

Research Article

Anti-Tumor Effect of a Local Delivery System; Hydroxyapatite-Alginate Beads of Paclitaxel

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Abstract This study was conducted to clarify direct anti-tumor effect of this local delivery system of paclitaxel. Rat's breast cancer cell line (CRL-1666) and Fischer 344 rat were used. When subcutaneous tumor (injected subcutaneously) size reached 10 mm in diameter, animals in the local treatment group were administered locally at dose of 5 mg/kg of paclitaxel-loaded HAp-alginate beads. Sham operation was performed in the control group. Tumor burden and body weights of the animals were checked at 3, 7, 10 days after treatment. Tumor burden at 10 days was a mean of 8.1 g and 17.2 g for the local treatment group and untreated group, respectively. Although local delivery of paclitaxel-loaded HAp-alginate beads did not inhibit the proliferation of subcutaneous tumor, proliferation time was significantly delayed in treated rats. Our studies revealed that local delivery of paclitaxel-loaded HAp-alginate beads delayed the time to progression of subcutaneous breast cancer in rats.

Keywords drug delivery system; paclitaxel; hydroxyapatite; tumor-bearing rats; local chemotherapy

1 Introduction

We reported a local delivery system of paclitaxel in the form of hydroxyapatite (HAp)-alginate composites, previously [6]. In our investigation, paclitaxel was released slowly from the composites for approximately 7 days *in vitro* [3]. The composites containing 2.4% (weight/weight, w/w) of paclitaxel showed cytotoxic activity against 20,000 of rat's breast cancer cell (CRL-1666) for 72 h [2]. Local treatment with paclitaxel-loaded HAp-alginate composites prolonged the time until the loss of ambulatory in spinal tumor-bearing rats [2,1,4]. However, anti-tumor effect of these therapeutic composites was not known. This study was conducted to clarify the anti-tumor effect of this local delivery system of paclitaxel.

2 Materials and methods

Rat's breast cancer cell line (CRL-1666, purchased from ATCC) and female Fischer 344 (8-weeks-old, 130–150 g) were used for this study. Strontium ion (2 M) was used for a cross-linker of HAp (10%)-alginate (1%) beads. Paclitaxel was loaded into the composites by a spray-drying technique [5]. Twelve animals were injected subcutaneously with 1×10^6 cells/0.1 ml of CRL-1666 cells. When tumor size reached approximately 10 mm in diameter (9 days after injection), animals were allocated to the local treatment group (treated group) and the control group (untreated group), each of which was made up of 6 animals. The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Somnopenil®, 32.5 mg per 1 kg body weight). After curved incision was made along with the margin of tumor, animals in the local treatment group were administered locally at dose of 5 mg/kg (12–13 beads per rat) of the paclitaxel-loaded HAp-alginate beads (Figure 1). On the other hand, unmodified HAp-alginate beads (without paclitaxel) were administered in the control group.

Anti-tumor activity of the paclitaxel-loaded HAp-alginate beads was evaluated by tumor burden, which was calculated using the following relation based on the volume of an ellipsoid, tumor weight (g) = $ab^2/2000$, where 'a' (mm) is the maximum diameter and 'b' (mm) is the minimum diameter (Figure 2). The diameters were measured with vernier caliper. Tumor burdens were checked at 3, 7, 10 days after treatment. The body weights were also monitored for rats. Any animal with persistent signs of malaise, or whose body weight loss, adjusted for the weight of the tumor burden, reached or exceed 10% of its starting body weight, was considered a treatment failure and the animal euthanized and tumor excised for gross examination. Both treated and untreated animals were designed with 6

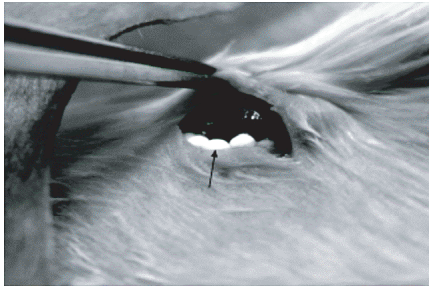


Figure 1: This figure shows photograph after local administration of the HAP-alginate beads containing 2.4% of paclitaxel (arrow). Local treatment was done at dose of 5 mg/kg (12-13 beads per rat)

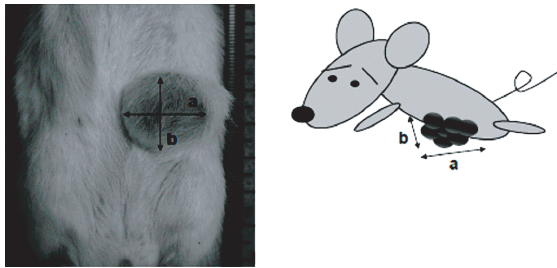


Figure 2: This figure shows schematic design of the calculation of the tumor burden, which was calculated using the following relation based on the volume of an ellipsoid, tumor weight (g) = $ab^2/2000$, where 'a' (mm) is the maximum diameter and 'b' (mm) is the minimum diameter.

rats. For analysis, body weights were adjusted by subtracting the estimated weight of the tumor burden and normalized to the individual animal's adjusted body weight at the time of treatment. At the end of the experiments, animals were euthanized by overdose of intraperitoneal (i.p.) injection. Statistical significance of tumor burden between the treated and untreated group were determined with the Mann-Whitney's U-test. All data were calculated with software (Stat mate, version 3, Japan). A value of $P < 0.05$ was defined statistically significant.

3 Results and discussions

Tumor burden at 0, 3, 7, 10 days after local treatment was a mean of 0.53 g, 1.99 g, 4.9 g, 8.12 g, respectively. Tumor burden at 0, 3, 7, 10 days after sham operation was a mean of 0.67 g, 1.63 g, 5.82 g, 17.2, respectively. The tumor burden of the local treatment group was significantly inferior to that of the untreated group at 10 days (Figure 3).

Although local delivery of paclitaxel-loaded HAP-alginate beads did not inhibit the proliferation of subcutaneous tumor, proliferation time of the local treatment group was significantly delayed than that of the untreated

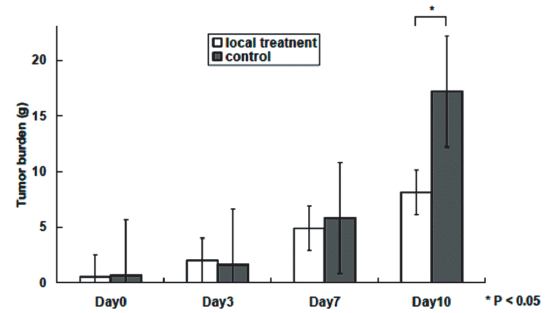


Figure 3: This figure shows the mean tumor burdens in 2 groups at day 0, 3, 7 and 10. There was a significant difference of tumor burden at Day 10 between the local treatment group and the control group.

group. The percent decrease of animal weight from the day of initial treatment to 10 days after treatment was 96.4% and 92.7% for the local treatment group and the control group, respectively. No compromise of skin by the administration of paclitaxel-loaded HAP-alginate beads was observed. According to the weight loss data, general toxicity was not evident in localized paclitaxel delivery using the HAP-alginate beads.

4 Conclusions

Our studies revealed that local delivery of paclitaxel-loaded HAP-alginate beads delayed the time to progression of subcutaneous breast cancer in rats without any sign of toxicity. Although further examination of the controlled release of paclitaxel is required, the HAP composite beads can be act as drug-delivery matrices for local chemotherapy.

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