Received: 4 April 2019 | Revised: 9 July 2019 | Accepted: 24 July 2019

DOI: 10.1111/cas.14145

ORIGINAL ARTICLE

Cancer Science WILEY

Bone invasion-targeted chemotherapy with a novel anionic platinum complex (3Pt) for oral squamous cell carcinoma

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Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 16K20234

Abstract

Cisplatin (CDDP) is an important drug for chemotherapy in patients with head and neck squamous cell carcinoma. Nephrotoxicity and lack of an effect on bone invasion are limitations of CDDP. To increase its antitumor effect on bone invasion and reduce toxicity problems, anionic Pt complex (3Pt) has been developed. The present study aimed to characterize the basis of the cytotoxicity of the novel platinum complex 3Pt in comparison with that of CDDP for oral squamous cell carcinoma. The ionic platinum complex was prepared to increase solubility and avoid platinum nephrotoxicity. Furthermore, 3Pt was designed to target bone hydroxyapatite and has germinal bisphosphonate moieties for drug delivery. In vitro antitumor activity was assayed in two oral squamous cell carcinoma cell lines. To investigate the antitumor and nephrotoxic effects of 3Pt, nude mice with OSC-19 were given 3Pt and CDDP. The in vitro growth-inhibitory effect of 3Pt was significantly less than that of CDDP. However, both 3Pt and CDDP showed equivalent antitumor effects in vivo. Mice injected with CDDP developed renal cell apoptosis; however, those injected with 3Pt were almost free of renal cell injury. In addition to similar in vivo antitumor effects, 3Pt decreased the volume of bone resorption compared to that with CDDP in a bone invasion model using OSC-19. In conclusion, considering the potential advantages in terms of noticeable antitumor activity on bone invasion and reduced nephrotoxicity, 3Pt represents a significant improvement in the development of bone-targeting platinum drugs.

KEYWORDS
bone invasion, cisplatin, drug delivery system, head and neck cancer, ionic platinum complex

1 | INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) arises in the oral cavity, oropharynx, larynx, or hypopharynx, and is the sixth leading cancer by incidence worldwide. It is likely that approximately 600 000 cases will arise this year worldwide and that only 40%-50% of these patients will survive for 5 years.[1] Cisplatin (cis-dichlorodiammineplatinum [CDDP]) plays a central role in cancer chemotherapy for the treatment of solid tumors,[2] and CDDP-based chemoradiotherapy regimens are being widely used in patients with HNSCC.[3] However, its administration has been hindered by its adverse reactions, such as nephrotoxicity.[4] A serious risk of nephrotoxicity frequently prevents the maximization of its antitumor activity using high doses, which can result in treatment failure.

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Head and neck squamous cell carcinoma, including oral cancer, nasal cavity and paranasal sinus cancer, and ear canal cancer, frequently involves adjacent bones. Oral cancer, which has an incidence of 7000 people per year in Japan, is the most common cancer among head and neck cancers. The prevalence of mandibular bone involvement in cases of oral squamous cell carcinoma (OSCC) has been reported to range from 12% to 56%.[5] The 5-year disease-specific survival rate has been shown to be 61% for patients with mandibular invasion and 80% for those without mandibular invasion.[5] Additionally, cases with mandibular invasion were found to be resistant to CDDP-based chemoradiotherapy.[6] Furthermore, medullar invasion was the only independent predictor of reduced survival.[7,8]

Recently, we designed a platinum-based chemotherapy agent using new concepts.[9] An anionic platinum complex was prepared to increase solubility and avoid platinum nephrotoxicity. Bisphosphonates, which are metabolically stable pyrophosphate analogs, are known for their bone-targeting properties and are used as therapeutic agents for bone diseases such as bone metastasis.[10,11] Bisphosphonates have a strong affinity for hydroxyapatite as bone component and show strong inhibition to osteoclastic resorption.[12] Therefore, we incorporated bisphosphonates into the platinum complex to target bone tissue. Our novel platinum drug was referred to as 3Pt ([Pt(Pt(1R,2R-diaminocyclohexane)(myo-inositol-1,2,3,4,5,6-hexakisphosphate))$_2$]) which contains 12 phosphate groups. The present study aimed to analyze the cytotoxicity of 3Pt using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay in OSCC cell lines. Additionally, in in vivo experiments, the antitumor and nephrotoxic effects of 3Pt were investigated and compared with those of CDDP. The antitumor effect of 3Pt was further evaluated to develop a target therapy for bone invasion in vivo, which has not been evaluated previously.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The animal study was conducted in accordance with the Guidelines on the Care and Use of Laboratory Animals issued by Kanazawa University. The protocol was approved by the ethics committee of Kanazawa University (permit number: AP-153670). All surgeries were carried out under three types of mixed anesthetic agent. All efforts were made to minimize animal suffering, decrease the number of animals used, and use possible alternatives to in vivo techniques.

2.2 | Cell culture and drugs

Human OSCC cell lines OSC-19 and OSC-20 were used for experiments. These cell lines were maintained in Eagle's minimum essential medium supplemented with 10% FBS. CDDP was obtained from Nippon Kayaku Co., Ltd (Tokyo Japan).

2.3 | 3Pt synthesis

For 3Pt synthesis, Ag(NO_3) (0.10 g, 0.6 mmol) was dissolved in 5 mL H_2O. To the solution, Pt(Pt(1R,2R-diaminocyclohexane (DACH)) (myo-inositol-1,2,3,4,5,6-hexakisphosphate (IP6))-10Na-14H_2O) (0.41 g, 0.3 mmol) aqueous solution 7.5 mL, and K_2PtCl_4 (0.06 g, 0.15 mmol) aqueous solution 7.5 mL were added sequentially. The mixture was stirred for 3 days at 55°C and, after removal of AgCl by filtration, the filtrate was concentrated by evaporation and MeOH was added to the residue. The off-white powder was collected and dried under a vacuum. The yield was 0.303 g (60%).

Elemental analysis: $C_{24}H_{40}N_4O_{48}P_{12}Pt_3Na_{12}H_6-51H_2O$ (Pt(Pt(DACH) IP6)$_2$12Na6H-51H_2O)
Observed: C, 8.62%; H, 4.25%; N, 1.66%
Calculated: C, 8.70%; H, 4.47%; N, 1.69%

The structure of 3Pt was estimated as shown in Figure 1 by the pH dependence of P-NMR,[9] where the PO_3 groups coordinated with Pt were not shifted by the protonation.

2.4 | In vitro growth-inhibition assay

Head and neck cancer cell lines were used to assess the antitumor effect of 3Pt in vitro. An MTS assay was carried out to assess the effect of cell proliferation using the CellTiter 96 Aqueous One Solution Cell

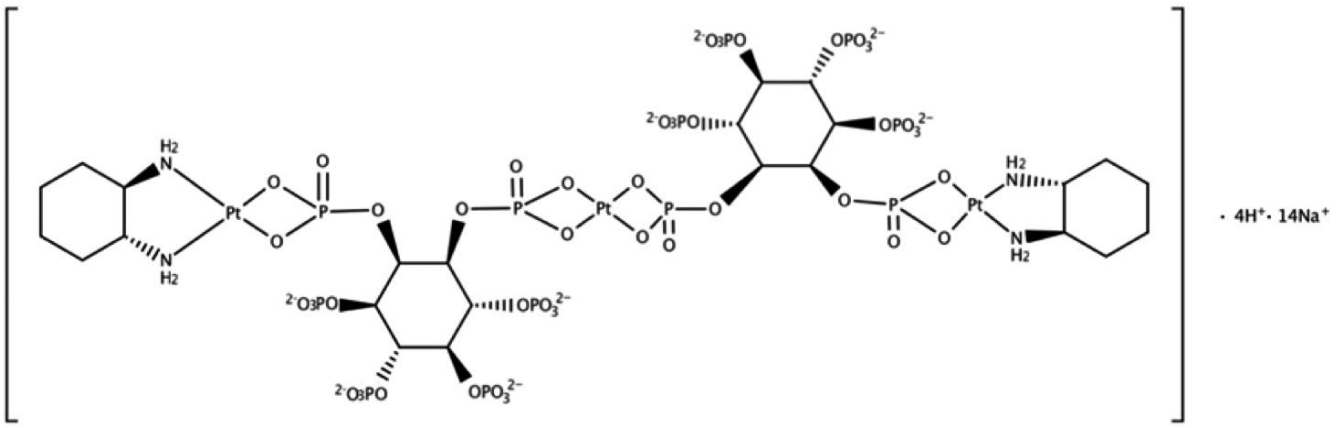

FIGURE 1 Structure of the novel platinum compound 3Pt

Proliferation Assay (Promega, Madison, WI, USA). Briefly, cells were seeded in 96-well culture plates at a density of 2×10^3 cells/well. After 24-hour incubation, 5% glucose (control) or a graded concentration of CDDP, or 3Pt was added to each well, and the plates were incubated for 48 or 72 hours. The MTS reagent was added, and after 2-hour incubation, the optical density was read with Microplate Reader Manager (Molecular Devices, San Jose, CA, USA) at a wavelength of 490 nm. The IC_{50} value represented the drug concentration that reduced the mean absorbance at 490 nm to 50% in the untreated control well.

2.5 | Evaluation of antitumor activity

For the evaluation of the antitumor activity of 3Pt, 1×10^6 OSC-19 cells were injected s.c. at the dorsal skin of BALB/c-nu/nu mice. After 14 days, the tumor diameters reached approximately 5 mm, and the tumor-bearing mice were randomly divided into the following three groups: control group, CDDP group, and 3Pt group. Drugs were injected i.v. into the tail vein each week, with three doses in total. Mice in the respective groups were given a single i.v. injection of 5% glucose solution (control group), CDDP (5 mg/kg; CDDP group), or 3Pt (an equivalent dose of 5 mg/kg CDDP; 3Pt group). The dose was determined according to our previous study.[13] At this dose, renal toxicity is unlikely to occur, so the efficacy could be stably evaluated. Tumor volume was calculated weekly using the following formula: tumor volume (mm^3) = $1/2 \times L \times W^2$ (where L is the major axis and W is the minor axis).

2.6 | Assessment of renal toxicity

A TUNEL assay was carried out for the detection and quantitation of kidney cell apoptosis.[14] The ApoAlert DNA Fragmentation Assay Kit obtained from Clontech (Mountain View, CA, USA) was used to assess apoptosis-induced nuclear DNA fragmentation by a fluorescence assay. On day 28 after drug administration, the mice mentioned above were killed. Both kidneys were removed and fixed in 10% formalin solution. Paraffin sections were deparaffinized. TUNEL-positive apoptotic cells were detected using a fluorescent microscope. Mean proportion of apoptotic cells per 100 cells and the mean proportion of apoptotic cells in five fields of tubule cells of the kidney were determined.

2.7 | Plasma platinum concentration and bone platinum accumulation

In the same method as described above, 6-week-old female BALB/c-nu/nu mice were s.c. injected with OSC-19 cells. After 14 days, a

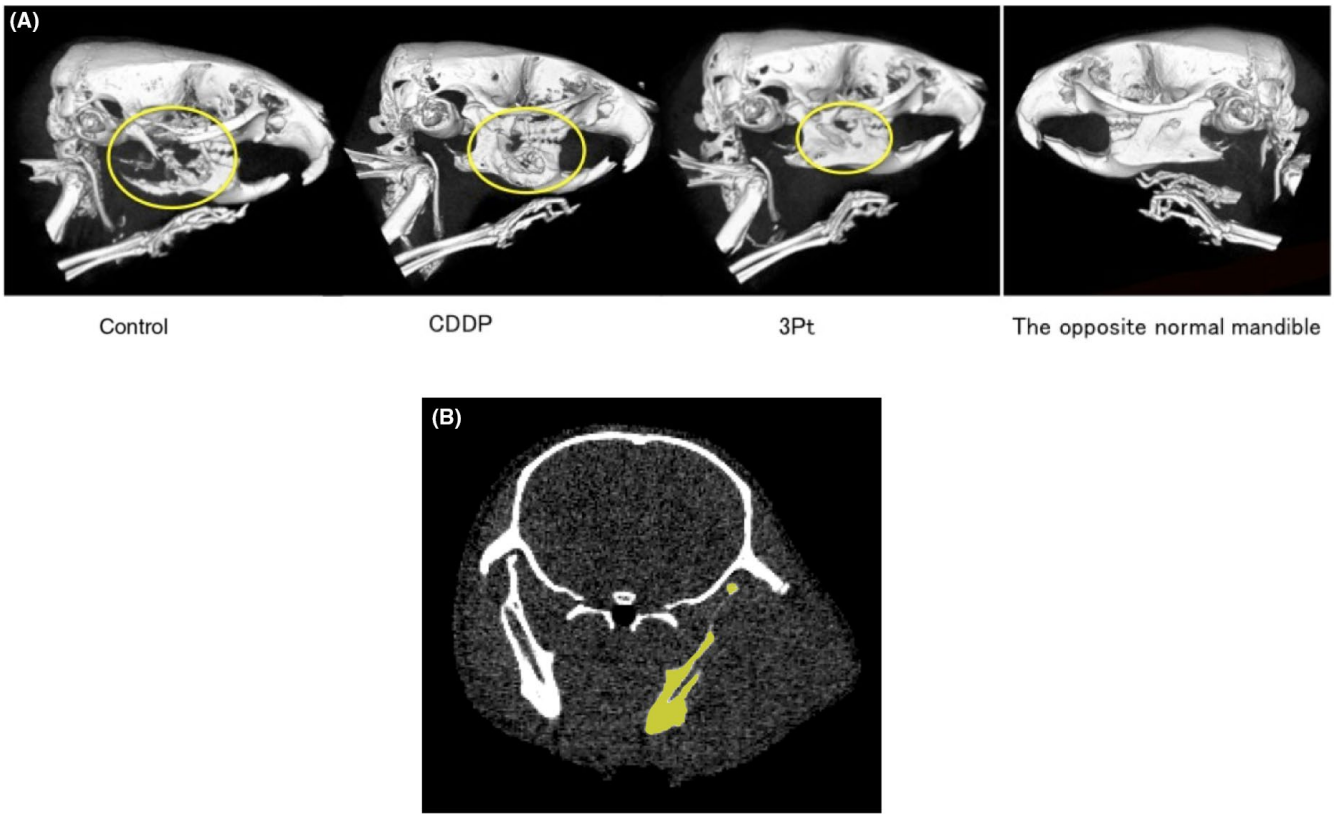

FIGURE 2 A, Mandibular bone resorption volume was calculated using microcomputed tomography sections and reconstruction was done with a reconstruction system. Bone invasion is indicated by circle. B, Volume measurements were carried out by mapping the bones in coronal section and stacking them. Yellow area on the right is the mandibular bone that has been dissolved by the tumor and the white area on the left is the normal mandible

single dose of either CDDP or 3Pt was given i.v. (5 mg/kg elemental Pt). Then, the mice were killed at defined time points (1, 24, 48, and 168 hours), and the right femoral bone and blood were collected. Blood samples were collected from the inferior vena cava, and were heparinized and centrifuged to obtain plasma. Fetal bone samples were treated with nitric acid. Plasma and bone Pt concentrations were measured using iCAP-6300 (Thermo Fisher Scientific, Waltham, MA, USA).

2.8 | Antitumor activity of 3Pt in a bone invasion model

A mouse model of bone invasion was developed through injection of OSCC cells in the masseter region.[15] A total of 1×10^6 OSC-19 cells were injected into the left masseter region. After 7 days, all mice showed tumor formation, and the tumor-bearing mice were randomly allocated to the following groups: control group, CDDP group, and 3Pt group. Drugs were injected into the tail vein each week, with two doses in total. Mice in the respective groups were given a single i.v. injection of 5% glucose solution (control group), CDDP (5 mg/kg; CDDP group), or 3Pt (an equivalent dose of 5 mg/kg CDDP; 3Pt group).

At the end of 3 weeks, microcomputed tomography (μ-CT) scans of the heads of all mice were carried out. Reconstruction was done with a reconstruction system (3-D slicer 4.6. Brigham and Women's Hospital Surgical Planning Lab) (Figure 2A). Volume measurements were carried out by mapping the bones in coronal section and stacking them. Yellow area on the right is the mandibular bone that has been dissolved by the tumor and the white area on the left is the normal mandible (Figure 2B).

Mandibular bone resorption volume was calculated using μ-CT sections and computer modeling (Aloka Latheta LCT-200; Hitachi, Tokyo, Japan) by comparing with the opposite normal mandible (Figure 3).

2.9 | Statistical analysis

Data are expressed as mean ± SD. Values were analyzed using one-way repeated-measures ANOVA followed by the Turkey post-hoc test to determine differences among groups. SPSS statistical software (Version 23; IBM, Armonk, NY, USA) was used for all analyses. *P*-value <.05 was considered statistically significant.

TABLE 1 Fifty per cent inhibitory concentration (IC_{50}) values of cisplatin (CDDP) and 3Pt (μmol/L)

	IC_{50}			
	48 h		72 h	
Cell line	CDDP	3Pt	CDDP	3Pt
OSC-19	19.4	53.6	17.4	47.6
OSC-20	8.0	17.3	19.1	39.2

3 | RESULTS

3.1 | Sensitivity of OSCC cells to CDDP and 3Pt

Cytotoxicity of 3Pt was evaluated in comparison with that of CDDP in oral carcinoma cell lines. IC_{50} values, calculated from dose-survival curves obtained after 48 and 72 hours of treatment in the MTS assay, were examined (Table 1). IC_{50} values for CDDP ranged from 8.0 to 19.4 μmol/L at 48 hours and from 17.4 to 19.1 μmol/L at 72 hours. 3Pt showed a growth inhibitory potency lower than that of CDDP, with IC_{50} values ranging from 17.3 to 53.6 μmol/L at 48 hours and from 39.2 to 47.6 μmol/L at 72 hours. The growth-inhibitory effect of CDDP in vitro was approximately 2.5-fold higher than that of 3Pt.

3.2 | Antitumor activity of 3Pt in an OSC-19 xenograft

The antitumor activity of 3Pt or CDDP was evaluated in oral carcinoma-bearing mice. All mice survived the experiment. In OSC-19 xenografts, the tumor started to grow on day 14. On day 35, mice treated with either CDDP or 3Pt showed 2.5- or 6.8-fold higher tumor growth inhibition, respectively, when compared to that in the control group (Figure 4). Reductions in tumor size after giving 3Pt or

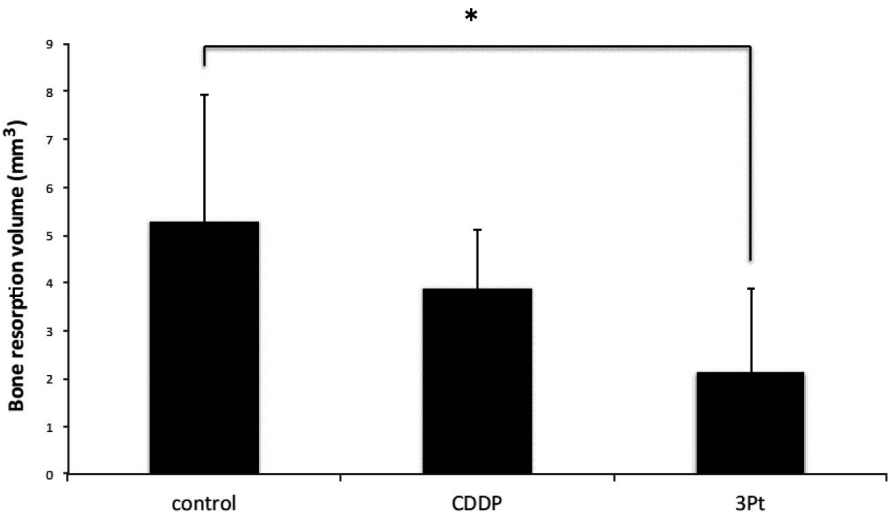

FIGURE 3 Antitumor activity of 3Pt in a bone invasion model. Dissolution volume was the largest in the control group (mean volume, 5.299 ± 2.636 mm^3), followed by the CDDP group (mean volume, 3.896 ± 1.224 mm^3) and 3Pt group (mean volume, 2.130 ± 1.768 mm^3). Mean dissolution volume was larger in the control group than in the 3Pt group (*P = .031)

CDDP were approximately 20%-29%, and there was no significant difference between the groups (P = .728).

3.3 | Decreased nephrotoxicity of 3Pt compared with CDDP in mice

Nude mice given CDDP or 3Pt at a dose of 10 mg/kg were used to evaluate the structural and functional consequences of CDDP-induced nephrotoxicity. In the control group, no apoptosis was detected in the tubule cells of the kidney. Drug-induced tubule cell apoptosis was recognized as TUNEL staining. Apoptotic cells from CDDP or 3Pt-treated mice were counted and scored. Number of apoptotic cells in 3Pt-treated mice was lower than that in CDDP-treated mice (P = .035) and was similar to that in control mice (Figure 5).

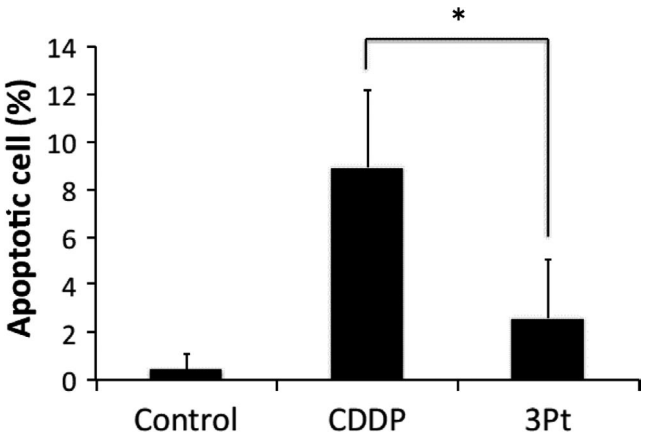

FIGURE 5 Low renal toxicity of 3Pt. Mice were treated with cisplatin (CDDP) (10 mg/kg) or 3Pt (an equivalent dose of 10 mg/kg CDDP), and kidney sections were obtained on day 28 after drug administration. Bars indicate standard deviations. *P = .035 for CDDP vs 3Pt. Each group included five mice

3.4 | Platinum concentration in the plasma and bone after 3Pt treatment

Time course of elemental Pt in mice plasma and bone after giving i.v. either CDDP or 3Pt was measured by inductively coupled plasma. After injection of CDDP or 3Pt, samples were collected at 1, 24, 72, and 168 hours (Figure 6).

Plasma concentration of Pt after giving CDDP or 3Pt decreased with time and Pt of 3Pt was cleared from circulation more rapidly than CDDP (Figure 6A). Bone Pt concentration of 3Pt peaked at 24 hours, and the mean Pt value was 2.5-fold higher than that with CDDP (Figure 6B). This was consistent with high affinity of 3Pt to hydroxyapatite. After 168 hours, Pt was almost cleared from the bone in both groups.

3.5 | Antitumor activity of 3Pt in the bone invasion model

Effect of 3Pt was analyzed using the bone invasion model in vivo. We analyzed bone invasion in each mouse group using 3-D reconstructions of the skull created from μ-CT sectioning as macroscopic observations by carrying out comparisons with the opposite normal mandible. Dissolution volume was the largest in the control group (mean volume, 5.299 ± 2.636 mm^3), followed by the CDDP group (mean volume, 3.896 ± 1.224 mm^3) and 3Pt group (mean volume, 2.130 ± 1.768 mm^3) (Figure 3). Mean dissolution volume was larger in the control group than in the 3Pt group (P = .031). However, the difference between the control and CDDP groups was not statistically significant (P = .394) (Figure 3).

4 | DISCUSSION

Cancer nanotechnology is a new field of interdisciplinary research aiming to enhance the methods of cancer diagnosis and treatment.[15] Micellarized cisplatin and liposomal cisplatin have made it possible to enhance efficacy and reduce side-effects, and accumulation at specific targets can be achieved by incorporating existing drugs. Recently, we reported the safety and translymphatic efficacy of NC-6004, which is cisplatin contained in micelles. NC-6004 has

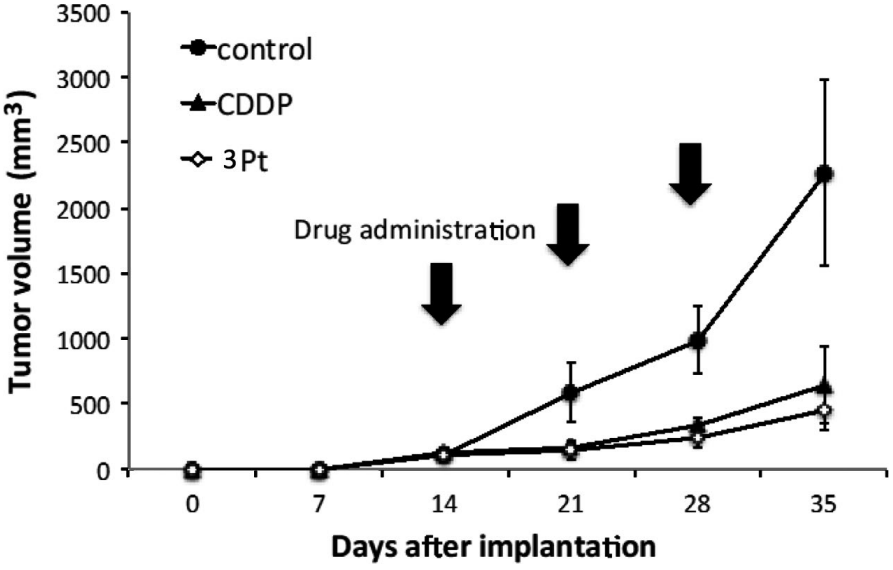

FIGURE 4 Effect of cisplatin (CDDP) or 3Pt in an OSC-19 mouse model. Each data point is the mean value (±SD) of six primary tumors

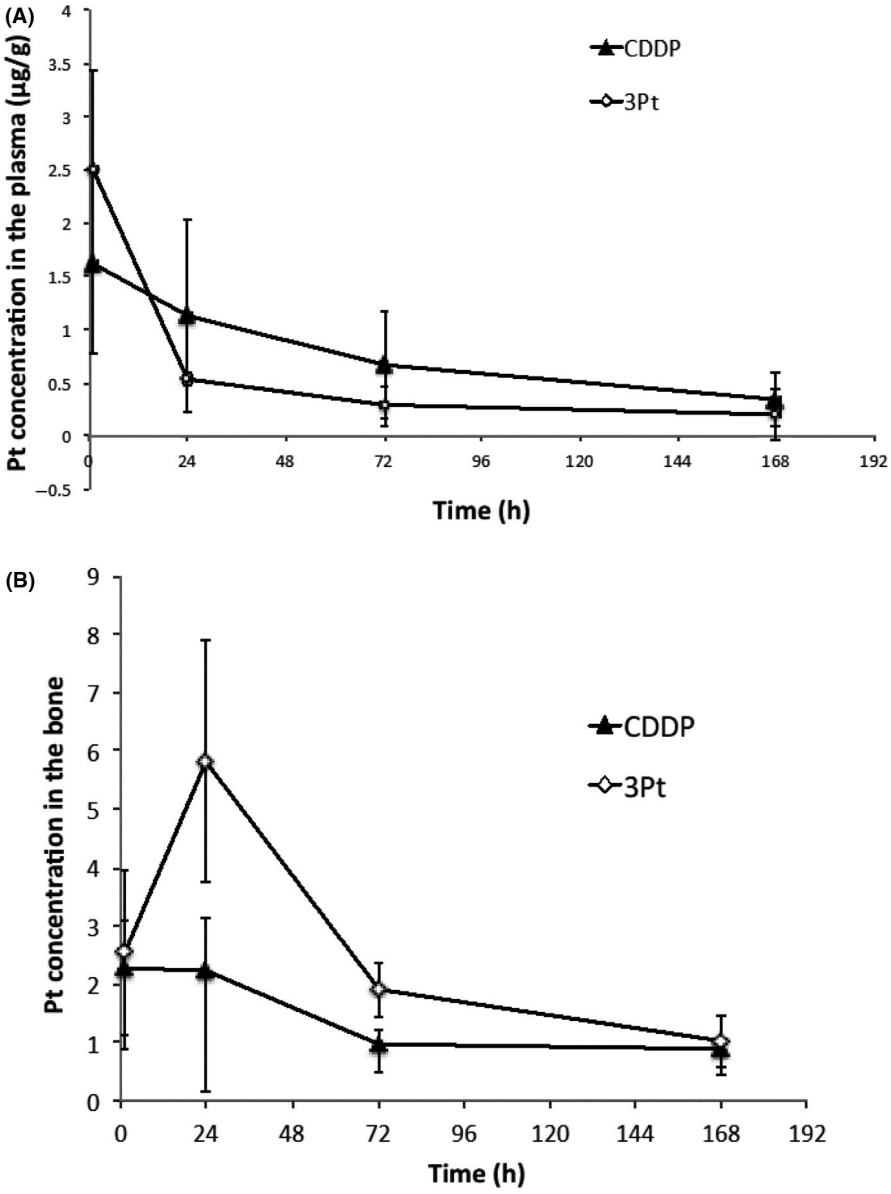

FIGURE 6 A and B, Time course of elemental platinum concentrations in the plasma (A) and bone (B) after giving i.v. CDDP (5 mg/kg) or 3Pt (an equivalent dose of 5 mg/kg CDDP). Each data point is the mean value (±SD) of six mice

progressed to a phase I clinical trial in the UK, and phase I/II trials are currently underway in East Asia.[13,16,17] In addition, we reported the safety, with regard to acute neuropathy, and antitumor efficacy of NC-4016, which is oxaliplatin contained in micelles.[18] Currently, various treatments, such as bisphosphonate preparations, are being used for bone-infiltrating cancer. Although these methods contribute to a reduction in bone pain and hypercalcemia, they do not contribute to good prognosis.[19] Thus, many new medicines, which are based on the concept of drug delivery system (DSS), have been developed and applied in the clinic, but no new drugs targeting bone invasion have been discovered so far. From the standpoint of DDS targeting solid carcinoma, a novel anionic platinum complex 3Pt was developed to achieve high accumulation at bone sites. 3Pt was shown to have potent antitumor effects on osteosarcoma cell lines and in an orthotopic mouse model. 3Pt may be an effective drug for the treatment of bone cancer because the PO_3 moiety of this complex has a high affinity for bone.[20]

The success of CDDP lies in its ability to arrest DNA synthesis, induce oxidative stress, and activate apoptotic pathways in tumor cells.[21] The in vitro growth-inhibiting effect of the anionic platinum complex 3Pt was weaker than that of cisplatin in OSC-19. In addition to the in vitro assay, we evaluated the in vivo antitumor activity of cisplatin and 3Pt in a mouse model of human carcinoma. As a result, there were no significant differences in the growth-inhibitory effect between CDDP and 3Pt. From these results, it was concluded that the efficacy of 3Pt against OSCC cell lines is similar to that of CDDP in vivo. The different results between these two drugs may be associated with the complex Pt structure. Some studies reported varied cytotoxicity levels depending on the complex Pt structure.[22] For example, the growth-inhibitory effects of NC-6004 and NC-4016 were significantly less than the effects of CDDP and oxaliplatin in vitro, but were not significantly different in vivo.[13,18]

Nephrotoxicity is one of the most significant adverse effects of CDDP.[23] CDDP accumulates in cells from all nephron segments, but

is preferentially taken up by highly susceptible proximal tubular epithelial cells, which experience the most damage. This nephrotoxicity limits the dose that can be given and reduces the potential efficacy of CDDP.[24] The number of apoptotic renal cells in 3Pt-treated mice was significantly lower than that in CDDP-treated mice by approximately 66% (CDDP, 8.9%; 3Pt, 2.6%) and was similar to that in control mice. In a recent study, 3Pt showed less creatinine rise compared to that with cisplatin.[9]Pt showed excellent aqueous solution solubility, which could avoid renal secretion.[9] Furthermore, the absorption of 3Pt to bone may serve as a buffer and reduce accumulation in the kidney, which may be one factor that reduces renal injury. These findings indicate that the efficacy of 3Pt against OSCC cell lines is similar to that of CDDP, but with much less renal toxicity in the host.

Clinically, head and neck cancer with bone invasion is resistant to chemoradiation therapy.[5] The present study showed that Pt concentration in the bone was higher with 3Pt than with CDDP, with a peak at 24 hours. This is important as anticancer agents that specifically accumulate in bone have not been developed so far. This accumulation is due to 12 PO_3 groups of 3Pt having high affinity with bone. The cytotoxicity of cisplatin shows concentration dependence and time dependence. From the standpoint of DDS targeting bone invasion carcinoma, higher Pt concentrations in the bone may be associated with a stronger antitumor effect with 3Pt than with cisplatin in an in vivo bone invasion mouse model. In our in vivo murine model, bone resorption volumes in the CDDP and 3Pt groups were approximately 26% and 60% less than the volume in the control group, respectively, with significant differences between the groups. The findings suggest that the antitumor effect of 3Pt against bone invasion is more potent than that of CDDP in vivo.

In conclusion, the present study showed that the safety and antitumor efficacy of 3Pt was superior to that of cisplatin in a bone invasion mouse model of human carcinoma cell line OSC-19.

Reductions in the nephrotoxicity of 3Pt might allow patients to undergo therapy without hospitalization for hydration and treatment of CDDP-related toxicity.[17] Patients who have had to avoid treatment with cisplatin owing to renal disorders or heart failure may have another treatment opportunity with 3Pt. 3Pt can be a potential treatment option for patients with solid cancer who have had bone invasion. In the present study, evaluation was carried out on bone invasion but, based on the possible mechanism, the effect can be expected for bone-metastasized cancer.

ACKNOWLEDGMENT

This work was supported by the Japan Society for the Promotion of Science KAKENHI (grant number 16K20234).

CONFLICTS OF INTEREST

Y. Kasahara received a research fund from the Japan Society for the Promotion of Science. Other authors have no conflicts of interest directly relevant to the content of this article.

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REFERENCES

1. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer*. 2011;11:9-22.
2. Boulikas T, Vougiouka M. Recent clinical trials using cisplatin, carboplatin and their combination chemotherapy drugs (review). *Oncol Rep*. 2004;11:559-595.
3. Seiwert TY, Salama JK, Vokes EE. The chemoradiation paradigm in head and neck cancer. *Nat Clin Pract Oncol*. 2007;4:156-171.
4. Pinzani V, Bressolle F, Haug IJ, Galtier M, Blayac JP, Balmes P. Cisplatin-induced renal toxicity and toxicity-modulating strategies: a review. *Cancer Chemother Pharmacol*. 1994;35:1-9.
5. Quan J, Newell WJ, Guangbiao Z, Peter GP, Glen MB, Jin G. Potential molecular targets for inhibiting bone invasion by oral squamous cell carcinoma: a review of mechanisms. *Cancer Metastasis Rev*. 2012;31:209-219.
6. Pignon J, Bourhis J, Domenge C, Designe L. Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: three meta-analyses of updated individual data. MACH-NC Collaborative Group. Meta-Analysis of Chemotherapy on Head and Neck Cancer. *Lancet*. 2000;355(9208):949-955.
7. Brown JS, Browne RM. Factors influencing the patterns of invasion of the mandible by oral squamous cell carcinoma. *Int J Oral Maxillofac Surg*. 1995;24:417-426.
8. Li X, Di B, Shang Y, Zhou Y, Cheng J, He Z. Clinicopathologic risk factors for distant metastases from head and neck squamous cell carcinomas. *Eur J Surg Oncol*. 2009;35:1348-1353.
9. Odani A. Metal complexes and anticancer agents comprising same as active ingredient. Patent WO 2011125911 A1. 2011; 13 October.
10. McClung M, Harris ST, Miller PD, et al. Bisphosphonate therapy for osteoporosis: benefits, risks, and drug holiday. *Am J Med*. 2013;126:13-20.
11. Holen I, Coleman RE. Bisphosphonates as treatment of bone metastases. *Curr Pharm Des*. 2010;16:1262-1271.
12. Jobke B, Milovanovic P, Amling M, Busse B. Bisphosphonate-osteoclasts: changes in osteoclast morphology and function induced by antiresorptive nitrogen-containing bisphosphonate treatment in osteoporosis patients. *Bone*. 2014;59:37-43.
13. Endo K, Ueno T, Kondo S, et al. Tumor-targeted chemotherapy with the nanopolymer-based drug NC-6004 for oral squamous cell carcinoma. *Cancer Sci*. 2013;104:369-374.
14. Supavekin S, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC, Devarajan P. Differential gene expression following early renal ischemia/reperfusion. *Kidney Int*. 2003;63:1714-1724.
15. Takeshi N, Takahiko S, Akira K, Shizuka M, Nobuo T. Establishment of a murine model of bone invasion by oral squamous cell carcinoma. *Oral Oncol*. 2007;43:257-262.
16. Manchun S, Dass CR, Sriamornsak P. Targeted therapy for cancer using pH-responsive nanocarrier systems. *Life Sci*. 2012;90:381-387.
17. Plummer R, Wilson RH, Calvert H, et al. A Phase I clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours. *Br J Cancer*. 2011;104:593-598.
18. Ueno T, Endo K, Hori K, et al. Assessment of antitumor activity and acute peripheral neuropathy of 1,2-diaminocyclohexane platinum (II)-incorporating micelles (NC-4016). *Int J Nanomedicine*. 2014;9:3005-3012.
19. Wong MH, Stockler MR, Pavlakis N. Bisphosphonates and other bone agents for breast cancer. *Cochrane Database Syst Rev*. 2012;2:CD003474.

20. Igarashi K, Yamamoto N, Hayashi K, Miwa S, Odani A, Tsuchiya H. Effectiveness of two novel anionic and cationic platinum complexes in the treatment of osteosarcoma. *Anticancer Agents Med Chem*. 2015;15:390-399.
21. Boulikas T, Vougiouka M. Cisplatin and platinum drugs at the molecular level (Review). *Oncol Rep*. 2003;10:1663-1682.
22. Hambley TW. The influence of structure on the activity and toxicity of Pt anti-cancer drugs. *Coord Chem Rev*. 1997;166:181-223.
23. Safirstein R, Winston J, Goldstein M, Moel D, Dikman S, Guttenplan J. Cisplatin nephrotoxicity. *Am J Kidney Dis*. 1986;8:356-367.
24. Townsend DM, Deng M, Zhang L, Lapus MG, Hanigan MH. Metabolism of cisplatin to a nephrotoxin in proximal tubule cells. *J Am Soc Nephrol*. 2003;14:1-10.

How to cite this article: Kasahara Y, Endo K, Ueno T, et al. Bone invasion-targeted chemotherapy with a novel anionic platinum complex (3Pt) for oral squamous cell carcinoma. *Cancer Sci*. 2019;110:3288–3295. https://doi.org/10.1111/cas.14145