An Experimental Study of Regional Chemotherapy Using CDDP-Loaded Microspheres for Esophageal Cancer

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Abstract

Purpose. The purpose of this study was to assess the antitumor effects of cisplatin-loaded microspheres (CDDP-MS) and the efficacy of the administration of CDDP-MS into the mediastinum.

Methods. To evaluate the antitumor effect, we first performed a paratumoral injection of CDDP-MS to FF6 tumor-bearing DA rats to compare its effect with that of the intraperitoneal injection of a CDDP solution at different doses.

Results. In the CDDP-MS groups the tumor growth was effectively delayed in proportion to the dosage of CDDP-MS. All rats treated with the CDDP solution at a dose of 10 mg/kg died within 1 week, while no rats treated with CDDP-MS even at a CDDP dose of 20 mg/kg were lost. In the second experiment, which was designed to determine the delivery of the microspheres-released CDDP to various organs, CDDP-MS was injected directly into the mediastinum via the diaphragm in male Wistar rats. In the CDDP-MS group, the plasma CDDP concentration stayed significantly lower than that in the CDDP solution (intravenous) group while the tissue CDDP concentration in the paratracheal lymph nodes was higher. Moreover, the lymph node-to-kidney platinum ratio was eight times higher in the rats given CDDP-MS intramediastinally than in those given the CDDP solution intravenously.

Conclusion. These results demonstrate that a high dose of CDDP can be administered with less systemic side effects by means of encapsulation in the microspheres, and that the administration of CDDP-MS into the mediastinum is more effective for delivering CDDP to the paratracheal lymph nodes. As a regional chemotherapy after esophageal cancer operation, the injection of CDDP-MS into the mediastinum for targeting of the lymph nodes thus promises to be an effective treatment.

Key words Microspheres · Cisplatin · Adjuvant chemotherapy · Esophageal cancer

Introduction

For effective postoperative chemotherapy for cancer of the digestive organs, it is essential to ensure that anticancer drugs are delivered from the site of application to the site of action. With the systemic administration of anticancer drugs in conventional chemotherapy, some anticancer drugs cannot be delivered effectively to the lymph nodes without side effects. Regional chemotherapy, on the other hand, with slow-release formulas containing anticancer drugs and targeting remnant cancer lesions in the space where the primary tumor has been resected, would be suitable for selective delivery to cancer lesions. Especially in the case of squamous cell carcinoma of the thoracic esophagus, micrometastasis of mediastinal lymph nodes or viable tumor cells may be overlooked during surgery. This is because a complete extensive lymphadenectomy cannot be achieved in conjunction with esophagectomy, and may thus result in recurrence at the primary site. In this study we used microspheres incorporating cisplatin (CDDP) which were designed to be released slowly, and to establish an effective administration route, we tried injecting the microspheres into the mediastinum in order to target the lymph nodes or viable tumor cells. We first evaluated the antitumor effects of the microspheres and then evaluated the lymphotrophic selectivity of CDDP-loaded microspheres after intramediastinal administration.

Materials and Methods

Glycolic Acid/ß-Lactic Acid Copolymer Microspheres Containing CDDP

CDDP-Loaded microspheres (CDDP-MS) were prepared after a modification of the method of Kyo et al.¹ Briefly, the microspheres containing CDDP were de-
signed with the aid of the solvent evaporation method using oil-in-oil emulsion. The weight-average molecular weight of glycolic acid/l-lactic acid copolymer (PGLA) (Wako Pure Chemical, Osaka, Japan) was 13,000 and CDDP was supplied by Nippon Kayaku, Tokyo, Japan. PGLA was dissolved in 5 ml of dimethyl formamide (DMF) containing 22 mg of CDDP. The PGLA/CDDP solution in DMF was added dropwise and under agitation at 25°C to 400 g of liquid paraffin containing 10 wt% Span 80. This emulsion was stirred by a disk-shaped stirring bar measuring 5 cm in diameter at a constant speed of 400 rpm, after which the temperature was gradually raised from 25°C to 40°C at a rate of 0.2°C/min, followed by a further 40 h at 40°C to evaporate the DMF. The resulting microsphere was collected by centrifugation, washed four times with n-hexane and once with 2-propyl alcohol, and then dried under reduced pressure until the residual DMF was nearly evaporated. The use of liquid paraffin was found to be effective for reducing CDDP loss during the dispersed phase of microsphere preparation. A weighted amount of the CDDP-MS was dissolved in chloroform followed by the extraction of CDDP during the dispersed phase of microsphere preparation. A weighted amount of the CDDP-MS was dissolved in chloroform followed by the extraction of CDDP during the water phase after the addition of water to the chloroform solution. The loading limit of CDDP into the CDDP-MS was set at 5.0 wt%. To morphologically observe the extent of accumulation of the microspheres administered via the mediastinum to lymph nodes, polylactide microspheres containing fluorescent dye, rhodamine 6GX (Nacalai Tesque, Kyoto, Japan), were also prepared using the solvent evaporation method.

**Properties of CDDP-MS**

According to Kyo et al., the in vitro profile of CDDP release from CDDP-MS showed that about 90% of the incorporated CDDP was released slowly from CDDP-MS over 20 days, and that the initial burst release of CDDP could be reduced to 25%. Scanning electron microscopy with a Hitachi Model S-450 (Hitachi, Tokyo, Japan) demonstrated the CDDP-MS to be spherical with a smooth surface without porosity or cracks and an average size of 15 µm, ranging from 5 to 40 µm (Fig. 1). The concentration of CDDP was measured at a wavelength of 265.9 nm with a flameless atomic absorption spectrophotometer (type Z-7000, Hitachi). The reported concentration of the 50% lethal dose (LD50) of CDDP-MS was 407 µg/mouse, five times higher than that of the CDDP solution.

**Animals**

Male DA rats weighing 200–250 g and male Wistar rats weighing 300–350 g were used in this study and maintained at the Institute of Laboratory Animals, Kobe University School of medicine. The animals were kept in a temperature-controlled room on a 12-h light/dark cycle and were fed standard rat chow and water ad libitum. The study protocol was approved by the Experimental Animals Committee of the Kobe University School of Medicine.

**Tumor Model and Assessment of Antitumor Effect**

A transplantable rat squamous cell carcinoma which originally arose spontaneously in the facial skin of a DA rat, FF6,4–6 was used. This carcinoma was maintained by successive transplantation into the subcutaneous tissue of the abdominal wall of DA rats. Such a transplantable tumor is useful for analyzing the mechanism of proliferation and the effect of anticancer drugs. About 1 month after the inoculation of a 1-mm cube of the tumor into the subcutaneous tissue, the tumor grows into a solitary and thumb-sized tumor with central necrosis.

The FF6 tumor-bearing DA rats were divided into seven groups of four rats each. In the first experiment, 2 days after inoculation into the abdominal subcutaneous