Cisplatin Suppository: Preparation, Release Characteristics and Clinical Evaluation

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Cisplatin (CDDP) has attracted attention as a chemotherapeutic agent for the treatment of uterine endometrial carcinoma but causes serious side effects, including renal toxicity. CDDP suppositories containing NaCl at different concentrations were prepared to enhance the efficacy and to reduce the side effects of CDDP. The release characteristics, melting point and viscosity of the suppositories were first studied. The rate of CDDP release increased as the NaCl concentration increased: it was 12% 12 h after administration of suppositories containing no NaCl, but 32% with 0.2% NaCl. The melting point was raised by addition of NaCl: 35.5°C without NaCl and 36.5°C with 0.2% NaCl. Addition of 0.2% NaCl doubled the viscosity. Clinically, the suppository containing 0.06% NaCl was given to 3 patients with endometrial carcinoma twice a week for 3 weeks to examine serum CDDP levels and endometrial absorption. Patients with endometrial carcinoma showed different peak plasma platinum (Pt) levels which were as low as 0.12, 0.06 and 0.22 μg Pt/ml with similar patterns of change in the level. Radiographic analysis revealed many Pt particles in sections of necrosed endometria after 21d of the treatment. No side effects of CDDP were found in biochemical testing or subjective symptoms.

Keywords cisplatin; sodium chloride; suppository; endometrial carcinoma

The incidence of uterine endometrial carcinoma has been rising steadily and diverse methods are used for its treatment. In particular, cisplatin (CDDP) has been reported to be effective against endometrial carcinoma and has attracted attention.1−3) Chemotherapy using CDDP is often combined with radiotherapy to enhance the effects of the latter and to improve the patient’s prognosis.4) However, CDDP caused serious renal toxicity in these studies. Therefore, we tried local chemotherapy with a CDDP suppository to enhance the effects of CDDP and to reduce its side effects. We measured the release and content of CDDP, the melting point and the viscosity of the suppository. In addition, a CDDP suppository was combined with radiotherapy in 3 patients with endometrial carcinoma to examine, serum CDDP concentration and endometrial absorption.

Experimental

Reagents CDDP powder was obtained by the following method. Commercially available CDDP for injection (Nippon Kayaku Co.) was dried using an evaporator. The powder so obtained was transferred to a beaker and was mixed thoroughly after adding water and then filtered. The same procedure was repeated five times to remove NaCl. Since CDDP is thought to be degraded by heat and light during the drying process in the evaporator, the powder was analyzed by high performance liquid chromatography (HPLC) for the presence of the degradation products. At the same time, the purity of the powder was measured to see if the desalting process was complete. The material which was completely desalted, as confirmed by HPLC, and which was devoid of degradation products was designated as the CDDP a powder. HPLC was carried out using a Hitachi L-6000 apparatus equipped with a Shodex OH pak B804 (500 x 8 mm i.d., Showa Denko) and Hitachi L-4000 ultraviolet (UV) monitor (210 nm). Saline solution employed as a mobile phase at a flow rate of 1.0 ml/min. Witepsol H-15 and E-75 were purchased from Marushi Seiyaku. All other reagents employed were commercial special-grade products.

Preparation of CDDP Suppositories CDDP powder and NaCl were dissolved in water and the solution was evaporated. The resultant solid was finely pulverized in an agate mortar, mixed with a base [a mixture of Witepsol H-15 and E-75 (6:4)] and melted at 50°C. The melted mixture was cooled to 45°C and then poured into a metal mold kept at 37°C to form a rod suppository. The content of CDDP in the suppository was 20 mg/g. Since Witepsol H-15 was used as a substrate, it was thought that free OH residues in the substrate could react with CDDP and could produce highly toxic CDDP hydrate products such as mono- and di-watercompounds, and that the concentration of Cl− had a great effect on the complex formation. Thus, NaCl was added to increase the stability of CDDP and to suppress the formation of complexes.

Melting Point and Viscosity The melting point was measured with a melting-point measuring device (MP-21, Yamato) by increasing temperature at the rate of 1°C/min. A rotating viscosity meter (Model ED, Tokyo Keiki) was used for determination of viscosity. A suppository was finely crushed and softened on a plate at 37°C for 10 min. The viscosity was measured at 37±0.1°C, and expressed as an average value obtained at various shear rates.

CDDP Release Test A suppository release tester (TMS-103, Toyama Sangyo) was used. Normal saline (100 ml) as a release solution was poured into a release cell, which was immersed in a thermostatic tank maintained at 37°C. A cell (nitrate cellulose membrane, pore size 3 μm) containing a suitable amount of the sample was immersed in this tank.

The contents of the cell were stirred (50 rpm) with a stirring rod, and aliquots of the solution were serially taken. The CDDP content in the release solution was measured with an atomic absorption spectrophotometer (Hitachi Z-8100).

Administration of CDDP Suppository and Blood Sampling Subjects were 3 patients with endometrial carcinoma: stage III (A: 41 kg, 76 years), stage I b-II (B: 72 kg, 74 years) and stage III (C: 65 kg, 68 years). A CDDP suppository was inserted into the uterus twice a week (9:00 a.m., Tuesday and Friday) for 3 weeks. Three milliliters of blood were collected from the brachial vein in a heparinized tube 0.5 and 1d after the administration, and then every day. Plasma was separated and used for the test. Radiotherapy was a combined treatment in the 3 patients: intravaginal radiation of 3 GY for 10d and extracorporeal radiation of 2 Gy for 30d in patient A; intrauterine radiation of 4 Gy for 10d and extracorporeal radiation of 2 Gy for 25d in patient B; intravaginal radiation of 4 Gy for 10d and extracorporeal radiation of 2 Gy for 30d in patient C. Plasma CDDP levels were determined using atomic absorption spectrophotometry.

Effects on liver and renal function, such as glutamate oxaloacetate transaminase (GOT) and creatinine, were studied with a biochemical analyzer (Vision analyzer).

Before the drug was administered, the doctor in charge fully explained to the patient the purpose, method, expected effects and dangers, and it was administered with the consent of the patient.

Plasma CDDP levels were determined 21d after administration was started and biochemical examination was evaluated until 30d afterwards.

CDDP in Endometrium Samples from the endometrium were endoscopically collected before and 21d after the treatment was started, fixed in phosphate buffered formalin, dried and embedded. Sections of 90 nm in thickness were prepared with a Cu grid. Sections thus prepared
were U-Pb double stained. All sections were observed using a trace element X-ray analyzer (JEM-1200EX, LINK 860-500J, Nihon Denshi) after carbon evaporation.

Cytology The endometrium was cytologically examined before and after the combination therapy of CDDP suppository with radiotherapy to evaluate the therapeutic effects.

Results

Melting Point and Viscosity Melting points and viscosity of the suppositories are listed in Table I. Addition of NaCl raised the melting point: 35.5°C when there was no NaCl and 36.5°C with 0.2% NaCl. Addition of 0.2% NaCl doubled the viscosity.

CDDP Release from Suppository The CDDP release profiles from suppositories at various NaCl concentrations are shown in Fig. 1. The time required for 50% release was about 84 h with no NaCl and 30 h with 0.2% NaCl. The rate of release after 72 h was 42%, 88%, 99% and 100% at concentrations of NaCl corresponding to 0%, 0.06%, 0.1% and 0.2%, respectively.

For clinical application, the suppository containing 0.06% NaCl was selected on the ground that it is used twice

Fig. 1. Effect of NaCl on the CDDP Release Profiles from the CDDP Suppository
Concentration of NaCl: ○, 0%; △, 0.06%; □, 0.10%; ●, 0.20%.

Fig. 2. Plasma CDDP Concentration after Administration of the CDDP Suppository
Patient: ○, (A); △, (B); □, (C). The arrow indicates the time at which the CDDP (20 mg) suppository was administered on Tuesday and Friday.

Fig. 3. Effect of the CDDP Suppository on the Liver and Renal Function
Patient: ○, (A); △, (B); □, (C).
TABLE I  Effect of NaCl on Melting Point and Viscosity

<table>
<thead>
<tr>
<th>Concentration of NaCl (%)</th>
<th>Melting point (°C)</th>
<th>Viscosity (η cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>35.5 ± 0.2</td>
<td>12.8 ± 0.3</td>
</tr>
<tr>
<td>0.06</td>
<td>35.9 ± 0.1</td>
<td>20.5 ± 0.2</td>
</tr>
<tr>
<td>0.10</td>
<td>36.1 ± 0.1</td>
<td>21.9 ± 0.3</td>
</tr>
<tr>
<td>0.20</td>
<td>36.5 ± 0.1</td>
<td>25.7 ± 0.4</td>
</tr>
</tbody>
</table>

Mean ± S.D. (n = 5).

A week and the CDDP release was 88% after 72 h and 100% after 96 h.

**Plasma CDDP Level and Biochemical Examination**

Figure 2 shows plasma CDDP levels after the suppository containing 0.06% NaCl was given. Plasma CDDP was detected in patient A on day 0.5, patient B, day 7, and patient C, day 5 after administration. The plasma levels differed among the patients and the peak levels were 0.12, 0.06 and 0.22 μg Pt/ml in patients A, B, and C, respectively.

The suppository did not influence the patient biochemical examination (Fig. 3).

**X-Ray Analysis of CDDP in the Endometrium**

Figure 4 shows electron microscopic pictures of the endometrium on patient A. (A) Transmission electron microscopy showed that the membranous structure and organellae had been destroyed by tissue necrosis. A nucleus and some microsomes are shown. (B) An enlarged image of area (a) in (A), showing many black spots. The spots were analyzed by X-ray.

Four Pt peaks (Mα, Lα, Lα + β, Lβ) were detected. CDDP Pt was also identified in other spots (Fig. 5).

**Cytological Examination**

Cytological examination showed therapeutic effects of the suppository: change from type 5 to type 2 in patient A, from type 3A to type 1 in patient B and from type 5 to type 2 in patient C.

**Discussion**

*In vitro*, the release of CDDP from the suppository was markedly increased by the addition of NaCl, possibly because of the enhanced solubility of CDDP, reduced substitution activity of Cl− ions in CDDP molecules, and inhibited hydrate formation. The melting point and viscosity were markedly affected by the NaCl content. Particularly, the viscosity was enhanced by the addition of NaCl, suggesting that added NaCl may prolong the intrauterine stay of the suppository.

*In vivo*, changes in the plasma CDDP level differed among the patients, possibly because of the difference in CDDP absorption in affected cells and in the distribution volume, which is dependent on the body weight. X-ray analysis showed a number of CDDP particles in necrotized endometrial cells collected 21 d after the administration was
started, and cytological examination revealed a therapeutic
effect of the suppository. Therefore, it was suggested that
the CDDP suppository enhanced the antitumor activity of
CDDP. Low plasma CDDP concentrations (0.12, 0.06 and
0.22 μg Pt/ml) and no abnormality in patients biochemical
examinations indicated that the suppository reduced the
side effects of CDDP. We will study the absorption process
in the future.

References
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