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The effects of cisplatin, an emetic agent, on behavior and plasma cortisol levels in goats

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Abstract

Ruminants are not considered to experience nausea because they do not possess the emetic reflex. This study examined the effects of administration of cisplatin (*cis*-diamminedichloro platinum (II): CDDP), a common emetic agent, on the behavior of goats. In Experiment 1, adult Shiba goats received intravenous (IV) administration of CDDP. CDDP-administered goats spent a shorter time feeding ($P < 0.01$), lied down earlier ($P < 0.01$), and spent a longer period lying down ($P < 0.01$) than the saline-administered control goats, and sometimes, they directed their face downward and close their eyes. These behavioral responses were followed by a significant ($P < 0.01$) increase in plasma cortisol (Cor) levels, which indicated that goats experienced stress. The dose dependency was found in the extent of the CDDP effects. In Experiment 2, the effects of pretreatment of ondansetron (Ond), an antiemetic agent, were examined. Pretreatment of Ond extended the latency of lying ($P < 0.01$), shortened the time spent lying ($P < 0.05$), and reduced the extent of the increase in plasma Cor levels ($P < 0.01$). These results suggested that CDDP administration generated some state of stress in goats via the similar physiological mechanisms as emesis-caused stress in emetic species.

KEYWORDS
behavior, cisplatin, cortisol, goats, ondansetron

1 | INTRODUCTION

From the viewpoint of the wellbeing of farm ruminants, the assessment for uncomfortable senses, such as pain, thermal stress, and psychological stress have been investigated in cattle, sheep, and goats. However, there were no reports that investigate the nausea, one of the most common uncomfortable senses, in ruminants. In humans, many works investigating nausea have been reported, and almost all of them observed emesis (vomiting) as the index of nausea, because emesis is the clearest behavioral response to nausea. Emesis is also observed in other mammalian species, such as nonhuman primates (Fukui et al., 1993), dogs (Kenward et al., 2015), ferrets (Minami et al., 1997), suncus (Ito et al., 1995), and pigs (Szelenyi et al., 1994).

Although other species, such as rodents, rabbits and horses, do not possess the emetic reflex (Andrews & Horn, 2006; Horn et al., 2013), rodents, however, have sometimes been used in studies of nausea. The abnormal feeding behavior called "pica," the consumption of kaolin (hydrated aluminum silicate; a nonnutritive substance) is considered an alternative index for nausea in rodents.

Pica in rodents is induced by the administration of emetic agents (Jonghe et al., 2009; Takeda et al., 1995), radiation exposure (Yamamoto et al., 2002), and unfamiliar motion stimuli (Takeda et al., 1995) that induce emesis in the emetic species (Andrews & Horn, 2006; Conder et al., 2008; King, 1988). The validity of pica as an alternative index for nausea is still controversial, but pica in rodents suggests that even nonemetic species may experience nausea, or the similar unpleasant sense.

Ruminants also are not emetic species (Horn et al., 2013); therefore, they are not considered to experience nausea. Meanwhile, pigs, which are classified as Artiodactyla like ruminants, are thought to experience nausea because they are an emetic species. Farm pigs vomit due to infectious diseases model (Jung & Saif, 2015; Kanitz et al., 2002), taking toxic substances such as mycotoxin (Smith & MacDonald, 1991), or motion sickness by road transportation (Bradshaw et al., 1999). Sometimes farm ruminants may experience these same unpleasant events, and some behavioral and physiological changes were observed in these situations (Aoyama et al., 2008; Borderas et al., 2008; Gallo et al., 2015; Takeuchi et al., 1995, 1997). If ruminants experience nausea in these situations, and if we can assess it, we may be able to offer more sophisticated treatments to relieve their aversion. The aim of this study is exploring the possibility of existence of nausea sensation in ruminants.

Cisplatin (*cis*-diamminedichloro platinum (II): CDDP) is an effective anticancer chemotherapy, but it evokes nausea and emesis as a side effect (Hesketh, 2008). Therefore, CDDP were used for the studies in the mechanisms of nausea in dogs (Kenward et al., 2014), ferrets (Minami et al., 1997), suncus (Ito et al., 1995), pigs (Szelenyi et al., 1994), and in rats (Takeda et al., 1993). In the present study, as the first step of an exploration into the existence of nausea in ruminants, we examined the effects of CDDP administration on behavior in goats. In addition, the effects of ondansetron (Ond) on the CDDP-administered goats were also examined. Ond is an antagonist for the serotonin receptor, and it is used as a medication for CDDP-induced nausea for cancer patients (Tyers, 1992).

2 | MATERIALS AND METHODS

2.1 | Animals

A total of 23 adult Shiba goats were used in the study. All experimental procedures and the care of animals were carried out according to "Guide for Care and Use of Laboratory Animals at Utsunomiya University." The experiments were approved by the Committee of Animal Experiments at Utsunomiya University (A10C-0018).

All experiments were conducted on the research farm at the Faculty of Agriculture of Utsunomiya University, located in Tochigi prefecture, Japan. The experiments were conducted during April to July or October to December in 2011–2015 at temperatures ranging between 9°C and 24°C.

2.2 | Experiment 1: The effects of cisplatin

2.2.1 | Animals and drug administration

A total of 15 adult Shiba goats (eight males; 25–35 kg, 2–4 years of age, and seven females; 20–30 kg, 2–5 years of age) were used. The animals were housed in individual pens (2.0 × 3.0 m) and fed daily with ad libitum timothy hay, and water was always available. Fresh hay and water were given, and the old ones removed, at 10:00 h each day.

The 15 goats were assigned to three experimental groups ($n = 6$, 7 and 2, respectively) balanced by sex and age. The first group ($n = 6$) was used for the "low-dose test" in which animals were administered *cis*-diammineplatinum (II) dichloride (cisplatin: CDDP) (P4394; Sigma- Aldrich, St. Louis, MO, USA) intravenously (IV) (0.5 mg/0.25 ml saline/kg BW) at 09:00 h. The CDDP was dissolved in saline at 70°C and cooled to 37°C before administration. The same volume of saline was administered to the same animal as the control test on another day, and at least 40 days separated control and CDDP administration. Three out of six goats received CDDP first, and the others received it second. The administration rate was 5.0–7.5 ml/min. One to 2 days before each test, a catheter was fitted to the jugular vein of each animal for IV administration and blood sampling.

The second group ($n = 7$) was used for the "high-dose test" in which animals were given 1.0 mg/0.5 ml saline/kg BW of CDDP. Three out of seven goats received CDDP first, and other four received it second. The administration rate was the same as the low-dose test.

The third group ($n = 2$) was used for the hyper-dose test (3.0 mg/1.5 ml saline/kg BW).

Each animal could see, hear, and smell other goats kept in the neighboring pens. Only one animal was used for the test per day.

2.2.2 | Behavior observation

The behavior of each goat was videotaped between 09:00 and 13:00 h for subsequent observation and analysis. We focused in particular on the behavior after 10:00 h, when goats were presented new hay and usually they started to feed immediately and kept feeding until the end of observation. The analyzed behavioral parameters were "latency from the administration to lying down after 10:00 h," "total time spent lying down between 10:00 and 13:00 h", and "total time spent feeding". If a goat never lied down throughout the observation, the latency was regarded as 240 min, because the observation period was 4 h. The latency from the administration to lying before 10:00 h was also observed. In this parameter, if a goat never lied down, the latency was regarded as 60 min.

2.2.3 | Cortisol assay

Plasma concentration of cortisol (Cor) was measured as the stress indicator. Blood samples were collected at 08:30, 09:00

(just before the administration), 10:00, 11:00, 12:00, and 13:00 h. Each sample was immediately transferred to a polypropylene tube containing sodium heparin and stored in an ice bath. Within 60 min from the collection, plasma samples were separated by centrifugation (1400*g*, 4°C, 10 min) and stored at −30°C. The detailed procedure for the Cor assay was presented in our previous article (Aoyama et al., 2009). In brief, before the assay, Cor was extracted from the plasma with diethyl ether. The Cor concentrations were measured by RIA using the anti-cortisol antibody (FKA-404; CosmoBio Co., Ltd., Tokyo, Japan) and [3H]-labeled hydrocortisone (NET-396; Perkin Elmer Inc., Waltham, MA, USA). After incubation at 4°C for 36–48 h, free Cor was removed by a dextran-coated charcoal solution containing dextran (Dextran T-70 V; Pharmacia Corporation, Peapack, NJ, USA) and charcoal (Norit sx-3; Wako Co., Ltd., Osaka, Japan). The intra- or inter-assay CV was 6.7% and 12.1%, respectively.

2.3 | Experiment 2: The effects of ondansetron

2.3.1 | Animals and the administration of drugs

Eight adult Shiba goats were used (four males; 25–40 kg, 3–5 years age, and four females; 20–35 kg, 2–5 years age). The source and usual managements for the animals were the same as in Experiment 1.

Based on the results obtained in Experiment 1, we adopted 1.0 mg/kg BW as the CDDP dose. The time frame of each test was almost the same as in Experiment 1. At 08:30 and 09:00 h (just after the first and second blood collection, respectively), the Ondansetron injection solution (Sandoz; Sandoz K. K., Tokyo, Japan) was given via IV to each goat. The dose of Ond was 0.1 mg/0.05 ml solution/kg BW for each administration; thus, the total dose was 0.2 mg/kg BW. The same volume of saline was administered on another day as the control. Each goat received four combinations of the drug treatments: saline and saline (Sa-Sa), Ond and saline (Ond-Sa), saline and CDDP (Sa-CDDP), and Ond and CDDP (Ond-CDDP). The order of the treatments was designed as the Latin-square design in each sex, and the interval of each test was longer than 40 days.

2.3.2 | Behavior observation and cortisol assay

All procedures were the same as in Experiment 1.

2.3.3 | Data analysis

In Experiment 1, the differences in each behavioral parameter between the CDDP- and saline-administered tests and the dose of CDDP were analyzed using repeated measures of two-way (treatment × dose) analysis of variance (ANOVA). Prior to the statistical analysis, the behavioral data were converted to common logarithmic values to fit normal distribution. The differences in plasma Cor levels between the CDDP- and saline-administered tests and the time periods were compared by repeated measures of two-way (treatment × time) ANOVA within each dose. The data in the hyper-dose test were not included in the analysis because only two goats were used in this test.

In Experiment 2, the effects of CDDP or Ond were analyzed using repeated measures of two-way (with or without CDDP × with or without Ond) ANOVA. To examine the effects of Ond on plasma Cor levels, the difference between Sa-Sa and Ond-Sa, or between Sa-CDDP and Ond-CDDP treatments, were analyzed using repeated measures of two-way (with or without Ond × time) ANOVA.

When a significant difference was found, Tukey's test was used for a post hoc test.

3 | RESULTS

There were no clear differences between sexes in any parameters, thus the results of both sexes were combined and analyzed together.

3.1 | Experiment 1: The effects of cisplatin

3.1.1 | The effects of cisplatin on behavior

The results of the behaviors in Experiment 1 are represented in Table 1. In the first hour after administration (09:00–10:00 h), goats'

TABLE 1 The effects of intravenous administration of cisplatin on behavior in goats

	0.5 mg/kg BW ($n = 6$)		1.0 mg/kg BW ($n = 7$)	
Dose of cisplatin	**Control**	**Cisplatin**	**Control**	**Cisplatin**
Latency to lying down before feed change (min)	34.5 ± 5.4	46.5 ± 6.4	32.9 ± 6.7	40.6 ± 5.37
Latency to lying down after feed change (min)	236.3 ± 2.3	196.0 ± 14.0*	231.3 ± 7.1	128.9 ± 10.3**##
Total time spent lying down (min)	2.7 ± 1.7	12.7 ± 4.1*	1.21 ± 1.1	47.7 ± 13.9**#
Total time spent feeding (min)	171.3 ± 2.8	107.5 ± 11.2	156.7 ± 17.0	51.6 ± 14.1**##

Note: Cisplatin was dissolved in saline at a concentration of 1.0 mg/0.5 ml. In the control, the same volume of saline was administered. Each value represents the average ± standard error.

*$P < 0.05$ (significant difference from the control within each dose). **$P < 0.01$ (significant difference from the control within each dose). #$P < 0.05$ (significant difference from the test of 0.5 mg/kg BW dose).

##$P < 0.01$ (significant difference from the test of 0.5 mg/kg BW dose).

behavior did not seem to differ among the treatments, and there were no difference in the latency to the lying down between saline and CDDP treatment in both of low and high dose test. Although we cannot represent as the numerical data, all goats of each treatment performed rumination in this period.

In the saline-administered control test, goats began to feed immediately after the new hay was given at 10:00 h and spent much of their time feeding. The CDDP administrated goats also started to feed on new hay at 10:00 h. However, after a while, goats stopped feeding and showed the specific behavioral responses represented in Figure 1. Sometimes goats stood looks normally but without feeding or ruminating (Figure 1a), but other times, they stood directed toward the wall (Figure 1b). Finally, almost all goats laid down, generally directing their faces down and closing their eyes (Figure 1c), and some goats did not stand up when we approached them for blood collection (Figure 1d).

In the control test, goats laid down little between 10:00 and 13:00 h, but all the CDDP-administered goats laid down except one female in the low-dose test. The latencies to lying down of the CDDP-administered goats were shorter than those of controls in both the low- ($P < 0.05$) and high- ($P < 0.01$) dose test. The goats that received a high dose of CDDP lay down significantly earlier than goats that received low dose ($P < 0.01$).

The total time spent lying down among CDDP-administered goats was significantly longer than those in the controls in both the low- ($P < 0.05$) and high- ($P < 0.01$) dose test, and the goats that received a high dose of CDDP spent a significantly longer time lying than goats that received a low dose ($P < 0.05$).

The time spent feeding among goats that received the low dose of CDDP did not differ statistically from that of the control, but the goats that received the high dose of CDDP spent a significantly shorter time feeding than the control goats ($P < 0.01$) and goats that received the low dose ($P < 0.01$) (Table 1).

We found that all goats in the high-dose test and some goats in the low-dose test breathed faster than usual when they stopped feeding and showed behavioral changes represented in Figure 1. Unfortunately, we could not always obtain the picture of the detailed facial expressions nor the accurate breathing rate from the video recording, so we analyzed only feeding and lying behavior statistically in this study.

In the low- and high-dose tests, all goats had recovered from the effects of CDDP by the next day; they showed normal feeding and rumination and no longer displayed the behavior represented in Figure 1. Meanwhile, in the hyper-dose test, in which goats received 3.0 mg/kg BW of CDDP, a male and female goat showed behavioral changes 70 and 45 min after the CDDP administration, respectively, and still had not recovered by the next day; they fed little on hay and performed little rumination for 2 to 3 days. We gave them an IV drip and decided to cease further hyper-dose testing for ethical reasons.

3.1.2 | The effects of cisplatin on plasma cortisol levels

The results of the plasma Cor levels are represented in Figure 2. In the first 60 min after administration, plasma Cor levels did not change by CDDP. In the low-dose test, plasma Cor levels at 120 min after CDDP administration still did not differ from the control, and it became significantly higher than the control at 180 and 240 min ($P < 0.01$) (Figure 2a). Meanwhile, in the high-dose test, plasma Cor levels in CDDP-administered goats had already increased significantly at 120 min after administration ($P < 0.01$) (Figure 2b). The remarkable increase in plasma Cor levels were seen even when the goats stood looks normally like represented in Figure 1a.

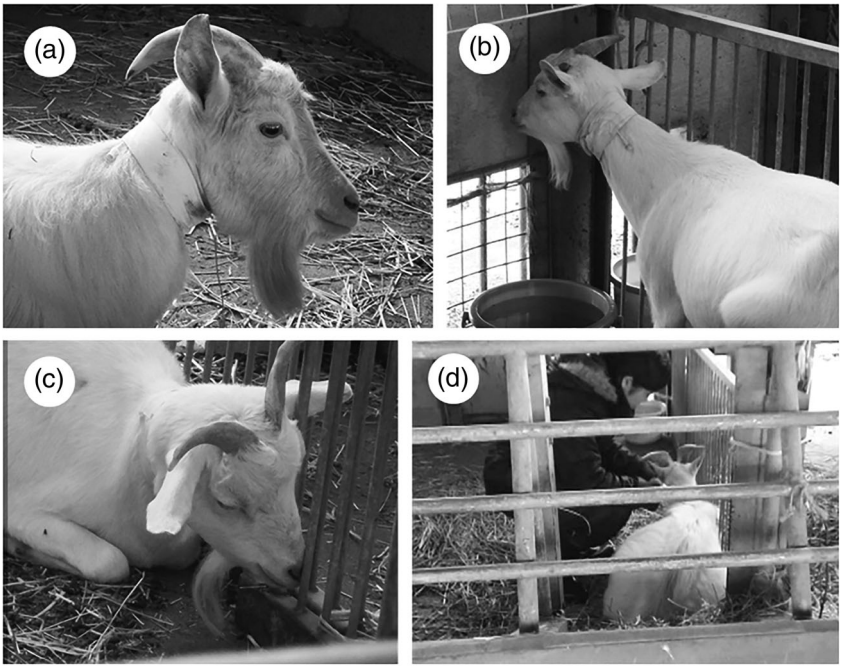

FIGURE 1 Examples of the behavioral changes induced by intravenous administration of cisplatin in goats. (a) A male goat stood looks normally but did not feed or ruminate. (b) A female goat stood without feeding or ruminating, directed toward the wall. (c) A female goat lay without ruminating, facing downward with eyes closed. (d) A male goat continued lying down even when a researcher approached him for blood collection

3.2 | Experiment 2: The effects of ondansetron

3.2.1 | The effects of ondansetron on behavior

The results of the behavior in Experiment 2 are represented in Table 2. There was no difference in the latency to lying down before 10:00 h among the treatments.

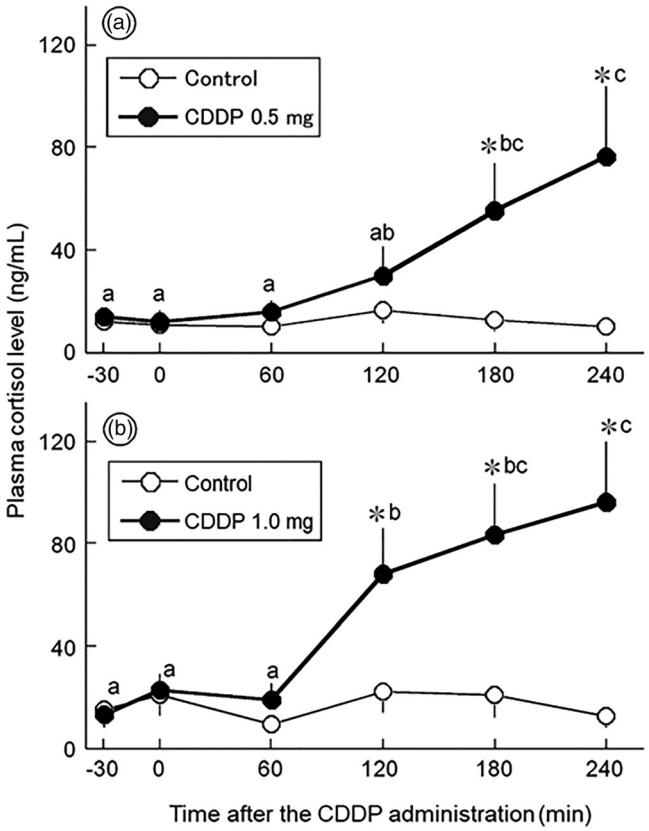

FIGURE 2 The effects of cisplatin (CDDP) administration on plasma cortisol levels in goats. The administered dose was 0.5 (a) or 1.0 mg/kg BW (b). The same volume of saline was administered for the control. Each value represents the average ± standard error of six (a) or seven (b) goats. *: Significant difference from the controls at the same time ($P < 0.05$). a, b, c: Lack of the same letter indicates a significant difference among sampling times within the test ($P < 0.05$)

Comparing the Sa-Sa and Ond-Sa treatments, no differences in any behavioral parameters were found throughout the observation periods.

CDDP-administered goats began to feed on hay immediately at 10:00 h, but they stopped feeding and displayed behavioral changes like Experiment 1 after a while. All behavioral parameters in the Sa-CDDP or Ond-CDDP treatments differed significantly from those in the Sa-Sa or Ond-Sa treatments, respectively ($P < 0.01$ for all parameters) (Table 2).

The latency to lying after 10:00 in the Ond-CDDP treatment was significantly longer than that in Sa-CDDP treatment ($P < 0.01$). The total time spent lying in the Ond-CDDP treatment was significantly shorter than that in the Sa-CDDP treatment ($P < 0.05$). The total time spent in feeding in the Ond-CDDP treatment was slightly longer than that in Sa-CDDP treatment, although the difference was not statistically significant.

3.2.2 | The effects of ondansetron on the plasma cortisol level

There were no significant changes in plasma Cor levels in the Sa-Sa nor Ond-Sa treatment throughout the observation period (Figure 3). CDDP administration increased plasma Cor levels significantly in both of Sa-CDDP and Ond-CDDP treatments. In the Sa-CDDP treatment, plasma Cor levels at 120 min were significantly higher than those before administration ($P < 0.01$), and elevated plasma Cor levels were maintained until 240 min. Meanwhile, in the Ond-CDDP treatment, plasma Cor levels at 120 min did not differ from those before administration and were lower than those in the Sa-CDDP treatment at the same time ($P < 0.01$). At 180 and 240 min after CDDP administration, plasma Cor levels in Ond-CDDP treatment were significantly increased ($P < 0.01$), although significantly lower than those in the Sa-CDDP treatment at the same time ($P < 0.01$).

4 | DISCUSSION

This study revealed that CDDP administration induces the remarkable behavioral changes in goats. There were no remarkable

TABLE 2 The effects of preadministration of ondansetron on cisplatin-induced behavioral changes in goats

Treatment	Sa-Sa	Ond-Sa	Sa-CDDP	Ond-CDDP
Latency to lying down before feed change (min)	42.8 ± 7.4	44.6 ± 8.0	33.6 ± 6.7	39.5 ± 8.1
Latency to lying down after feed change (min)	240.0 ± 0.0	240.0 ± 0.0	115.4 ± 7.3*	156.1 ± 8.0*##
Total time spent lying down (min)	0.0 ± 0.0	0.0 ± 0.0	61.8 ± 8.5*	31.3 ± 6.5*#
Total time spent feeding (min)	159.4 ± 8.7	160.5 ± 8.1	50.0 ± 7.7*	75.0 ± 8.2*

Note: Ondansetron (Ond) was administered at 30 min and just before cisplatin (CDDP) administration. The dose of each Ond administration was 0.1 mg/0.05 ml solution/kg BW, and the dose of CDDP was 1.0 mg/kg BW. The same volume of saline (Sa) was used as the control for Ond and CDDP. Each treatment was represented as the combination of the administered substances. Each value represents the average ± standard error ($n = 8$).
*$P < 0.01$ (significant difference from the Sa-Sa or Ond-Sa treatment). #$P < 0.05$ (significant difference from the Sa-CDDP treatment).
##$P < 0.01$ (significant difference from the Sa-CDDP treatment).

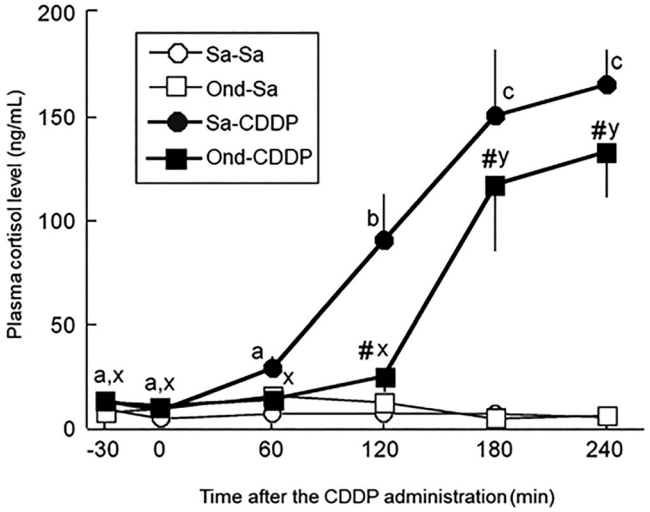

FIGURE 3 The effects of intravenous preadministration of ondansetron (Ond) on the cisplatin (CDDP)-induced increase in plasma cortisol levels in goats. Ond was administered at 30 min and just before CDDP administration, and the dose of each Ond administration was 0.1 mg/kg BW. The dose of CDDP was 1.0 mg/kg BW. The same volume of saline (Sa) was used as the control for ondansetron or cisplatin. Each treatment was represented as the combination of the administered substances. Each value represents the average ± standard error of eight goats. a, b, c: Lack of the same letter indicates a significant difference among sampling times within Sa-CDDP treatment ($P < 0.05$). x, y: Lack of the same letter indicates a significant difference among sampling times within Ond-CDDP treatment ($P < 0.05$). #: Significant difference from the Sa-CDDP treatment at the same time ($P < 0.05$)

behavioral changes during the first 1 h after the administration, but approximately 120 min after the 1.0 mg/kg BW of CDDP administration, all goats had stopped feeding on hay and lied down, whereas the control goats remained in a standing position and continued to feed. When CDDP-administered goats had stopped feeding, they sometimes showed the specific condition (lowered head, closed eyes: Figure 1c) and rapid breathing. The movements of goats became slower, and the extent of their responses to external stimuli became lower, such that some goats did not stand up even when we touched them for blood collection (Figure 1d). Kenward et al. (2015) indicated some nausea-induced behavioral changes other than emesis, such as "rapid breathing," "dropping the head," and "closing the eyes" in dogs. Although we did not obtain as the analytic data, some responses of goats to CDDP administration might resemble to those in dogs.

At the same time as the behavioral changes, CDDP administration increased plasma Cor levels significantly. A similar increase in plasma glucocorticoids levels was also reported in dogs and rats: Kenward et al. (2014) reported that CDDP administration to dogs increased plasma Cor levels simultaneously with the vomiting. Similarly, Tohei et al. (2011) reported that the administration of cyclophosphamide, another emetic agent, induced pica, and a significant increase in plasma corticosterone levels in rats. The blood levels of corticoids are the most popular physiological indicator of the stress. The major system that increases the plasma glucocorticoid level is the hypothalamus–pituitary–adrenal (HPA) axis: when some stimulus that induce stress are given to animals, corticotropin releasing hormone (CRH) is released from the hypothalamus, and adrenocorticotropic hormone (ACTH) released from the pituitary, and consequently, glucocorticoids is released from the adrenal cortex. We hypothesized that the administration of CDDP might induce some unpleasant state to goats, and consequently, the behavioral changes and the activation of the HPA axis were induced. Meanwhile, Malik et al. (2006) reported that CDDP administration failed to affect the hypothalamic CRH mRNA expression in rats. This report is opposes to the report by Tohei et al. (2011) in which cyclophosphamide significantly increase the plasma corticosterone levels in rats. The failure in the increasing hypothalamic CRH mRNA expression might be the relatively longer period from the administration to the sampling: in Maliks' report (2006), the samples were corrected no earlier than 2 days after CDDP administration. The expression of the hypothalamic CRH mRNA at the earlier period might be increased.

Although it is still unclear the pharmacological mechanisms of administrated CDDP in goats, there were some similarities among the results in this study and the previous works investigating the CDDP-induced emesis in emetic animals or pica in rats.

Firstly, previous reports have indicated that CDDP administration induced emesis in a dose-dependent manner. The number of emetic episodes per animal and latency to the first emesis depended on the dose of CDDP in dogs (Kenward et al., 2015), ferrets (Endo et al., 1993), and pigs (Szelenyi et al., 1994). Similarly, in rats, the dose of CDDP influenced the amount of kaolin consumed (Takeda et al., 1993). Similar dose dependency in responses to CDDP administration was also seen in this study; the low dose of CDDP (0.5 mg/kg BW) was effective, although weaker, and it required a longer time to be expressed comparing with the high dose test.

Secondly, in our study, the pretreatment of Ond significantly relieved (at least, delayed) the CDDP-induced behavioral changes and the increase in plasma Cor levels in goats. Ond is a medicine to relieve CDDP-induced nausea for cancer patients (Tyers, 1992). The previous reports indicated that the pretreatment of Ond reduced the number of animals that showed emesis, reduced the number of the emetic episodes per animal, and elongated the latency to the first emesis in CDDP-administered dogs (Fukui & Yamamoto, 1999), ferrets (Rudd & Naylor, 1994), suncus (Sam et al., 2003), and pigs (Szelenyi et al., 1994). Additionally, Ond reduced the amount of kaolin intake in CDDP-administered rats (Takeda et al., 1993). CDDP and some other emetic agents are thought to induce emesis by stimulating serotonin (5-hydroxytryptamine; 5-HT) release from the enteroendocrine cells in the digestive tract (Hesketh, 2008; Minami et al., 2003). The increased-5-HT release is thought to stimulate the afferent projection of the vagal nerve that terminates to the brain stem to generate emesis. Ond is an antagonist of the serotonin 5-HT type 3 receptor, and it is believed that this substance relieves CDDP-induced nausea by blocking 5-HT binding to the receptor in the small intestine

(Hesketh, 2008; Tyers, 1992). Our results suggested that the CDDP-induced responses in goats might be generated through the similar physiological mechanisms as emesis in emetic species.

Thirdly, CDDP administration required a certain latency to induce the behavioral and physiological changes. In this study, CDDP-induced changes in goats did not occur just after the administration, but it took about 120 min to express. Previous reports indicated that CDDP has a relatively longer latency to induce emesis compared with other emetic agents; although it depended on the dose and dosage, the reported latencies from CDDP administration to emesis were 70–90 min in dogs (Kenward et al., 2014; Yamakuni et al., 2002), 60–100 min in ferrets (Minami et al., 1997; Yamakuni et al., 2002), 20–120 min in suncus (Ito et al., 1995), and 36–84 min in pigs (Szelenyi et al., 1994). Other emetic agents, such as cyclophosphamide or copper sulfate, induced emesis within the shorter latencies in ferrets (Minami et al., 1997), and suncus (Ito et al., 1995). As we discussed previously, CDDP and some other emetic agents are thought to induce emesis by stimulating 5-HT release from the enteroendocrine cells in the digestive tract (Hesketh, 2008; Minami et al., 2003). Some emetic agents, including CDDP, stimulate 5-HT release by increasing biosynthesis enzyme activity and inhibiting metabolic enzymes activity in the ileum (Endo et al., 1993), but the efficiency of these effects differed among the agents, and CDDP may require a longer period in order to accumulate a sufficient amount of 5-HT compared with other emetic agents. Although we have not examine the effects of other emetic agents, the latency from CDDP administration to the onset of behavioral and physiological changes in goats was similar to that in previous reports regarding emetic species.

These similarities in the effects among the results in this study and the previous reports suggest that the CDDP-induced behavioral changes in goats might be evoked via the similar physiological mechanisms as emesis in emetic species, at least in parts.

Some previous reports indicated that CDDP could induce not only the nausea but also some other harmful conditions, such as cardiovascular diseases (Bano et al., 2013), renal failure (Miller et al., 2010), or auditory disorder (Rybak et al., 2007). In addition, the direct action of CDDP on the brain might be possible. Charest et al. (2013) reported the uptake of CDDP in the brain was observed by IV administration of CDDP in rats. These other effects of CDDP do not seem to be the major cause of the goats' responses to CDDP in this study because there were no reports indicating the effects of Ond treatment on these other CDDP-induced effects, but we could not perfectly exclude the possibility of these other effects. These other effects might contribute to the CDDP-induced changes in goats in this study, at least partially. Ond treatment had failed to inhibit the effects of CDDP perfectly, even if it was effective.

The behavioral changes induced by CDDP in goats in this study partially resembled those observed in sick animals. For example, some of the behavioral and physiological responses, such as the increase in inactive lying, reduction in the feeding, or increase in plasma Cor levels, to administration of lipopolysaccharide (LPS) to goats (Takeuchi et al., 1995, 1997) or calves (Borderas et al., 2008) were similar to those induced by CDDP in goats. LPS is the molecules in the cell membrane of the gram-negative bacteria, and its administration could mimic infection disease in animals. The nausea is thought to be one of the symptoms induced by LPS, because its administration induced emesis in dogs (Yu et al., 2012) and pigs (Kanitz et al., 2002). It is still unclear whether the CDDP-induced sense to goats was similar to nausea or not, because LPS might induce some other unpleasant state. But the similar senses that induce emesis in pigs or dogs might be induced also in goats, and it might contribute the behavioral changes and the increase in the plasma Cor in some degree.

Despite the fact that some similarities were seen between CDDP-induced emesis in emetic species and the CDDP-induced behavioral changes in goats, whether or not goats truly experience nausea is still unknown. The behavioral aspects induced by CDDP might also resemble to the behavior induced by pain. In goats, pain induced behavioral changes, such as lying down or standing and looking depressed, lack of appetite, rapid breathing, and increase in plasma Cor level (Alvarez et al., 2015; Hendrickson et al., 1996). It may be difficult to discriminate between nausea and pain perfectly only by the behavior. Furthermore, some relationships between nausea and visceral pain were suggested (Andrews & Horn, 2006). Further studies are required to reveal what type of sensation goats experience with CDDP administration.

4.1 | Conclusions

CDDP administration did not induce vomiting in goats, but it evoked some specific behavioral changes and a significant increase in plasma Cor levels, which indicates they might experience some stress. These CDDP-induced responses could be relieved by preadministration of Ond. These results indicated that CDDP administration might generate some kind of unpleasant state in goats via the similar physiological mechanisms as emesis in emetic animals. Although further studies are required to confirm this, CDDP administration may cause goats to experience nausea or some similar senses.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest that could be perceived as prejudicing the impartiality of the researches reported.

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