



Original Article

## A 6-week repeated intranasal dose toxicity study of TTA-121, a novel oxytocin nasal spray, in rats

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**ABSTRACT** — Autism spectrum disorder (ASD) is a highly prevalent neurodevelopmental disorder. Although there is no established treatment for the core symptoms of ASD, recent research has indicated a potentially therapeutic effect of intranasally administered oxytocin. TTA-121 is a novel oxytocin nasal spray with high bioavailability and is expected to increase oxytocin delivery to the brain by adjusting osmolality and viscosity of the formulation. As nonclinical safety studies to support the conduct of the Phase 1 and Phase 2 studies of TTA-121, a 6-week repeated intranasal dose toxicity study of TTA-121 in rats was conducted. In the present study, TTA-121 was administered intranasally to male and female rats at 0 (placebo), 1.2, 6, and 30 U/body/day once daily for 6 weeks followed by a 4-week recovery period to evaluate potential toxicity and systemic exposure to oxytocin. The toxicokinetic analysis indicated that systemic exposure of oxytocin increased with a dose ranging from 1.2 to 30 U/body/day at the first and last dosing. No deaths or morbidity were observed. There were no toxicologically significant changes in clinical signs, functional observational battery, body weight, food consumption, water consumption, ophthalmology, urinalysis, hematology, blood chemistry, organ weights, necropsy or histopathology at any dose during the dosing or recovery period. Based on these results, the no-observed-adverse-effect level of TTA-121 was 30 U/body/day for male and female rats, suggesting that there is a sufficient safety margin. We believe that TTA-121 is expected to be sufficiently safe to treat males and females with ASD.

**Key words:** Autism spectrum disorder, Oxytocin, Novel nasal spray, TTA-121, Repeated dose toxicity

### INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder. Its core symptoms include deficits in social interactions and communication as well as restricted and repetitive behaviors (Yamasue and Domes, 2018). Although ASD is highly prevalent, affecting 1/100 in the general population, there is no established treatment for the core symptoms of this disorder (Lai *et al.*, 2014).

Oxytocin, a neuropeptide hormone primarily synthe-

sized in the hypothalamus and released by the pituitary gland, is expected to be a potential therapeutic resource for the social core symptoms of ASD, as this neuropeptide has an important role in social behavior and cognition (Donaldson and Young, 2008). Recent research has suggested a potential therapeutic effect of intranasally administered oxytocin on core social symptoms of ASD (Andari *et al.*, 2010; Gordon *et al.*, 2013; Harris and Carter, 2013; Ma *et al.*, 2016; Watanabe *et al.*, 2014; Yamasue, 2016). These studies used Syntocinon (Novartis,

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Switzerland), which is an oxytocin nasal spray approved for enhancing lactation in Europe.

TTA-121 is a novel oxytocin nasal spray with high bioavailability expected to increase oxytocin delivery to the brain by adjusting osmolality and viscosity of the formulation (WO 2017/073798 A1). Phase 1 and Phase 2 investigator-initiated trials of TTA-121 have been conducted; these studies were funded by the Japan Agency for Medical Research and Development (AMED). A Phase 1 study of TTA-121 in healthy individuals has been completed (UMIN000025922, Sakanaka *et al.*, 2018). In the Phase 1 study, single (5-200 U/mL, 0.1 mL/spray/day) and multiple doses (30-200 U/mL, 0.1 mL/spray x 9days, Day 1, 9 qd, Day 2-8 bid) of TTA-121 were safe and well tolerated. Dose proportional increases of plasma concentrations of oxytocin were observed after intranasal administration of TTA-121. A Phase 2 study of TTA-121 on individuals with ASD is currently underway (NCT03466671/UMIN000031412).

In advance of the investigator-initiated trials of TTA-121, the need for nonclinical safety studies of TTA-121 was investigated via a literature review for safety information on oxytocin. To date, there are no published reports evaluating the toxicity of oxytocin when administered intranasally to animals. Safety information regarding intravenous infusion of oxytocin and the use of intranasal oxytocin with humans is available. Evidence from intravenous infusion of oxytocin suggests possible cardiovascular effects including tachycardia and bradycardia (MacDonald *et al.*, 2011), and there have been cases of water intoxication from excessive doses of intranasal oxytocin together with a large volume of fluid (Mayer-Hubner, 1996). However, this safety information is largely derived from females treated for a short period in the purpose of obstetric delivery, although TTA-121 is intended for use for a longer period in males and females with ASD. In addition, based on recent research using Syntocinon, the anticipated therapeutic dose range for ASD is considered higher than the dose used for enhancing lactation (Andari *et al.*, 2010; Gordon *et al.*, 2013; Harris and Carter, *et al.*, 2013; Ma *et al.*, 2016; Watanabe *et al.*, 2014; Yamasue, 2016). Therefore, there was a need to evaluate the toxicity of TTA-121 before investigator-initiated trials are conducted. As nonclinical safety studies to support the conduct of the Phase 1 and Phase 2 studies of TTA-121, the 6-week repeated intranasal dose toxicity studies of TTA-121 in rats and monkeys were performed. In this paper, we show the results of the toxicity study in rats.

## MATERIALS AND METHODS

The present study was conducted in compliance with Good Laboratory Practice Standards (MHW, Ordinance No. 21, “Standards for Conduct of Nonclinical Studies on the Safety of Drugs”) at LSI Medience Corporation (Ibaraki, Japan). The study protocol was reviewed by the Institutional Animal Care and Use Committee and approved by the General Manager of the test facility according to the Guidelines for Animal Studies (Nonclinical Research Center). The test facility was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International.

### Test article

TTA-121 (48, 240 and 1200 U/mL of oxytocin) and placebo were prepared and supplied by Teijin Pharma Limited (Tokyo, Japan). The test article was confirmed to be stable during the experimental period.

### Animals

Male and female Crl:CD(SD) rats (100 males and 100 females) at 6 weeks of age were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). All animals were acclimated for 2 weeks before the first dose. Administration of the test article was initiated at 8 weeks of age. The ranges in body weight for males and females on initiation of dosing were 245.2-295.8 g and 165.8-228.3 g, respectively. Animals were housed in controlled temperature (19-25°C), humidity (35-75%), ventilation (6-20 times/hr with all fresh air) and lighting (07:00-19:00) conditions throughout the study. The animals were fed a pellet diet (CR-LPF, Oriental Yeast Co., Ltd., Tokyo, Japan) and given tap water *ad libitum*.

### Selection of dose levels

Dose levels were selected based on the results obtained from a 14-day repeated intranasal dose toxicity study of TTA-121 in rats and information regarding test article solubility (unpublished data). In the 14-day intranasal dose toxicity study, single cell necrosis and flattening of the olfactory epithelium were observed in the right-side nasal cavity, the administered site, in the control (placebo) and TTA-121 treated groups (2.4, 12, and 60 U/body/day), and there was a possibility that these changes were caused by mechanical stress associated with administration of the large dose volume (50  $\mu$ L) to the right-side nasal cavity of animals. Therefore, in the present study, the dose volume was set at 25  $\mu$ L/body, and the high dose was set at 30 U/body/day as a maximum feasible dose for

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6-week repeated dosing. The middle and low doses were set at 6 and 1.2 U/body/day, respectively, at a common ratio of 5.

### Experimental design

TTA-121 was administered intranasally at 0 (placebo), 1.2, 6, and 30 U/body/day once daily for 6 weeks to male and female rats. The main group consisted of 10 animals/sex/group. The recovery group consisted of 6 animals/sex/group in the placebo control and 30 U/body/day groups. The dosing formulation (25  $\mu$ L/body) was drawn into a micropipette and administered into the right nasal cavity. The animals were held in the same position for 5 to 10 sec until the dosing formulation was inhaled through the nostril. On the day after the last dosing, 10 animals/sex/group in the main group were euthanized for hematology, blood chemistry, organ weight, necropsy, and histopathologic examination. After the dosing period, the remaining 6 animals/sex/group in the placebo control and 30 U/body/day groups in the recovery group were kept without treatment for 4 weeks to evaluate reversibility of toxicity.

Plasma concentrations of oxytocin were monitored at the first and last (42nd) dosing in the toxicokinetic (TK) group. The TK group consisted of 4 animals/sex/group in the placebo control group, and 12 animals/sex/group in the TTA-121-treated groups.

### TK analysis

Blood samples were collected from animals in the TK group without anesthesia before dosing, 5, 15, and 30 min, and 1 and 2 hr after dosing at the first dosing and last dosing. Blood samples were put into a tube with preliminary weighed EDTA-2K (approximately 1.8 mg) and 1, 10-phenanthroline (approximately 4 mg), and mixed by end-over-end rotation and a rolling mixer. Then, the blood sample was immediately cooled on ice. Plasma samples were obtained by centrifugation ( $1870 \times g$  for 10 min at  $4^{\circ}\text{C}$ ) immediately after the blood sampling. Plasma concentrations of oxytocin were measured using liquid chromatography-mass spectrometry and tandem mass spectrometry (LC-MS/MS). Plasma samples were treated using solid phase extraction and resultant solutions were injected to an LC-MS/MS system. The LC-MS/MS system consisted of a Nexera X2 system (Shimadzu, Kyoto, Japan) and a Triple Quad 5500 (Sciex, MA, USA). The analyte and internal standard were separated using a reverse phased HPLC column under high pressure gradient elution. The positive ions of oxytocin and internal standard formed by electrospray ionisation were detected using multiple reaction monitoring method.

TK parameters including maximum plasma concentration of the drug ( $C_{\text{max}}$ ), time to maximum plasma concentration ( $t_{\text{max}}$ ), and area under the concentration-time curve to 2 hr after dosing ( $\text{AUC}_{0-2\text{hr}}$ ) were calculated.

### Observations

The day of first dosing was designated as day 1. Days 1 to 7 were designated as week 1. Weeks 1 to 6 were designated as the dosing period and weeks 7 to 10 were designated as the recovery period.

All animals were observed daily for clinical signs. A functional observational battery (FOB, detailed clinical observations and function tests) was performed in 5 males and 5 females in each group of the main group on the day before the start of dosing (in the morning) and during week 1 (day 2: 15 minutes and 2 hr after dosing). Detailed clinical observations were carried out in the cage, on the hand, and in the open field. Function tests were carried out for sensory reactivity to stimuli and grip strength. Body weight, food consumption, and water consumption were recorded once a week during the dosing and recovery periods.

Ophthalmologic examination was performed on all animals before the start of dosing and at week 6 and week 10. After the examination of the light pupillary reflex, a mydriatic (Mydrin-P ophthalmic solution, Santen Pharmaceutical Co., Ltd., Osaka, Japan) was instilled and the anterior portion, optic media, and ocular fundus were examined under dim light.

Urinalysis was performed on all animals at week 6 and week 10. Fresh urine samples were collected within 3 hr in the morning (before dosing during the dosing period) without food or drinking water. From the afternoon (after dosing during the dosing period, around 13:00) on the day of fresh urine sampling to the next morning (around 9:00), 20-hr urine samples were collected while providing food and drinking water. Paper test (pH, protein, glucose, ketones, bilirubin, occult blood) and sediment tests were examined using fresh urine samples. Urine volume, color, osmotic pressure, and electrolytes (sodium [Na], potassium [K], and chlorine [Cl]) were assessed using 20-hr urine samples.

On the day after the end of dosing and recovery periods, blood samples were collected from the posterior vena cava under anesthesia with sodium pentobarbital 50 mg/kg (Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan) through intraperitoneal injection after overnight fasting. One portion of the blood sample was anticoagulated with EDTA-2K and examined for hematology parameters, including red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular vol-

ume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count, white blood cell count, and differential leukocyte ratio and count using a XT-2000iV (Sysmex Corporation, Hyogo, Japan). Another blood sample was anticoagulated with 3.2 w/v% trisodium citrate solution, and then plasma samples were obtained by centrifugation ( $12000 \times g$  for 3 min at  $4^{\circ}\text{C}$ ) to determine prothrombin time (PT) and activated partial thromboplastin time (APTT) using CA-510 (Sysmex Corporation).

Serum from the remaining portion of the blood was analyzed for blood chemistry, including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, urea nitrogen, creatinine, glucose, total cholesterol, phospholipid, triglyceride, total protein, albumin, albumin/globulin ratio, calcium, inorganic phosphorus, sodium, potassium, and chlorine using TBA-200FR (Canon Medical Systems Corporation, Tochigi, Japan).

After blood collection, all animals were euthanized by exsanguination from the abdominal aorta, and then subjected to necropsy. The heart, mandibular and mesenteric lymph nodes, thymus, spleen, trachea, lungs/bronchus, tongue, esophagus, stomach, duodenum, jejunum, ileum (including Peyer's patch), cecum, colon, rectum, salivary gland, liver, pancreas, kidney, urinary bladder, testis, epididymis, seminal vesicle, prostate, pituitary, thyroid/parathyroid, adrenal, femur (including bone marrow, left), sternum (including bone marrow), skin/mammary gland, eyeball/optic nerve/Harderian gland, brain (cerebrum, cerebellum, medulla oblongata, olfactory bulb), spinal cord (neck, thoracic and lumbar part), ovary, uterus, vagina, aorta, skeletal muscle/sciatic nerve (left) and nasal cavity were then removed and fixed in 10% phosphate-buffered formalin. The testes were fixed with Bouin's solution and then stored in 10% phosphate-buffered formalin. The eyeballs including the optic nerve and Harderian gland were fixed with Davidson's solution and then stored in 10% phosphate-buffered formalin.

The heart, thymus, spleen, lungs/bronchus, salivary gland, liver, kidney, testis, epididymis, prostate, pituitary, thyroid/parathyroid, adrenal, brain, ovary, and uterus were weighed using the AW120 electronic balance (Shimadzu Corporation, Kyoto, Japan). Bilateral organs were weighed separately, and total weights were calculated. The relative organ weights were calculated from the ratio of organ weights to body weight on the day of necropsy.

All fixed organs and tissues were routinely prepared and stained with hematoxylin and eosin, and histopathologic examination was conducted on both the placebo

control and 30 U/body/day groups. Level I, II, and III in the nasal cavity (National Toxicology Program) were examined on the administered site and non-administered site.

### Statistical analysis

Comparisons between the placebo control and TTA-121-treated groups during the dosing period were performed as follows: Numerical data such as detailed clinical observation (rearing), grip strength of the forelimb and hindlimb, body weight, food consumption, water consumption, urinalysis (osmotic pressure, urine volume, and electrolytes), hematology, blood chemistry, and absolute and relative organ weights were first analyzed by Bartlett's test for homogeneity (significance level: 1%). When the group variance was homogeneous, Dunnett's test (significance level: 1% and 5%, two-tailed test) was performed to compare values with the mean value of the placebo control group. When the group variance was heterogeneous, Bartlett's test was performed again after logarithmic conversion of data. According to the results, when the variances were homogeneous, Dunnett's test was performed after logarithmic conversion of data. When the variances were heterogeneous, Steel test (significance level: 1% and 5%, two-tailed test) was performed to compare values with the mean value of the placebo control group after rank transformation of the data.

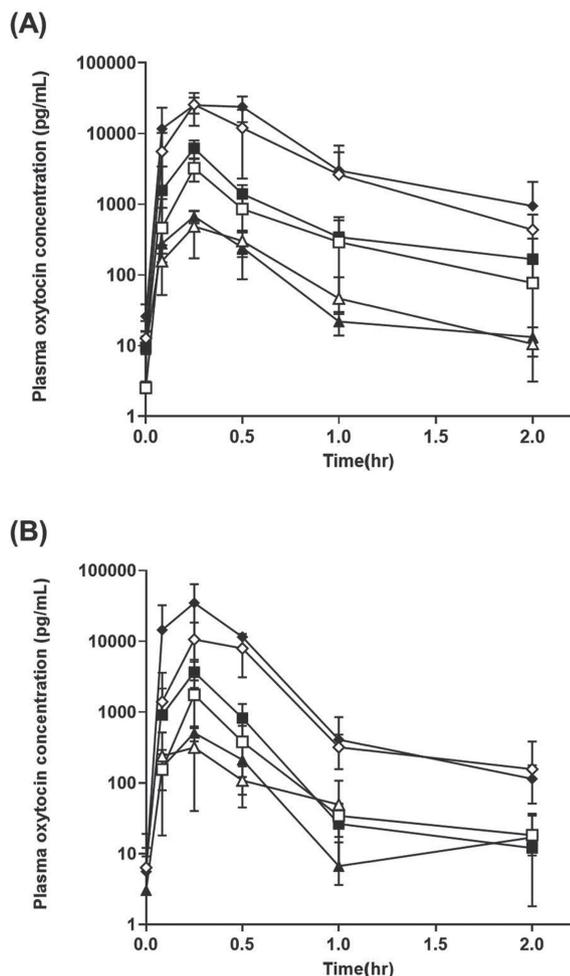
Comparisons between the placebo control and TTA-121-treated groups during the recovery period were performed as follows: Numerical data such as body weight, food consumption, water consumption, urinalysis (osmotic pressure, urine volume, and electrolytes), hematology, blood chemistry, and absolute and relative organ weights was tested by the F test (significance level: 5%) for homogeneity of variance. When the variances were homogeneous, the Student t-test (significance level: 1% and 5%, two-tailed test) was used, and when the variances were heterogeneous, the Aspin-Welch t-test (significance level: 1% and 5%, two-tailed test) was performed.

## RESULTS

The plasma concentrations of oxytocin in rats treated with TTA-121 are shown in Fig. 1. The  $C_{\max}$  and  $AUC_{0-2\text{hr}}$  values increased with the dose ranging from 1.2 to 30 U/body/day at the first and last dosing (Table 1). There were no apparent changes in toxicokinetic parameters after repeated dosing. In addition, there were no apparent gender differences in toxicokinetic parameters.

No deaths or moribundity were observed in any group during the dosing or recovery period. There were no

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**Fig. 1.** Concentration-time course of oxytocin in plasma of rats treated with TTA-121. TTA-121 was administered intranasally at 1.2, 6, and 30 U/body/day once daily for 6 weeks to male and female rats. Plasma concentrations of oxytocin at the first (A) and last dose (B) were determined as described in the Materials and Methods section (Open diamonds: males 30 U/body/day; Open squares: males 6 U/body/day; Open triangles: males 1.2 U/body/day; Closed diamonds: females 30 U/body/day; Closed squares: females 6 U/body/day; Closed triangles: females 1.2 U/body/day). Data represent mean  $\pm$  S.D. ( $n = 4$ ).

changes in clinical signs in any group during the dosing or recovery period. In the FOB, there were no changes in any group during the dosing period (data not shown).

The body weight and daily food consumption in rats treated with TTA-121 are shown in Fig. 2 and Fig. 3, respectively. No test article-related changes in body weight or daily food consumption were noted in any group during the dosing or recovery period. Statistically

significant low mean body weight (day 36) and decrease in food consumption (day 15-22 and 29-36) were noted in females at 30 U/body/day. However, the changes were not considered to be test article-related because they were transient, and the differences were slight.

There were no test article-related changes in water consumption, ophthalmology, hematology, blood chemistry, organ weights, or necropsy in any group during the dosing or recovery period (data not shown).

In the urinalysis, test article-related changes, as stated below, were noted in urine volume and urinary Na, K, and Cl during the dosing period (Table 2). Decreases in urine volume and urinary Cl were noted in males at 30 U/body/day and females at 6 U/body/day or more. A decrease in urinary Na was noted in males at 6 U/body/day or more and in females at 1.2 U/body/day or more. A decrease in urinary K was noted in females at 6 U/body/day or more. These changes were not observed during the recovery period (Table 2).

In terms of histopathology, treatment-related changes were observed in the right-side nasal cavity, the administered site, in females in both the placebo control group and 30 U/body/day group (Table 3). The changes were restricted to the olfactory epithelium of Level II, and included single cell necrosis of the olfactory epithelium and flattening of the olfactory epithelium (Fig. 4). Single cell necrosis of the olfactory epithelium was noted in 2 animals each in the female in the placebo control and 30 U/body/day groups. Flattening of the olfactory epithelium was seen in 2 and 3 females in the placebo control and 30 U/body/day group, respectively. These changes were not observed in the right-side nasal cavity in males. There were no test article-related changes in other organs or tissues.

## DISCUSSION

TTA-121 was administered intranasally (25  $\mu$ L/body) to male and female rats at 0 (placebo), 1.2, 6, and 30 U/body/day once daily for 6 weeks followed by a 4-week recovery period to evaluate the potential toxicity and systemic exposure to oxytocin.

There were no test article-related changes in clinical signs, FOB, water consumption, ophthalmology, hematology, blood chemistry, organ weights, or necropsy at any dose during the dosing or recovery period.

There were no test article-related changes in body weight or food consumption at any dose during the dosing or recovery period. Although recent studies have shown that peripheral or central administration of oxytocin results in sustained weight suppression by reduced

**Table 1.** Toxicokinetic parameters of oxytocin in rats treated with TTA-121 for 6 weeks.

Dose level (U/body/day)	Day of dosing	Male			Female		
		t <sub>max</sub> (hr)	C <sub>max</sub> (pg/mL)	AUC <sub>0-2hr</sub> (pg·hr/mL)	t <sub>max</sub> (hr)	C <sub>max</sub> (pg/mL)	AUC <sub>0-2hr</sub> (pg·hr/mL)
1.2	1	0.3	485	274	0.3	672	289
	42	0.3	316	173	0.3	507	219
6	1	0.3	3230	1310	0.3	6210	2360
	42	0.3	1760	563	0.3	3670	1210
30	1	0.3	25700	12700	0.3	25200	18400
	42	0.3	10600	5670	0.3	34800	13700

Data are shown as the mean value (n = 4).

**Table 2.** Urinalysis findings in rats treated with TTA-121 for 6 weeks.

Dose (U/body/day)	Dosing period (week 6)				Recovery period (week 10)	
	0	1.2	6	30	0	30
<b>Male</b>						
No. of animals	16	10	10	16	6	6
Urine volume (mL)	11.33 ± 4.19	10.64 ± 3.36	11.46 ± 3.17	8.50 ± 1.85*	10.85 ± 3.74	11.43 ± 3.11
Na (mmol)	1.475 ± 0.277	1.389 ± 0.330	1.103 ± 0.341*	1.024 ± 0.328**	1.442 ± 0.658	1.719 ± 0.495
K (mmol)	3.297 ± 0.300	3.249 ± 0.417	3.145 ± 0.252	3.102 ± 0.335	3.552 ± 0.942	3.786 ± 0.830
Cl (mmol)	2.279 ± 0.321	2.281 ± 0.354	2.024 ± 0.307	1.842 ± 0.278**	2.262 ± 0.777	2.632 ± 0.599
<b>Female</b>						
No. of animals	16	10	10	16	6	6
Urine volume (mL)	9.89 ± 2.53	8.56 ± 2.37	6.84 ± 4.05*	6.94 ± 2.38*	10.87 ± 4.20	13.27 ± 5.59
Na (mmol)	1.255 ± 0.188	1.001 ± 0.254**	0.933 ± 0.170**	0.787 ± 0.104**	1.185 ± 0.309	1.098 ± 0.297
K (mmol)	2.699 ± 0.354	2.421 ± 0.501	2.278 ± 0.341*	2.015 ± 0.336**	2.703 ± 0.498	2.767 ± 0.533
Cl (mmol)	1.859 ± 0.272	1.623 ± 0.381	1.542 ± 0.260*	1.322 ± 0.200**	1.761 ± 0.279	1.737 ± 0.425

Data represents mean ± S.D.

\*, \*\*: There are significant differences compared with the placebo control group.

(\*: p < 0.05, \*\*: p < 0.01, Dunnett's test).

**Table 3.** Histopathological findings of Level II in the nasal cavity in rats treated with TTA-121 for 6 weeks.

Dose (U/body/day)	Male		Female	
	0	30	0	30
No. of animals	10	10	10	10
<b>Level II in the nasal cavity</b>				
<u>Administration site:</u>				
Cell infiltration; squamous epithelium, inflammatory, focal (minimal)	1	1	0	0
Single cell necrosis; olfactory epithelium (minimal)	0	0	2	2
Flattening; olfactory epithelium (minimal)	0	0	2	3
<u>Non-administration site:</u>				
Cell infiltration; squamous epithelium, inflammatory, focal (minimal)	0	1	1	0

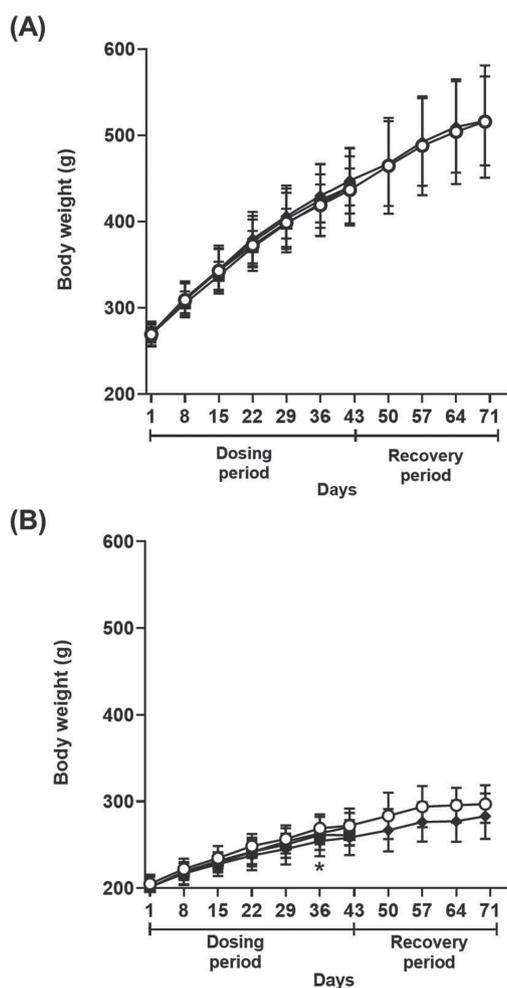
Values are number of animals with findings.

food intake in diet-induced obese rats (Deblon *et al.*, 2011; Morton *et al.*, 2012), there were no such changes in the present study.

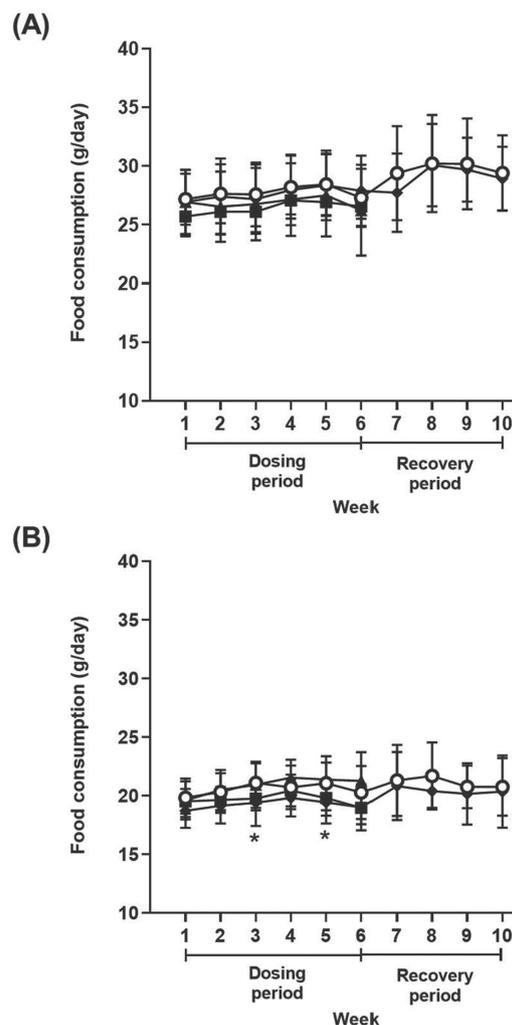
In the urinalysis, decreases in urine volume and urinary Na, K, and Cl were noted during the dosing period. As the rare adverse reaction of water intoxication following oxytocin administration in humans (Mayer-Hubner, 1996) is considered to be related to the close similarity in chemical structure between oxytocin and the antidiuretic hormone,

vasopressin (Legros, 2001; MacDonald *et al.*, 2011; Potter, 1964), the decreases in urine volume and urinary Na, K, and Cl in the present study may be related to the antidiuretic effect of oxytocin. However, they were not considered to be toxicologically significant because almost all the individual values were within the range of the historical control data of the test facility (in-house data of the test facility), no changes were noted in serum electrolytes in blood chemistry, and there were no corre-

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**Fig. 2.** Mean body weight in rats treated with TTA-121. Body weight for male (A) and female (B) rats were measured as described in the Materials and Methods section (Open circles: control; Closed triangles: 1.2 U/body/day; Closed squares: 6 U/body/day; Closed diamonds: 30 U/body/day). Data represent mean  $\pm$  S.D. ( $n = 10$  or 16 [dosing period], 6 [recovery period]). Significant differences from the placebo control group are indicated (\*:  $P < 0.05$ , Dunnett's test).

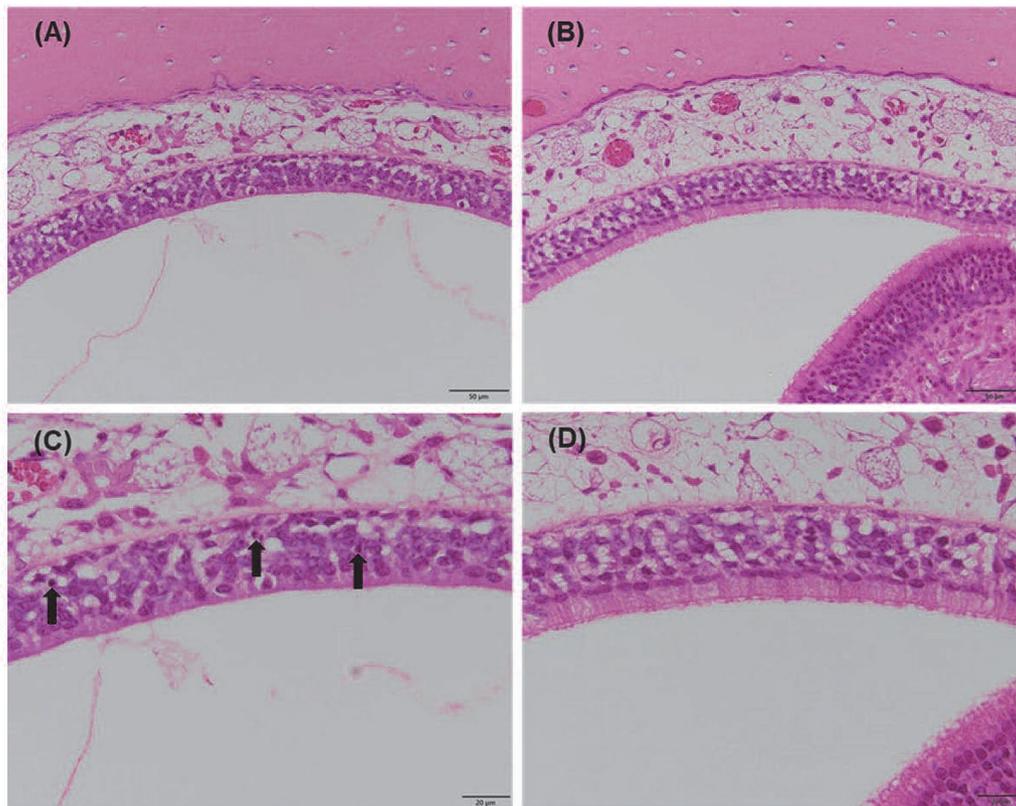


**Fig. 3.** Mean daily food consumption in rats treated with TTA-121. Daily food consumption for male (A) and female (B) rats was measured as described in the Materials and Methods section (Open circles: control; Closed triangles: 1.2 U/body/day; Closed squares: 6 U/body/day; Closed diamonds: 30 U/body/day). Data represent mean  $\pm$  S.D. ( $n = 10$  or 16 [dosing period], 6 [recovery period]). Significant differences from the placebo control group are indicated (\*:  $P < 0.05$ , Dunnett's test).

sponding histopathologic changes in the kidney in these groups.

In terms of histopathology, single cell necrosis and flattening of the olfactory epithelium were observed in the right-side nasal cavity, the administered site, in females in both the placebo control group and 30 U/body/day group. The changes were restricted to the olfactory epithelium of Level II. The incidence of the changes was low (2 of

10 females in each group) and the severity was minimal in both groups. Single cell necrosis was characterized by the presence of necrotic cells within the olfactory epithelium. The origin of the necrotic cells was not clear but might be olfactory cells. Flattening was characterized by a diffuse reduction in the width of the olfactory epithelium. However, the tissue structure of the olfactory epithelium was normal. These changes were not associated



**Fig. 4.** Representative photographs of Level II in the nasal cavity in rats treated with TTA-121. Histopathologic examination of the nasal cavity was performed as described in the Materials and Methods section. Administration site (A and C) and non-administration site (B and D) of Level II in the nasal cavity in the 30 U/body/day group are shown. The arrows identify the single cell necrosis. The scale bar represents 50  $\mu\text{m}$  (A and B) or 20  $\mu\text{m}$  (C and D).

with any lesions such as inflammation in the nasal cavity. In addition, our supplementary mechanistic study revealed that intranasal administration of the placebo used in the present study at 50  $\mu\text{L}$  for 14 days induced minimal goblet cell hyperplasia at Level I of the administered site and minimal single cell necrosis of the olfactory cells and flattening of olfactory epithelium at Level II of the administered site in 4 of 5 female rats while administration of the placebo at 12.5 and 25  $\mu\text{L}$  did not induce any changes (unpublished data), suggesting that these changes detected in the olfactory epithelium could result from mechanical stress related to the large dose volume and liquid characteristics (osmolality and viscosity) of the placebo. Therefore, the single cell necrosis and flattening of the olfactory epithelium observed in the present study were not considered to be toxicologically significant. In the 6-week repeated intranasal dose toxicity study of TTA-121 in monkeys, there were no treatment-related changes in the nasal cavity up to 672.0 U/body/day (Matsumoto *et al.*, 2019). On a U/cm<sup>2</sup> nasal surface area

basis, the high dose levels in the rat (2.1 U/cm<sup>2</sup>) and monkey (10.8 U/cm<sup>2</sup>) studies produced 17 and 86-fold safety margins, respectively compared to the high dose level of 20 U/body (0.125 U/cm<sup>2</sup>) in the Phase 1 study of TTA-121 (nasal surface area: rats; 14 cm<sup>2</sup>, monkeys; 62 cm<sup>2</sup>, human; 160 cm<sup>2</sup>) (Erdő *et al.*, 2018). These results suggest that there is no clinically relevant concern with regard to the local effect of TTA-121.

In the TK analysis, the  $C_{\text{max}}$  and  $\text{AUC}_{0-2\text{hr}}$  values increased with the dose ranging from 1.2 to 30 U/body/day. Compared to the plasma oxytocin concentrations at the high dose group (20 U/body) in the Phase 1 study of TTA-121 (Day 9:  $C_{\text{max}}$ ; 85.712 pg/mL,  $\text{AUC}_{0-6\text{hr}}$ ; 47.683 pg·hr/mL) (UMIN000025922, Sakanaka *et al.*, 2018), the dose levels of 1.2, 6, and 30 U/body/day in the present study produced 3.7 to 5.9, 21 to 43, and 124 to 406-fold margin respectively based on the  $C_{\text{max}}$ , and 3.6 to 4.6, 12 to 25, and 119 to 287-fold margin respectively based on the AUC. The results of the TK analysis indicated that sufficiently high systemic exposures of oxytocin were

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achieved in the present study. As mentioned earlier, TTA-121 is a novel oxytocin nasal spray with high bioavailability expected to increase oxytocin delivery to the brain. Our pharmacokinetic study using rabbits demonstrated that TTA-121 achieved remarkably higher oxytocin concentrations in both plasma and cerebrospinal fluid (CSF) than a comparative formulation prepared by adding oxytocin to Syntocinon to adjust the oxytocin concentrations (WO 2017/073798 A1). Furthermore, in a social interaction test using rats, TTA-121 (0.0125 to 1.25 U/body) promoted social interaction, indicative of central nervous system (CNS) activity of oxytocin, more potently than the comparative formulation prepared by adding oxytocin to Syntocinon to adjust the oxytocin concentrations (unpublished data). Therefore, CNS exposures of oxytocin were expected to be also sufficiently high in the present study.

As oxytocin has CNS activity, its abuse potential was preliminary assessed based on the results of the present study and a literature search. In the present study, there were no withdrawal signs in clinical signs or body weight during the recovery period, suggesting that oxytocin is unlikely to cause physical dependence. Although known drugs of abuse with binding affinity to receptors, transporters and ion-gated channel systems associated with abuse potential (e.g., dopamine, serotonin, gamma-aminobutyric acid, opioids, etc.) were reported to cause abuse-related effects such as stimulant or sedative properties in mice or rats (FDA, 2017; Himmel, 2008), there were no such changes in the clinical signs or FOB in the present study. Therefore, these results suggest that oxytocin is unlikely to cause abuse-related effects resulting from the above-mentioned neuronal systems known to be associated with abuse potential.

In conclusion, no toxicologically significant changes were observed in males or female rats up to 30 U/body/day under the conditions of the present study. The no-observed-adverse-effect level of TTA-121 was 30 U/body/day for males and female rats, suggesting that there is a sufficient safety margin. The Phase 2 study of TTA-121 on individuals with ASD is currently underway (NCT03466671/UMIN000031412). We believe that TTA-121 is expected to be sufficiently safe to treat males and females with ASD, which is currently untreatable with pharmacotherapy.

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**Conflict of interest----** The authors declare that there is no conflict of interest.

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