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Apatite cement containing *cis*-diamminedichloroplatinum implanted in rabbit femur for sustained release of the anticancer drug and bone formation

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Abstract To treat malignant bone tumors, anticancer drugs are administered systemically, simultaneously with surgical therapy. However, drugs administered systemically have considerable invasive action on bone and other organs, and are also associated with various side effects. A bone-cementing material that can maintain high concentrations of anticancer drug at local sites and which can improve local structural weakness after tumor resection would constitute an ideal therapeutic means of treating malignant bone tumors. We therefore applied the concept of a drug delivery system and developed an implant containing calcium phosphate cement and the anticancer drug, *cis*-diamminedichloroplatinum (CDDP). The results of a sustained release test showed that the in-vitro cumulative release ratio of an implant containing 20% CDDP was over 60%, and a release rate of 0.1 mg/day was maintained. Experiments in vivo, using adult rabbits implanted with 10% CDDP, showed that the platinum (Pt) concentration in local bone marrow was an average 3200 μg/tissue·g 6 weeks after implantation. The concentration of Pt in the systemically administered group was 0.2 μg/tissue·g at 6 weeks. The Pt concentrations in other organs of the implanted group were: 3 μg/tissue·g or less in the kidney, and 2 μg/tissue·g or less in liver. These values were lower than those in the systemically administered group (3.5 and 2.1 μg/tissue·g, respectively). Local bone formation was observed by 12 weeks after implantation. Our implant maintained high Pt concentrations at local sites and the bone that formed reinforced the implant.

Key words *cis*-Diamminedichloroplatinum · Apatite cement · Intraosseous implantation · Drug delivery system · Anticancer drug

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Introduction

In the treatment of malignant bone tumors, the anticancer drug systemically administered simultaneously with surgical therapy. However, the systemic administration of drugs has high invasive action on bone and other organs and also causes severe side effects. Therefore, a new bone cementing material that could maintain a high concentration of an anticancer drug at local sites and which could improve local structural defects after tumor resection would represent an ideal therapeutic strategy with which to treat malignant bone tumors. We therefore applied the concept of a drug delivery system (DDS) to achieve the sustained release of an anticancer drug at high concentration at local sites and to reconstruct bone in a defective region after the resection of a lesion. In the present study, we chose to implant rabbits with calcium phosphate cement (hydroxyapatite; HAP) containing *cis*-diamminedichloroplatinum (CDDP), as Uchida et al.[13] (1992) previously reported the sealing of CDDP in HAP, and Wu et al.[14] (1990) reported the sustained release of CDDP into soft tissues from HAP containing adriamycin and CDDP. Shinto et al.[12] (1991) evaluated the formation of osseous union after implanting the implant prepared by Uchida et al. into the rat tibia. Kitamoto et al.[3] (1994) studied bone conduction potency by embedding CDDP in HAP. The following factors are important for evaluating the anti-tumor effects of CDDP at local sites: the effects of the sustained release of CDDP on the bone marrow around the implant; distance of 1 cm from the implant; and the duration of sustained release by which CDDP can be delivered to bone tissues. To date, these factors have

not been studied extensively. Polylactic acid (PLA) has been used as the substrate material for the sustained release of anticancer drugs in previous studies,[2,10] but it has poor bone conduction potency.[6,11] Accordingly, in the present study, we utilized HAP as the substrate material, because it affords sustained drug release, has a good affinity with bone, and is as strong as cancellous bone.[5,8,9] We examined the sustained release of CDDP and bone formation in vitro and in vivo, and we also evaluated bone formation from a histological viewpoint.

Materials and methods

Materials

The implant comprised calcium phosphate hydroxide cement[2] (powdered tribasic calcium phosphate 75%, tetrabasic calcium phosphate 20%, and dibasic calcium phosphate cement), CDDP powder, and curing solution (sodium chondroitin sulfate 5%, sodium succinate 12%, and water 83%). The ratio of calcium phosphate hydroxide cement to the curing solution was 4.5:1.

Male Japanese white rabbits, weighing 2.6 to 3.3 kg (average weight, 3.0 kg), were housed individually in cages and provided with water and solid feed ad libitum. One hundred rabbits were separated into three groups that were either systemically administered with 0.5 mg CDDP in 1 ml of physiological saline (systemic group; $n = 24$), provided with an implant (implant group; $n = 48$), or examined for bone formation (bone formation group; $n = 28$).

Implant preparation

Powdered CDDP and calcium phosphate hydroxide cement were mixed, then the curing solution was added and the mixture was kneaded. The mixture was placed in a Teflon tube (5-mm diameter) and left for 24 h at 37°C and 50% relative humidity for curing. The cylindrical implants (5-mm diameter × 4-mm height) had a porosity of 50% and weighed 150 mg. The implants contained 0 mg (0% w/w), 7.5 mg (5%), 15 mg (10%), or 30 mg (20%) of CDDP.

In-vitro test of sustained release of Pt (CDDP) from the implant

Each implant was kept stationary in 100 ml of phosphate buffer (pH 7.4) in a chamber maintained at 37°C, and sustained release was tested. Ten ml of the phosphate buffer was collected weekly for 4 weeks, and the platinum concentration in each sample was determined, using a plasma emission spectrochemical analyzer, in order to calculate the release of CDDP from the implant. After each sampling, the phosphate buffer was replenished (10 ml). The cumulative sustained release ratio and sustained release rates were determined.

CDDP administration

The systemic group was administered with CDDP, as described by Komatsu.[4] With a single dose to humans as a reference, a dose of 3 mg/kg was injected via the auricular vein. Starting with 6 rabbits in the first week, the number of rabbits injected was increased by 6 each week for a period of 4 weeks. That is, in the fourth week, all 24 rabbits were injected.

Ketamine hydrochloride (Ketalar; Sankyo Yell, Tokyo, Japan); 1.0 ml was given intramuscularly to the implant group, followed by 1 ml of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL, USA); injected via the auricular vein. The rabbit was fixed on a surgical platform and the right posterior limb was shaved, the skin was sterilized with 0.05% chlorhexidine gluconate solution (Hibitane concentrate; Sumitomo Pharmaceuticals, Osaka, Japan). Local anesthesia was induced with 2 ml of 1% lidocaine hydrochloride (Xylocaine; Astra Zeneca, Osaka, Japan) applied to the distal medial site of the right femur. A longitudinal incision of about 2 cm was made, and then the periosteum was separated from the distal metaphysis of the femur. A hole of 5-mm diameter was made in the bone, using a drill, and then the implant was implanted manually (Fig. 1a,b and Fig. 2). The subcutaneous tissues and skin were sutured, and the site was sprayed with Nobectan L spray (Mitsubishi Pharma, Osaka, Japan). Implants, containing 0%, 5%, 10%, and 20% (w/w) CDDP were each implanted in 2 rabbits per week for a period of 6 weeks (48 rabbits in total).

Measurement parameters were body weight; Pt concentrations in bone marrow, kidney, and liver; and bone formation. The animals were weighed each week (on a counter scale with pans for small animals) for 4 weeks (systemic group) or 6 weeks (implant group).

Determination of Pt concentrations in bone marrow, kidney, and liver

In the systemic group, 6 rabbits were killed each week for 4 weeks (24 rabbits in total). In the implant group, four pairs of rabbits with implants of the same concentration were killed each week for 6 weeks (48 rabbits in total). Rabbits were killed by the injection of 10 ml of sodium pentobarbital into the auricular vein. The femur was exposed and cut from the middle to about halfway towards the lower end. A portion distal to the cutting site was dissected at the knee joint, after which the distal femur was longitudinally split, using nippers. In the implant group, bone marrow (0.2 g) was collected from

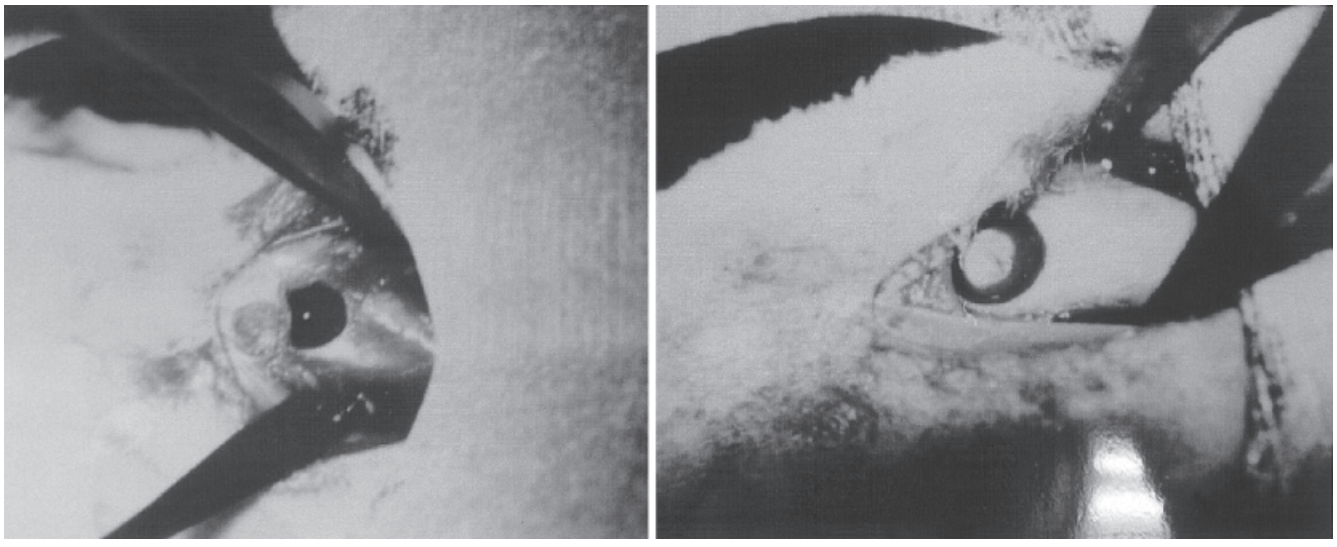

Fig. 1a,b. Implanting. **a** Femur bone hole; **b** implanting

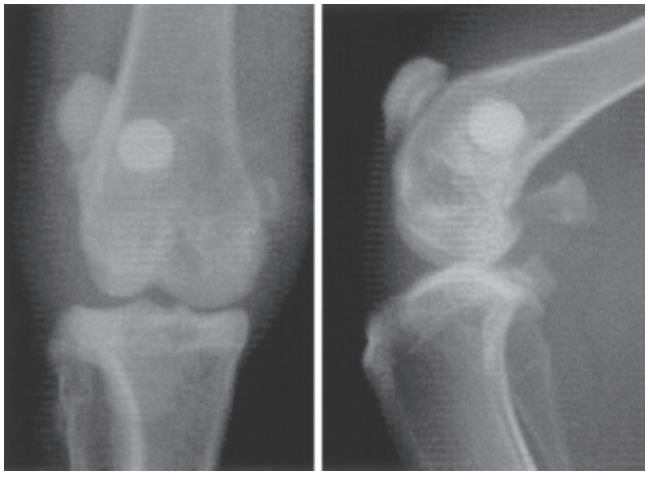

Fig. 2. X-ray after implanting

around the implant and from a site 1 cm from the implant. In the systemic group, bone marrow (0.2 g) was collected from the distal metaphysis of the femur; this area corresponded to the region surrounding the implant. The abdomens of animals in both groups were longitudinally incised after the femurs had been collected, and small slices (0.5 g) were removed from the kidneys and liver. The Pt concentrations in these samples were determined using atomic absorption spectrometry.

Results were statistically analyzed using the *t*-test ($P < 0.05$).

Evaluation of bone formation

Implants were implanted into the distal metaphysis of the rabbit femur, as described above. The femurs of 4 rabbits, 2 each implanted with 0% and 5% CDDP, were exposed at weeks 2, 4, and 6 (12 rabbits in total). The femurs of 4 rabbits, 2 each implanted with 10% and 20% CDDP, were exposed at weeks 2, 4, 6, and 12 (16 rabbits in total). The femur was cut as described above and fixed immediately in 70% ethanol. A block ($1.5 \times 1.5 \times 0.5$ cm) cut from the center of the implant was stained with Villanueva bone stain. After dehydration with 70%, 80%, 90%, and 100% ethanol, the block was defatted with acetone, and implanted with methyl methacrylate (MMA) resin, using a vacuum system based on acetone substitution. The sample was cured in a constant-temperature chamber at 60°C for 24 h, sliced with a crystal cutter, type I (MC-411; Maruto, Tokyo, Japan), manually polished, and ground to a thickness of about 20 μm. Bone formation was examined histologically under a light microscope.

Results

Sustained release test in vitro

The cumulative Pt release ratios ranged from 11% after 24 h (5% implant) to 62% after 4 weeks (20% implant) (Fig. 3a).

The Pt release rate of the 5% implant decreased from about 0.81 mg/day after 24 h to about 0.02 mg/day after 4 weeks. The Pt release rate of the 10% implant decreased from 1.00 mg/day after 24 h to 0.03 mg/day after 4 weeks, and that of the 20% implant decreased from 2.80 mg/day after 24 h to 0.10 mg/day after 4 weeks. Thus, the Pt cumulative release ratios and release rates of the 20% and 10% implants were acceptable (Fig. 3a,b).

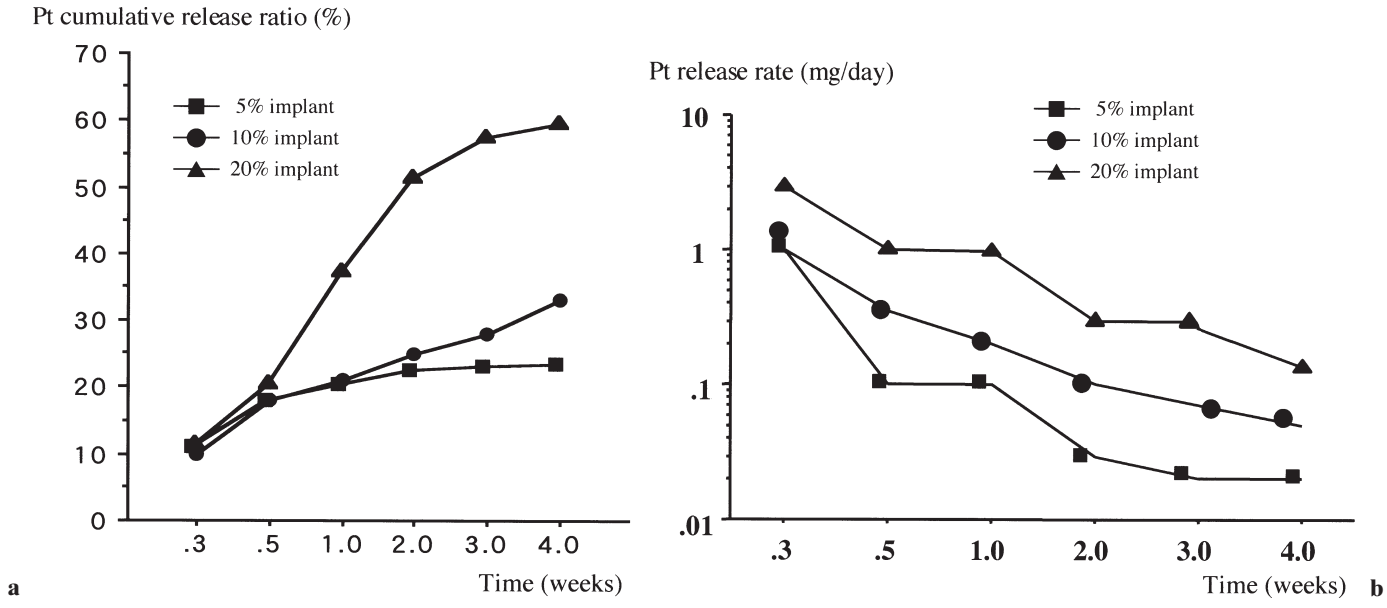

Fig. 3a,b. In-vitro sustained release test. **a** Pt cumulative release ratio; **b** Pt release rate

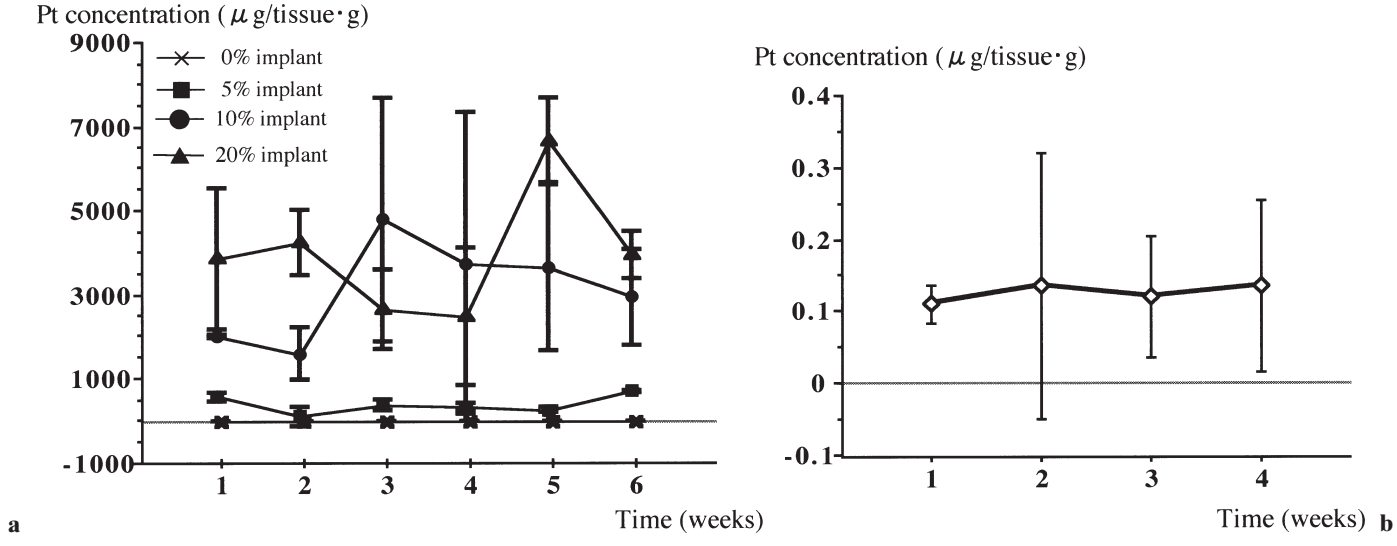

Fig. 4a,b. Pt concentration in bone marrow. **a** Implanted group (around implant); **b** systemic group

Sustained release test in vivo

Platinum concentration in bone marrow around the implant. The Pt concentration was 0μg/tissue·g (wet weight [w/w]) for the 0% implant throughout the experiment, and 1000μg/tissue·g (w/w) for the 5% implant. With the 10% implant, the Pt concentration was 2050μg/tissue·g (w/w) after 1 week, 1800μg/tissue·g (w/w) after 2 weeks, and 5040μg/tissue·g (w/w) after 3 weeks. Thereafter, the value gradually decreased until week 6, after which an average of 3200μg/tissue·g (w/w) was maintained.

The 20% implant released 3810μg/tissue·g (w/w) during weeks 1 and 2, and this value had decreased by 50% at week 4. However, the peak concentration of 6730μg/tissue·g (w/w) was reached at week 5. By week 6, the concentration was similar to that at weeks 1 and 2 (Fig. 4a).

Platinum concentration in bone marrow in the systemic group. The Pt concentration in bone marrow in the systemic group never exceeded 0.2μg/tissue·g (w/w) during the course of the study. In addition, the Pt con-

centration in bone marrow was much lower than that in the implant group (Fig. 4b).

The Pt concentration in the systemic group was significantly lower than those in all implanted groups at week 1, the 10% and the 20% implant groups at week 2, and the 5% implant group at weeks 3 and 4.

At week 1, the Pt concentration in the 5% and 10% implant groups was significantly higher than that in the 0% implant group. The Pt concentration for the 10% implants was higher than that for the 5% implants in week 1. By week 2, the values for the 20% implants were higher than those for the 0% and 5% implants.

In week 3, the values for the 5% implants were higher than those for the 0% implants. By week 4, the values for the 5% implants were significantly higher than those for the 0% implants.

Platinum concentration in bone marrow 1 cm from the implant. For the 0% implant, the Pt concentration was 0 μg/tissue·g (w/w) during the course of the study, whereas that for the 5% implant was not more than 500 μg/tissue·g (w/w). The 10% and 20% implants released Pt at a concentration of more than 500 μg/tissue·g (w/w) and 2000 μg/tissue·g (w/w), respectively at week 6 (Fig. 5).

Platinum concentrations in other organs

Kidney. The Pt concentration in the systemic group averaged 3.5 μg/tissue·g (w/w) at week 1. This value subsequently decreased to 1.1 μg/tissue·g (w/w) by week 4 (Fig. 6a).

The Pt concentration was 0 μg/tissue·g in kidneys from rabbits with 0% implants and 2.1 μg/tissue·g (w/w) for the rabbits with 5% implants during the course of the study. In kidneys from rabbits with 10% implants, the Pt concentration was 3 μg/tissue·g (w/w) or less. Kidneys from rabbits with 10% implants and implants with a lower CDDP content contained less Pt than the kidneys of the systemic group during the course of the study. In kidneys from the rabbits with 20% implants, the Pt concentration reached 7.5 μg/tissue·g (w/w) during week 1, then decreased to a value higher than that in the systemic and other groups (Fig. 6b).

The Pt concentration was significantly higher in the 20% implant group than in the systemic group at weeks 1 and 3. An intra-group comparison of the implant group showed that the value for the 20% group

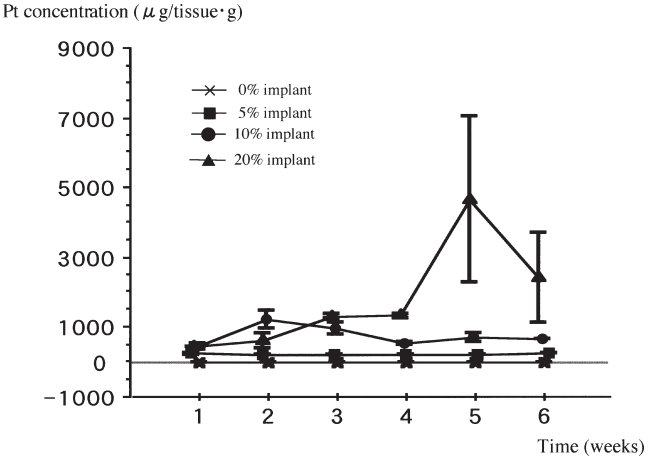

Fig. 5. Pt concentration in bone marrow 1 cm from implant

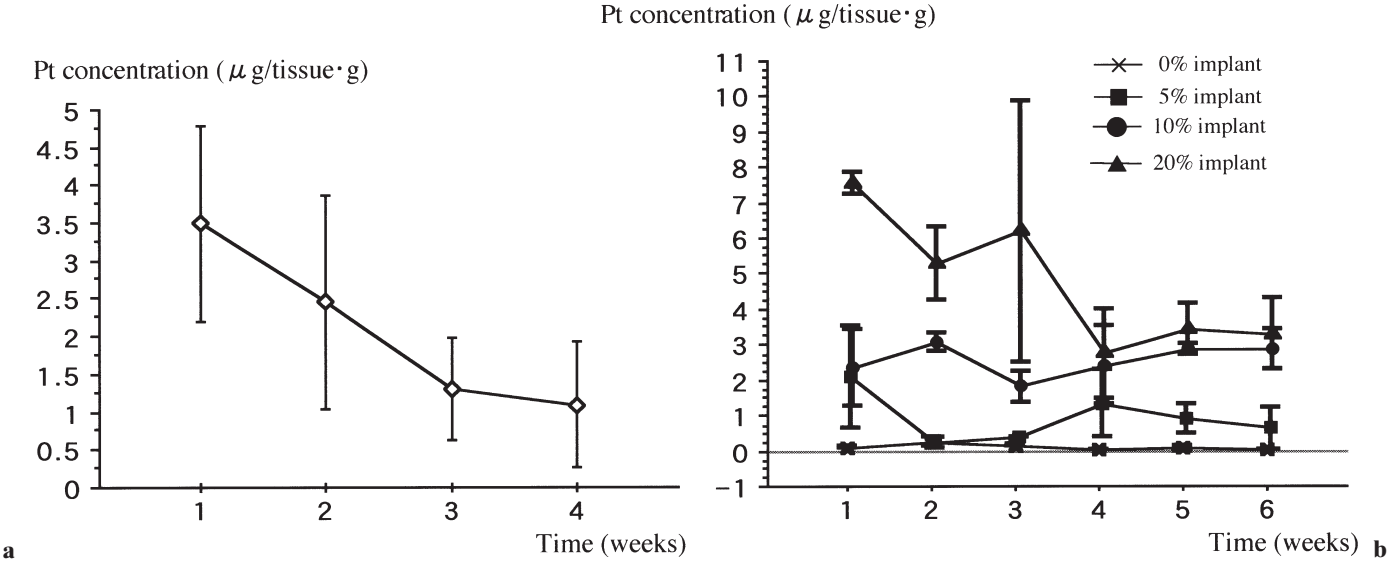

Fig. 6a,b. Pt concentration in kidney. **a** Systemic group; **b** implanted group

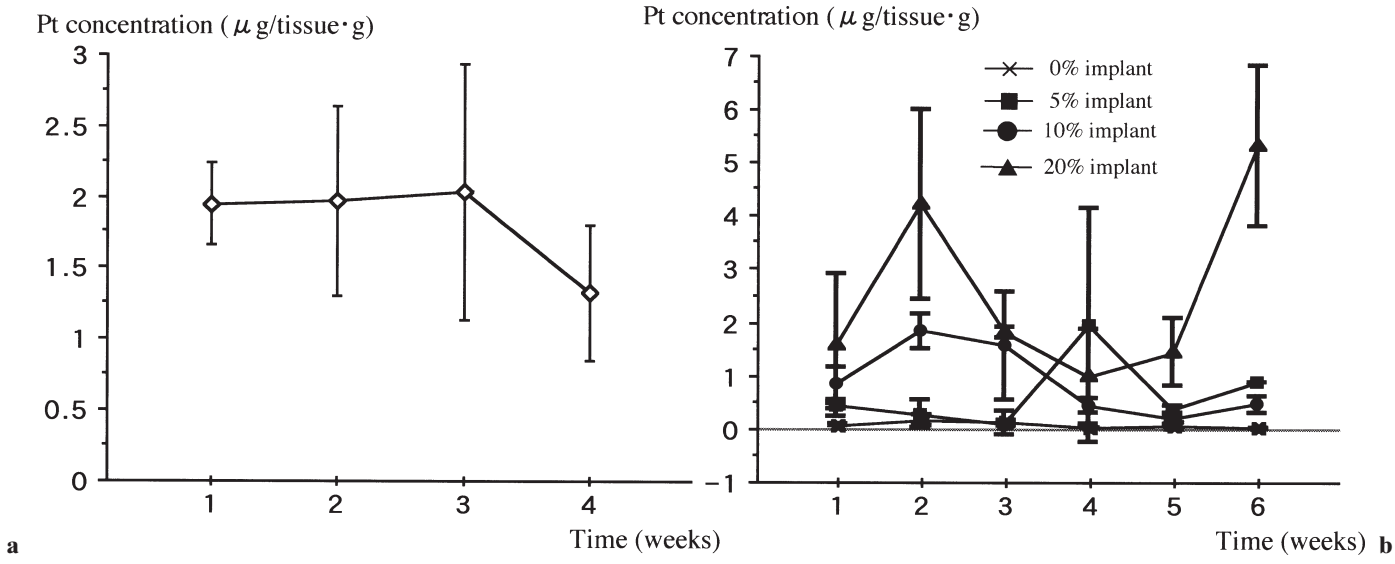

Fig. 7a,b. Pt concentration in liver. **a** Systemic group; **b** implanted group

was significantly higher than those for all other implants at week 1. By week 2, the values for the 10% and the 20% implants were higher than that for the 5% implant. By week 3, the 10% and 20% implants released significantly higher Pt concentrations into the kidney than the 5% implants.

Liver. The Pt concentration in the liver of the systemic group increased until week 3 (1.9–2.1 μg/tissue·g [w/w]), and had declined (1.3 μg/tissue·g [w/w]) at week 4 (Fig. 7a).

For the 5% and 10% implants, the Pt concentration in the liver was 0 μg/tissue·g (w/w) for 0% implants and 2.0 μg/tissue·g (w/w) or less throughout the study. These values were lower than those for the systemic group. The value for the 20% implant increased until week 2 (2.1–4.4 μg/tissue·g [w/w]), then decreased until week 5, and increased once more (5.3 μg/tissue·g [w/w]) at week 6. These values were higher than those for the systemic group (Fig. 7b).

We compared the Pt concentrations in the livers of the systemic and implant groups. At week 2, only the 20% implant group had a concentration that was significantly higher than that of the systemic group. An intra-group comparison of the implant groups showed that the livers of the 5% and 10% implant groups contained significantly more Pt than those of the 0% implant group at week 1, and the values for the 10% implants were significantly higher than those for the 0% and 5% implants at week 2. By week 3, the values for the 20% implants were significantly higher than those for the 0% and 5% implants. By week 4, the livers from rabbits with 10% implants contained significantly more Pt than those from rabbits with 0% implants.

Body weight

The body weight of the systemic group decreased by a maximum of 1 kg and by an average of 0.56 kg up to week 2. Thereafter, body weight tended to return to pre-administration values (Fig. 8a).

The body weight of the implant group decreased for up to 3 weeks (maximum, 4 kg; average, 0.23 kg). The rate of this decrease was lower than that in the systemic group (Fig. 8b).

A comparison of the systemic and implant groups showed that an implanted rabbit weighed significantly more than a rabbit from the systemic group at week 1. The rabbits with 0%, 5%, and 10% implants weighed more than systemic group rabbits at week 2, and rabbits with 0% and 10% implants weighed more than systemic group rabbits at week 3. An intra-group comparison of the implant group showed that the body weight of rabbits with 0% implants was significantly higher than that of rabbits with 20% implants at week 1.

By week 3, the rabbits with 5% and 10% implants weighed significantly more than those with 20% implants.

Histopathological findings at the implant site

The results of Villanueva bone staining showed that the gap between the bone and the 0% implant had disappeared by week 2 and new bone had formed (Fig. 9). The gap between the bone and the 5% implant had disappeared by week 4, and new bone had formed by week 6 (Fig. 10). The gap between the bone and the 10% implant had disappeared by week 6, and new bone had formed by week 12 (Fig. 11). The gap between the

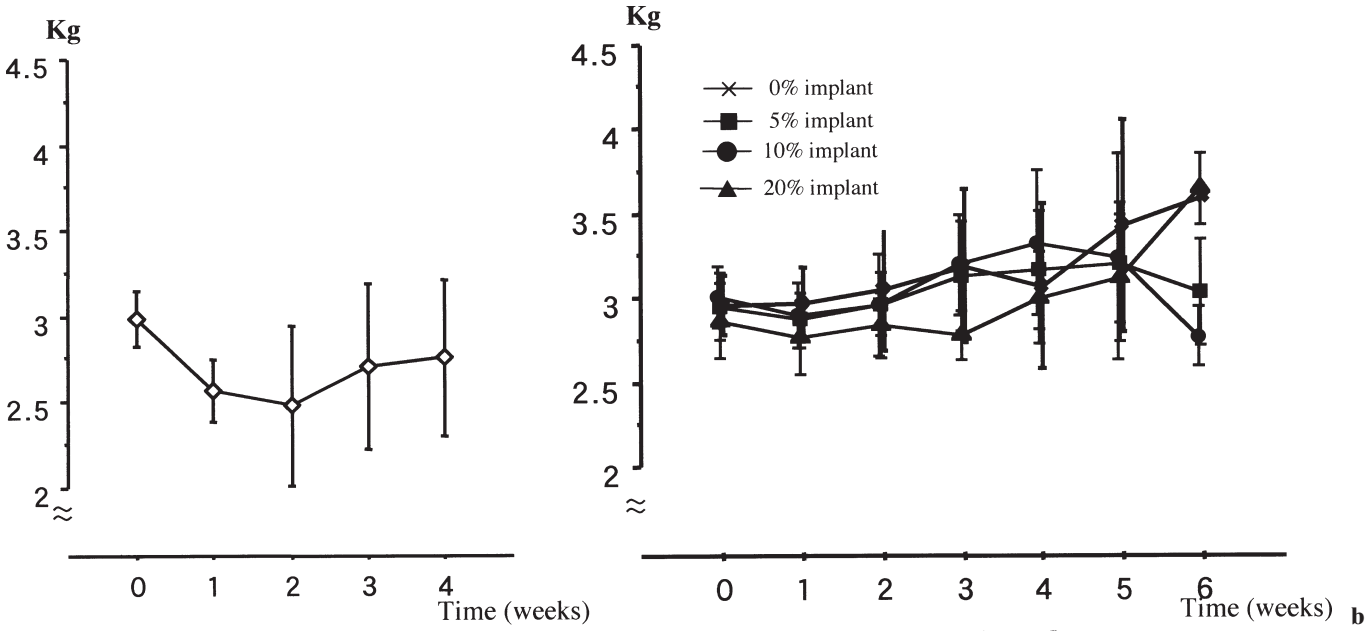

Fig. 8a,b. Variations in body weight. **a** Systemic group; **b** implanted group

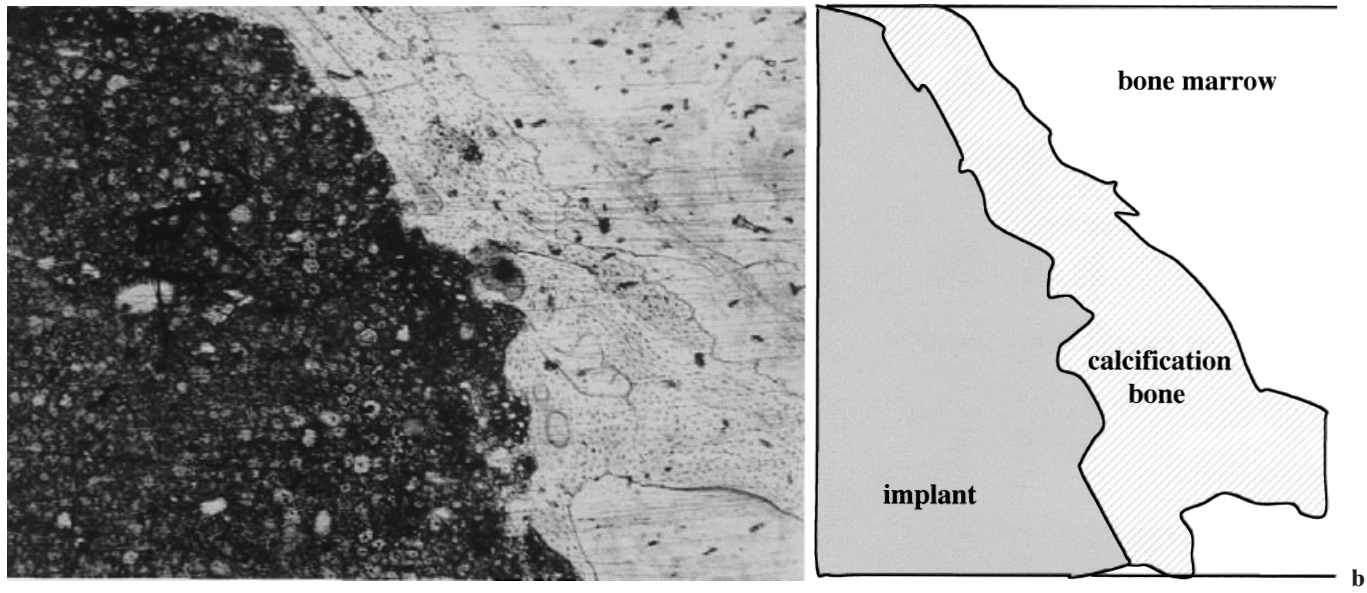

Fig. 9a,b. Histopathological findings 2 weeks after implantation; calcification bone (new bone) formation was detected along the surfaces of the 0% implant. Villaneueva bone stain, ×40

bone and the 20% implant remained at week 12, and new bone had not formed by that time.

Summary of results

1. The Pt cumulative release ratios and release rates in vitro were highest for the 20% implants.
2. For the 20% implants, the Pt concentration in the bone marrow immediately adjacent to the implant was higher than the bone marrow concentration in the systemic group and was higher than all concentrations measured in vitro. However, the Pt concentrations in the kidneys and liver were also high.
3. The body weight of the systemic group was markedly decreased compared with implanted group.

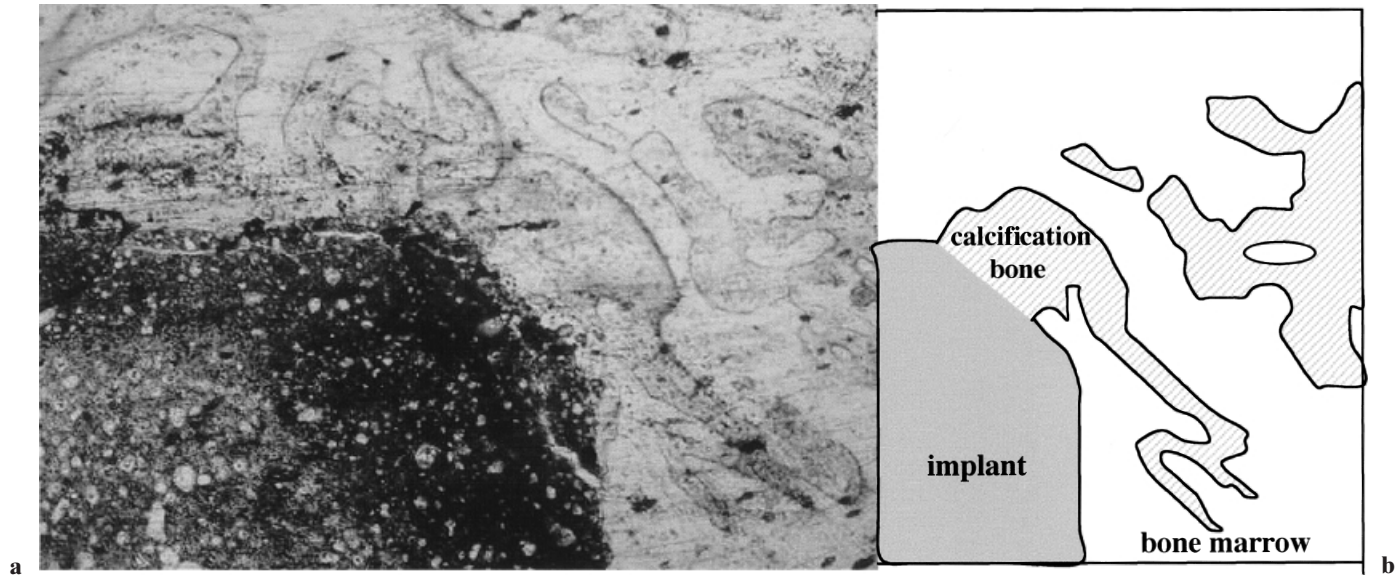

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Fig. 10a,b. Histopathological findings 6 weeks after implantation; calcification bone (new bone) formation was detected along the surfaces of the 5% implant. Villaneueva bone stain, ×40

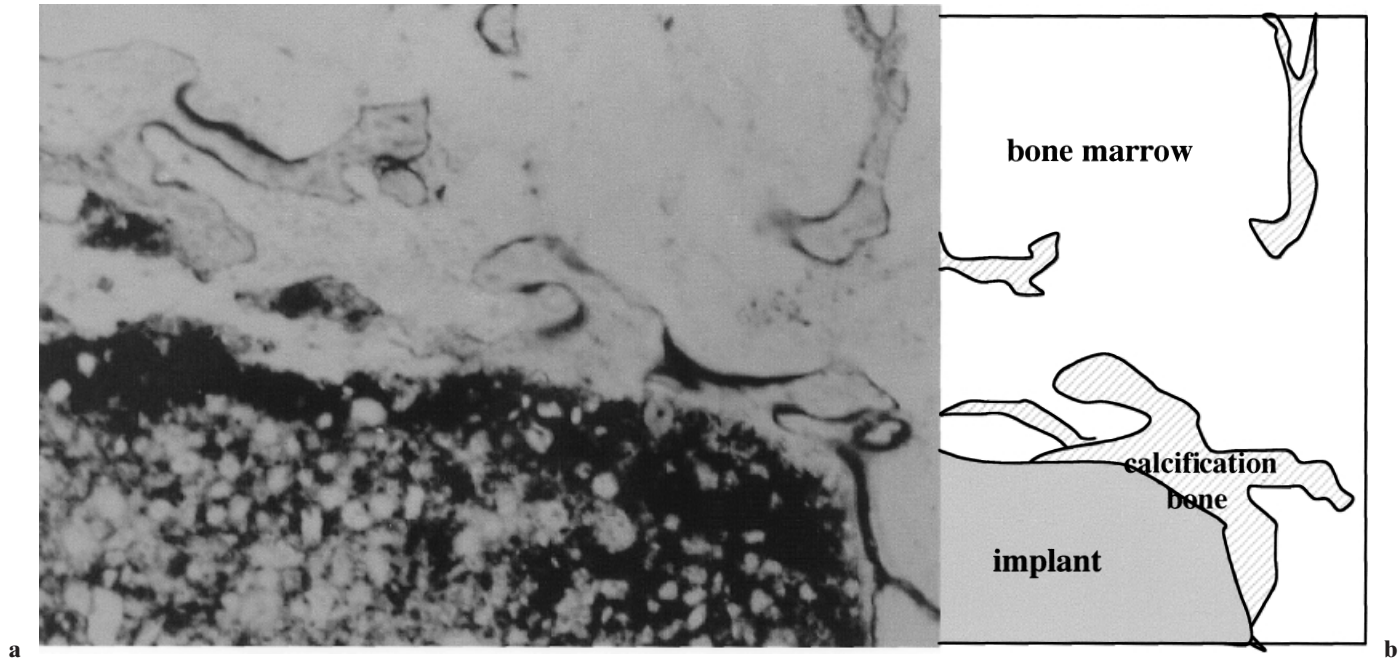

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Fig. 11a,b. Histopathological findings 12 weeks after implantation; calcification bone (new bone) formation was detected along the surfaces of the 10% implant. Villaneueva bone stain, ×40

4. Bone formed for up to 12 weeks after the implantation of the 0%,5% and 10% implants, but did not form in rabbits with 20% implants.
5. The Pt concentration in bone marrow immediately adjacent to the 10% implant was higher than the bone marrow concentration in the systemic group, and was higher than the concentration in bone marrow immediately adjacent to the 0% and 5% implants. The Pt concentrations in the kidneys and liver in the 10% implant group were lower than those in the 20% implant group.

Discussion

We applied the concept of a drug delivery system to an implant containing an anticancer drug, and tested its ability to deliver platinum and repair bone in an animal model.

We used HAP implants containing the anticancer drug cis-diamminedichloroplatinum to fill defective bone regions after resecting tumor tissues. We then examined their effects at local sites and their effect on bone formation.

The anticancer drug CDDP is currently the leading drug in cancer chemotherapy. Bone and soft tissue tumors are highly sensitive to platinum, and CDDP is not metabolized during implant preparation.

The implants in the present study contained 0%, 5%, 10%, or 20% (weight %) CDDP. In vitro, the 20% implant showed the most satisfactory results with respect to both cumulative release ratio and release rates, and results indicate that the 20% implant has sufficient antitumor effects at local sites. We also evaluated the effects of these implants on organs and their bone formation potency in vivo.

To assess the systemic influence of CDDP, we weighed the animals over a period of several weeks. Body weight decreased markedly in the systemic group, but was less affected in the implant group. These result indicate that local implantation with an anticancer drug has less systemic influence than systemic administration.

In the systemic group, 3 of the 24 rabbits showed symptoms such as vomiting during week 1, and 1 rabbit had diarrhea. This implied that CDDP affected the digestive organs. None of the animals in the implanted groups showed signs of vomiting or diarrhea.

A CDDP content of 500–1000 μg/tissue·g (w/w) bone was maintained from 1 to 6 weeks after implantation with the 5% implant, and a content of more than 2000 μg/tissue·g (w/w) was maintained by the 10% and 20% implants. These results show that the sustained release of a high CDDP concentration can be achieved for at least 6 weeks by the implant used in the present study.

The value in bone marrow immediately adjacent to the implant was below 1000 μg/tissue·g (w/w) for the 5% implant and more than 2000 μg/tissue·g (w/w) for the 10% and 20% implants. At bone marrow sites 1 cm from the implant, the value was 500 μg/tissue·g (w/w) for the 10% and 20% implants; this result has important clinical implications.

Uchida et al.[13] and Kitamoto et al.[3] experimented with implants into soft tissues and reported that the CDDP concentration in the kidney and liver was in the range of one-fifth to one-tenth of that in the implanted site. Shinto et al.[12] reported a range of CDDP concentrations in the kidney that was 1/100 to 1/1000 of the concentration at the implanted site. Obviously, there is a great disparity between these results. Moreover, in none of these three studies were the findings compared with those in a group given systemic drugs. The present study showed that the Pt concentration in the kidneys and livers of animals implanted with HAP containing 20% CDDP was 0.02% of that at the implanted site. The concentration in other organs also appeared to be relatively low. The concentration of CDDP in the kidney and liver of animals with the 20% implants was 1.5 times higher than that in the kidneys and livers of the systemic group. This may be because the 20% implant contained 3.33 times more CDDP than the amount administered to the systemic group. However, the Pt concentration in the kidneys and livers of the 10% implant group was slightly lower than that in the systemic group, even though the implant contained 1.67 times more CDDP than the amount administered to the systemic group. The 5% implant contained 0.833 times more CDDP than that administered to the systemic group, but the Pt concentrations in the kidneys and livers of the 5% implant group were lower than those in the systemic group. These results show that the 5% and 10% implants have lower sustained release rates than the 20% implant, and that the effect of the 5% and 10% implants on the kidney and liver was less significant than that of the systemic administration or the 20% implant.

Bone was not formed in rabbits with the 20% implant, even by week 12. New bone was formed by weeks 12 and 6 with the 10% and 5% implants, respectively. This suggests that high concentrations of CDDP inhibit bone formation, as reported by Nakayama et al.[7] However, the effects of different CDDP concentrations have not yet been elucidated. The present study demonstrated that, in order to achieve early bone formation, the CDDP content in an implant should not exceed 10% (w/w). Implants with 10% CDDP content have good sustained release, and are suitable as a bone cementing material for the sustained release of anticancer drugs.

Conclusions

We conclude that HAP containing 10% CDDP (weight %) is the ideal implant, because it achieves sustained drug release and new bone formation, as well as showing minimal organ involvement.

The present results indicate that this system is applicable to the treatment of patients with various types of bone tumors.

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