Antitumor Effects of a Novel Lipophilic Platinum Complex (SM-11355) against a Slowly-Growing Rat Hepatic Tumor after Intra-Hepatic Arterial Administration

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The antitumor effects of cis-[[1(R,2R)-1,2-cyclohexanediamine-N,N’bis(myristato)] platinum(II)] (SM-11355) were evaluated in a rat hepatic tumor model, and were compared with those of cisplatin (CDDP). A novel slowly-growing rat hepatic tumor model was established by the successive transplantation of rat AH109A tumor into the liver. The drugs, which were suspended in Lipiodol, were administered into the proper hepatic artery of tumor-bearing rats. Tumor growth was suppressed in the group that received SM-11355 suspended in Lipiodol (SM-11355/Lipiodol). Mean tumor growth rates in the groups administered 20 μl of Lipiodol containing 0, 0.02, 0.04, 0.1, 0.2, or 0.4 mg of SM-11355 were 244, 86, 110, 81, 51, and 40%, respectively, 1 week after treatment. Those in the groups administered 20 μl of Lipiodol containing 0.1, 0.2, or 0.4 mg of CDDP were 240, 110, and 45%, respectively. In the groups administered 0.2 and 0.4 mg of SM-11355 or 0.4 mg of CDDP, massive necrosis was observed in the tumor tissue 1 week after drug administration, and the tumors disappeared 4 weeks after drug administration. Serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels were measured as markers of liver damage one day after the drug was administered into the hepatic artery of rats. The minimum toxic dose, which raised serum GOT and GPT levels significantly compared with Lipiodol alone, was 0.2 mg for SM-11355/Lipiodol and 0.1 mg for CDDP/Lipiodol, respectively. The results demonstrated that SM-11355/Lipiodol exerted antitumor activity at a dose that showed no hepatic toxicity in the rat model, but CDDP/Lipiodol did not.

Key words lipophilic platinum; Lipiodol; hepatic tumor; arterial administration; cisplatin (CDDP)

Intra-hepatic arterial administration of anticancer drugs has been widely used to treat hepatocellular carcinoma. Lipiodol, an oily lymphographic agent, is used in this therapy as a carrier for anticancer drugs, because it selectively remains in tumour tissue when injected into the hepatic artery.1 Various anticancer drugs such as styrene maleic acid neocarzinostatin (SMANCS),2,3 5-fluoro-2'-deoxyuridine derivative,4 mitomycin C,5 doxorubicin,6,8 and cisplatin,7,9 suspended or emulsified in Lipiodol, have been used in cancer patients. Cisplatin (CDDP) is one of the most effective drugs for the treatment of various malignancies. Sasaki et al. showed the antitumor effect of CDDP against hepatic cancer using a method in which Lipiodol, CDDP and a gelatin sponge were delivered individually.8 Shibata et al. have reported that intra-hepatic arterial administration of CDDP powder suspended in Lipiodol (CDDP/Lipiodol) resulted in prolonged survival of hepatic cancer patients.9,10 cis-[[1(R,2R)-1,2-cyclohexanediamine-N,N’bis(myristato)] platinum(II)] (SM-11355) is a lipophilic platinum complex. Although CDDP/Lipiodol is an unstable suspension, and CDDP is released rapidly from Lipiodol to the aqueous phase, SM-11355 suspended in Lipiodol (SM-11355/Lipiodol) is a stable and colloidal suspension and acts as a sustained release preparation.11

SM-11355/Lipiodol showed antitumor effects in animal models.11,12 In our previous study,11 SM-11355/Lipiodol inhibited the growth of a VX-2 tumor transplanted in liver by injection into the hepatic artery of rabbits, but its effects were inferior to those of CDDP/Lipiodol. VX-2, which has been widely used in studies on experimental hepatic arterial chemotherapy,2,3,13,14 is a rapidly growing tumor which grows by 6 fold in one week. It was reported that the doubling time of most hepatocellular carcinoma in man was more than 30 d.15,16 Thus a sustained release preparation such as SM-11355/Lipiodol would be better evaluated in a slowly-growing tumor model to predict its clinical efficacy.

In the present study, we established a novel rat hepatic tumor model using ascite hepatoma AH109A cells, which grew more slowly than VX-2 did. Using this model, SM-11355/Lipiodol or CDDP/Lipiodol was injected into the hepatic artery, and the antitumor effects and hepatic toxicity were examined.

MATERIALS AND METHODS

Chemicals cis-[[1(R,2R)-1,2-cyclohexanediamine-N,N’bis(myristato)] platinum(II)] (SM-11355), was prepared by Sumitomo Pharmaceuticals Co. (Osaka) following the method by Maeda.17 The structure is illustrated in Fig. 1. Cisplatin (CDDP) was a product of Sigma Chemical Co., U.S.A. Lipiodol (Lipiodol Ultra-Fluid, Laboratoire Guerbet, Villepinte, France) was purchased from Kodama Co., Ltd., Tokyo, Japan.

Preparation of the Drug Suspension in Lipiodol

![Chemical Structure of SM-11355](image)

Fig. 1. Chemical Structure of SM-11355

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11355 was mixed with Lipiodol (SM-11355/Lipiodol). It forms a stable and colloidal suspension in Lipiodol. The CDDP powder was finely micronized and suspended in Lipiodol using a mortar and pestle (CDDP/Lipiodol).

**Animals** Donryu male rats were purchased from Charles River Japan, Inc. (Kanagawa) and were maintained under standard laboratory conditions. Rats were 3—4 months old when used in the experiments.

**Tumor** A rat ascite hepatoma cell line, AH109A, was kindly provided by Dr. M. Nakano (Kumamoto University Hospital, Kumamoto). Tumor cells, taken from ascite of a rat, were transplanted subcutaneously in a second rat. The resulting tumor mass was resected and cut into about $1 \times 1 \times 1$ mm cubes, and each cube was transplanted into the subcapsular parenchyma of the liver of another rat. The hepatic tumor model was established by successive transplantation into the rat liver.

**Evaluation of Antitumor Effects** Rats were anesthetized by intraperitoneal injection of sodium pentobarbital before laparotomy. About $1 \times 1 \times 1$ mm cube of the AH109A tumor, which was maintained by successive transplantation into the rat liver, was transplanted into the subcapsular parenchyma of the liver. About twenty days after the transplantation, when the tumor size reached about 5—20 mm in length along its major axis, the drug was administered. A catheter (polyethylene tube with an outside diameter of 0.61 mm) was inserted into the gastroduodenal artery under laparotomy and the head of the tube was fixed at the branching point between the common hepatic artery and the proper hepatic artery (Fig. 2). Twenty μl of SM-11355/Lipiodol, CDDP/Lipiodol, or Lipiodol alone was injected into the proper hepatic artery via the catheter. The length of the major and minor axes of the tumor was measured on the day of administration (day 0), and 7 or 8 days (day 1w) and 27 or 28 days (day 4w) after administration. The effect of the drug on the tumor growth was evaluated by comparing the size of the tumor expressed as the product of the length of the major and minor axes of the tumor. The tumor growth rate was calculated as follows:

$$\text{growth rate} (%) = \frac{\text{tumor size on day 1 w or 4 w}}{\text{tumor size on day 0}} \times 100$$

Because only about 10 rats could be treated in one experi-

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**Table 1. Growth Rates of AH109A Tumor in Rat Liver of Rats at 1 and 4 Weeks after Treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
</tr>
<tr>
<td>Non-treatment</td>
<td>240 ± 56</td>
</tr>
<tr>
<td>Lipiodol</td>
<td>244 ± 62</td>
</tr>
</tbody>
</table>

Lipiodol was injected into the hepatic artery at the volume of 20 μl.

a) The treatment was carried out about 20 d after tumor transplantation. Values are the mean ± S.D. (n = 5 — 15).

b) Not tested.

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**RESULTS**

**Establishment of the AH109A Hepatic Tumor Model**

The AH109A tumor was successfully transplanted into the rat liver. The tumor size increased by 2.4 fold in 1 week about 20 d after tumor transplantation as shown in Table 1. The tumor growth in the group administered Lipiodol was similar to that of the untreated control group. The tumor growth rates in the rats administered Lipiodol alone were 100—400% and 600—1900%, 1 week and 4 weeks after treatment, respectively.

**Antitumor Effects of the Drugs on AH109A Tumor**

The antitumor effects of SM-11355/Lipiodol and CDDP/Lipiodol were examined 1 week after administration. Figure 3A shows that the tumor growth was inhibited by SM-11355/Lipiodol. Tumor growth rates 1 week after treatment in the groups administered 20 μl of Lipiodol containing 0, 0.02, 0.04, 0.1, 0.2, or 0.4 mg of SM-11355 were 244, 86, 110, 81, 51, and 40%, respectively. Massive necrosis was macroscopically observed in the tumor tissues of rats treated with 0.2 and 0.4 mg of SM-11355/Lipiodol, but it was not observed in tumor tissues of rats treated with Lipiodol alone. Figure 3B shows that CDDP/Lipiodol also inhibited tumor growth. Tumor growth rates 1 week after treatment in the groups administered 20 μl of Lipiodol containing 0.1, 0.2, or 0.4 mg of CDDP were 240, 110, and 45%, respectively. When the antitumor effects of SM-11355/Lipiodol and CDDP/Lipiodol were examined on day 4 weeks, the tumors in the groups administered 0.2 mg of SM-11355/Lipiodol or 0.4 mg of CDDP/Lipiodol almost disappeared as shown in Fig. 4, and scar tissue was observed at the tumor site. The tumor in the group administered 0.1 mg of SM-11355/Lipiodol did not grow from day 0 to day 4 weeks. Although tumor growth was suppressed on day 1 week in the groups administered 0.04 mg of SM-11355/Lipiodol or 0.2 mg of CDDP/Lipiodol, the tumors of those groups had grown by more than 6 fold on
day 4 weeks.

Effects on Serum GOT and GPT Levels after the Intra-Hepatic Arterial Administration of the Drugs  The influence of SM-11355/Lipiodol and CDDP/Lipiodol on liver function was evaluated. Table 2 shows the serum GOT and GPT levels one day after the drug administration. CDDP/Lipiodol at 0.1 mg significantly elevated GOT and GPT levels when compared to Lipiodol alone. In the case of SM-11355/Lipiodol, a significant elevation in GOT and GPT lev-

![Graph A](image1)

![Graph B](image2)

Fig. 3. Growth Rates of the AH109A Tumor in the Rat Liver at 1 Week after Administration of (A) SM-11355/Lipiodol and (B) CDDP/Lipiodol.

The drugs were injected into the hepatic artery at a volume of 20 μl. Tumor size was measured on day 0 and day 1 week. Values are the means±S.D.

![Graph A](image3)

![Graph B](image4)

Fig. 4. Growth Rates of AH109A Tumor in the Rat Liver at 4 Weeks after Administration of (A) SM-11355/Lipiodol and (B) CDDP/Lipiodol.

Drugs were injected into the hepatic artery at a volume of 20 μl. Tumor size was measured on the day 0 and day 4 weeks. Values are the means±S.D.

Table 2. GOT and GPT Levels in the Rat Serum 24 Hours after Drug Administration into the Hepatic Artery

A. SM-11355/Lipiodol

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/head)</th>
<th>GOT (IU/l)(^a)</th>
<th>GPT (IU/l)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
</tr>
<tr>
<td>Lipiodol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM-11355/Lipiodol</td>
<td>0.1</td>
<td>156±20</td>
<td>139±5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>174±13</td>
<td>153±14</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>219±9(^b)</td>
<td></td>
</tr>
</tbody>
</table>

B. CDDP/Lipiodol

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/head)</th>
<th>GOT (IU/l)(^a)</th>
<th>GPT (IU/l)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
</tr>
<tr>
<td>Lipiodol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDDP/Lipiodol</td>
<td>0.04</td>
<td>153±14</td>
<td>129±13</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>196±17(^b)</td>
<td>145±15</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>341±40(^b)</td>
<td>205±8(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Values are the means±S.D. (n=3).  
\(^b\) Significantly different from the group given Lipiodol alone, p<0.05.
el was observed at a dose of 0.4 mg and 0.2 mg, respectively.

**DISCUSSION**

The antitumor effect of SM-11355/Lipiodol was examined and compared to that of CDDP/Lipiodol using a slowly-growing hepatic tumor model. The hepatic tumor was established by the successive transplantation of a rat ascitehepatoma, AH109A, into rat liver. The AH109A tumor grew 2.4 fold within 1 week, and the tumor growth was observed for 4 weeks after Lipiodol administration. It shows that the tumor grows about 3 times more slowly than rabbit VX-2 hepatic tumor which is widely used in studies on experimental hepatic arterial chemotherapy. Thus the AH109A hepatic tumor model is suitable to predict the clinical efficacy of a sustained release preparation.

Although SM-11355/Lipiodol could not sufficiently inhibit the growth of VX-2 transplanted into a rabbit liver in our previous study, both SM-11355/Lipiodol and CDDP/Lipiodol exhibited a strong antitumor effect on AH109A in the rat liver. In the groups administered 0.2 mg (0.26 μmol) of SM-11355/Lipiodol and 0.4 mg (1.3 μmol) of CDDP/Lipiodol, the tumors regressed on day 1 week and disappeared by day 4 weeks, indicating that SM-11355/Lipiodol was more potent than CDDP/Lipiodol against AH109A hepatic tumor. These findings suggest that SM-11355/Lipiodol shows an antitumor effect against hepatocellular carcinoma in humans as well as CDDP/Lipiodol.

Raoul et al. reported that arterial administration of a Doxorubicin-Lipiodol emulsion lowered the peak plasma concentration and increased the intratumoral concentration and half-life of doxorubicin. CDDP is known to exert an antitumor effect dependent on AUC (area under the concentration-time curve), and the administration of a sustained release preparation of CDDP into a hepatic tumor should increase the AUC for the tumor tissue and have a more pronounced antitumor effect. In the present study, 0.2 mg of SM-11355/Lipiodol exhibited a similar antitumor effect to 0.4 mg of CDDP/Lipiodol. SM-11355 barely inhibits cell growth because it is water-insoluble. We have found that SM-11355/Lipiodol also inhibited the growth of AH109A cells in vitro, but its activity was not superior to that of CDDP/Lipiodol (paper in preparation). Thus the difference in the in vivo effect between SM-11355/Lipiodol and CDDP/Lipiodol is not due to the sensitivity of AH109A cells to each drug. It was shown in our previous studies that SM-11355/Lipiodol released platinum into the aqueous phase more slowly than CDDP/Lipiodol in vitro. These findings suggest that active platinum compounds were slowly released from SM-11355/Lipiodol, localized in the tumor blood vessels and the extracapillary space of the tumor tissue, for a longer period compared with the case of CDDP/Lipiodol.

It was reported that serum GOT and GPT levels in hepatic cancer patients were elevated 3 days after CDDP/Lipiodol administration and they returned to pretherapy levels within 1 to 2 weeks, but hematological and renal toxicities were unusual. It was also shown in animal models that GOT and GPT levels were elevated transiently after an intraarterial administration of SMANCS and these higher levels correlated with histological changes in the normal liver tissue. A transient elevation of serum GPT and GPT levels after drug administration was also observed in our rat model. SM-11355/Lipiodol and CDDP/Lipiodol increased the serum GOT and GPT levels on day 1 in a dose dependent manner. The minimum dosage that elevates serum GOT and GPT levels was 0.2 mg for SM-11355/Lipiodol and 0.1 mg for CDDP/Lipiodol, respectively. Thus SM-11355/Lipiodol inhibited the tumor growth on day 4 weeks at a dose of 0.1 mg without any hepatic toxicity. But CDDP/Lipiodol could not inhibit the tumor growth on day 1 and 4 weeks even at a dose of 0.1 mg with hepatic toxicity.

Most antitumor platinum compounds are water-soluble. Among them, only CDDP has been examined for its antitumor effect by intra-hepatic arterial administration using Lipiodol as a carrier. However, CDDP/Lipiodol is an unstable suspension and CDDP is released rapidly from Lipiodol to the aqueous phase because it is water-soluble. On the other hand, SM-11355, which is a lipophilic platinum complex, forms a stable colloidal suspension in Lipiodol and acts as a sustained release preparation. In this report SM-11355/Lipiodol showed a higher antitumor activity than CDDP/Lipiodol. These findings suggest that SM-11355 is suitable for intra-hepatic arterial administration therapy using Lipiodol. It is known that a lipophilic platinum complex, cis-bis-neodecanato-trans-R,R-1,2-diaminocyclohexane platinum (II) (NDDP) entrapped in liposome, has antitumor activity in vitro and in vivo. NDDP in liposome was converted to active compounds when reconstituted in saline. Similarly, active platinum compounds may be released from SM-11355/Lipiodol. Further studies are necessary to clarify the mechanism of its antitumor effect.

In conclusion, SM-11355/Lipiodol showed more antitumor activity and less hepatic toxicity than CDDP/Lipiodol in the rat intra-hepatic arterial chemotherapy model. SM-11355 is now under clinical investigation.

**REFERENCES**
