Jpn. J. Cancer Res. **91**, 99–104, January 2000

In vitro Antitumor Activity, Intracellular Accumulation, and DNA Adduct Formation of *cis*-[((1*R*,2*R*)-1,2-Cyclohexanediamine-*N*,*N*′)bis(myristato)] Platinum (II) Suspended in Lipiodol

Shuichi Kishimoto,[1] Kenji Miyazawa, Shoji Fukushima and Yoshikazu Takeuchi

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Arise Igawadani-cho, Nishi-ku, Kobe 651-2180

SM-11355, *cis*-[((1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′)bis(myristato)] platinum (II), is a lipophilic platinum complex under clinical development that targets primary hepatocellular carcinoma using Lipiodol as a carrier. SM-11355 was compared with cisplatin (CDDP) using an *in vitro* evaluation system capable of examining the release characteristics and the cytotoxicity of drugs suspended in Lipiodol. SM-11355 suspended in Lipiodol (SM-11355/Lipiodol) and CDDP suspended in Lipiodol (CDDP/Lipiodol) showed cytotoxic activity against rat ascites hepatoma AH-109A cells in a dose-dependent manner. Their IC_{50} values following 7-day exposure were 22.3 and 0.40 μg/ml, respectively. Following the subsequent 7-day exposure, from day 7 to day 14 after preparation of the suspension, SM-11355/Lipiodol showed an almost equivalent activity, but CDDP/Lipiodol did not show any activity at all. SM-11355/Lipiodol showed a sustained release into the culture medium over the course of a 14-day exposure. Following the exposure to CDDP/Lipiodol, the platinum concentration in the medium was at its maximum on the first day and remained constant thereafter. Intracellular platinum uptake and formation of platinum-DNA adducts were dependent on the release characteristics of each drug suspension. For SM-11355/Lipiodol, the drug release, intracellular drug uptake, and formation of platinum-DNA adducts over the course of the subsequent 7-day exposure were similar to those observed during the first 7 days. DPC, one of the compounds released from SM-11355/Lipiodol, was taken up by cells and showed formation of platinum-DNA adducts. Thus, this study suggests that SM-11355/Lipiodol may release active platinum compound(s) that bind to nuclear DNA and mediate the cytotoxic activity of SM-11355/Lipiodol.

Key words: Lipiodol — Cisplatin — Hepatocellular carcinoma — Sustained release

Primary hepatocellular carcinoma therapy has improved as a result of advances made in hepatic arterial infusion chemotherapy.[1–3] As Lipiodol, an oily lymphographic agent, is selectively retained in hepatic tumor tissue when injected into the hepatic artery, it has been used both in the diagnosis and therapy of hepatic cancer.[4] Numerous studies have reported that Lipiodol selectively delivers various antitumor drugs to tumors.[5–9] SMANCS, amphipathic polymer styrene maleic acid neocarzinostatin, was developed as an anticancer drug for use in this therapy.[5, 10–12] The widespread clinical use of SMANCS has revealed areas of effectiveness and a few problems. CDDP, cisplatin, is reportedly highly effective against various carcinomas, and when suspended in Lipiodol (CDDP/Lipiodol) is effective against hepatocellular carcinoma.[7, 13] However, therapeutic problems associated with CDDP/Lipiodol include an insufficient anticancer effect and adverse effects resulting from both inadequate stability of the suspension and rapid release of the drug.

SM-11355 has been developed as a lipophilic platinum complex that has the added feature of lipophilicity, which makes this anticancer drug applicable for therapy using Lipiodol.[14, 15] SM-11355 suspended in Lipiodol (SM-11355/Lipiodol) showed an antitumor effect at a dose that produced no hepatic toxicity in the rat model. This result suggests that SM-11355 has markedly better characteristics in Lipiodol formulation than CDDP. However, the mechanism underlying the cytotoxic activity has not been elucidated. SM-11355/Lipiodol showed a sustained release of platinum compounds into saline. DPC, cyclohexane-1,2-diamineplatinum (II) dichloride, was detected as one of the compounds released from SM-11355/Lipiodol in saline. The cytotoxic activity of the release media was consistent with that of DPC. As the uptake behavior of tumor cells approximately parallels the release behavior of SM-11355/Lipiodol, it is conceivable that the released compound is taken up by tumor cells and subsequently shows antitumor activity. However, whether or not the

[1] To whom correspondence should be addressed.
E-mail: skisimot@pharm.kobegakuin.ac.jp

Abbreviations: SM-11355, *cis*-[((1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′)bis(myristato)] platinum (II); CDDP, cisplatin; Lipiodol, ethyl esters of iodized oil fatty acids, Lipiodol Ultra-Fluid.

compound that is taken up by the tumor cells induces formation of platinum-DNA adducts to the same extent as CDDP has not been elucidated.

In the present study, we investigated the formation of platinum-DNA adducts in AH-109A cells treated with SM-11355/Lipiodol using an assay system capable of examining the release characteristics and the cytotoxicity of drugs suspended in Lipiodol.

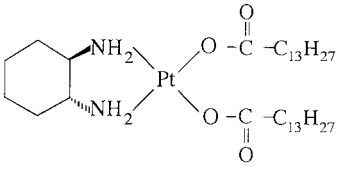
MATERIALS AND METHODS

Materials *cis*-[((1*R*,2*R*)-1,2-Cyclohexanediamine-*N*,*N*′)-bis(myristato)] platinum (II) (SM-11355) and cyclohexane-1,2-diamineplatinum (II) dichloride were prepared by Sumitomo Pharmaceuticals Co.(Osaka). The structures of these compounds are illustrated in Fig. 1. Cisplatin (CDDP) was a product of Sigma Aldrich Japan K.K. (Tokyo). Lipiodol Ultra-Fluid (Lipiodol), Laboratorie Guerbert (Paris, France), was purchased from Mitsui Pharmaceuticals Co., Ltd. (Tokyo).

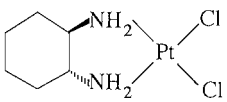
Preparation of suspension SM-11355 suspended in Lipiodol was prepared by adding Lipiodol to SM-11355 and shaking. CDDP suspended in Lipiodol was prepared by adding Lipiodol to CDDP powder and mixing in a mortar.

Cell culture Rat ascites hepatoma AH-109A cells were kindly provided by Dr. M. Nakano (Kumamoto University Hospital, Kumamoto). AH-109A cells were serially transplanted by intraperitoneal injection in Donryu rats (Charles River Japan, Inc., Kanagawa) and the cells taken from ascites were grown in RPMI-1640 medium containing 20% fetal bovine serum (FBS). The cells were cultured at 37°C in humidified 5% CO_2 air.

Cytotoxicity assay AH-109A cells were seeded into 6-well plates at 8×10^5 cells/well. Falcon cell culture inserts (Becton Dickinson and Co., Franklin Lakes, NJ), equipped with a 0.4 μm pore membrane on their bottom, were used to test drugs suspended in Lipiodol. On day 0, 2 ml of drug suspended in Lipiodol was added to the inserts. Following incubation for 7 days from day 0 to 7, the inserts containing the drug suspended in Lipiodol were transferred to a new well, and after incubation for 7 days from day 7 to 14, the cytotoxic activity was again determined. On days 7 and 14, trypsinized cells were collected and the number of cells was determined using a hemocytometer. The treated/control ratio (T/C(%)) was calculated by means of the following formula: T/C(%) = mean cell number of drug-treated wells/mean cell number of control wells. The concentration necessary to inhibit the cell growth by 50% (IC_{50}) was calculated from a plot of T/C(%).



SM-11355



DPC

Fig. 1. Chemical Structures of SM-11355 and DPC.

Release, intracellular accumulation, and DNA adduct formation for drug suspension To examine the release characteristics of SM-11355 or CDDP suspended in Lipiodol, 2 ml of drug suspended in Lipiodol was added to Falcon cell culture inserts in wells containing 2 ml of the culture medium, and the plates were incubated in humidified 5% CO_2 air at 37°C. Following incubation for a predetermined length of time, the medium in each well was collected and frozen until platinum measurement.

Cell treatments for drug uptake and DNA adduct studies were performed using AH-109A cells. AH-109A cells were incubated with SM-11355 suspended in Lipiodol at a concentration of 200 μg/ml or with CDDP suspended in Lipiodol at 20 μg/ml under the same conditions as for the growth inhibition assay described above. At the indicated time points, the cells were collected and washed three times with cold phosphate-buffered saline (PBS(−)). The resulting cell pellets were frozen until preparation for platinum measurement.

Intracellular accumulation and DNA adduct formation for drug solution DPC and CDDP were dissolved in dimethylformamide or saline and diluted with culture medium at 10 μg/ml. AH-109A cells were seeded into 6-well plates at 8×10^5 cells/2 ml/well on day 0. A 200 μl aliquot of each drug solution was added to the cell suspension ten times on days 1 and 2. On day 3, the cells were collected and washed three times with cold PBS(−). The resulting cell pellets were frozen until preparation for platinum measurement.

Determination of platinum uptake in cells Following drug incubation, the cell pellets were lysed using digestion buffer (10 m*M* Tris-HCl, 10 m*M* EDTA, 0.15 *M* NaCl, and 0.5% sodium lauryl sulfate) in the presence of 0.1 mg/ml proteinase K for 1 h at 55°C, then overnight at 37°C. The amount of platinum in the samples was determined by flameless atomic absorption spectrometry (FAAS).

Determination of platinum-DNA adducts Following drug incubation, high-molecular-weight DNA was isolated from the cell pellets by the following standard procedure. The pellets were lysed at 55°C for 1 h and at 37°C over-

night with digestion buffer in the presence of 0.1 mg/ml proteinase K. DNA was extracted with equal volumes of TE (10 m*M* Tris-HCl and 1 m*M* EDTA) saturated phenol and 25:24:1 TE saturated phenol:chloroform:isoamyl alcohol, and then precipitated with isopropanol. The pellet was dissolved in TE buffer at 37°C overnight. The sample was then treated with 2 μl of 10 mg/ml RNase A at 37°C for 1 h. DNA was again extracted with an equal volume of 25:24:1 TE saturated phenol:chloroform:isoamyl alcohol and then precipitated with isopropanol. The pellet was washed with cold 80% ethanol. The DNA was dissolved in 2 *N* HCl. The DNA content was assessed spectrophotometrically at 260 nm and the amount of platinum was determined by FAAS.

Platinum determination The platinum content was analyzed quantitatively at 265.9 nm by FAAS (Z-9000, Hitachi). The limit of quantification was 5 ng/ml and the sample volume was 0.01 ml. Argon gas was used to purge the furnace.

RESULTS

Antitumor activity The antitumor activities of SM-11355/Lipiodol and CDDP/Lipiodol were examined against rat ascites hepatoma AH-109A cells following incubation for 7 days (Fig. 2). The IC_{50} values, defined as the concentration of drug in Lipiodol required to inhibit the growth of AH-109A cells by 50%, of SM-11355/Lipiodol and CDDP/Lipiodol were 22.3 and 0.40 μg/ml, respectively. Furthermore, to assess the duration of the release of active compounds from SM-11355/Lipiodol, after the first 7 days of exposure the SM-11355/Lipiodol Falcon cell culture inserts were transferred to new wells, and the antitumor activity was determined during the subsequent 7 days of exposure from day 7 to day 14. The IC_{50} value for SM-11355/Lipiodol at this point was 23.9 μg/ml and the cytotoxic activity had been maintained. However, no CDDP/Lipiodol activity could be detected.

Release characteristics The release characteristics for SM-11355/Lipiodol and CDDP/Lipiodol were examined using Falcon cell culture inserts. The amount released from suspension was determined as the platinum concentration in RPMI-1640 medium containing 20% FBS (Fig. 3). CDDP/Lipiodol (20 μg/ml) caused a rapid increase in the platinum concentration in the medium after a 1-day exposure, with the concentration at almost the same level (6.5±1.2 μg/ml) on day 3 of exposure. SM-11355/Lipiodol (200 μg/ml) produced a gradual increase in the platinum concentration of the medium over the course of a 7-day exposure from day 0 to day 7. The concentration reached approximately 1.6 times (10.2±5.1 μg/ml) that for CDDP/Lipiodol after 7 days of exposure. Similarly, the amount released was determined after the next 7 days of incubation from day 7 to day 14. In addition, during this period, SM-11355/Lipiodol showed a sustained release and the platinum concentration in the medium was almost at the same level as during the first 7 days of exposure.

Intracellular accumulation and formation of platinum-DNA adducts The level of platinum uptake by cells and that of platinum-DNA adducts were determined after exposure of AH-109A cells to SM-11355/Lipiodol or

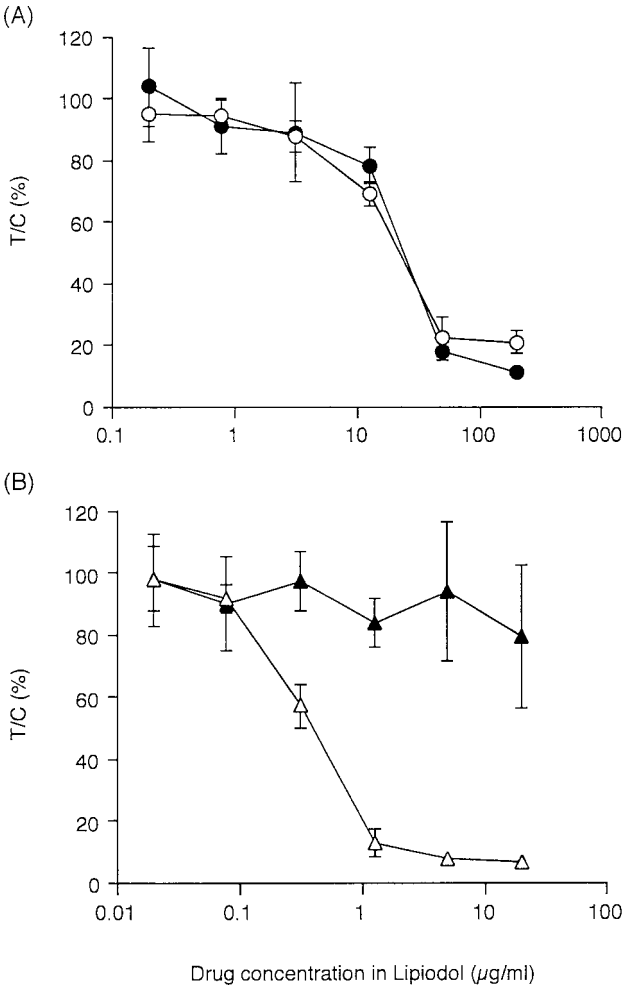


Fig. 2. Cytotoxic activity of SM-11355/Lipiodol (A) and CDDP/Lipiodol (B) in AH-109A tumor cells. On day 0, AH-109A cells were seeded, and 2 ml of Lipiodol containing a drug was added to Falcon cell culture inserts. Following incubation for 7 days, the inserts containing the drug were transferred to new wells, and further incubated for 7 days. Cytotoxicity was expressed as T/C (%) relative to the cell number of the Lipiodol-treated control group after 7 days of drug exposure. The symbols used are as follows: ○ and △, first 7 days of exposure from day 0 to day 7; ● and ▲, second 7 days of exposure from day 7 to day 14. Dose is expressed as the amount of drug in 2 ml of drug suspension. Data represent the mean±SD for two or three individual experiments, each conducted using triplicate cultures.

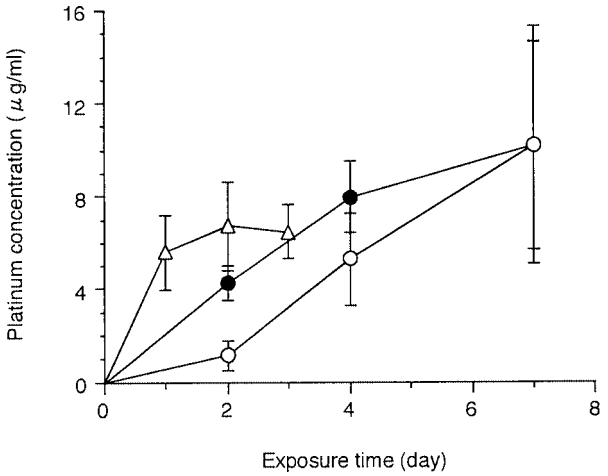


Fig. 3. Release characteristics of SM-11355/Lipiodol (○, ●, 200 μg/ml) and CDDP/Lipiodol (△, 20 μg/ml) into RPMI-1640 containing 20% FBS. On day 0, 2 ml of Lipiodol containing drug was added to Falcon cell culture inserts in wells containing 2 ml of the culture medium. Following incubation for 7 days, the inserts containing the drug were transferred to new wells, and further incubated for 7 days. The symbols used are as follows: ○ and △, first 7 days of exposure from day 0 to day 7; ●, second 7 days of exposure from day 7 to day 14. Data represent the mean±SD for three to five individual experiments, each conducted using triplicate cultures.

Table I. Intracellular Platinum Accumulation and Formation of Platinum-DNA Adduct following Treatment with SM-11355/Lipiodol in AH-109A Cells

Estimation period	Exposure time (day)	Intracellular Pt (ng/10^6 cells)	Pt-DNA adducts (pg/μg DNA)
day 0–7	2[a)]	14±10	30[d)]
	4[a)]	193±73	52[e)]
	7[b)]	634±487	208±110[f)]
day 7–14	2[c)]	93±25	41[d)]
	4[c)]	283±37	43[d)]
	7[b)]	642±394	99±34[g)]

On day 0, AH-109A cells were seeded, and 2 ml of SM-11355/Lipiodol (200 μg/ml) was added to Falcon cell culture inserts. Following incubation for 7 days, the inserts containing drugs were transferred to new wells, and further incubated for 7 days. At the indicated time points, the cells were collected. Data represent the mean±SD for three to five individual experiments.
a) Three individual experiments were done.
b) Five individual experiments were done.
c) Four individual experiments were done.
d) n=1. *e*) n=2. *f*) n=4. *g*) n=3.

Table II. Intracellular Platinum Accumulation and Formation of Platinum-DNA Adduct following Treatment with CDDP/Lipiodol in AH-109A Cells

Exposure time (day)	Intracellular Pt (ng/10^6 cells)	Pt-DNA adducts (pg/μg DNA)
1	57±45	344±223
2	68±50	384±242
3	47±18	306±186

On day 0, AH-109A cells were seeded, and 2 ml of CDDP/Lipiodol (20 μg/ml) was added to Falcon cell culture inserts. At the indicated time points, the cells were collected. Data represent the mean±SD for five individual experiments.

CDDP/Lipiodol for predetermined periods (Tables I and II). In cells treated with CDDP/Lipiodol (20 μg/ml), the values peaked after one day of exposure. In contrast, in cells treated with SM-11355/Lipiodol (200 μg/ml), the values increased in a time-dependent fashion. Additionally, SM-11355/Lipiodol caused similar levels of platinum uptake by cells and similar levels of platinum-DNA adducts following the subsequent 7-day period. With each drug treatment, the behavior of platinum uptake by cells and formation of platinum-DNA adducts was dependent on the drug release profile.

The effects of DPC, one of the compounds released in saline, and CDDP on AH-109A cells were compared with those of SM-11355/Lipiodol. Intermittent exposure to each drug was performed to mimic the sustained release from SM-11355/Lipiodol. The amounts of intracellular platinum were 25 and 48 ng/10^6 cells for DPC and CDDP, respectively. The levels of platinum-DNA adducts were 51 and 195 pg/μg DNA for DPC and CDDP, respectively.

DISCUSSION

Previous studies have suggested that SM-11355/Lipiodol has a more selective antitumor effect and less hepatic toxicity than CDDP/Lipiodol after intraarterial administration in the rat hepatic tumor model. Although CDDP/Lipiodol has been reported to be extremely effective against primary hepatocellular carcinoma, in experimental studies it has shown a rapid release and a negative influence on normal liver function.[13)] The selective antitumor effect of SM-11355/Lipiodol may be attributable to tumor-selective retention of Lipiodol and the sustained release of the active compound from SM-11355/Lipiodol. The previous study findings have suggested that the released compound is associated with the expression of cytotoxic activity, given that the intracellular uptake behavior correlated well with the release profile. However, the interaction of the released compound with DNA remains to be clarified. The present study evaluated the relation between the sustained release of SM-11355/Lipiodol and the duration of the

effect on tumor cells, as well as the interactions between the compound released from SM-11355/Lipiodol and the tumor cells and DNA.

Various characteristics of SM-11355/Lipiodol were examined in an *in vitro* assay system using Falcon cell culture inserts. The assay system consecutively estimates the characteristics through transfer of the inserts containing drugs to new plates following incubation for a predetermined period. The cytotoxic activity was evaluated by placing the inserts in contact for 7 days with rat ascites hepatoma AH-109A cells (used previously in an *in vivo* assessment). SM-11355/Lipiodol and CDDP/Lipiodol showed concentration-dependent cytotoxic activity. An approximately 60-fold difference in IC_{50} values was detected. This difference may in part be due to the release characteristics of the drugs. CDDP/Lipiodol enables a high concentration of the active compound to come in contact with the tumor cells and inhibits the tumor growth within a short period of time following exposure. To evaluate duration of action, the cytotoxic activity in the 7-day period beginning on day 7 after preparation was determined through transfer of the Falcon cell culture inserts containing the drugs to new plates, after an initial evaluation of 7 days. Although CDDP/Lipiodol did not show any activity at all at that point, SM-11355/Lipiodol showed an almost equivalent activity to that observed during the first 7 days.

The evaluation of the release characteristics was done using a culture medium with 20% FBS added. The drug concentrations used in the experiment, CDDP/Lipiodol at 20 μg/ml and SM-11355/Lipiodol at 200 μg/ml, were those that inhibited the growth of AH-109A cells to a similar extent. SM-11355/Lipiodol, unlike CDDP/Lipiodol, showed a continuous sustained release over the course of the 2 weeks. CDDP/Lipiodol might generate a high concentration of active compounds *in vitro* due to its rapid release into the medium. SM-11355/Lipiodol might require a longer exposure time and higher drug concentration than CDDP/Lipiodol for inhibition of tumor growth in this *in vitro* assay due to its continuous sustained release. It is well known that the antitumor effects of platinum compounds are dependent on both the drug concentration and the exposure time. Thus, the effect of platinum compound showing sustained release may appear weak *in vitro*. The difference in the release characteristics of drug suspensions is probably one factor that influences the antitumor effects and side effects *in vivo*.

Intracellular uptake of platinum compounds was found to be dependent on the release characteristics of each drug suspension. SM-11355/Lipiodol showed formation of platinum-DNA adducts that was dependent on the release characteristic, in the same manner as CDDP/Lipiodol. Also, the decrease in the viability of cells treated with these drug suspensions correlated well with the formation of platinum-DNA adducts (data not shown). Therefore, it was suggested that the active compound released from SM-11355/Lipiodol can bind to DNA. SM-11355/Lipiodol may show a sustained release of active compounds, resulting in the level of platinum-DNA adducts increasing with exposure time to SM-11355/Lipiodol. The levels of intracellular uptake and platinum-DNA adducts following the first 7 days of incubation were equal to those observed in the subsequent 7 days of incubation from day 7 to day 14. A similar tendency was observed in the cytotoxic activity.

Like CDDP, DPC is taken up by tumor cells and generates platinum-DNA adducts. The above findings suggest that the platinum compound released from SM-11355/Lipiodol has the ability to bind to DNA. Similarly, Han *et al*. reported that liposomal NDDP, which has a structure similar to that of SM-11355, forms DPC as the active compound via an interaction of NDDP with liposome constituent lipid.[16] Although the concentration of DPC in the present evaluation was lower than the concentration of platinum released from SM-11355/Lipiodol, the level of platinum-DNA adducts in cells treated with DPC was comparable to that in cells treated with SM-11355/Lipiodol. In contrast, the amount of intracellular platinum in cells treated with SM-11355/Lipiodol was relatively high compared to that in cells treated with DPC or CDDP/Lipiodol. The large amount of intracellular platinum obtained by the present treatment may reflect the sustained release of DPC from SM-11355/Lipiodol. Another possibility is that SM-11355 may interact with amino acids contained in culture medium or serum to generate inactive platinum compounds that are taken up by cells. The large amount of intracellular platinum produced by SM-11355/Lipiodol may be linked to cytotoxic mechanisms other than platinum-DNA adduct formation. A follow-up study using cell lines that show different sensitivity to SM-11355/Lipiodol or resistance to CDDP is being carried out to clarify the mechanism of SM-11355/Lipiodol cytotoxicity.

In conclusion, SM-11355/Lipiodol interacts with DNA as a target for cytotoxicity, as well as CDDP does. SM-11355/Lipiodol shows a sustained release of compounds that bind to DNA and produce a cytotoxic effect.

(Received August 2, 1999/Revised October 18, 1999/Accepted October 27, 1999)

REFERENCES

1) Minoyama, A., Yoshikawa, M., Ebara, M., Saisho, H., Sugiura, N. and Ohto, M. Study of repeated arterial infusion chemotherapy with a subcutaneously implanted reservoir for advanced hepatocellular carcinoma. *J. Gastro-*

enterol., **30**, 356–366 (1995).
2) Rougier, P., Munck, J. N., Elias, D., Herait, P., Bongel, C., Gosse, C. and Lasser, P. Intra-arterial hepatic chemotherapy with pirarubicin. Preclinical and clinical studies. *Am. J. Clin. Oncol.*, **13**, S1–S4 (1990).
3) Vexler, A. M., Mou, X., Gabizon, A. A. and Gorodetsky, R. Reduction of the systemic toxicity of cisplatin by intra-arterial hepatic route administration for liver malignancies. *Int. J. Cancer*, **60**, 611–615 (1995).
4) Iwai, K., Maeda, H. and Konno, T. Use of oily contrast medium for selective drug targeting to tumor: enhanced therapeutic effect and X-ray image. *Cancer Res.*, **44**, 2115–2121 (1984).
5) Konno, T. Targeting cancer chemotherapeutic agents by use of lipiodol contrast medium. *Cancer*, **66**, 1897–1903 (1990).
6) Yamashita, Y., Takahashi, M., Bussaka, H., Fukushima, S., Kawaguchi, T. and Nakano, M. Intraarterial infusion of 5-fluoro-2-deoxyuridine-C8 dissolved in a lymphographic agent in malignant liver tumors. A preliminary report. *Cancer*, **64**, 2437–2444 (1989).
7) Shibata, J., Fujiyama, S., Sato, T., Kishimoto, S., Fukushima, S. and Nakano, M. Hepatic arterial injection chemotherapy with cisplatin suspended in an oily lymphographic agent for hepatocellular carcinoma. *Cancer*, **64**, 1586–1594 (1989).
8) Bayraktar, Y., Balkanci, F., Kayhan, B., Uzunalimoglu, B., Gokoz, A., Ozihik, Y., Gurakar, A., Van Theil, D. H. and Filat, D. A comparison of chemoembolization with conventional chemotherapy and symptomatic treatment in cirrhotic patients with hepatocellular carcinoma. *Hepatogastroenterology*, **43**, 681–687 (1996).
9) Hirashima, N., Sakakibara, K., Itazu, I., Hirai, T., Nemoto, A., Matsuura, H., Kumada, K., Nojiri, O. and Kano, H. Zinostatin stimalamer-transcatheter arterial embolization for hepatocellular carcinoma: a comparison with Lipiodol-transcatheter arterial embolization. *Semin. Oncol.*, **24**, S6-91–S6-96 (1997).
10) Konno, T., Maeda, H., Iwai, K., Tashiro, S., Maki, S., Morinaga, T., Mochinaga, M., Hiraoka, T. and Yokoyama, I. Effect of arterial administration of high-molecular-weight anticancer agent SMANCS with lipid lymphographic agent on hepatoma: a preliminary report. *Eur. J. Cancer Clin. Oncol.*, **19**, 1053–1065 (1983).
11) Konno, T., Kai, Y., Yamashita, R., Nagamitsu, A. and Kimura, M. Targeted chemotherapy for unresectable primary and metastatic liver cancer. *Acta Oncol.*, **33**, 133–137 (1994).
12) Seymour, L. W., Olliff, S. P., Poole, C. J., De Takats, P. G., Orme, R., Ferry, D. R., Maeda, H., Konno, T. and Kerr, D. J. A novel dosage approach for evaluation of SMANCS [poly-(styrene-co-maleyl-half-*n*-butylate)-neocarzinostatin] in the treatment of primary hepatocellular carcinoma. *Int. J. Oncol.*, **12**, 1217–1223 (1998).
13) Fukushima, S., Kishimoto, S., Hayashi, Y., Kaneko, M. and Nakano, M. Intra-hepatic artery infusional chemotherapy with cisplatin suspension in lipiodol (LPS) of hepatocellular carcinoma (I)—preparation of LPS and its anticancer effect on a rabbit liver cancer model after injection into the hepatic artery. *J. Jpn. Soc. Cancer Ther.*, **23**, 2743–2749 (1988).
14) Maeda, M., Uchida, N. A. and Sasaki, T. Liposoluble platinum (II) complexes with antitumor activity. *Jpn. J. Cancer Res.* (Gann), **77**, 523–525 (1986).
15) Kishimoto, S., Fukui, M., Fukushima, S. and Nakano, M. Application of a lipophilic platinum derivative contained in an oily lymphographic agent to intra-hepatic arterial chemotherapy. *Reg. Cancer Treat.*, **1-2**, 25–29 (1992).
16) Han, I., Khokhar, A. R. and Perez-Solar, R. Intraliposomal conversion of lipophilic *cis*-bis-carboxylato-*trans*-R,R-1,2-diaminocyclohexane-platinum (II) complexes into *cis*-bis-dichloro-*trans*-R,R-1,2-diaminocyclohexane-platinum (II). *Cancer Chemother. Pharmacol.*, **39**, 17–24 (1996).