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Influence of exposure and infusion times on the cytotoxicity and pharmacokinetics of *cis*-malonato[(4*R*, 5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]platinum(II)

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Abstract The effect of exposure time on the in vitro cytotoxicity of a new platinum complex, *cis*-malonato-[(4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]platinum(II) (SKI 2053R) and cisplatin (CDDP) toward two human lung-adenocarcinoma cell lines (PC-9, PC-14) and two human stomach-adenocarcinoma cell lines (KATO III, MKN-45) was investigated by variation of the exposure time (1, 4, 12, and 24 h) and drug concentration to yield a constant product of drug concentration times exposure time ($C \times T$). Exposure of cancer cells to low concentrations of SKI 2053R for 12 or 24 h resulted in a greater killing effect than did 1- or 4-h exposure to 24- or 6-fold higher concentrations; the inhibitory effects of SKI 2053R on the colony formation of all tumor cell lines except for KATO III were significantly increased with increasing exposure time ($P < 0.05$). However, the inhibitory effects of CDDP against all tumor cell lines tested except for PC-14 were inversely correlated with increasing exposure time ($P < 0.05$). The intracellular accumulation of SKI 2053R and CDDP was measured under the same conditions used in the cell-survival assay using MKN-45 cells. The amount of platinum accumulated from SKI 2053R into MKN-45 cells was greater for the treatment involving low concentrations and long-term exposures (12 and 24 h) than for that using high concentrations and short-term exposures (1 and 4 h) at the constant $C \times T$ values; however, the increased accumulation of CDDP was more prominent as the concentration was increased, even if the exposure time became shorter. The pharmacokinetics studies of SKI 2053R following 1-, 4-, 12-, and 24-h infusions were performed in beagle dogs. A single dose of SKI 2053R (5.0 mg/kg) was successively given over various infusion periods to three beagle dogs at 3-week intervals. The peak levels of ultrafiltrable platinum observed for SKI 2053R at the 1-, 4-, 12-, and 24-h infusions were 3.10 ± 0.49 (mean ± SD), 1.24 ± 0.06, 0.43 ± 0.07, and 0.25 ± 0.04 μg/ml, respectively. The mean binding ratios of platinum from SKI 2053R to plasma protein at the end of 1-, 4-, 12-, and 24-h infusions were approximately 91%, 73%, 53%, and 51%, respectively. The steady-state level of free platinum was maintained during long-term infusions (12 and 24 h) after short periods (1–3 h) from the start of the infusion. This study strongly suggests that the therapeutic efficacy of SKI 2053R given by continuous long-term infusion should be investigated in future clinical studies.

Key words SKI 2053R · In vitro cytotoxicity · Pharmacokinetics

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Introduction

cis-Malonato[(4*R*,5*R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]platinum(II) (SKI 2053R) is a new antitumor platinum complex [11]. SKI 2053R was selected for further development on the basis of its high in vitro and in vivo antitumor activity against a variety of murine and human tumor cell lines as well as its excellent in vivo antitumor activity against cisplatin (CDDP)-resistant L1210 leukemia and the difference in its toxicity profile from that of CDDP [11–14]. A phase I trial of SKI 2053R showed that the pharmacokinetic patterns and toxicity profiles of SKI 2053R were similar to those of carboplatin (CBDCA) [15, 28]. Currently, phase II studies of SKI 2053R given by 1-h i.v. infusion are being

conducted in patients with stomach cancer, non-small-cell lung cancer, and small-cell-lung cancer.

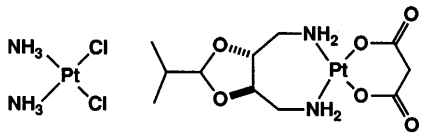
The efficacy of anticancer drugs may depend on the schedule of administration as well as the delivered dose. Most, if not all, anticancer agents can efficiently kill tumor cells when these are in an active phase of the cell cycle. In general, solid tumors are mostly composed of cells that are in the quiescent phase of the cell cycle. It is therefore conceivable that prolonged infusion of a cytotoxic agent allows drug exposure to all tumor cells, which subsequently enter the cell cycle, as opposed to short-term treatment, which allows only a small proportion of cells that are in an active phase of the cycle at that point to be affected. Since the protein-unbound fraction exerts the anticancer effects of platinum-based anticancer drugs [6, 9], exposure of more tumor cells to an adequate level of the free drug by extension of the exposure time could theoretically produce a greater cell kill. Recent clinical investigations have suggested that continuous infusion of platinum compounds may be beneficial by either enhancing their antitumor activity or reducing their severe side effects [7, 20, 22, 24, 25]. For SKI 2053R with its properties of low-level plasma protein binding and rapid elimination [15], short-term infusion may lower its actual antitumor efficacy. Most evidences suggests that the transport of platinum drugs across tumor cell membranes may occur by passive diffusion [8, 23]. This transport may be dependent not only on the concentration of the drug but also on the exposure time. Mauldin et al. [19] demonstrated that the intracellular accumulation rate of a platinum complex possessing a bidentate leaving ligand such as malonate was much slower than that of a platinum complex with chloride leaving ligands in vitro and that the uptake of the malonate compound into the cell increased linearly with the exposure time, the kinetics of which is different from that of the chloro counterpart. Therefore, it is strongly suggested that continuous infusion of SKI 2053R may enhance its antitumor activity.

The goals of this study were to evaluate the in vitro cytotoxicity of SKI 2053R by varying the exposure time at a constant product of drug concentration times exposure time ($C \times T$) and to determine the pharmacokinetic behavior of SKI 2053R in dogs infused with an identical dose over different infusion periods for the purpose of investigating differences that could be important in optimization of clinical drug administration schedules.

Materials and methods

Cancer cells and animals

Two human lung-adenocarcinoma cell lines (PC-9, PC-14) and two human stomach-adenocarcinoma cell lines (MKN-45, KATO III) were used. These cell lines were kindly donated by Dr. N. Saijo, National Cancer Center Hospital, Japan. All cell lines were propagated in RPMI 1640 medium (Gibco, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), penicillin (100 units/ml), and streptomycin (100 μg/ml; RPMI-FBS) in a highly humidified incubator containing 5% CO_2 at 37 °C. The doubling times of PC-9, PC-14, MKN-45, and KATO III are 26, 28, 30, and 36 h, respectively. Male beagle dogs were purchased from Marshall Farms (North Rose, N.Y.), and animals weighing 9.0–11.0 kg were used in this pharmacokinetics study.



CDDP **SKI 2053R**

Fig. 1 Structures of SKI 2053R and CDDP

Drugs

SKI 2053R was synthesized at the Life Science Research Center of Sunkyong Industries, Korea, as previously described [11] (Fig. 1). CDDP was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). The molecular weights of SKI 2053R and CDDP are 471.37 and 300.06 Dal, respectively. SKI 2053R is quite stable in solution, showing very little decomposition (< 1%) in water and 5% aqueous dextrose solution, respectively, over a period of 6 months at 4 °C. For the cell-survival assay we prepared stock solutions of these drugs by dissolving them in distilled water and stored them at −70 °C. Immediately before each experiment, these stock solutions were diluted with RPMI 1640 medium to the required concentrations. For the pharmacokinetics study, SKI 2053R was dissolved in sterile 5% aqueous dextrose solution just before use.

Cell-survival assay

A modified human tumor clonogenic assay, as originally described by Hamburger and Salmon [10], was used to evaluate the in vitro cytotoxicity of SKI 2053R and CDDP against four human cancer cell lines under the condition of varying exposure time at constant $C \times T$ values. In brief, single-cell suspensions were prepared by mechanical disaggregation for PC-9, PC-14, MKN-45, and KATO III. After viability had been confirmed as being over 95% by trypan-blue dye exclusion, cells were counted using a hemocytometer, diluted with RPMI-FBS, and transferred into a 25-cm^2 culture flask (Nunc, Denmark) at final concentrations (5 ml/flask) of 1×10^5, 2×10^5, 2×10^5, and 2×10^5 cells/flask for PC-9, PC-14, MKN-45, and KATO III, respectively. Cells were incubated with various concentrations of the drugs and were harvested at preset incubation times of 1, 4, 12, and 24 h to yield a desired, constant $C \times T$ value. The employed $C \times T$ values ranged from 600 to 3600 μg min ml^{-1} for SKI 2053R and from 150 to 1200 μg min ml^{-1} for CDDP as based on their clinically achievable areas under the concentration-time curve (AUCs) derived from the ultrafiltrable platinum from each drug [15, 27]. The range of concentrations was 0.417–60.0 μg/ml for SKI 2053R and 0.104–20.0 μg/ml for CDDP, respectively, and the incubation times were correspondingly decreased from 1440 to 60 min.

After incubation for preset incubation times, cells were centrifuged at 600 *g* for 10 min and supernatants were discarded. After being washed twice with Hanks' balanced salt solution (HBSS, Gibco), cells were dispersed in drug-free culture medium containing 0.3% agar (Bacto, Difco, Detroit, Mich., USA), and 1 ml tumor cell suspension was pipetted onto 1 ml underlayer in a 35-mm flat-bottomed well of a tissue-culture multiwell plate (Nunc, Denmark) at final concentrations of 2×10^4, 4×10^4, 4×10^4, and 4×10^4 cells/well for PC-9, PC-14, MKN-45, and KATO III, respectively. Control cultures consisted of cells treated in exactly the same way as the test cells without receiving drugs. The underlayer contained 1 ml McCoy's 5A medium (Gibco) supplemented with 10%

heat-inactivated fetal bovine serum, 5% heat-inactivated fetal horse serum (Gibco), 0.02% sodium pyruvate (Gibco), 2 m*M* glutamine (Gibco), 0.004% serine (Gibco), penicillin (100 units/ml), and streptomycin (100 μg/ml). The plates were incubated at 37 °C in a highly humidified incubator containing 5% CO_2 for 9–14 days. Plating numbers and incubation periods were determined from the growth curve generated for each cell line. Colonies of > 50 cells were counted with an automatic colony counter (Model 880, Artek Systems Corporation, USA). The percentage of clonogenic survival was determined as the mean number of colonies obtained from three test wells relative to untreated control values. Each experiment was performed in triplicate and repeated three times. Cytotoxicity was expressed as $I(C \times T)_{50}$, the $C \times T$ value required to reduce the number of colonies by 50% relative to the untreated control, which was determined from dose-response curves plotted with probit analysis.

Intracellular drug accumulation

Accumulation studies were performed using MKN-45 cells under conditions identical to those described for the cell-survival assay. In all, 5 ml of cell suspensions (at a final concentration of 1×10^6 cells/ml) in a 25-cm^2 culture flask (Nunc) was incubated in a highly humidified incubator containing 5% CO_2 at 37 °C with the drugs at various concentrations and then harvested at preset incubation times of 1, 4, 12, and 24 h to yield two constant $C \times T$ values for each drug: 1200 and 2400 μg min ml^{-1} for SKI 2053R and 600 and 1200 μg min ml^{-1} for CDDP. After incubation for the preset periods, cells were chilled and centrifuged at 4 °C for 10 min, and then supernatants were saved for determination of the amount of total platinum from SKI 2053R and CDDP in the media. Cells were washed twice with cold HBSS, and cell pellets were frozen at −70 °C until sample preparation for platinum measurement. The cell pellets were dissolved in 1 ml 65% HNO_3 (Merck, FRG) on a hot plate, and the amount of platinum in the samples was measured as described below. Each experiment was performed in duplicate and repeated three times.

Determination of platinum in culture media

In parallel with the accumulation studies, following initial centrifugation the supernatants of cultures with a $C \times T$ value of 2400 μg min ml^{-1} for SKI 2053R and 1200 μg min ml^{-1} for CDDP were saved for determination of the amount of total platinum from SKI 2053R and CDDP. Platinum unbound to the protein in the culture media was obtained by centrifugation of the supernatant through an Amicon CF 4104 filter (Amicon Corporation, Danvers, Mass., USA) at 1500 *g* for 20 min. The protein-free ultrafiltrates and aliquots of the supernatants for platinum analysis were stored at −70 °C until analysis.

Drug administration

A single dose of 5.0 mg/kg SKI 2053R dissolved in 5% aqueous dextrose solution was given to dogs in a volume of 6 ml/kg. The drug was given with a syringe pump (Model 341, Sage Instruments, USA) over various infusion periods of 1, 4, 12, and 24 h. All three dogs were repeatedly used in these infusion studies. The animals first received SKI 2053R by 1-h infusion and then successively received the drug in the order of 4-, 12-, and 24-h infusion periods, with a 3-week washout period for recovery being introduced between infusions. Toxicological monitoring of these animals was performed during and after all of the infusion studies. No significant change in hematology or serum chemistry parameters was observed.

Blood-sample preparation

Blood was sampled through an indwelling i.v. cannula placed in the cephalic vein into a heparin-containing syringe prior to drug administration, at the end of infusion, and at 5 and 30 min as well as 1, 2, 4, 6, 8, 12, and 24 h after the end of each infusion. In addition, blood samples were drawn during infusion at 30 min after the start of the 1-h infusion; at 1, 2, and 3 h after the start of the 4-h infusion; at 1, 2, 4, 6, 8, and 10 h after the start of the 12-h infusion; and at 1, 2, 4, 6, 8, 12, 16, and 20 h after the start of the 24-h infusion. Plasma was immediately separated by centrifugation at 1000 *g* for 15 min. As soon as plasma had been prepared, part of it was passed through an Amicon CF 4104 filter by centrifugation at 1500 *g* for 20 min to obtain protein-free ultrafiltrate. The protein-free ultrafiltrate and aliquots of whole plasma for platinum analysis were stored at −70 °C until analysis.

Platinum determination

Platinum contents in whole plasma, ultrafiltrable plasma, and tumor cells as well as in the supernatants of culture media and the filtered supernatants were quantitatively analyzed at 265.9 nm with a flameless atomic absorption spectrophotometer (Varian SpectrAA 300, Zeeman, Australia). The detection limit for platinum was approximately 0.02 μg/ml.

Pharmacokinetic analysis

The concentrations of total and ultrafiltrable platinum from SKI 2053R in plasma were best characterized by a biexponential decline; thus, a biexponential equation was fitted to the plasma platinum levels. The pharmacokinetic parameters were calculated by a two-compartment model using the computer program PCNONLIN. The area under the plasma concentration-time curve from the start of the infusion to 24 h after the end of infusion ($AUC_{0 \to 24}$) was calculated using the trapezoidal rule. The remaining $AUC_{24 \to \infty}$ was computed by extrapolation using the equation $AUC_{24 \to \infty} = C_{24}/\beta$, where β is the terminal elimination rate constant. $AUC_{0 \to \infty}$ is the sum of the measured $AUC_{0 \to 24}$ and $AUC_{24 \to \infty}$. Estimated plasma half-lives for each phase of the plasma concentration-time curves were calculated by division of 0.693 into the respective elimination rate constants. The total clearance (CL_T) was estimated as the dose divided by $AUC_{0 \to \infty}$. The steady-state volume of distribution (Vd_{ss}) was calculated as the total clearance divided by β. The terminal half-life ($t_{1/2}\beta$), CL_T, and Vd_{ss} of total platinum from the drug were omitted because the estimation of these parameters is inaccurate due to the short sampling time (up to 24 h from the end of the infusion). The AUC for total platinum from the drug was calculated up to the latest measured time point (24 h) because the AUC percentage, which is added by extrapolation to infinity, is > 20%.

Statistical analysis

The mutual dependency between the duration of exposure and the $I(C \times T)_{50}$ value for each drug against each cell line was analyzed by the one-tailed Spearman rank-correlation coefficient test, and statistical analysis of the pharmacokinetics data was determined by Student's *t*-test and the Kruskal-Wallis test using the personal computer program SigmaStat (version 2.0; Jandel Corporation, USA). A P value of < 0.05 was considered to be statistically significant.

Results

Effect of exposure time on the in vitro cytotoxicity of SKI 2053R and CDDP at a constant $C \times T$ value

Figure 2 illustrates the dose-response curves generated for four tumor cell lines exposed at several $C \times T$ values

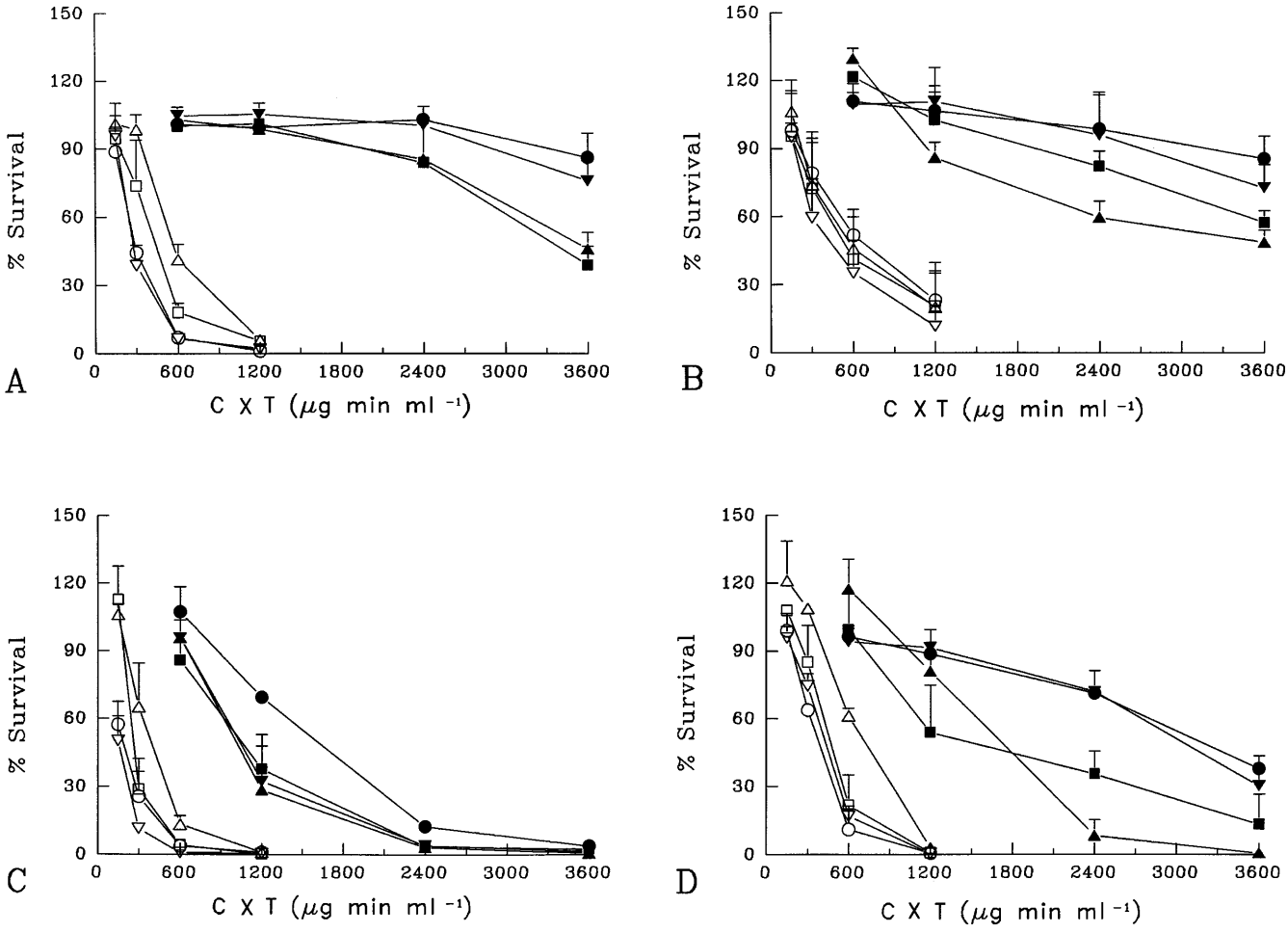


Fig. 2A–D Effect of exposure time on the in vitro cytotoxicity of SKI 2053R (*black symbols*) and CDDP (*white symbols*) at a constant C × T value as determined by a human tumor clonogenic assay (*Circles* 1-h exposure time, *inverted triangles* 4-h exposure time, squares 12-h exposure time, *upright triangles* 24-h exposure time). Human lung-adenocarcinoma cell lines **A** PC-9 and **B** PC-14 and human stomach-adenocarcinoma cell lines **C** KATO III and **D** MKN-45 were used as target cells. Each point and bar represents the mean value ± SD for three independent experiments. Bars were omitted if the SD was smaller than the size of the point

to SKI 2053R and CDDP under the condition of variation of both exposure time and concentration; the $I(C \times T)_{50}$ values determined for the drugs against each cell line at each exposure time are summarized in Table 1. The inhibitory effects of SKI 2053R and CDDP on the colony formation of tumor cell lines were dependent on the intensity of the C × T value. Exposure of cancer cells to low concentrations of SKI 2053R for 12

Table 1 $I(C \times T)_{50}$ values determined for SKI 2053R and CDDP against two human lung-adenocarcinoma (PC-9, PC-14) and two human stomach-adenocarcinoma (MKN-45, KATO III) cell lines by clonogenic assay[a]

Exposure time (h)	PC-9		PC-14		MKN-45		KATO III	
	SKI 2053R[b]	CDDP[c]	SKI 2053R[b]	CDDP	SKI 2053R[b]	CDDP[c]	SKI 2053R	CDDP[c]
1	8795 ± 1940	297 ± 1.0	6105 ± 979	378 ± 57	3281 ± 229	310 ± 24	1348 ± 135	188 ± 84
4	7927 ± 2010	258 ± 4.5	5789 ± 1113	329 ± 18	3147 ± 645	385 ± 66	1098 ± 214	159 ± 30
12	3350 ± 1940	419 ± 121	3733 ± 979	375 ± 16	1788 ± 858	408 ± 11	1049 ± 406	240 ± 55
24	3599 ± 848	507 ± 41	3207 ± 424	395 ± 44	1494 ± 549	579 ± 41	945 ± 371	324 ± 34

[a]$I(C \times T)_{50}$ the inhibitory C × T value required to reduce the number of colonies by 50% in relation to the untreated control, was determined from dose-response curves generated for each tumor cell line exposed to several constant C × T values attained by adjustment of drug concentrations at each exposure time. Data represent mean values ± SD derived from three independent experiments run in triplicate, expressed in μg min ml^{-1}

[b]There is a tendency for $I(C \times T)_{50}$ values of the drug to decrease with increasing duration of exposure ($P < 0.05$, one-tailed Spearman rank-correlation coefficient test)

[c]There is a tendency for $I(C \times T)_{50}$ values of the drug to increase with increasing duration of exposure ($P < 0.05$, one-tailed Spearman rank-correlation coefficient test)

or 24 h resulted in a greater killing effect than did 1- or 4-h exposure to 24- or 6-fold higher concentrations. The inhibitory effects of SKI 2053R on the colony formation of all tumor cells except for KATO III were significantly increased with increasing exposure time and decreasing $I(C \times T)_{50}$ value ($P < 0.05$). However, the maximal inhibitory effects of CDDP were observed mostly in cells treated at high concentrations for either a 1- or a 4-h exposure period as compared with those treated at low concentrations for long periods (12 or 24 h); $I(C \times T)_{50}$ values for CDDP against all tumor cell lines tested except for PC-14 were significantly increased with increasing exposure time ($P < 0.05$). Under the same $C \times T$ value, two human stomach-cancer cell lines (KATO III, MKN-45) were more sensitive to SKI 2053R than were two human lung-cancer cell lines (PC-9, PC-14 cells) as compared with CDDP.

Platinum measurement in cells and culture media

The intracellular accumulation of platinum from SKI 2053R and CDDP was determined in MKN-45 cells (Fig. 3). The intracellular accumulation of platinum from SKI 2053R and CDDP increased with the intensity of the $C \times T$ value, except for the long-term periods of exposure (12- and 24-h periods) to SKI 2053R. The amount of accumulated platinum from SKI 2053R was greater for the treatment involving low concentrations and long-term exposure (12- and 24-h periods) than for that involving high concentrations and short-term exposure (1- and 4-h periods) at constant $C \times T$ values, whereas the increased accumulation of CDDP was more prominent as the concentrations were increased, even if the exposure time became shorter. For 1- and 4-h exposure times a substantially greater level of platinum was present in cells treated with a smaller amount of CDDP as compared with that of SKI 2053R. The amount of platinum accumulated by cells treated with SKI 2053R was higher than or equal to that of CDDP at long exposure times of 12 and 24 h.

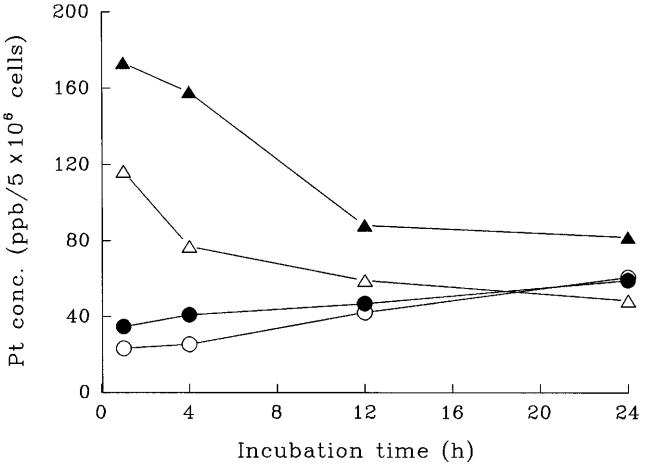


Fig. 3 Intracellular accumulation of platinum (*Pt*) in MKN-45 cells treated with SKI 2053R and CDDP at a constant $C \times T$ value by varying incubation time and concentration. In all, 5 ml of cell suspensions (final concentration 1×10^6 cells/ml) in a culture flask was incubated with the drugs at various concentrations and harvested at preset incubation times of 1, 4, 12, and 24 h to yield two constant $C \times T$ values for each drug: 1200 (*white circles*) and 2400 (*black circles*) μg min ml^{-1} for SKI 2053R and 600 (*white triangles*) and 1200 (*black triangles*) μg min ml^{-1} for CDDP. Each point represents the mean value for three independent experiments

In vitro protein binding of SKI 2053R and CDDP in RPMI 1640 culture medium was determined in parallel with the intracellular accumulation studies as described above. The binding ratio of both drugs to protein in culture media increased slightly with increasing incubation time. Less than 15% of either drug became bound to protein in culture medium after the 24-h exposure time. The difference observed in the binding ratio of SKI 2053R and CDDP to protein in culture medium between the 1- and the 24-h incubation times was 11% and 5%, respectively.

Pharmacokinetics of SKI 2053R following 1-, 4-, 12-, and 24-h infusions

The concentration versus time curves generated for total and ultrafiltrable platinum in plasma obtained from beagle dogs receiving an identical dose of SKI 2053R over different infusion periods are shown in Fig. 4. The pharmacokinetic parameters derived from these curves are presented in Table 2. After infusion, the plasma concentrations of total and ultrafiltrable platinum from SKI 2053R declined in a biexponential fashion. The peak levels of ultrafiltrable platinum observed for SKI 2053R at the 1-, 4-, 12-, and 24-h infusions were 3.10 ± 0.49, 1.24 ± 0.06, 0.43 ± 0.07, and 0.25 ± 0.04 μg/ml, respectively. The mean CL_T and Vd_{ss} values for ultrafiltrable platinum from SKI 2053R were similar for the 1-, 4-, 12-, and 24-h infusion studies; no statistically significant difference between the pharmacokinetic parameters obtained from these infusion studies was observed. The mean binding ratios of platinum from SKI 2053R to plasma protein at the end of 1-, 4-, 12-, and 24-h infusions were approximately 91%, 73%, 53%, and 51%, respectively. Ultrafiltrable platinum from SKI 2053R was detected for up to 8 h after the end of the 1- and 4-h infusions and for 4 h after the end of the 12- and 24-h infusions. In the 12- and 24-h infusion studies, the steady-state level of free platinum was maintained until the end of the infusion, beginning at 4 h after the initiation of the infusion.

Discussion

The rationale for continuous-infusion chemotherapy is provided by cytokinetic and pharmacokinetic considerations [2, 5]. Tumor cells in the resting state would be kinetically resistant to a short-term exposure to anticancer drugs. This consideration is clearly of importance

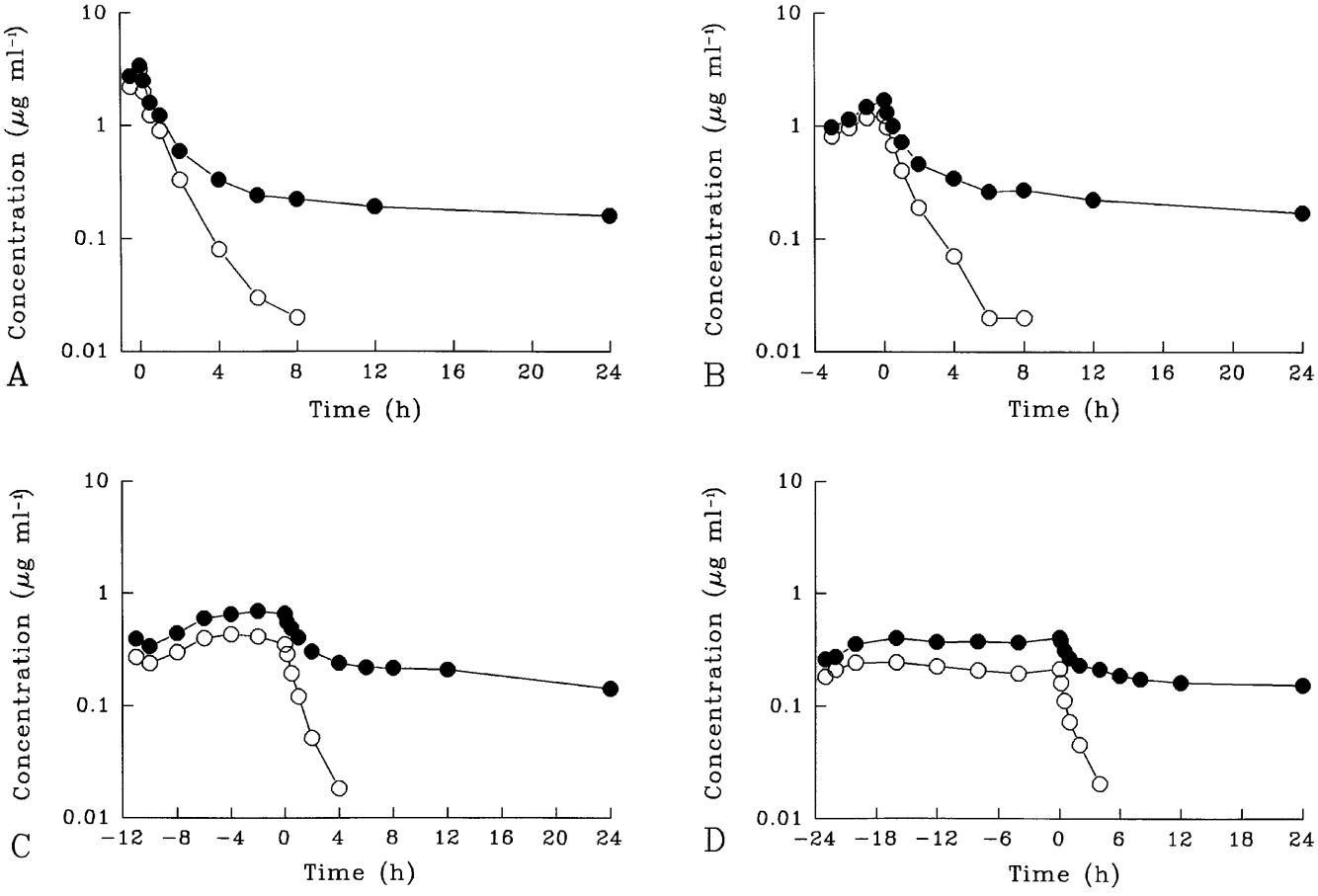


Fig. 4A–D Concentration versus time curves generated for total and ultrafiltrable platinum (*Pt*) in the plasma of beagle dogs given a single i.v. infusion of 5.0 mg/kg SKI 2053R over **A** 1, **B** 4, **C** 12, and **D** 24 h. Each point represents the mean value obtained from three animals (*Black circles* Total Pt from SKI 2053R, *white circles* ultrafiltrable Pt from SKI 2053R)

for schedule-dependent drugs such as methotrexate, vinblastine, and bleomycin [2, 5, 17]. An important characteristic of schedule-dependent drugs is the exposure-time-dependent reduction in tumor cell survival.

Although CDDP, a representative of platinum drugs, has been known not to have schedule dependency in tumor cell killing [1, 17], some in vitro studies suggest that CDDP has schedule-dependent cytotoxic effects on tumor cells [3, 18]. However, no distinct advantage for either short-term or continuous infusion of CDDP has been shown on the basis of survival or toxicity data in mice [21]. Continuous infusion of platinum compounds has been considered to possess some advantages in enhancing their activity and/or ameliorating their toxicities [7, 20, 22, 24, 25], even though increased clinical responses

Table 2 Pharmacokinetic parameters of total and ultrafiltrable platinum derived from SKI 2053R after 1-, 4-, 12-, and 24-h i.v. infusions of 5.0 mg/kg to dogs[a]

Infusion period (h)	Total platinum		Ultrafiltrable platinum				
	$t_{1/2}\alpha$ (h)	$AUC_{0\rightarrow 24}$ (μg h ml^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	CL_T (ml min^{-1} kg^{-1})	$AUC_{0\rightarrow\infty}$ (μg h ml^{-1})	Vd_{ss} (1/kg)
1	0.54 ± 0.12[b]	10.86 ± 1.95	0.37 ± 0.05	1.39 ± 0.17	17.60 ± 1.46	5.46 ± 1.08	1.00 ± 0.01
4	0.64 ± 0.11	11.86 ± 0.65	0.36 ± 0.06	1.36 ± 0.18	15.33 ± 0.21	5.41 ± 0.01	1.09 ± 0.03
12	0.66 ± 0.24	11.57 ± 0.01	0.40 ± 0.16	2.87 ± 2.01	20.41 ± 2.14	4.57 ± 0.12	1.61 ± 0.29
24	0.39 ± 0.03	12.95 ± 0.67	0.23 ± 0.03	1.81 ± 0.20	15.24 ± 1.25	5.45 ± 0.66	1.18 ± 0.31

[a]Three beagle dogs were repeatedly used in these infusion studies. The animals first received SKI 2053R by 1-h infusion and then successively received the drug in the order of 4-, 12-, and 24-h infusion periods, with a 3-week washout period for recovery being introduced between infusions

[b]Each figure indicates the mean value ± SD obtained from three animals

for either CDDP or CBDCA given by continuous infusion have not yet been confirmed by clinical trials [2].

To find out whether a new anticancer drug is likely to possess schedule dependency for its antitumor activity, drugs have been tested at a single set of concentrations or at the same concentration for both 1-h exposure and continuous exposure [1, 4, 7, 18]. A more valuable assay would use clinically achievable and identical $C \times T$ values for the agent at various exposure times if pharmacokinetic results for the agent were available, since the use of an unrealistically high concentration over a long period of exposure is not clinically relevant. Therefore, we designed this study and showed that the susceptibility of tumor cells to SKI 2053R was increased by extension of the period of exposure to the drug at an identical $C \times T$ value, whereas the in vitro cytotoxicity of CDDP seemed to be more dependent on the exposure concentration than on the exposure time. This finding is consistent with the previous investigators' report on CDDP's action, in which the longest period of exposure to CDDP resulted in the lowest cell killing in vitro [26].

The reason for this difference in the in vitro cytotoxicity of SKI 2053R and CDDP can probably be explained by the difference in the kinetic patterns of their cellular uptake and intracellular metabolism. It is shown in this report that the level of accumulation of platinum derived from both drugs was strongly related to the in vitro cytotoxic effects of SKI 2053R and CDDP. Although the amount of intracellular accumulation is not a true measure of cytotoxicity, the greater accumulation of SKI 2053R at longer exposure times could be an explanation for the greater degree of cytotoxicity observed in the cell-survival assay. It has been reported that the rates of metabolism to active species as well as the intracellular uptake of platinum complexes containing a bidentate leaving ligand such as malonate and 1,1-cyclobutanedicarboxylate were lower than those of platinum complexes having chloride as a leaving ligand [16, 19]. It was observed in our preliminary study that the rate of uptake of SKI 2053R was slower than that of CDDP for both 1- and 4-h exposure times (data not shown). Even under the condition that tumor cells are being treated when they are in an active phase of the cell cycle and a sufficient level of drug is achieved around tumor cells, the magnitude of cell killing would finally be reduced if drug transport across the tumor cell membrane were slow.

However, there is a possibility that continuous infusion of SKI 2053R would be less effective if the effective drug concentration were not reached. It is difficult to know which concentration of a drug in plasma can be maintained long enough to be therapeutic in patients due to the diversity of tumor types as well as to differences in the pharmacokinetic behavior of the drug between patients. The drug concentration within solid tumors may vary quite widely and be impossible to simulate precisely in vivo.

An accurate comparison of the cytotoxicity data we obtained in the cell-survival assay between SKI 2053R and CDDP following prolonged drug exposure seems to be difficult due to the known rapid and extensive binding capacity of CDDP to biological fluids [6, 9]. However, one result obtained in this cell-survival assay represents a meaningful in vitro pharmacological finding for both drugs, since the majority most of the platinum ($> 85\%$) from both drugs was shown to be unbound to protein in culture media until the end of the exposure periods (24 h). In addition, the difference observed in the binding ratio of CDDP between the 1- and the 24-h incubation times was approximately 5%. However, this in vitro study has some restrictions, such as the absence of renal clearance data and the use of a logarithmically growing cell culture instead of solid tumors.

The enhanced in vitro cytotoxicity of SKI 2053R noted for long-term exposure led us to determine its pharmacokinetic behavior over different infusion periods in dogs. The pharmacokinetic parameters obtained for total and ultrafiltrable platinum from SKI 2053R were nearly identical for the various infusion periods. Although the plasma binding ratio of SKI 2053R during the infusion gradually increased with the infusion period, more than 50% of the SKI 2053R in plasma remained filtrable at the end of the 24-h infusion. On the other hand, the level of free platinum from CDDP in patients was $< 10\%$ at the end of a 24-h infusion [27]. The peak levels of ultrafiltrable platinum from SKI 2053R measured after a 24-h infusion were only about 12-fold lower than those obtained following a 1-h infusion. It is very promising that a steady state for a relatively high level of free platinum was maintained during long-term (12- and 24-h) infusion after short periods (1–3 h) from the start of the infusion. Since free platinum is the major component possessing antitumor activity in platinum-based anticancer drugs [6, 9, 14], the prolonged steady state achieved for free platinum from SKI 2053R could allow more tumor cells in the active phase of the cell cycle to be exposed to the drug in vivo.

In conclusion, the pharmacological and pharmacokinetic findings described in this report strongly suggest that the therapeutic efficacy of SKI 2053R given by continuous long-term infusion should be investigated in future clinical studies.

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