Anticancer Effects of Adriamycin-Loaded Hydroxyapatite Implants Determined in a Swarm Rat Chondrosarcoma Model

Keiko Yamamura¹, Hisashi Iwata², Takashi Osada¹, Toshihisa Yotsuyanagi³ and Toshitaka Nabeshima¹

¹Department of Hospital Pharmacy and ²Department of Orthopedic Surgery, Nagoya University School of Medicine, Tsuruma-cho, Showa-ku, Nagoya 466, Japan
³Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan

Received June 1, 1994 Accepted June 9, 1994

ABSTRACT—We investigated the antitumor effects of adriamycin (ADR)-loaded hydroxyapatite (HAP) beads in a Swarm rat chondrosarcoma model. When one ADR-loaded HAP bead (ADR: 0.8 mg/bead) was implanted into the central portion of a rat bearing tumor, the ADR-loaded HAP beads showed good therapeutic effects, increasing the life span (ILS) by 90%. Significantly, leukopenia and diarrhea were not observed. These results suggest that HAP delivery offers an interesting and a potentially effective method.

Keywords: Chondrosarcoma (Swarm rat), Adriamycin, Hydroxyapatite

In general, initial applications included en bloc resection or local excision with curettage as the basic therapy of malignant bone tumors (1). However, the possibility of remaining tumor cells persisted since surgery with curettage is often difficult. Patients, therefore, had received systemic postoperative adjuvant chemotherapy for controlling local recurrence and metastasis to bone and lung (2). Systemic administration of anticancer drugs may be ineffective since chondrosarcoma, which comprises about 20% of all primary malignant bone tumors, shows low sensitivity to most anticancer drugs (3). Adriamycin (ADR) is an anthracycline antibiotic possessing antitumor activity against a variety of cancers (4). Its systemic use, however, is limited by severe side effects that include dose-limiting myelosuppression and cardiotoxicity (5). To reduce systemic toxicity while retaining or increasing the tumoricidal effect, the tumor should ideally be selectively exposed to the anticancer drug. Therefore, it may be useful to fill the bony defect after excision with an active anticancer drug incorporated into hydroxyapatite (HAP) ceramics used in orthopedics as an aid in bone grafts for controlling local recurrence. On the other hand, to evaluate the therapeutic potential of anticancer drugs, it is important to use a selective tumor cell model. The Swarm rat chondrosarcoma was isolated from a tumor that originally arose spontaneously in an 18-month-old female Sprague-Dawley rat (6). Its histological features such as moderate cellularity and mild nuclear hyperchromasia (7) are similar to those of well-differentiated (grade I) human chondrosarcoma. No references concerning chemotherapy on a chondrosarcoma model with anticancer drug-loaded hydroxyapatite were observed. Therefore, we investigated the antitumor effects of ADR-loaded HAP delivery using Swarm rat chondrosarcoma as an experimental chondrosarcoma.

ADR injection (Adriacin® 10 mg potency/vial) and ADR-HCl were supplied by Kyowa Hakko Kogyo Co., Ltd., Tokyo. Porous HAP beads were a gift from NGK Spark Plug Co., Ltd., Nagoya. The physical data of the beads (XVC-56), given by the manufacture, are as follows: sintering temperature, 1100°C; diameter, 8.48 mm; weight of one bead, 531 ± 0.7 mg; bulk density, 1.66; true density of the material, 2.97; and Ca/P, 1.68. All other chemicals were of reagent grade. The drug amount loaded in the bead was estimated from the mean full displacement of the pore spaces with water (0.140 ± 0.001 ml, n = 10). Preparation of the ADR-HAP beads (ADR: 0.8 mg/bead) was carried out as follows: HAP beads were placed in ADR aqueous solution (5.71 mg/ml) at 51°C under atmospheric pressure and allowed to remain for 1 hr. HAP beads containing drug solution were lightly wiped with a filter paper and lyophilized in a freeze-drying apparatus (8). Four-week-old male Sprague-Dawley (SD) rats weighing 90 ± 5 g were obtained from Japan SLC, Ltd., Hamamatsu. In this study, the Swarm rat chondrosarcoma was maintained by subcutaneous transplantation into SD rats in our laboratory. When tumor cell suspension (10⁷ cells) was inoculated s.c. into four-week-old...
SD rats, the tumor size reached about ~1.0 cm³ in 1 week postinoculation and ~196 cm³ in 4 weeks. Tumor cell suspensions (10⁷ cells/ml) in 0.9% NaCl solution were prepared by mincing tumor surgically obtained through a stainless steel sieve (pore size of 1-mm²). The tumor pieces were digested sequentially with 0.5% trypsin and 0.5% collagenase for about 90 min each. The concentration of cells released was determined by using a counter for bio cells (Elzone® Model 80). Tumor cell suspensions (1 ml) were inoculated s.c. into the armpit sites of SD rats on Day 0. All rats used in a single experiment (6 rats/group) were inoculated on the same day using cells from the same tumor. Rats were divided as follows: (i) controls without receiving chemotherapy; (ii) ADR i.v. injection: ADR in saline was given by a single i.v. injection (0.8 mg/body) on Day 6 postinoculation; (iii) ADR-HAP: Under ether anesthesia, one ADR-HAP bead (0.8 mg ADR/bead) was implanted into the central portion of the tumor; (iv) drug-free HAP beads: Drug-free HAP beads were implanted in the same manner to determine if the HAP beads themselves have any effects.

Tumor growth of rats was determined by measuring the volumes with a caliper every 3 to 4 days postinoculation. Tumor volume was calculated by the formula 1/2ab², where a is the long diameter and b is the short diameter (9). The antitumor effect was evaluated by comparing the mean survival time of the treated group (T) with that of the control group (C) and expressing it as an increase in life span (ILS).

\[
ILS = (T/C - 1) \times 100
\]

The statistical significance of differences between experimental results was tested by Student's t-test. A P value of < 0.05 was considered significant. The results of local ADR on this tumor system are shown in Table 1. The ADR-HAP beads showed higher tumor inhibition against Swarm rat chondrosarcoma compared with i.v. administration. The ADR-HAP beads showed chemo-

therapeutic effects of 90% (P < 0.05) ILS, and single i.v. injection showed effects of 66% ILS. No effect for tumor growth was observed in drug-free HAP beads compared with the untreated control. The changes in leukocyte counts are shown in Table 2. Relative to the controls, the i.v. injected rats were leukopenic at 5 days after administration (reduced to 46%, P < 0.05), while in rats implanted with ADR-HAP beads, there was little change in the leukocyte number (reduced to 94%). Neither administration produced significant changes in hemoglobin (data not shown). There is evidence that toxicity is more related to the peak serum levels of ADR cumulative dose (10). In the present study, the ADR-loaded HAP delivery system showed higher antitumor effects but less systemic toxicity to an equal dose given by i.v. injection. These findings indicated that the use of ADR-loaded HAP delivery as a means of transporting anticancer agents is more effective in the delivery of high drug doses into the neoplasm with low serum levels than i.v. injection. In conclusion, this study suggests that the HAP drug delivery system offers an interesting and potentially effective method of anticancer drug delivery with low systemic toxicity following local excision with the bony curettage of bone tumors.

| Table 1. Antitumor effects of adriamycin against Swarm rat chondrosarcoma |
|-----------------------------|----------------|--------------|--------------|----------------|
| Drugs                      | Route         | Drug dose   | MST³ (days) | ILS (%)       | Body weight change³ (g) |
| Control                    |_route         | (mg/body)   |             |              |                         |
| Drug free-HAP              | implant       | 0.8         | 50±3.6       | 66*           | 32.4±4.2               |
| ADR-HAP⁵                   | implant       | 0.8         | 57±4.1       | 90*           | 41.5±3.1               |
| ADR³                       | i.v.          | 0.8         | 32.4±4.2     |               |                         |

Swarm rat chondrosarcoma cells (10⁷/rat) were transplanted into SD rats (6 rats/group) on Day 0. *Mean survival time of deceased rat. **Difference in body weight (g) between Days 6 and 16. ^Adriamycin loaded into HAP beads were implanted on Day 6. i.v. injection was made on Day 6. *Values represent mean±S.E. (n = 5–6). There was no statistically significant difference between the control group and the group implanted with HAP alone. **P < 0.05 vs. control group (Student's t-test), ^P < 0.05 vs. ADR i.v. group (Student's t-test).
REFERENCES


