Regular Article

Oxaliplatin Alters Expression of T1R2 Receptor and Sensitivity to Sweet Taste in Rats

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As one of the adverse effects of oxaliplatin, a key agent in colon cancer chemotherapy, a taste disorder is a severe issue in a clinical situation because it decreases the quality of life of patients. However, there is little information on the mechanism underlying the oxaliplatin-induced taste disorder. Here, we examined the molecular and behavioral characteristics of the oxaliplatin-induced taste disorder in rats. Oxaliplatin (4–16 mg/kg) was administered to Sprague-Dawley (SD) rats intraperitoneally for 2 d. Expression levels of mRNA and protein of taste receptors in circumvallate papillae (CP) were measured by real-time quantitative polymerase chain reaction (PCR) and immunohistochemistry, respectively. Taste sensitivity was assessed by their behavioral change using a brief-access test. Morphological change of the taste buds in CP was evaluated by hematoxyline–eosin (HE) staining, and the number of taste cells in taste buds was counted by immunohistochemical analysis. Among taste receptors, the expression levels of mRNA and protein of T1R2, a sweet taste receptor subunit, were increased transiently in CP of oxaliplatin-administered rats on day 7. In a brief-access test, the lick ratio was decreased in oxaliplatin-administered rats on day 7 and the alteration was recovered to the control level on day 14. There was no detectable alteration in the morphology of taste buds, number of taste cells or plasma zinc level in oxaliplatin-administered rats. These results suggest that decreased sensitivity to sweet taste in oxaliplatin-administered rats is due, at least in part, to increased expression of T1R2, while these alterations are reversible.

Key words taste disorder; oxaliplatin; chemotherapy; adverse effect; T1R2

Taste sensing plays a critical role in nutrition in mammals, and taste is perceived as five basic tastes, sweet, umami, salty, sour and bitter. The taste perception system is localized to taste buds in papillae such as circumvallate papillae (CP), fungiform papillae and foliate papillae of the tongue. In taste buds, various taste receptors are expressed by taste cells and their stimulation by specific tastants is transmitted by taste nerves to the brain, where the tastes are perceived.

Taste cells are classified into four types, types I–IV, according to their morphological characteristics.1,2 Among them, type II taste cells express sweet taste receptors T1R2/T1R3, umami taste receptors T1R1/T1R3 and metabotropic glutamate receptor 4 (mGluR4), and bitter taste receptors T2Rs, these receptors being involved in recognition of the corresponding tastants.3–7 Type III taste cells are thought to be concerned in taste signal transduction.8) Homeostasis of this taste-sensing system is important for maintenance of the physiological condition, especially the nutrition status. Onoda et al.9 reported decreased T2R expression in the tongue tissues of patients with hypogeusia, suggesting that altered expression levels of taste receptors may be a determinant of taste sensitivity.

It is well-known that a deficiency of zinc, an essential trace element, induces a taste disorder.10 In addition to inadequate intake of zinc from food, a zinc-chelatable drug, n-penicillamine, is reported to induce a taste disorder.11 Watanabe et al.12 found that the zinc levels in saliva of patients with taste disorders were lower than those in healthy subjects. Thus, in clinical situations, zinc is prescribed to patients with taste disorders, but some of such patients do not respond to zinc supplementation,13 implying that a zinc-independent mechanism might be involved in dysfunction of the taste-sensing system.

A taste disorder is one of the adverse effects of cancer chemotherapy, resulting in a loss of appetite, malnutrition and a decrease in the quality of life of patients.14 A taste disorder induced by cyclophosphamide, an anticancer drug,15,16 has been demonstrated to be due to a decreased number of taste cells and atrophy of taste buds in mice.17,18 Oxaliplatin, a platinum anticancer drug, is used for the treatment of colon cancer19 and is known to induce a taste disorder,14 but the mechanism underlying the taste disorder is unknown.

To determine the molecular and behavioral characteristics of the oxaliplatin-induced taste disorder, in the present study, we examined the effect of oxaliplatin on the expression levels of taste receptors, taste sensitivity, plasma zinc levels, morphology of taste buds and number of taste cells in oxaliplatin-administered rats.

MATERIALS AND METHODS

Chemicals Collagenase D (#1088858) and dispase II (#4942078) were purchased from Roche Applied Science (Tokyo, Japan). A trypsin inhibitor (#T9128) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). All other chemicals and reagents were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), except where otherwise noted.

Animals and Treatment Male Sprague-Dawley (SD) rats (200–300 g; Japan SLC, Hamamatsu, Japan) were housed with food and water available ad libitum in a controlled environment with a 12h/12h light/dark cycle. All experiments were

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RNA was extracted and reverse transcribed with a NucleoSpin. Total RNA later® solution (Sigma-Aldrich) and kept at −20°C until use. In control rats, the same volume of the 5% glucose solution was injected i.p. with the same dosing schedule.

Exfoliation of Epithelial Tissue Including Rat CP  Rats were perfused transcardially with saline under deep anesthesia (pentobarbital sodium, 25 mg/kg, i.p.). As reported previously, rat lingual epithelial tissues containing CP were exfoliated from the tongue by injection of an enzyme cocktail comprising 2.5 mg/mL dispase II, 1.0 mg/mL collagenase D, and 1.0 mg/mL trypsin inhibitor for 30 min at room temperature (r.t.), and then the epithelial tissues were treated with an RNAlater® solution (Sigma-Aldrich) and kept at −20°C until use.  

Reverse Transcription (RT) and Real-Time Quantitative Polymerase Chain Reaction (PCR) Analyses  Total RNA was extracted and reverse transcribed with a NucleoSpin RNA® XS kit (Macherey-Nagel, Düren, Germany), and a PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Shiga, Japan) according to the manufacturer's instruction manuals. Real-time quantitative PCR was conducted with an ABI PRISM 7500 Real-time PCR System (Life Technologies, Tokyo, Japan) using SYBR Premix Ex Taq (TaKaRa). The primer sets are shown in Table 1. All reactions for real-time quantitative PCR were carried out with the following parameters: 94°C for 5 min, and 40 cycles of 94°C for 30 s, 60°C for 60 s, and 72°C for 15 s. Negative control experiments were performed using DNase-free water instead of the template DNA, and their specific amplification was confirmed using dissociation curves.

Tissue Preparation  Animals were perfused transcardially with 4% paraformaldehyde in 0.15 m phosphate buffer (pH 7.4) containing 0.2% picric acid under deep anesthesia (pentobarbital sodium, 25 mg/kg, i.p.), and then their tongues were removed. The tongues were sectioned at 40 µm thickness with a freezing microtome (Leica CM1850; Leica, Nussloch, Germany), and then the sections were subjected to immunohistochemical analysis and hematoxylin–eosin (HE) staining.

Immunohistochemical Analysis  The expression levels of antigens were investigated by free-floating immunohistochemistry. The free-floating sections were immunoreacted with primary antibodies (Table 2) in phosphate buffered saline.

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Table 1. Primers Used for Quantitative Real-Time PCR

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Table 2. Antibodies Used for Immunohistochemistry

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<td>NTPDase2</td>
<td>Sheep anti-NTPDase2 Ab (1:200; AF5797, R&amp;D Systems)</td>
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<td>PLC-β2</td>
<td>Rabbit anti-PLC-β2 Ab (1:1000; sc206, Santa Cruz Biotechnology)</td>
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<td>AADC</td>
<td>Rabbit anti-AADC Ab (1:200; BML-AZ1030-0050, Enzyn)</td>
<td>Donkey anti-rabbit IgG conjugated with Alexa Fluor® 488 (1:1000; #A21206, Life Technologies)</td>
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<td>5-HT</td>
<td>Rabbit anti-5-HT Ab (1:50; S5545, Sigma-Aldrich)</td>
<td>Donkey anti-rabbit IgG conjugated with Alexa Fluor® 488 (1:1000; #A21206, Life Technologies)</td>
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(PBS) containing 1% donkey or goat serum, 0.3% Triton-X-100, 0.3% BSA and 0.05% sodium azide for 3 d at 4°C, followed by incubation for a day at 4°C with secondary antibodies (Table 2) in the same buffer as that for the primary antibodies. For all immunostaining, a negative control, which was prepared by omitting the primary antibodies, was prepared, and the reproducibility of immunostaining was confirmed by assessing sections from 3 or 4 rats per immunostaining. The sections were mounted on glass slides and then enclosed using a Prolong® antifade kit (Life Technologies). Photomicrographs were obtained under a confocal laser microscope (LSM510META; Carl Zeiss, Jena, Germany). Fluorescence intensity was measured using the histogram program of the Photoshop software (Adobe Systems, San Jose, CA, U.S.A.).

**Brief-Access Test** We adopted the brief-access test to evaluate the effects of oxaliplatin on behavioral responses to tastants in rats. Rats were randomly divided into the two groups, control and oxaliplatin ones. All training and test sessions were performed during the light phase of the light/dark cycle. The rats had restricted access to water for more than 20 h before each training or test session. A training session was performed to obtain a stable lick number and was performed for 6 d before initiation of a test session. On the first day of a training session, so that it would get used to the experimental apparatus, a rat was placed in a test box (a black box to shield from light; width 36.5 cm, depth 21.5 cm and height 25.5 cm) and was given free-access to distilled water for 15 min from a polypropylene tube via an elliptical window (major axis: 15 mm, minor axis: 10 mm). A lick counter system (INECK, Kyoto, Japan) was set up between the edge of the tube and the window, and the lick number was determined automatically by recording the number of interceptions of the sensor beam by the tongue of the rat as it licked the solution from the edge of the tube. On the second day of the training session, the rat was trained to drink distilled water on an interval schedule, consisting of a 10-s period of presentation of distilled water and a 20 s inter-presentation interval, and this schedule was repeated 15–25 times. On the third day, the training was performed with the same procedure as that for a test session and this schedule was repeated 15–25 times. On the fourth day, the training was performed with the same procedure as for a test session and this schedule was repeated 15–25 times. On the third day of a training session, so that it would get used to the experimental apparatus, a rat was placed in a test box (a black box to shield from light; width 36.5 cm, depth 21.5 cm and height 25.5 cm) and was given free-access to distilled water for 15 min from a polypropylene tube via an elliptical window (major axis: 15 mm, minor axis: 10 mm). A lick counter system (INECK, Kyoto, Japan) was set up between the edge of the tube and the window, and the lick number was determined automatically by recording the number of interceptions of the sensor beam by the tongue of the rat as it licked the solution from the edge of the tube. On the second day of the training session, the rat was trained to drink distilled water on an interval schedule, consisting of a 10-s period of presentation of distilled water and a 20 s inter-presentation interval, and this schedule was repeated 15–25 times. On the third day, the training was performed with the same procedure as on the second day except for the use of a 0.3 M sucrose solution instead of distilled water. On the fourth day, the 0.3 M sucrose solution and a 1 mM quinine hydrochloride (QHCl) solution were presented to the rat for 10 s alternatively with 10-s-presentation of distilled water as an interval between the two taste solutions. On the 5th or 6th day, the training was performed with the same procedure as that for a test session described below, and the lick numbers obtained in this session were used as the base data (day 0). After a 20 h-deprivation period, rats were allowed to drink distilled water for 2 min freely to quench their excess thirst. Then, each rat was subjected to the test session. In the test session, the lick numbers were determined 3, 7, 10 and 14 d after initiation of oxaliplatin administration. The test session was designed to detect the concentration-dependent response to sucrose or QHCl for quantification of sweet or bitter taste sensitivity.23,24 The taste solutions used in the experiments were prepared as mixtures of a concentration range of sucrose (0.01 to 1 mM) and 1 mM QHCl or a single taste solution of a concentration range of QHCl (0.001 to 1 mM). The use of a mixture of sweet and bitter taste solutions has been reported to allow sensitive detection of a change in sweet taste sensitivity, because a rat, which can not detect the sweet tastant in the mixture, perceives only the bitter taste, and thus avoids drinking the mixture.25 A series of sucrose–QHCl mixtures was presented with a descending order of sucrose concentrations in the mixtures, while QHCl solutions were presented with an ascending order of QHCl concentrations. During the test session, the taste solution and distilled water were alternatively presented to the rat for 10 and 5 s, respectively, presentations of distilled water being designed to rinse the oral cavity. Data were expressed as lick ratios as a quantitative index of taste sensitivity, which were calculated by dividing the lick number for a taste solution in 10 s by that for distilled water. This is because the lick number is affected by the differences in motivation to drink solutions among rats. We excluded the data for rats that could not accomplish a series of lick tests on each test day.

**Sampling of Saliva** Saliva collection was performed following the method reported previously.21 In brief, rats were anesthetized with pentobarbital sodium (25 mg/kg, i.p.), and then injected with pilocarpine (1.25 mg/kg, i.p.) for complete excretion of saliva from the salivary glands. The excreted saliva was collected using sterilized cotton for 20 min, and then the cotton was weighed, and used for determination of the zinc and platinum contents.

**Determination of Zinc and Platinum Concentrations** According to a previous report,25 we measured the zinc and platinum concentrations in saliva and plasma, both of which were collected on days 3 and 7 after oxaliplatin administration. Samples were subjected to a wet-ashing process involving nitric acid, perchloric acid and hydrogen peroxide at 200–250°C, and the ashed samples were dissolved in 5 mL of 5% nitric acid followed by the addition of 6 µL of 40 ppm indium solution as an internal standard. The zinc and platinum levels were measured using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700x ICP-MS; Agilent Technologies, Santa Clara, CA, U.S.A.). Standard curves for determination of zinc and platinum levels were prepared by dilution of the multi-element standard solution BM (Wako) and platinum standard solution (Wako), respectively.

![Fig. 1. Effect of Oxaliplatin on mRNA Expression of Taste Receptors in Rat CP](image)

After administration of oxaliplatin at the total dose of 8 mg/kg, tissues containing CP were removed on days 3, 7 and 14, and then used for quantification of the mRNA expression levels of T1R1, T1R2, T1R3, T2R107 and mGluR4 in the CP by real-time PCR. Data are given as % of PLCβ mRNA expression. Each bar represents the mean±S.D. (N=3–4). The differences between control and oxaliplatin groups were compared by Mann–Whitney U test (*p<0.05).
Statistical Analysis All data were expressed as the mean±standard deviation (S.D.). The Mann–Whitney U test was used to evaluate the difference between control and oxaliplatin-administered rats. Tukey–Kramer test was used to compare the differences in fluorescence intensity between control and oxaliplatin-administered rats in immunohistochemistry of which the population was considered to be normal distribution.

Comparison of more than two groups was performed by means of the Steel test. A p-value of 0.05 or less was considered statistically significant.

RESULTS

General Toxicity To evaluate the general toxicity in oxaliplatin-administered rats, their body weight was measured on days 1 and 14. As shown in Table 3, there was no difference in their body weight between control and oxaliplatin (4–16 mg/kg)-administered rats. In addition, on visual inspection, there was no apparent change in the condition of their coats or stools.

Expression of Taste Receptors To evaluate the expression levels of taste receptors in CP, we determined the expres-
sion levels of mRNAs for T1R1, T1R2, T1R3, T2R107 and mGluR4 (Fig. 1). All of the examined mRNAs were detected in the rat CP. Compared to in the control rats, among these receptors, the expression level of T1R2 mRNA was approximately 2-fold greater in oxaliplatin-administered rats on days 3 and 7, but there was no difference in it on day 14. On im-

Fig. 3. Effect of Oxaliplatin on Sensitivity to Sweet and Bitter Taste Solutions in Rats

The lick ratios for 0.01–1.0 mM sucrose with 1 mM QHCl (A–E), and for 0.001–1.0 mM QHCl (F–H) were determined in control and oxaliplatin-administered rats on days 0, 3, 7, 10 or 14. Each point or bar represents the mean±S.D. (N=4–6). The differences between control and oxaliplatin groups were compared by Mann-Whitney U test (*p<0.05).

Fig. 4. Effect of Oxaliplatin on Morphology of Taste Buds in Rat CP

Representative photomicrographs of HE staining of rat CP from 8 mg/kg oxaliplatin-administered rats on days 3 and 7. Scale bar=50 µm.
munohistochemistry, immunoreactivity for T1R2 was detected in CP, and its fluorescence intensity was significantly greater in oxaliplatin-administered rats on day 7 than the intensity in the control and oxaliplatin-administered rats on days 3 and 14, and the control ones on day 7, indicating the alteration of T1R2 expression was transient (Figs. 2A, B). Next, we examined the dose-dependent effect of oxaliplatin administration on T1R2 expression. Although there was no change in the expression of phospholipase C-β2 (PLC-β2), a type II taste cell marker (Figs. 2C, D), the expression level of T1R2 was increased by administration of oxaliplatin at the doses of 8 and 16 mg/kg (Figs. 2C, E). These results indicated that the increase of the expression level of T1R2 in the rat CP was specifically due to the oxaliplatin administration, and that this alteration was transient.

**Sweet Taste Sensitivity** Since oxaliplatin increased T1R2 expression in the rat CP, we assessed sweet taste sensitivity in rats. On day 0, for which data were obtained on the 5th or 6th day of the training session, the lick ratios for a sucrose solution mixed with 1 mM QHCl exhibited a sucrose concentration-dependent increase, and there was no difference between the control and oxaliplatin groups (Fig. 3A). On days 3 and 7, the lick ratios were lower in oxaliplatin-administered rats than in control ones with 0.3 and 1 M, and 0.1 and 0.3 M sucrose, respectively (Figs. 3B, C), but there was no difference in their lick ratios on days 10 and 14 (Figs. 3D, E).

In this experiment, we used the sucrose and QHCl mixture as the sweet taste solution, and thus there is a possibility that

![Fig. 5. Effect of Oxaliplatin on Numbers of Taste Cells in Rat CP](image-url)
the decrease in the lick ratio for the mixture in the oxaliplatin group might have been due to a change in the bitter taste sensitivity of the rats. However, as shown in Figs. 3F, G and H, there were no differences in the lick ratios between the control and oxaliplatin-administered rats on days 3 and 7 as the same on day 0. Based on these results, it is suggested that oxaliplatin administration induces a transient decrease in the sweet taste sensitivity in rats.

**Morphology of Taste Buds and Number of Taste Cells**

To confirm the effect of oxaliplatin on the morphology of taste buds in CP, we performed histological analysis of them by HE staining. As shown in Fig. 4, an apparent morphological alteration such as atrophy was not detected in the taste buds of oxaliplatin-administered rats on days 3 and 7. Next, we examined as to whether the numbers of taste cells in the CP were altered by counting types II and III taste cells by immunostaining for PLC-β2 and α-gustducin,27,28 and aromatic acid decarboxylase (AADC) and 5-hydroxytriptamine (5-HT),2,29 respectively. Oxaliplatin-administration had no effect on the numbers of PLC-β2- and α-gustducin-positive type II taste cells (Figs. 5A–F), or AADC and 5-HT-positive type III taste cells (Figs. 5G–L). These results imply that oxaliplatin has no or only a negligible effect on the number of taste cells.

**Zinc and Platinum Levels in Plasma and Saliva**

As shown in Figs. 6A, B, there was no difference in the zinc levels in either plasma or saliva between control and oxaliplatin-administered rats on days 3 and 7. The platinum levels in plasma had increased dose-dependently by day 3, and a similar tendency was observed on day 7, but the platinum levels on day 7 were apparently lower than those on day 3 (Fig. 6C). In saliva, platinum was detected on day 3 in the oxaliplatin-administered group with the doses 8 and 16 mg/kg, but on day 7, it was under the detection limit of the assay (Fig. 6D).

**DISCUSSION**

In this study, we examined the effect of oxaliplatin on taste function in rats, and obtained the following results: in oxaliplatin-administered rats, (1) the expression level of T1R2 was transiently increased, (2) the sweet taste sensitivity was decreased, (3) their alteration was reversible, and (4) there was no alteration in the morphology of the taste buds, number of the taste cells or plasma zinc levels. Together, it was suggested that the oxaliplatin-induced taste disorder as to sweet taste was reversible.

Appropriate intake of food provides us with not only nutrients but also pleasure, and thus taste perception is important to maintain the nutrition status and regulate the emotional status. In patients receiving cancer chemotherapy, decrease of appetite is frequently induced by alteration of taste sensitivity on administration of anticancer drugs, which leads to malnutrition and deterioration of the physical condition with decreased quality of life, resulting in discontinuance of the chemotherapy.30

In our study, we found that oxaliplatin administration to rats increased the expression level of T1R2 in CP, and that their sensitivity to sweet taste was unexpectedly decreased (Figs. 1–3). In general, increase of expression of taste receptors is associated with increase of taste sensitivity.31 In fact, very recently, Tsutsumi et al. reported that suppression of T1R3 gene expression induced by chemotherapy including cisplatin resulted in the hypoperception of umami and sweet tastes in patients with head and neck cancer.32 In addition, oxaliplatin is reported to increase sweet taste perception in patients.33 There is an apparent conflict in profiles in platinum anticancer drug-induced alteration of sweet taste perception between human and rodents. Jiang et al.33 indicated that there were species differences in sweet taste perception. Shimizu et al.34 reported that human T1R3 required co-expression...
of human T1R2 for its membrane trafficking, while such an interaction between mouse T1R3 and T1R2 was not found. Thus, alteration profiles in platinum anticancer drug-induced sweet taste perception might be different between human and rat. On the other hand, Tsutsumi et al. revealed that chemotherapy-induced increase of gene expression of T2R5, a bitter taste receptor, had no detectable effect on bitter taste perception in patients.\(^{32}\) Together, it is suggested that expression levels of taste receptor are a determinant of taste sensitivity, but other factors such as change in taste signaling system are also involved in alteration of taste perception. To obtain definitive findings, detail investigations are now in progress in our laboratory.

Taste cells in taste buds undergo turnover for about 10 days.\(^{35,36}\) In this study, increase of T1R2 receptor and decrease of sweet taste sensitivity were recovered to the control level on day 14. The times for recovery of them in oxaliplatin-administered rats and the turnover of the taste cells were almost identical, suggesting that taste cells with increased expression of T1R2 might be replaced by the newborn ones with normal expression of it. In general, chemotherapy-induced taste disorder is relieved by termination of the drug administration in clinical situations.\(^{37,38}\) It was reported that cisplatin-containing chemotherapy induced alteration of sweet taste sensitivity 2 weeks after the administration, and then the alteration was returned to pretreatment levels at 4 weeks posttreatment.\(^{39}\) Therefore, these findings imply that transient alteration of sweet perception might be common to platinum anticancer drugs, while profiles of the onset and/or disappearance and degree of the symptom are considered to differ among the drugs with different pharmacokinetic/pharmacodynamic characteristics.

Zinc plays an important role in cell proliferation and differentiation.\(^{39}\) In humans, hypogeusia is one of the zinc-deficiency symptoms,\(^{10}\) and zinc levels in saliva were decreased in patients with a taste disorder.\(^{12}\) Chronic zinc-deficiency in rats caused morphological changes in taste buds, and decreases of taste cell numbers and taste sensitivity.\(^{10,40–43}\) Additionally, it has been reported that zinc chelation by drugs is one of the mechanisms of a drug-induced taste disorder.\(^{10}\) In this study, oxaliplatin administration did not affect the zinc level in either plasma or saliva (Fig. 6), the morphology of taste buds (Fig. 4), or the numbers of taste cells (Fig. 5), indicating that the oxaliplatin-induced taste disorder was not due to zinc-deficiency. Previously, atrophy of the taste buds and a decrease of the taste cells were detected in cyclophosphamide-administered mice\(^{7}\) and X-ray-irradiated ones.\(^{44}\) Because such observations were not made in oxaliplatin-administered rats, suppression of taste cell proliferation is not considered to be involved in the alteration of the sweet taste sensitivity in rats.

Salivary excretion of platinum anti-cancer drugs sometimes causes a metallic taste as an adverse effect.\(^{14}\) As shown in Fig. 6D, we detected platinum excretion in saliva of oxaliplatin-administered rats with the doses of 8 and 16 mg/kg on day 3, but not on day 7, on which the alteration of sweet taste sensitivity was observed. Thus, we think that platinum excretion into the saliva does not contribute to the alteration of taste sensitivity in oxaliplatin-administered rats.

CONCLUSION

In this study, we demonstrated that oxaliplatin administration to rats transiently increased the expression of T1R2 and decreased the sensitivity to sweet taste, while there was no alteration in the morphology of taste buds or the numbers of taste cells in them. These findings suggest that oxaliplatin induces a taste disorder as to sweet taste and this alteration is due, at least in part, to the alteration of expression of sweet taste receptors, but that these alterations are reversible.

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Conflict of Interest The authors declare no conflict of interest.

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