-methyl)-L-methionine. Symbols and bars represent the mean and standard deviation of 6 rats. ○, tumor DAR; △, non-tumor region DAR; ●, tumor AIF; △ non-tumor region AIF.

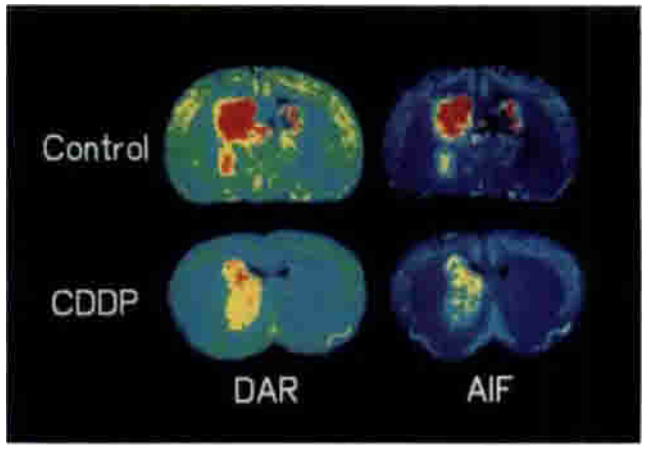

FIGURE 2 – Representative DAR and AIF autoradiographs using (^{14}C-methyl)-L-methionine in C6 inoculated brain tumors with and without (control) CDDP treatment. The tracer uptake is markedly high in the tumor lesion, and decreases after CDDP treatment.

TABLE I – UPTAKE OF (^{14}C-METHYL)-L-METHIONINE IN RAT BRAIN TUMORS WITH CDDP TREATMENT

	Tumor	Non-tumor	T/NT
DAR			
Control	1.93 (0.37)	0.83 (0.14)	2.32 (0.15)
with CDDP	1.68 (0.26)	1.01 (0.14)	1.59 (0.09)[1]
AIF			
Control	1.26 (0.22)	0.37 (0.06)	3.43 (0.25)
with CDDP	0.98 (0.08)	0.43 (0.07)	2.30 (0.32)[1]

Data denote the mean value (standard deviation) of 6 rats.
T/NT: tumor/non-tumor (contralateral gray matter) ratio.
[1] $p < 0.01$, significantly different from the control group, as determined by the Mann-Whitney U-test.

L-methionine (Fig. 1). Each time-point included data from 6 rats. We chose 15 min as the time-point for comparison. Rats that received 30-min CDDP treatment underwent autoradiographic procedures 15 min after the tracer injection. DAR and AIF were likewise calculated on autoradiographic images of unwashed (DAR) and washed sections (AIF). Figure 2 shows representative autoradiographs of DAR and AIF with and without CDDP treatment. The DAR and AIF images clearly delineate the extent of the tumor, which corresponded to the lesion confirmed by histology.

The 2 parameters of DAR and AIF were compared in the brain tumors and in normal brain treated with or without CDDP (Table I). Each group consisted of 6 rats. Statistical analysis was carried out by the Mann-Whitney non-parametric test, which used the ranks of the measurements.

The arithmetic mean (±standard deviation) tumor DAR of (^{14}C-methyl)-L-methionine was 1.93 ± 0.37, more than 2-fold that of the non-tumor region ($p < 0.01$). The tumor AIF was significantly higher than that of the non-tumor region ($p < 0.01$). The AIF comprised 65% (tumor) and 45% of the DAR (non-tumor).

With CDDP treatment, the tumor DAR decreased by 23% in the mean value, whereas the non-tumor DAR was unchanged. The tumor/non-tumor ratios of the DAR and the AIF decreased significantly ($p < 0.01$), suggesting that the decrease in DAR and AIF values was selective for the tumor. The injected dose (5 mg/kg) of CDDP had no effect on tumor size, histological alternation or body weight as compared with non-treated control during the observed time.

DISCUSSION

Methionine, an essential amino acid, penetrates into cerebral parenchyma through the blood-brain barrier and incorporates into protein, resulting in a high uptake index in the brain (Oldendorf, 1971). Although brain tumors possess variable enzyme activities and metabolism, (^{11}C-methyl)-L-methionine uptake, indicated by clinical positron-emission tomography (PET) studies, increases in cerebral gliomas and head-and-neck cancer, and is useful for diagnosing tumor lesions and for assessing response to the treatment. (Derlon *et al.*, 1989; Lindholm *et al.*, 1995). Restriction of methionine required for tumor growth is an alternative treatment. CDDP is an effective agent for inhibiting methionine uptake *in vitro* and *in vivo*, and is expected to increase 5-fluorouracil cytotoxicity through metabolic activation (Scanlon *et al.*, 1986, 1988; Shirasaka *et al.*, 1993). Methionine-deficient nutrition suppresses tumor growth and offers a synergistic therapeutic effect with 5-fluorouracil in human tumors (Hoshiya *et al.*, 1995; Goseki *et al.*, 1995).

We used a C6 tumor model to acquire the basic knowledge of metabolic effects by CDDP treatment. In earlier studies (Mineura *et al.*, 1990, 1991), we tested *in vitro* and *in vivo* drug sensitivity of tumor cells, such as C6 cells, 9L cells (another rat glioma cell line) and HeLa S3 cells, to a variety of chemotherapeutic agents. C6 cells were more sensitive to CDDP than 9L cells. Xenografts of C6 cells into nude mice responded to CDDP with a transient retardation of growth. Cytotoxicity tests have shown that the presence of CDDP enhanced cytotoxicity of 5-fluorouracil by more than 3-fold that of 5-fluorouracil alone in C6 cells, whereas no enhancement was noted in 9L cells (Mineura and Kowada, 1996).

A stereotactically inoculated brain-tumor model and (^{14}C-methyl)-L-methionine tracers were employed to elucidate the

metabolic modulation of exogenous methionine by CDDP treatment. The tumor DAR was more than twice as high as normal-brain DAR. The lesions revealed by high accumulation of the tracers on the DAR images corresponded to the extent of the tumor revealed by histological examination. Since (^{14}C-methyl)-L-methionine is the same chemical as (^{11}C-methyl)-L-methionine, except for a radioactive isotope decaying at a different time, the DAR images appear practically equivalent to PET images of (^{11}C-methyl)-L-methionine.

The kinetics of DAR and AIF after the tracer injection were determined in tumors and in non-tumor regions. The time course of DAR and AIF was much less variable at points between 15 and 90 min after the tracer injection. The shorter period more accurately reflects blood clearance and metabolism of CDDP, and is more practical for clinical PET studies. We therefore studied the effects of CDDP on methionine uptake at the time-point of 15 min.

The uptake of (^{14}C-methyl)-L-methionine, representative as DAR and AIF, was measured up to 90 min after the start of i.v. CDDP. DAR corresponds to the total sum of incorporated (^{14}C-methyl)-L-methionine as protein-free plus protein-bound fractions, whereas AIF represents the amount of a protein-bound fraction. The tumor protein-bound fraction was higher than the protein-free fraction, in comparison with normal brain. After CDDP treatment, the percentage of AIF decreased to almost the same level as that of normal tissue. CDDP inhibited methionine uptake more selectively at the process of incorporation into the protein fraction of C6 tumors, indicating inhibition of protein synthesis. Thus, CDDP may be a potent chemical modulator in the chemotherapy of brain tumors. Further experiments are needed, using a variety of brain-tumor cell lines, a lineage of doses and different modalities of CDDP administration in order to maximize the effects.

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REFERENCES

BENDA, P., LIGHTBODY, J., SATO, G., LEVINE, L. and SWEET, W., Differentiated rat glial-cell strain in tissue culture. *Science,* **161,** 370–371 (1968).

BUSWELL, L., RECHT, A., CLARK, J., PAVIKUMAR, T., BUSSE, P.M. and COLEMAN, C.N., Combined-modality therapy of esophageal cancer with radiotherapy, etanidazole and cisplatin-fluorouracil, with or without surgery: neurotoxicity, other toxicities and outcome. *Int. J. Radiat. Oncol. Biol. Phys.,* **29,** 535–540 (1994).

DERLON, J.M., BOURDET, C., BUSTANY, P., CHATEL, M., THERON, J., DARCEL, F. and SYROTA, A., [^{11}C]L-methionine uptake in gliomas. *Neurosurgery,* **25,** 720–728 (1989).

FORASTIERE, A.A., Cisplatin and radiotherapy in the management of locally advanced head-and-neck cancer. *Int. J. Radiat. Oncol. Biol. Phys.,* **27,** 465–470 (1993).

GOSEKI, N., YAMAZAKI, S., SHIMOJYU, K., KANDO, F., MARUYAMA, M., ENDO, M., KOIKE, M. and TAKAHASHI, H., Synergistic effect of methionine-depleting total parenteral nutrition with 5-fluorouracil on human gastric cancer: a randomized, prospective clinical trial. *Jap. J. Cancer Res.,* **86,** 484–489 (1995).

HOSHIYA, Y., GUO, H., KUBOTA, T., INADA, T., ASANUMA, F., YAMADA, Y., KOH, J., KITAJIMA, M. and HOFFMAN, R.M., Human tumors are methionine-dependent *in vivo*. *Anticancer Res.,* **15,** 717–718 (1995).

LINDHOLM, P., LESKINEN-KALLIO, S., GRENMAN, R., LEHIKOINEN, P., NAGREN, K., TERAS, M., RUOTSALAINEN, U. and JOENSUU, H., Evaluation of response to radiotherapy in head-and-neck cancer by positron-emission tomography and [^{11}C]methionine. *Int. J. Radiat. Oncol. Biol. Phys.,* **32,** 787–794 (1995).

MAHALEY, M.S., Neuro-oncology index and review (adult primary brain tumors). *J. Neurooncol.,* **11,** 85–147 (1991).

MARRIAN, D.H. and MAXWELL, D.R., Tracer studies of potential radiosensitizing agents. Tetrasodium 2-^{14}C-methyl-1:4-naphthohydroquinone diphosphate. *Brit. J. Cancer,* **10,** 575–582 (1956).

MINEURA, K., FUSHIMI, S., KOWADA, M., ISOWA, G., ISHIZAKI, K. and IKENAGA, M., Linkage between O^6-methylguanine-DNA-methyltransferase (O^6-MT) activity and cellular resistance to anti-tumor nitrosoureas in cultured rat-brain-tumor cell strains. *Acta neurochir. (Wien),* **103,** 62–66 (1990).

MINEURA, K. and KOWADA, M., Enhancement of 5-fluorouracil cytotoxicity by cisplatin in brain-tumor cells. *Cell Biol. Int.* (1996). (In press).

MINEURA, K., KUWAHARA, N., WATANABE, K. and KOWADA, M., O^6-methylguanine-DNA-methyltransferase activity as an index of drug responsiveness to anti-tumor chloroethylnitrosoureas in xenografted brain tumors. *In:* K. Tabuchi (ed.), *Biological aspects of brain tumors,* pp. 266–272, Springer, Tokyo (1991).

MINEURA, K., SASAJIMA, T., KUWAHARA, N., KOWADA, M., MURAKAMI, M. and UEMURA, K., (^{14}C-methyl)-L-methionine uptake in rat brain tumors before and after treatment with the protein-synthesis inhibitor cycloheximide. *J. Neurooncol.,* **15,** 229–233 (1993).

OLDENDORF, W.H., Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Amer. J. Physiol.,* **221,** 1629–1639 (1971).

SCANLON, K.J. and KASHANI-SABET, M., Elevated expression of thymidylate-synthase-cycle genes in cisplatin-resistant human ovarian-carcinoma A2780 cells. *Proc. nat. Acad. Sci. (Wash.),* **85,** 650–653 (1988).

SCANLON, K.J., NEWMAN, E.M., LU, Y. and PRIEST, D.G., Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian-carcinoma cells. *Proc. nat. Acad. Sci. (Wash.),* **83,** 8923–8925 (1986).

SHANI, J. and WOLF, W., Modalities of cisplatin administration to brain tumors. *Cancer Invest.,* **7,** 571–579 (1989).

SHIRASAKA, T., SHIMAMOTO, Y., OHSHIMO, H., SAITO, H. and FUKUSHIMA, M., Metabolic basis of the synergistic anti-tumor activities of 5-fluorouracil and cisplatin in rodent tumor models *in vivo*. *Cancer Chemother. Pharmacol.,* **32,** 167–172 (1993).

SPENCE, A.M., BERGER, M.S., LIVINGSTON, R.B., ALI-OSMAN, F. and GRIFFIN, B., Phase-II evaluation of high-dose intravenous cisplatin for treatment of adult malignant gliomas recurrent after chloroethylnitrosourea failure. *J. Neurooncol.,* **12,** 187–191 (1992).