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Original Article

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

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ABSTRACT — 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 monkeys was conducted. In the present study, TTA-121 was administered intranasally to male and female cynomolgus monkeys once daily for 6 weeks at 0, 26.88, 134.4, and 672.0 U/body/day followed by a 4-week recovery period to investigate the toxicity of oxytocin and systemic exposure to oxytocin. No animal died or was euthanized due to moribundity. There were no test article-related changes in clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology, blood chemistry, necropsy, organ weights, or histopathology at any dose level during the dosing or recovery periods. The toxicokinetic analysis indicated that systemic exposures of oxytocin increased with the dose ranging from 26.88 to 672.0 U/body/day on the first and final day of dosing. Based on these results, the no-observed adverse effect level (NOAEL) of TTA-121 was 672.0 U/body/day.

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

INTRODUCTION

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 (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). A Phase 2 study of TTA-121 on individuals with Autism spectrum disorder (ASD) is currently underway (NCT03466671/UMIN000031412).

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 conducted (Namekawa *et al.*, this issue). In this paper, we show the results of the toxicity study in monkeys.

MATERIALS AND METHODS

The present study was conducted at Shin Nippon Biomedical Laboratories, Ltd. (Kagoshima, Japan) in compliance with the Good Laboratory Practice regulations (Ministry of Health and Welfare Ordinance No. 21, March 26, 1997, and as amended, Ministry of Health, Labour and Welfare Ordinance No. 114, June 13, 2008). This study was approved by the Institutional

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Animal Care and Use Committee and performed in accordance with the animal welfare bylaws of Shin Nippon Biomedical Laboratories, Ltd., Drug Safety Research Laboratories, which is accredited by 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). All test articles and placebo were stored in a refrigeration room (2 to 8°C), and protected from light. The stability of the test article from the same lot stored under the same conditions as those in the present study was confirmed.

Animals

Twenty-one male and 21 female purpose bred cynomolgus monkeys (*Macaca fascicularis*) were purchased from Angkor Primates Center Inc. (Kampong Thom Province, Cambodia). All animals were acclimated to the testing environment for 9 days prior to the first dosing. At the end of acclimation, all the monkeys were 3 to 4 years of age with body weights that ranged from 3.37 to 5.45 kg for males and 2.78 to 3.99 kg for females. The animals were housed individually in stainless steel cages (680 × 620 × 770 mm) and kept in an environmentally controlled room with conditions as follows: temperature, 23 to 29°C; humidity, 30 to 70%; ventilation, 15 times/hr; and lighting, 12-hr day (lights on/off, 07:00/19:00). Approximately 108 g of solid food (HF Primate J 12G 5K9J, Purina Mills, LLC, Arden Hills, MN, USA) was provided to each animal once daily between 14:00 and 16:00. Water was available *ad libitum* from an automatic water supply.

Selection of dose levels

Dose levels were selected on the basis of the results obtained from a 2-week repeated intranasal dose toxicity study of TTA-121 in cynomolgus monkeys and the soluble data of the test article. The high dose level in the present study was set at 672.0 U/body/day, which was considered to be the maximally practicable dose. The middle and low dose levels were set at 134.4 and 26.88 U/body/day, respectively, in a common ratio of 5.

Experimental design

TTA-121 was administrated intranasally once daily at 0 (placebo control), 26.88, 134.4 and 672.0 U/body/day for 6 weeks to male and female cynomolgus monkeys. Three males and 3 females were allocated to all the administered groups as the main group, while the recovery group was comprised of 2 males and 2 females in the high dose and control groups. The dosing formulation was sprayed into the left and right nasal cavities (560 μL/body, 280 μL/nasal cavity). The animal's chin was held up in order to keep the head tilted backward for approximately 1 min. On the day after the final dosing, 12 males and 12 females from the main group were euthanized for evaluation of the organ weights, and macroscopic and microscopic findings. After the dosing period, the remaining 4 males and 4 females were kept without treatment for 4 weeks as the recovery group and then fully examined similar to the main group.

Observation

The first day of 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 mortality and clinical signs throughout the study period. Body weight was recorded once a week throughout the study period. Food consumption was recorded daily, with the mean daily food consumption then calculated for each week.

Ophthalmologic examinations were performed before the start of dosing (acclimation period), and at weeks 6 and 10. Gross observations and pupillary light reflex examinations were performed using a portable slit lamp (SL-15, Kowa Co., Ltd., Nagoya, Japan). The anterior ocular segment and optic media were examined with a portable slit lamp (SL-15, Kowa Co., Ltd.), and the ocular fundi was examined with an indirect ophthalmoscope (IO-αSmall Pupil, Neitz Instruments Co., Ltd., Tokyo, Japan) under ketamine hydrochloride anesthesia (10 mg/kg, Supriya Lifescience Ltd., Maharashtra, India) by an intramuscular injection after instillation of a mydriatic agent (Mydrin-P ophthalmic solution, Santen Pharmaceutical, Co., Ltd., Osaka, Japan).

Electrocardiography was performed before the start of dosing and at weeks 6 and 10 (at a time corresponding to between immediately and 30 min after the dosing). Electrocardiograms (leads I, II, III and aVR, aVL, aVF) were measured in restrained conscious monkeys with an ECG processor (SP2000, Softron Co., Ltd., Tokyo, Japan) via an electrocardiograph for animals (Cardisuny α6000AX-D or Cardisuny D500, Fukuda M·E Kogyo Co., Ltd., Tokyo, Japan). Heart rate, PR interval, QRS duration, QT interval and QTc (Bazzett's formula) were analyzed using averaged continuous waveforms from lead II for 8 seconds.

Urinalysis was performed before the start of dosing, and at weeks 6 and 10. Urine within 2 hr and urine for

approximately 16 hr were collected for the fresh and preserved urine samples, respectively. Color and sediments in the fresh urine were examined visually or microscopically. Ketone bodies, bilirubin, occult blood, urobilinogen, protein, glucose and pH in the fresh urine were analyzed using an automated urine chemistry analyzer (Clinitek Atlas XL, Sparton Medical Systems, Inc., Schaumburg, IL, USA) or an automatic analyzer (JCA-BM6070, JEOL Ltd., Tokyo, Japan). Urine volume, specific gravity, sodium, potassium and chloride in preserved urine were analyzed using a measuring cylinder, a urinary refractometer (Uricon-JE, Atago Co., Ltd., Tokyo, Japan) or an automatic analyzer (JCA-BM6070, JEOL Ltd.).

Hematology and blood chemistry were performed before the start of dosing, and at weeks 6 and 10. Blood was collected from the femoral vein. One mL of blood was treated with an anticoagulant, EDTA-2K and examined for hematologic parameters, such as erythrocyte count, leukocyte count, hematocrit value, hemoglobin concentration, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes, and differential leukocytes using a hematology system (ADVIA120, Siemens Healthcare Diagnostics Manufacturing Ltd., Dublin, Ireland). Another 1 mL of blood was treated with 3.8 w/v% sodium citrate, and plasma obtained by centrifugation (room temperature, 1710 × *g*, 3000 rpm, 10 min) was examined for blood coagulation parameters, such as prothrombin time and activated partial thromboplastin time using an automated blood coagulation analyzer (CS-5100, Sysmex Corporation, Kobe, Japan). Additional 2 mL of blood was left at room temperature for 20 to 60 min, and serum obtained by centrifugation (room temperature, 1710 × *g*, 3000 rpm, 10 min) was examined for blood chemistry, such as aspartate transaminase, alanine transaminase, alkaline phosphatase, creatine kinase (CK), total bilirubin, total protein, albumin, globulin, A/G ratio, total cholesterol, triglycerides, glucose, blood urea nitrogen, creatinine, inorganic phosphorus, calcium, sodium (Na), potassium and chloride (Cl) using an automatic analyzer (JCA-BM6070, JEOL Ltd.).

At the end of the dosing period and recovery period, all animals were weighed, anesthetized by an intravenous injection of sodium pentobarbital solution (64.8 mg/mL, 0.4 mL/kg, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) into the cephalic vein, and euthanized by exsanguination. External appearance, and following organs and tissues were examined macroscopically: trachea, lungs (including bronchi), nasal tissue, tongue, submandibular glands, pharynx, larynx, tonsils, esophagus, stomach (body/pylorus), duodenum, jejunum, ileum (including Peyer's patch), cecum, colon, rectum, pancreas, liver, gallbladder, aorta, heart, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, vagina, brain (cerebrum, cerebellum, pons, medulla oblongata and olfactory bulb), spinal cord, sciatic nerves, sternum/sternal bone marrow, femurs/femoral bone marrow, submandibular lymph nodes, mesenteric lymph nodes, spleen, thymus, pituitary, thyroids/parathyroids, adrenals, eyeballs/optic nerves, lacrimal glands, skeletal muscle, mammary glands/skin. The lungs, submandibular glands, liver, heart, kidneys, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, brain, spleen, thymus, pituitary, thyroids/parathyroids and adrenals were weighed with an electronic balance (HR-200, FX-3000N, GF-3000, or HF-3000, A&D Co., Ltd., Tokyo, Japan). Relative organ weights per kg of body weight were calculated from the body weight on the day of necropsy. In the case of bilateral organs that were weighed separately, the total bilateral weight was calculated for each of these organs. The eyeballs and optic nerves were fixed in a mixture of 3% glutaraldehyde and 2.5% formalin, while the testes were fixed in a formalin-sucrose-acetic acid solution. Other organs and tissues were fixed in 10% neutral buffered formalin. The trachea, sternum, sternal bone marrow, femur, and femoral bone marrow were decalcified with Kalkitox (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The pharynx, larynx, nasal tissue including vestibular, respiratory, olfactory and paranasal sinuses areas were decalcified with formic acid-formalin. The fixed organs and tissues were trimmed, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and then examined histopathologically. The frontal planes of vestibular, respiratory, olfactory and paranasal sinuses areas of nasal tissue were examined appropriately on histopathology of nasal tissue.

Blood sampling for toxicokinetic (TK) analysis was performed before dosing and 10, 20 and 40 min, and 1, 2 and 4 hr after dosing on days 1 and 42. Blood was collected from the femoral vein, treated with EDTA-2K and 1, 10-phenanthroline, and cooled on ice. Plasma was immediately obtained by centrifugation (4°C, 1870 × *g*, 10 min), with all plasma samples then stored in a deep freezer (below -70°C). Plasma concentrations of oxytocin were determined using liquid chromatograph-tandem mass spectrometer (LC-MS/MS). Plasma samples were treated using solid phase extraction and the resultant solutions were injected to the LC-MS/MS system. The LC-MS/MS system consisted of a Nexera X2 system (Shimadzu corporation, Kyoto, Japan) and a Triple Quad 5500 (Sciex, Framingham, 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 the internal standard formed by electrospray ionization were detected using a multiple reaction monitoring method. The TK parameters which included the maximum plasma concentration of the drug (C_{max}), time to maximum plasma concentration (t_{max}), and area under the concentration-time curve to 4 hr after dosing ($AUC_{0\text{-}4hr}$) were calculated.

Data analysis

Data obtained on the body weight, food consumption, electrocardiography, urinalysis (quantitative data except electrolyte concentration), hematology (except differential leukocyte ratio), blood chemistry, and organ weights (absolute and relative) during the acclimation and dosing periods were analyzed for homogeneity of variance by Bartlett's test. When the variance was homogeneous, Dunnett's test was performed for multiple comparison between the control group and each test article group. When the variance was heterogeneous for Bartlett's test, a Dunnett-type test (Miller's test) was performed for multiple comparison between the control group and each test article group. For the urinalysis, gradable data were analyzed by Wilcoxon's rank sum test, while the urine color was analyzed by Fisher's exact test between the control group and each test article group. The MiTOX System (Mitsui Zosen Systems Research Inc., Chiba, Japan) was used for the statistical analyses at a significance level of 5% for Bartlett's test or at a two-sided significance level of 5% for the other tests.

RESULTS AND DISCUSSION

In order to investigate the toxicity of oxytocin and systemic exposure to oxytocin, TTA-121 was administered intranasally (560 μL/body) to male and female cynomolgus monkeys once daily for 6 weeks at 0, 26.88, 134.4, and 672.0 U/body/day followed by a 4-week recovery period.

No animal died or was euthanized due to moribundity at any dose level during the dosing or recovery period. There were no test article-related changes in clinical signs at any dose level during the dosing or recovery period (data not shown).

The body weight and food consumption are shown in Fig. 1 and Fig. 2, respectively. There were no test article-related changes in body weight and food consumption at any dose level during the dosing or recovery period.

In ophthalmology, there were no abnormalities at any dose level during the dosing or recovery period (data not shown).

In electrocardiography, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown). Although a statistically significant shortening of QT interval was noted in males at 26.88 U/body/day at week 6 (QT intervals for the control, 26.88, 134.4 and 672.0 U/body/day group were 187.8 ± 10.2, 164.3 ± 7.1, 172.0 ± 15.4 and 168.8 ± 11.8 ms, respectively), this change was not considered to be test article-related because there was no dose dependency.

In urinalysis, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown). A positive occult blood reaction (Grade 3+ or 4+) and/or erythrocytes in urinary sediments were noted in 1 female at 134.4 U/body/day and in 1 female at 672.0 U/body/day at week 6, and in 1 female at 672.0 U/body/day at week 10. These changes were considered to be related to menstruation, which was observed in the animal around the same period as the urine collection day.

In hematology, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown).

In blood chemistry, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown). The following statistically significant changes were noted at week 6: an increase in Cl in females at 26.88 and 672.0 U/body/day, an increase in CK in females at 134.4 U/body/day and an increase in Na in males at 134.4 U/body/day (Table 1). These were not considered to be test article-related because there was no dose dependency, no clear differences were noted when compared with the pre-dosing values (Cl in female at 26.88 and 672.0 U/body/day group; 110.3 ± 2.1 mEq/L and 110.0 ± 1.6 mEq/L, respectively, CK in female at 134.4 U/body/day group; 872.7 ± 714.3 IU/L, Na in male at 134.4 U/body/day group;149.3 ± 4.0 mEq/L), or they were within the range of the background data (in-house data of the test facility).

In necropsy, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown).

In organ weights, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown). The following statistically significant changes were noted in females at the end of the dosing period: decreases in absolute and relative thyroid weight at 26.88, 134.4 and 672.0 U/body/day, and an increase in absolute lung weight at 26.88 U/body/day (Table 2). These were not considered to be test article-related because there was no dose dependency, no corresponding histopathological lesions were observed, or they

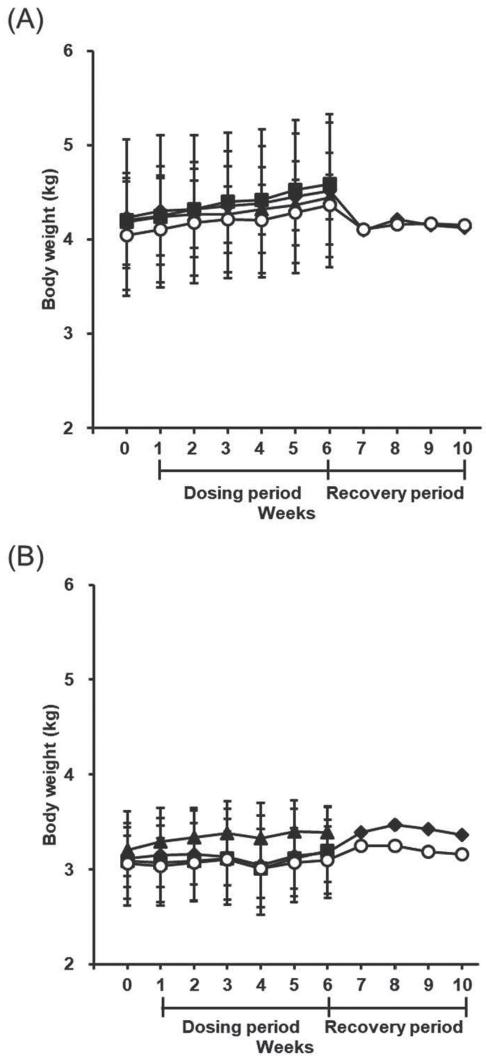

Fig. 1. Body weights of cynomolgus monkeys treated with TTA-121. Body weights for male (A) and female (B) cynomolgus monkeys were measured as described in the Materials and Methods section (open circles: control; closed triangles: 26.88 U/body/day; closed squares: 134.4 U/body/day; closed diamonds: 672.0 U/body/day). Values of the dosing period are presented as the mean ± S.D. (n = 3 or 5), while the values of the recovery period are presented as the mean (n = 2).

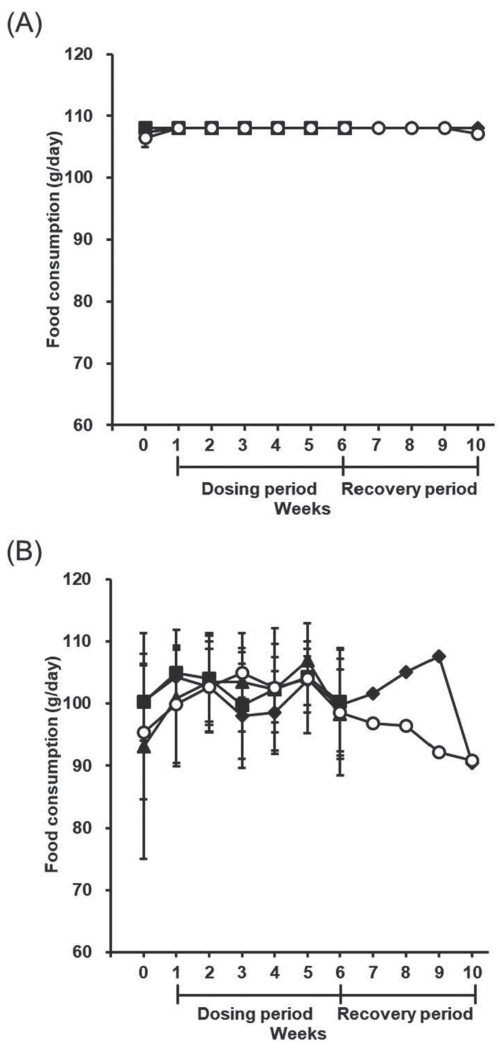

Fig. 2. Mean daily food consumption in cynomolgus monkeys treated with TTA-121. Mean daily food consumption for male (A) and female (B) cynomolgus monkeys was measured as described in the Materials and Methods section (open circles: control; closed triangles: 26.88 U/body/day; closed squares: 134.4 U/body/day; closed diamonds: 672.0 U/body/day). Values of the dosing period are presented as the mean ± S.D. (n = 3 or 5), while the values of the recovery period are presented as the mean (n = 2).

were within the range of the background data (in-house data of the test facility).

In histopathology, there were no test article-related changes at any dose level during the dosing or recovery period (data not shown). In terms of nasal cavity, no treatment-related changes were noted in vestibular, respiratory, olfactory and paranasal sinuses areas up to 672.0 U/body/day. On a U/cm^2 nasal surface area basis, the high dose level of 672.0 U/body/day (10.8 U/cm^2) in the present study produced a 86-fold safety margin compared to the high dose level of 20 U/body (0.125 U/ cm^2) in the Phase 1 study of TTA-121 (nasal surface area: mon-

Table 1. Summary of statistically significant changes in blood chemistry in cynomolgus monkeys treated with TTA-121 for 6 weeks.

Dose (U/body/day)	Dosing period (week 6)				Recovery period (week 10)	
	0	26.88	134.4	672.0	0	672.0
Male						
No. of animals	5	3	3	5	2	2
CK (IU/L)	317.0 ± 147.2	619.0 ± 741.3	324.3 ± 72.2	239.6 ± 83.3	143.0	216.5
Na (mEq/L)	145.2 ± 0.8	147.3 ± 1.5	148.7 ± 1.2 **	146.0 ± 1.6	146.0	146.5
Cl (mEq/L)	108.2 ± 3.2	110.3 ± 0.6	108.7 ± 0.6	109.4 ± 2.3	106.0	106.0
Female						
No. of animals	5	3	3	5	2	2
CK (IU/L)	128.6 ± 27.9	282.3 ± 213.2	595.0 ± 251.7*	158.6 ± 95.6	228.0	574.0
Na (mEq/L)	145.0 ± 1.6	147.0 ± 1.0	147.7 ± 3.8	148.0 ± 2.9	146.5	147.0
Cl (mEq/L)	107.0 ± 2.1	110.7 ± 0.6*	110.3 ± 2.1	110.2 ± 1.6*	108.0	110.5

Values of the dosing period are presented as the mean ± S.D.

Values of the recovery period are presented as the mean.

* There are significant differences compared with the control group at $p < 0.05$ by Dunnett's test or Miller's test.

** There are significant differences compared with the control group at $p < 0.01$ by Dunnett's test.

Table 2. Summary of statistically significant changes of organ weights (absolute and relative) in cynomolgus monkeys treated with TTA-121 for 6 weeks.

Dose (U/body/day)	Dosing period (week 6)				Recovery period (week 10)	
	0	26.88	134.4	672.0	0	672.0
Male						
No. of animals	3	3	3	3	2	2
Body weight (kg)	4.360 ± 0.638	4.307 ± 0.237	4.380 ± 0.603	4.613 ± 0.946	4.100	4.075
Lung (g)	19.00 ± 3.40	20.27 ± 3.44	19.60 ± 1.08	18.50 ± 4.25	18.25	17.85
Lung (g/kg)	4.363 ± 0.606	4.693 ± 0.654	4.523 ± 0.580	4.007 ± 0.462	4.415	4.390
Thyroid (g)	0.440 ± 0.098	0.480 ± 0.020	0.500 ± 0.229	0.940 ± 0.557	0.505	0.465
Thyroid (g/kg)	0.1023 ± 0.0301	0.1117 ± 0.0076	0.1110 ± 0.0350	0.2133 ± 0.1268	0.1255	0.1115
Female						
No. of animals	3	3	3	3	2	2
Body weight (kg)	2.847 ± 0.068	3.273 ± 0.315	3.053 ± 0.311	2.857 ± 0.142	3.100	3.295
Lung (g)	14.30 ± 0.75	17.33 ± 1.29 *	14.73 ± 1.72	15.80 ± 0.62	16.10	17.00
Lung (g/kg)	5.030 ± 0.364	5.320 ± 0.527	4.867 ± 0.847	5.533 ± 0.174	5.265	5.145
Thyroid (g)	0.640 ± 0.115	0.383 ± 0.103*	0.397 ± 0.042*	0.343 ± 0.061**	0.410	0.360
Thyroid (g/kg)	0.2243 ± 0.0365	0.1160 ± 0.0217**	0.1300 ± 0.0052**	0.1200 ± 0.0160**	0.1315	0.1095

Values of the dosing period are presented as the mean ± S.D.

Values of the recovery period are presented as the mean.

* There are significant differences compared with the control group at $p < 0.05$ by Dunnett's test.

** There are significant differences compared with the control group at $p < 0.01$ by Dunnett's test.

Table 3. TK parameters of oxytocin in cynomolgus monkeys treated with TTA-121 for 6 weeks.

Day	Dose level (U/body/day)	C_{max} (pg/mL)		T_{max} (hr)		$AUC_{0\text{-}4hr}$ (pg·hr/mL)	
		Males	Females	Males	Females	Males	Females
Day 1	26.88 [a)]	406	635	0.2	0.2	221	340
	134.4 [a)]	2780	3090	0.2	0.3	1570	4460
	672.0 [b)]	14200	20200	0.2	0.3	12000	15900
Day 42	26.88 [a)]	437	535	0.2	0.2	260	313
	134.4 [a)]	2110	3760	0.2	0.2	1230	2700
	672.0 [b)]	12100	33900	0.2	0.2	9770	19300

Values are presented as the mean.

a) n = 3, b) n = 5

keys; 62 cm^2, human; 160 cm^2) (Erdő *et al*., 2018). These results suggest that there is no clinically relevant concern with regard to the local effects of TTA-121.

In TK analysis, Fig. 3 shows the time-concentration profiles of oxytocin in monkey plasma. The mean of C_{max} and $AUC_{0\text{-}4hr}$ values increased with dose ranging from 26.88 to 672.0 U/body/day on days1 and 42 (Table 3). There were no apparent changes in the TK parameters after repeated dosing. Also, there were no apparent gender differences in the TK parameters. 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_{max}; 85.712 pg/mL, $AUC_{0\text{-}6hr}$; 47.683 pg·hr/mL) (Sakanaka *et al*., 2018), the dose levels of 26.88, 134.4, and 672.0 U/body/day in the present study produced a 5.1 to 6.2, 25 to 44, and 141 to 396-fold margin, respectively, based on the C_{max}, and a 5.5 to 6.6, 26 to 57, and 205 to 405-fold margin, respectively, based on the AUC. Thus the TK analysis results indicated that sufficiently high systemic exposures of oxytocin were achieved in the present study.

In conclusion, there were no test article-related changes in male or female monkeys up to 672.0 U/body/day under the conditions of the present study. The no-observed adverse effect level (NOAEL) of TTA-121 was 672.0 U/body/day.

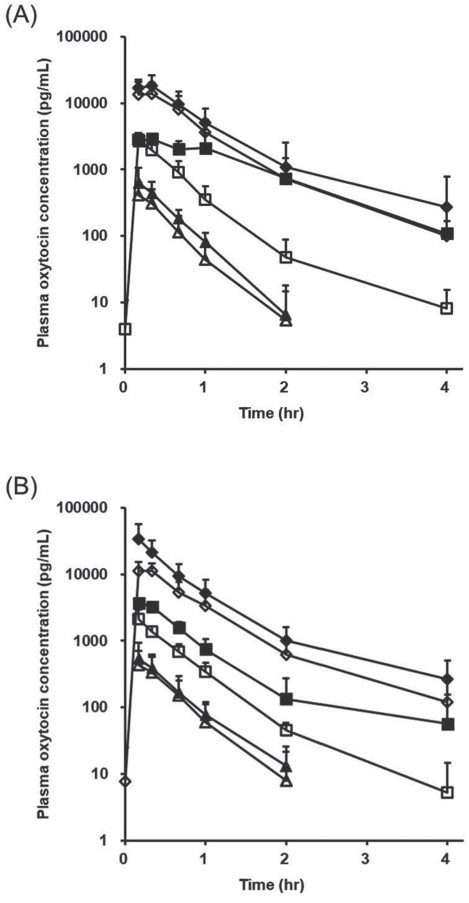

Fig. 3. Time-concentration profile of oxytocin in plasma of cynomolgus monkeys treated with TTA-121. TTA-121 was administrated intranasally to male and female cynomolgus monkeys once daily at 26.88, 134.4 and 672.0 U/body/day for 6 weeks. Plasma concentrations of oxytocin on the first (A) and final (B) day of dosing were determined as described in the Materials and Methods section (open triangles: males 26.88 U/body/day; open squares: males 134.4 U/body/day; open diamonds: males 672.0 U/body/day; closed triangles: females 26.88 U/body/day; closed squares: females 134.4 U/body/day; closed diamonds: females 672.0 U/body/day). Values are presented as the mean + S.D. (n = 3 or 5).

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The histopathological findings of the present study were reviewed by John Curtis Seely, D.V.M., Diplomate, ACVP (Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, USA).

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

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