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Full Length Manuscripts

Delivery of Acetaminophen to the Central Nervous System and the Pharmacological Effect after Intranasal Administration with a Mucoadhesive Agent and Absorption Enhancer

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Abstract

Acetaminophen, a central antipyretic and analgesic drug, is one of the most commonly used drugs among individuals of all ages throughout the world. This study pharmacokinetically and pharmacodynamically investigated the transport of acetaminophen to the central nervous system and systemic circulation after intranasal (*i.n.*) administration, and evaluated the potential of a transnasal acetaminophen formulation in comparison to other routes of administration. Direct transport to the brain and the pharmacological effect after the *i.n.* administration of acetaminophen with polyvinylpyrrolidone (PVP; a mucoadhesive agent) and poly-L-arginine (PLA; an absorption enhancer) were investigated to improve retention of the dosage solution in the olfactory epithelium region and enhance the transfer of acetaminophen to the brain. The transport of acetaminophen to the brain was rapid, and the concentration in the brain, especially the olfactory bulb, was higher after *i.n.* administration, resulting in a greater antipyretic effect in comparison to other routes of administration. The delivery system using PVP and PLA produced a high and prolonged antipyretic effect by enhancing the transfer of acetaminophen to the brain through suppression of the transfer to systemic circulation. Thus, this transnasal drug delivery system using PVP and PLA may be a promising method for transporting acetaminophen to the brain.

Key words: acetaminophen, intranasal administration, direct transport to the brain, antipyretic effect, polyvinylpyrrolidone (PVP), poly-L-arginine (PLA)

1. Introduction

The transport of drugs from the systemic circulation to the brain after oral administration or injection is determined by permeability through the blood-brain barrier (BBB). The BBB is composed of vascular endothelial cells, and the tissue surrounding the cells strictly controls the function of the capillaries; it therefore has a very tight cell sheet structure and the properties of a lipid membrane (Pardridge, 2003). Consequently, drug transfer to the brain via the BBB is generally high for lipophilic compounds, but low for hydrophilic compounds (Pardridge, 2012). However, the transfer of even lipophilic compounds to the brain may be restricted by efflux transporters, such as P-glycoprotein, which is expressed in the capillary endothelial cells. Thus, while the BBB has a very important barrier mechanism that selectively limits the entry of exogenous substances and transports nutrients to the brain by influx transporters, it presents a major obstacle to the delivery of therapeutic drugs for diseases of the central nervous system (CNS), and the development of technology to efficiently deliver drugs to the brain is actively underway. One method for delivering drugs to the brain is intranasal (*i.n.*) administration. Anatomically, the nasal cavity is located close to the brain. The cribriform plate of the skull, which separates the nasal region from the brain region, has numerous holes. The olfactory epithelium is connected to the olfactory bulb by olfactory nerves, which pass through the holes (Illum, 2003, Lochhead and Thorne, 2012, Crowe *et al*., 2018). Thus, numerous reports have suggested that drugs may be transported directly to the olfactory bulb via the olfactory epithelium and nerves after *i.n.* administration (Thorne *et al*., 2004, Uchida *et al*., 2011, Gartziandia *et al*., 2015). In addition, it has been suggested that the drugs may be transferred to the brainstem and spinal cord behind the brain via the trigeminal pathway (Johnson *et al*., 2010, Kanazawa *et al*., 2013). Therefore, *i.n.* administration is considered to be an excellent route for delivering drugs to the brain.

However, drug solutions that are administered intranasally are rapidly removed from the nasal cavity by mucociliary and physical clearance (Lochhead and Thorne, 2012, Crowe *et al*., 2018, Gänger and Schindowski, 2018). Thus, there is a need for processes that prolong the retention time of drug solutions in the olfactory epithelium region and which enhance the direct transport of drugs to the brain. Recently, studies have been conducted using absorption enhancers (e.g., chitosan, Tat peptide) and mucoadhesive substances (e.g., chitosan and polyethyleneglycol) (Uchida at al., 2011, Kanazawa *et al*., 2013, Gartziandia *et al*., 2015, Kanazawa *et al*., 2017., Li *et al*., 2018., Wang *et al*., 2019). These mucoadhesive substances improved the intranasal retentivity by increasing the viscosity of dosage solution (Dharmala *et al.*, 2008, Kamiya *et al.*, 2017). These absorption enhancers promoted the nasal absorption by opening the intercellular spaces since they were cationic compounds (Dodane *et al*., 1999. Zhong *et al.*, 2012. Li *et al*., 2018). Prolonged retention time and the transfer of increased amounts of drug to the brain have been achieved with their use.

Acetaminophen is a central antipyretic and analgesic drug that has long been used throughout the world. Although the detailed pharmacological mechanism is still unclear, it is thought to exert its antipyretic effects through activity in the thermoregulatory center in the hypothalamus, and to exert its analgesic effects by altering the pain threshold in the thalamus and cerebral cortex (Anderson, 2008). It is also thought that pharmacological effects are exhibited by the activation of the descending inhibitory pathways via cannabinoid receptors and serotonin, in addition to central cyclooxygenase (COX) inhibition (Seo *et al*., 2008, Smith, 2009, Ghanem *et al*., 2016). The World Health Organization (WHO) has listed acetaminophen as a basic medicine for the treatment of cancer pain, and as a first-line medicine in the three-stage pain relief ladder (WHO, 1996). It has similar antipyretic and analgesic effects to aspirin; however, it is not considered to

be associated with any side effects (e.g., damage to the gastrointestinal mucosa and kidneys), since it has almost no inhibitory effect on COX-1, which is an enzyme that produces prostaglandins, which show protective effects (Jóźwiak-Bebenista and Nowak, 2014). For these reasons, acetaminophen is considered a safe antipyretic and analgesic drug for elderly individuals. In addition, it is listed as a first-line antipyretic for children and is recommended as an antipyretic for patients with influenza. Moreover, its administration during pregnancy is considered relatively safe because the drug that penetrates through the placenta is efficiently metabolized by the fetus (Zutshi *et al*., 2016, Mian *et al*., 2020). Thus, acetaminophen, which is available in injectable, oral, and suppository formulations, is widely used by patients with various conditions. However, the use of injectable and oral formulations may lead to low compliance due to the invasiveness of injections and the difficulty in taking oral medicines experienced by patients with dysphagia. There may also be problems with suppositories, such as low bioavailability according to the insertion technique. Thus, an acetaminophen formulation that can easily be administered by patients and which is rapidly transferred to the CNS and expected to exert a rapid therapeutic effect after administration would be considered useful. In this study, the transport of acetaminophen, a central antipyretic and analgesic drug, to the CNS and systemic circulation after *i.n.* administration was pharmacokinetically and pharmacodynamically investigated, and the potential of a transnasal acetaminophen formulation was evaluated in comparison to other routes of administration. Furthermore, the direct transport to the brain and the pharmacological effect of acetaminophen after *i.n.* administration with polyvinylpyrrolidone (PVP) as a mucoadhesive agent and poly-L-arginine (PLA) as an absorption enhancer were investigated in order to improve the retention of acetaminophen solution in the olfactory epithelium region and enhance the transfer of acetaminophen to the brain. PVP was

chosen among many candidates of mucoadhesive agent because it was widely used as pharmaceutical and cosmetic materials, and had a high safety (Yeh *et al*., 2006). PLA was also used since it was cationic polymer such as chitosan and Tat peptide, and the absorption enhancing effect was demonstrated almost without cytotoxicity to mucosa (Ohtake *et al.*, 2002, Yamaki *et al.*, 2013).

2. Materials and methods

2.1. Materials

Acetaminophen (*p*-acetamidophenol, MW 151.17), polyvinylpyrrolidone K25 (PVP, MW 35 kDa) and diethyl ether were purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). Poly-L-arginine (PLA, MW 44.3 kDa) and Yeast Brewers (Lot: SLBC5519V) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isoflurane for inhalation was purchased from Pfizer Inc. (Tokyo, Japan). All other reagents used were of reagent grade.

2.2. Animals

Male Wistar rats (8 weeks old, weight: 250-300 g) were supplied by Sankyo Labo Service Co., Ltd. (Tokyo, Japan). Rats were housed at 3 or 4 animals/cage with *ad libitum* access to food and water and were kept under a 12-h light-dark cycle. The animals were fasted for 17 hours before the experiments. All of the experiments were performed in accordance with the guidelines for animal use of the Institutional Animal Care and Use Committee at the Life Science Research Center of Josai University and were performed in accordance with the guidelines stipulated by the same committee (JU 19009).

2.3. Preparation of dosage solution

Acetaminophen was dispersed in phosphate buffered saline (PBS) and heated in a water bath at 80°C for 4 hours to obtain acetaminophen-PBS. PLA was dissolved in prepared acetaminophen-PBS, and acetaminophen-PBS containing 0.5% PLA was obtained. PVP was dissolved in prepared acetaminophen-PBS, and acetaminophen-PBS containing 5.0% PVP was obtained. In addition, PLA was dissolved in the prepared acetaminophen-PBS containing 5.0% PVP, and acetaminophen-PBS containing 0.5% PLA and 5.0% PVP was obtained.

2.4. Determination of viscosity in various solutions

Acetaminophen-PBS, acetaminophen-PBS containing 5.0% PVP, acetaminophen-PBS containing 0.5% PLA or acetaminophen-PBS containing 0.5% PLA and 5.0% PVP were poured into a cone-plate type rotational viscometer (RE-215L, Toki Sangyo Co.,Ltd, Tokyo, Japan) equipped with an attached rotor (0.8° × R24), and the rotational speed was continuously changed to obtain the flow curves (0→ 100→ 0 rpm, 20 minutes, 34°C). The viscosity was calculated from the regression line of the obtained flow curves.

2.5. Evaluation of *in vitro* retentivity in various solutions

The retentivity of various solutions were evaluated by an *in vitro* inclined plate test (Kamiya *et al.*, 2017) with modification. A stainless-steel plate (SSP) was washed with methanol. After drying the SSP completely, it was inclined to an angle of 30° to the horizontal plane. Various solutions (50 μL) were added dropwise on the inclined SSP, and the time taken to flow down 5 cm was measured as retention time.

2.6. Anesthesia

In pharmacokinetic and pharmacological studies of acetaminophen, rats were anesthetized by inhalation of isoflurane (1.5-5%) and diethyl ether, respectively.

2.7. Administration of acetaminophen

2.7.1. Intranasal (*i.n.*) administration

After the induction of anesthesia, the head of the rat was inclined to an angle of 30° to the horizontal plane. Acetaminophen-PBS, acetaminophen-PBS containing 5.0% PVP, acetaminophen-PBS containing 0.5% PLA, or acetaminophen-PBS containing 0.5% PLA and 5.0% PVP (20 mg/mL, 4 mg/kg) was administered to the left nasal cavity (15 mm from the nasal inlet) using a micro syringe with a polyethylene tube attached to the tip. Immediately after administration, the left nostril was closed with an instant adhesive and the state at the time of administration was maintained for 5 minutes. The rats were returned to the cage after awakening.

2.7.2. Intravenous (*i.v.*), peroral (*p.o.*) or intrarectal (*i.r.*) administration

After the induction of anesthesia, acetaminophen-PBS (10 mg/mL, 4 mg/kg) was administered into the tail vein (*i.v.*), and acetaminophen-PBS (4 mg/mL, 4 mg/kg) was administered, using a feeding needle, to 80 mm from the oral cavity (*p.o.*) or 15 mm from the anus (*i.r.*). The state at the time of administration was then maintained for 5 minutes and the rats were returned to the cage after awakening.

2.8. Collection of samples

2.8.1. Collection of plasma

Rats were treated with the insertion of a catheter into the left jugular vein before administration. After the administration of acetaminophen via various routes, 0.2 mL of blood was collected from the left jugular vein via the catheter at predetermined times (2, 5, 10, 20, 30, 60, 90 and 120 minutes after administration) using heparinized syringes. The collected blood was immediately centrifuged (4°C, 20,400 ×*g*, 5 minutes) to obtain a 0.1 mL plasma sample.

2.8.2. Collection of the brain

Approximately 150 mL of PBS was infused into the left ventricle and the rats were sacrificed at 10, 30 and 60 minutes after the administration of acetaminophen via various routes. Immediately, the whole brain was removed and washed with PBS. The olfactory bulb (I), cerebral cortex (II) and hypothalamus (III) were obtained. The brain samples were frozen in liquid nitrogen and stored at -80°C until measurement. Since the brain was collected only once per rat, the pharmacokinetic analysis of acetaminophen in the brain was a population analysis.

2.9. The quantitative analysis of acetaminophen

2.9.1. Determination of the acetaminophen concentration in plasma

Two hundred microliters of acetonitrile containing *m*-acetaminophenol as the internal standard (I.S.) was added to 100 μL of the obtained plasma, and the mixture was stirred for 30 seconds. One hundred microliters of the supernatant obtained by centrifugation (4°C, 20,400 ×*g*, 5 minutes) was poured into a microtube and the solvent was removed at 40°C using nitrogen gas. One hundred microliters of the mobile phase were added to the

residue, and the solution was centrifuged (4°C, 20,400 ×*g*, 5 minutes). Thirty-five microliters of the supernatant was injected into an HPLC apparatus and the acetaminophen concentration was measured. The HPLC apparatus that was used included a solvent low-pressure gradient pump (PU-2089), an intelligent UV detector (UV-2070/2075), a chromatographic A/D interface (LC-NET II), an intelligent sampler (AS-2055), an intelligent column oven (CO-2067), a chromatography data station (Chrom NAV, Ver.1.18 Cor later) (JASCO Corp., Tokyo, Japan) and an HPLC column (XBridge™ C18, 5 μm, 4.6 mm i.d. × 250 mm, Waters Corp., Milford, MA, USA). The mobile phase was eluted with 0.1% phosphatic solution and methanol (9:1) at a flow rate of 1.0 ml/min. The UV detector was operated at 245 nm, and the column temperature was maintained at 40°C.

2.9.2 Determination of the acetaminophen concentration in the brain

PBS (30 mL/g) was added to the olfactory bulb (I) and the hypothalamus (III). PBS (10 mL/g) was added to the cerebral cortex (II). The mixtures were homogenized using a homogenizer (AHG-160A, 8 mm shaft HT1008, AS ONE Co., Ltd., Osaka, Japan) under ice cooling for 60 seconds, and 1 mL of the mixture was taken up in a microtube. After centrifugation (4°C, 20,400 ×*g*, 5 minutes), 300 μL of the obtained supernatant was poured into a microtube, 100 μL of 1 mol/L hydrochloric acid was added and the mixture was stirred for 2 minutes. One thousand microliters of ethyl acetate containing I.S. was added to the mixture, and stirred for 5 minutes. Eight hundred microliters of the organic layer, which had been separated by centrifugation (4°C, 20,400 ×g, 5 minutes), was poured into another microtube and the solvent was removed at 40°C using nitrogen gas. Seventy-five microliters of the mobile phase was added to the residue, and the solution was centrifuged (4°C, 20,400 ×*g*, 5 minutes). Thirty-five microliters of the supernatant

was injected into an HPLC apparatus and the acetaminophen concentration was measured as described above.

2.10. Evaluation of the antipyretic effect of acetaminophen

The usual rectal temperature (T_u) of the rats was measured using an interchangeable probe and a small digital thermometer (Bio Research Center Co., Ltd., Nagoya, Japan), 17 hours before performing the study (Reza *et al*., 2014, Abotsi *et al.*, 2017). Yeast Brewers was suspended in physiological saline to a concentration of 25 w/v%. The suspension (10 mL/kg) was administered subcutaneously to the dorsum. Elevation of the rectal temperature (T_f) was confirmed by the above-described procedure 17 hours after the administration. Acetaminophen was then administered intravenously, nasally, orally and rectally as described above. PBS alone was also administered via each route as a control. After the administration of acetaminophen or PBS via various routes, the rectal temperature (T_n) was measured at predetermined times (10, 20, 30, 60, 90 and 120 minutes after administration) by the above-described procedure.

2.11. Data Analysis

The plasma data for acetaminophen after *i.v.* administration was analyzed by a non-linear least squares regression program (Algorithm: Damping Gauss-Newton method). The maximum plasma or brain concentrations ($C_{max,\ plasma}$ or $C_{max,\ brain}$) and the times of the maximum plasma or brain concentrations ($T_{max,\ plasma}$ or $T_{max,\ brain}$) were determined from the plasma or brain concentration-time curve after the administration of acetaminophen via various routes. The area under the plasma or brain concentration-time curve ($AUC_{plasma\ 0\text{-}120}$ or $AUC_{brain\ 0\text{-}60}$) was calculated using the trapezoidal formula. The bioavailability ($F_{0\text{-}120}$) was also calculated from $AUC_{plasma\ 0\text{-}120}$.

The ΔT (°C) value after each administration of acetaminophen was calculated from:

$$\Delta T\ (°C) = T_n - T_u \quad \text{——} \quad (1)$$

where T_u is the usual rectal temperature, and T_n shows the rectal temperature each time after the administration of acetaminophen. The ΔT (°C) value is calculated by subtracting the usual rectal temperature (T_u) from the rectal temperature each time after the administration of acetaminophen (T_n).

The % antipyretic effect value was calculated from:

$$\%\ \text{antipyretic effect} = \frac{\Delta T_m - \Delta T}{\Delta T_m} \times 100 \quad \text{——} \quad (2)$$

where ΔT_m (°C) is the value obtained by subtracting the usual rectal temperature (T_u) from the rectal temperature immediately before the administration of acetaminophen (T_f). The maximum antipyretic effect ($E_{max,\ effect}$) and the time of the maximum antipyretic effect ($T_{max,\ effect}$) was determined from the % antipyretic effect-time profile obtained with each route of administration. The area under the antipyretic effect-time curve ($AUC_{effect\ 0\text{-}120}$) was calculated using the trapezoidal formula.

The results were analyzed using Student's *t*-test. *P* values of < 0.05 were considered to indicate statistical significance.

3. Results

3.1. The transfer of acetaminophen to systemic circulation after *i.n.* administration

Table 1 shows the pharmacokinetic parameters of acetaminophen calculated from plasma data after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration in rats. The pharmacokinetics of acetaminophen after *i.v.* administration followed the two-compartment model. The *i.n.* administration of acetaminophen-PBS resulted in a significantly higher $C_{max,\ plasma}$ ($p < 0.05$) and an earlier $T_{max,\ plasma}$ of 5 minutes in comparison to *p.o.* or *i.r.* administration.

The plasma acetaminophen concentration after *i.n.* administration, even at 2 minutes after *i.n.* administration, was significantly higher in comparison to the concentration after *p.o.* or *i.r.* administration ($p < 0.05$, data not shown). In contrast, the plasma acetaminophen concentrations at 20 and 30 minutes after *i.n.* administration were lower in comparison to those after *i.r.* administration (data not shown). Consequently, $AUC_{plasma\ 0\text{-}120}$ after *i.n.* administration was almost equivalent to that after *i.r.* administration, and $F_{0\text{-}120}$ was 86.3%.

3.2. The transfer of acetaminophen to the brain after *i.n.* administration

Figure 1 shows the acetaminophen concentrations in the brain after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration in rats, and Table 2 summarizes the obtained pharmacokinetic parameters of acetaminophen in the brain. The $T_{max,\ brain}$ in each brain region was early (within 10 minutes), except for after *p.o.* administration. After *i.v.* administration, acetaminophen was distributed at an almost constant concentration to each brain region, and the $C_{max,\ brain}$ values ranged from 1.59 μg/g tissue (olfactory bulb, I) to 2.13 μg/g tissue (cerebral cortex, II). The $AUC_{brain\ 0\text{-}60}$ values ranged from 74.9 μg·min/g tissue (olfactory bulb, I) to 81.8 μg·min/g tissue (cerebral cortex, II). After *i.n.* administration, $C_{max,\ brain}$ in the olfactory bulb (I) was 5.05 μg/g tissue, which was more than three times higher than that after *i.v.* administration and significantly higher than that after administration via other routes ($p < 0.01$). In addition, $C_{max,\ brain}$ in the cerebral cortex (II), which was considered to be the region related to the analgesic effect, was 1.16 μg/g tissue after *i.n.* administration, which was significantly higher than that after *p.o.* (0.255 μg/g tissue) or *i.r.* (0.745 μg/g tissue) administration ($p < 0.01$, $p < 0.05$, respectively). Also, $C_{max,\ brain}$ in the hypothalamus (III), which was considered to be the region related to the antipyretic effect, was 3.36 μg/g tissue after *i.n.* administration, which was significantly higher than after administration by other routes ($p < 0.01$).

3.3. The antipyretic effect of acetaminophen after *i.n.* administration

In the antipyretic tests, diethyl ether was used as an anesthetic in consideration of the decrease in body temperature caused by anesthetics such as isoflurane. The pharmacokinetics of acetaminophen after isoflurane and diethyl ether were not significantly different (data not shown). Figure 2 shows the percentage antipyretic effect-time curves after the *i.v.*, *p.o.*, *i.r.* or *i.n.* administration of acetaminophen in rats, and Table 3 summarizes the pharmacodynamic parameters of acetaminophen that were obtained. $T_{max,\ effect}$ after the *i.n.* administration of acetaminophen occurred within 10 minutes, which was earlier in comparison to after *i.v.* or *p.o.* administration (20 minutes). $E_{max,\ effect}$ after the *i.n.* administration of acetaminophen was 82.8%, which was significantly higher than that after *p.o.* (29.6%) or *i.r.* (35.2%) administration ($p < 0.05$ and $p < 0.01$, respectively). In addition, $AUC_{effect\ 0\text{-}120}$ (1657%·min) after the *i.n.* administration of acetaminophen was higher than that after *p.o.* (1163%·min) or *i.r.* (738.3%·min) administration, and the antipyretic effect was equal to or greater than that observed after *i.v.* administration (1479%·min). Regarding the analgesic effect obtained by the tail-immersion test, $E_{max,effect}$ after the *i.n.* administration of acetaminophen was also higher than that after *p.o.* or *i.r.* administration, and was similar to that after *i.v.* administration (data not shown). Furthermore, the analgesic effect after *p.o.* or *i.r.* administration was low, while $AUC_{effect\ 0\text{-}120}$ was high after the *i.n.* administration of acetaminophen.

3.4. Viscosity of each dosage solution

In each dosage solution, the shear stress was increased almost linearly as the shear rate was increased, and showed a plastic flow that was close to Newtonian flow (data not shown). There was no difference in the viscosity when 0.5% PLA (0.857 ± 0.0114 mPa·s)

was added to acetaminophen-PBS (0.771 ± 0.00840 mPa·s), but the viscosity was significantly increased by adding 5.0% PVP to acetaminophen-PBS (1.49 ± 0.0272 mPa·s). The viscosity, after the addition of 0.5% PLA and 5.0% PVP (1.64 ± 0.0642 mPa·s) was slightly increased in comparison to acetaminophen-PBS containing 5.0% PVP alone.

3.5 Retentivity of each dosage solution

The retention time of each dosage solution was prolonged as the viscosity of the solution was increased (data not shown). There was no significant difference between the retention times in acetaminophen-PBS with 0.5% PLA (1.10 ± 0.207 s) and acetaminophen-PBS (0.990 ± 0.156 s), but the retention time was significantly prolonged in acetaminophen-PBS with 5.0% PVP (3.08 ± 0.397 s) ($p < 0.01$). The retention time, in acetaminophen-PBS with 0.5% PLA and 5.0% PVP (4.50 ± 0.652 s) has a tendency to be further prolonged in compared to acetaminophen-PBS containing 5.0% PVP alone.

3.6. Effects of PVP and/or PLA on the transfer of acetaminophen to systemic circulation after *i.n.* administration

Table 4 summarizes the pharmacokinetic parameters of acetaminophen calculated from the plasma data after *i.n.* administration with PVP and/or PLA. $C_{max,\ plasma}$ and $AUC_{plasma\ 0\text{-}120}$ were decreased after the *i.n.* administration of acetaminophen-PBS with 5.0% PVP or 0.5% PLA in comparison to after the *i.n.* administration of acetaminophen-PBS alone; in particular, $AUC_{plasma\ 0\text{-}120}$ was significantly decreased ($p < 0.05$). $C_{max,\ plasma}$ and $AUC_{plasma\ 0\text{-}120}$ were also decreased after the *i.n.* administration of acetaminophen-PBS with 0.5% PLA and 5.0% PVP; in particular, $AUC_{plasma\ 0\text{-}120}$ was significantly decreased ($p < 0.05$). $F_{0\text{-}120}$ was 86.3% after the *i.n.* administration of acetaminophen-PBS alone,

but it decreased as the viscosity and the retentivity in olfactory epithelium region of the dosage solution increased. $F_{0\text{-}120}$ was 60.4% after the *i.n.* administration of acetaminophen-PBS with 0.5% PLA and 5.0% PVP, which was the lowest among the *i.n.* administration with each additive.

3.7. The effects of PVP and/or PLA on the transfer of acetaminophen to the brain after *i.n.* administration

Figure 3 shows the acetaminophen concentrations in the brain after *i.n.* administration with PVP and/or PLA in rats, and Table 5 summarizes the obtained pharmacokinetic parameters of acetaminophen in the brain. The $C_{max,\ brain}$ values in each region after *i.n.* administration with 5.0% PVP and those after the *i.n.* administration of acetaminophen-PBS did not differ to a statistically significant extent. However, acetaminophen concentrations in the olfactory bulb (I) at 30 and 60 minutes after *i.n.* administration with 5.0% PVP were significantly increased in comparison to that after *i.n.* administration of acetaminophen-PBS alone ($p < 0.01$ and $p < 0.05$, respectively). The acetaminophen concentration in the hypothalamus (III) was also significantly increased at 30 minutes after *i.n.* administration with 5.0% PVP in comparison to that after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.01$). $C_{max,\ brain}$ in the olfactory bulb (I) was significantly increased after *i.n.* administration with 0.5% PLA in comparison to that after the *i.n.* administration of acetaminophen-PBS ($p < 0.05$). Consequently, the $AUC_{brain\ 0\text{-}60}$ was also increased in comparison to that after the *i.n.* administration of acetaminophen-PBS alone. After *i.n.* administration with 0.5% PLA and 5.0% PVP, $C_{max,\ brain}$ in the olfactory bulb (I) was increased in comparison to that after the *i.n.* administration of acetaminophen-PBS alone, and the acetaminophen concentrations were also significantly increased at 30 minutes after administration ($p < 0.05$); as a result $AUC_{brain\ 0\text{-}60}$ was

increased approximately 1.7 times. Furthermore, $C_{max, brain}$ was significantly increased in the cerebral cortex (II) in comparison to that after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.05$), and the $AUC_{brain\ 0\text{-}60}$ was approximately 1.5 times higher than that after the *i.n.* administration of acetaminophen-PBS alone. The acetaminophen concentration in the hypothalamus (III) at 30 minutes after *i.n.* administration with 0.5% PLA and 5.0% PVP was significantly higher than that after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.01$).

3.8. The effect of PVP and/or PLA on the antipyretic effect of acetaminophen after *i.n.* administration

Figure 4 shows the percentage of the antipyretic effect-time curves after *i.n.* administration of acetaminophen with PVP and/or PLA in rats, and Table 6 summarizes the obtained pharmacodynamic parameters of acetaminophen. The percentage antipyretic effects at 20 and 30 minutes after *i.n.* administration with 5.0% PVP were significantly greater than those after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.05$), as a result, $AUC_{effect\ 0\text{-}120}$ increased approximately 1.5 times. The percentage antipyretic effect ($E_{max, effect}$) at 10 minutes after *i.n.* administration with 0.5% PLA was significantly higher than that after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.05$), resulting in an almost 2 times increase in the $AUC_{effect\ 0\text{-}120}$ ($p < 0.01$). In addition, the percentage antipyretic effects at 10–30 minutes after *i.n.* administration with 0.5% PLA and 5.0% PVP were significantly higher than those after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.05$), resulting in a significant increase in the $AUC_{effect\ 0\text{-}120}$ ($p < 0.01$) and a prolonged antipyretic effect. Although the analgesic effect was also observed in a tail-immersion test, it was lower than the antipyretic effect. However, the analgesic effect showed the same trend as the antipyretic effect (data not shown).

4. Discussion

Several preliminary studies have been conducted to determine the optimal dosing position in the nasal cavity and the optimal head angle for *i.n.* administration in rats to achieve the efficient transport of drugs to the brain, especially the olfactory bulb. The results suggested that it was possible to concentrate the drug solution near the olfactory epithelium region and to transport it efficiently to the brain, especially the olfactory bulb, by administering the drug at a position of 15 mm from the nasal cavity entrance when the head was inclined to an angle of 30° to the horizontal plane (data not shown). After *i.n.* administration, acetaminophen was more rapidly transferred to the systemic circulation in comparison to after *p.o.* or *i.r.* administration, which are the routes that are used in actual clinical practice. The acetaminophen concentrations in plasma after *i.n.* administration were also higher in comparison to after *p.o.* or *i.r.* administration. The *i.n.* administration of acetaminophen resulted in faster $T_{max,\ plasma}$ values and higher $C_{max,\ plasma}$ values in comparison to *p.o.* or *i.r.* administration. Although the bioavailability after *i.n.* administration was comparable to that after *i.r.* administration, in all brain regions the $C_{max,brain}$ and $AUC_{brain\ 0\text{-}60}$ values after *i.n.* administration were significantly higher in comparison to those after *i.r.* or *p.o.* administration, especially in the olfactory bulb (I). The $C_{max,brain}$ values in the olfactory bulb (I) and hypothalamus (III) after the *i.n.* administration of acetaminophen-PBS were significantly higher than those after *i.v.* administration ($p < 0.01$).

The pharmacological effect of acetaminophen was obtained according to the pharmacokinetic results. In the antipyretic study using Yeast Brewers febrile rats, the time of the maximum antipyretic effect ($T_{max,\ effect}$) after the *i.n.* administration of acetaminophen-PBS was rapid (occurring within only 10 minutes), and the maximum antipyretic effect ($E_{max,\ effect}$) was significantly higher in comparison to after *p.o.* or *i.r.*

administration ($p < 0.05$ and $p < 0.01$, respectively), indicating that it had the same effect as *i.v.* administration. Thus, the area under the antipyretic effect-time curve ($AUC_{effect\ 0\text{-}120}$) after the *i.n.* administration of acetaminophen-PBS was higher than that after *p.o.* or *i.r.* administration, indicating that the antipyretic effect was higher than that after *i.v.* administration. In the analgesic study using a tail-immersion test, the analgesic effect was low after the *p.o.* or *i.r.* administration of acetaminophen-PBS, which are the administration routes used in clinical practice, whereas the effect was sustained after the *i.n.* administration of acetaminophen-PBS and the $E_{max,effect}$ was equivalent to that after *i.v.* administration (data not shown). These results suggested that *i.n.* administration could transport acetaminophen rapidly and maintain a higher concentration in the brain in comparison to *p.o.* or *i.r.* administration, and that it might exhibit a higher pharmacological effect than *i.v.* administration.

It is noteworthy that the brain concentration of acetaminophen was not sustained after *i.n.* administration, since the acetaminophen solution that was administered intranasally was removed from the nasal cavity by mucociliary and physical clearance. Thus, PVP was used as a mucoadhesive agent to prolong the time that the acetaminophen solution was retained in the nasal cavity, especially the olfactory epithelium region. PLA was also used as an absorption enhancer to increase the direct transfer of acetaminophen to the brain. PVP is a polymer compound obtained by the polymerization of *N*-vinyl-2-pyrrolidone, and is also used as in additives (excipients and binders) for cosmetic and pharmaceutical products (Yeh *et al*., 2006, Burnett, 2017). Orally administered PVP is poorly absorbed from the gastrointestinal tract, and it is clear that it has no genotoxicity or acute toxicity at the usual doses. Thus, it was used in this study. The transfer of acetaminophen to the brain was investigated using the several concentrations of PVP in the preliminary study. As a result, the transfer of acetaminophen to the brain was increased up

to 5.0% PVP, but it was almost the same as 5.0% in 10% PVP. The viscosity of the 10% PVP solution was 3.42 mPa·s, and was higher than that in the other solutions. The retention time in 10% PVP solution was 5.71 s, which was longer than that in the other solutions. It seemed that the transfer of acetaminophen to the brain was limited by reducing the contact surface area between the mucosa and the solution due to excessively high viscosity, regardless of the prolonged retention time on the mucous membrane (Irie *et al*., 2009).

PLA is known to be a cationic absorption enhancer. It was used to enhance the direct transport of acetaminophen to the brain from the olfactory epithelium region in the nasal mucosa by opening the intercellular spaces. Although the detailed mechanism of enhancement is still not clear, it has been determined that PLA induces some ionic interactions on the surface of the cell membrane to internalize tight junction-related proteins from the intercellular space into the cell, thereby transiently opening the tight junctions (Yamaki *et al*., 2013). It was clarified that 0.5 to 1.0% PLA showed the sufficient absorption enhancing effect almost without cytotoxicity to the rat nasal mucosa (Miyamoto *et al*., 2001, Ohtake *et al*., 2002). The transfer of acetaminophen to the brain was investigated using 0.5% or 1.0% PLA in the preliminary study. As a result, there was no significant difference between them. Therefore, 0.5% PLA was used. In the present study, the combination of PVP as a mucoadhesive agent and PLA as an absorption enhancer was a novel approach that achieved the efficient delivery of drugs from the nasal cavity to the brain.

The plasma acetaminophen concentration after the *i.n.* administration of acetaminophen-PBS alone was similar to that after *i.r.* administration. In contrast, the plasma acetaminophen concentration after *i.n.* administration with each additive decreased as the viscosity and the retentivity in olfactory epithelium region of the dosage solution

increased, with the acetaminophen concentration after *i.n.* administration with 0.5% PLA and 5.0% PVP being the lowest among the *i.n.* administration with each additive. The bioavailability after *i.n.* administration with 0.5% PLA and 5.0% PVP was 60.4%. On the other hand, the acetaminophen concentration in the brain after *i.n.* administration with each additive tended to increase. The concentrations of acetaminophen in the olfactory bulb (I) at 30 and 60 minutes after *i.n.* administration with 5.0% PVP were significantly higher than those after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.01$ and $p < 0.05$, respectively), which resulted in a slight increase in $AUC_{brain\ 0\text{-}60}$. The acetaminophen concentration in the hypothalamus (III) 30 minutes after *i.n.* administration with 5.0% PVP was higher than that after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.01$), and showed a slight increase in $AUC_{brain\ 0\text{-}60}$. Consistent with these results, the pharmacological effect was maintained up to 30 minutes after *i.n.* administration with 5.0% PVP. This result seemed to be due to the improved retention of the acetaminophen solution in the olfactory epithelium by increasing the viscosity of the solution with PVP. $C_{max,\ brain}$ in the olfactory bulb (I) after *i.n.* administration with 0.5% PLA was remarkably increased in comparison to that after the *i.n.* administration of acetaminophen-PBS alone. Similarly, $C_{max,\ brain}$ in the cerebral cortex (II) after *i.n.* administration with 0.5% PLA was increased in comparison to that after the *i.n.* administration of acetaminophen-PBS alone ($p < 0.05$). Therefore, a dramatic pharmacological effect was produced early after *i.n.* administration with 0.5% PLA. This result seems to indicate that PLA promotes the translocation of acetaminophen to the brain by opening the tight junction, since the expression of tight junction-related proteins (ZO-1 and Occludin) is observed in the olfactory epithelium region (Wolburg *et al*., 2008, Mistry *et al*., 2009), resulting in rapid pharmacological action. The translocation of acetaminophen to the brain, especially to the olfactory bulb (I) was enhanced by *i.n.*

administration with 5.0% PVP and 0.5% PLA in comparison to other administration groups. In addition, the acetaminophen concentration in each brain region was maintained until at least 30 minutes after *i.n.* administration with 5.0% PVP and 0.5% PLA in comparison to *i.n.* administration with each additive. Consistent with these results, a significant pharmacological effect was obtained early after the *i.n.* administration of acetaminophen with 5.0% PVP and 0.5% PLA, and tended to persist. Thus, a transnasal delivery system with PVP and PLA, which can produce a significant pharmacological effect by increasing the translocation of acetaminophen to the brain by suppressing its translocation to the systemic circulation, may inhibit translocation to the peripheral tissues, which is not related to the efficacy and reduce the frequency of side effects. Moreover, this system could be expected to have a rapid and sustained pharmacological effect that compares favorably to *i.v.* administration without the associated pain. These results suggest that a transnasal delivery system for acetaminophen may be a promising alternative to other routes of administration. However, the acetaminophen concentration in the brain region related to the antipyretic effect may not be sufficiently correlated with the effect. Thus, further study on acetaminophen concentrations in cerebrospinal fluid may be needed to evaluate the transportation of acetaminophen to the brain in detail. Moreover, a pharmaceutical study may also be needed to optimize the delivery of the drug to the brain and improve the ease of administration.

5. Conclusion

The transport of the central antipyretic analgesic acetaminophen to systemic circulation and CNS after *i.n.* administration was investigated pharmacokinetically and pharmacodynamically, and the possibility of a transnasal delivery system was evaluated by comparing *i.n.* administration to other routes of administration. The results suggested

that acetaminophen could be delivered more rapidly into the brain after *i.n.* administration than after *p.o.* or *i.r.* administration, with the same pharmacological effect as *i.v.* administration. Furthermore, the usefulness of a transnasal delivery system for acetaminophen using PVP as a mucoadhesive substance and PLA as an absorption enhancer was investigated. This system produced a significant pharmacological effect by enhancing the transfer of acetaminophen to the brain by suppressing the transfer of acetaminophen to the systemic circulation. Thus, it was suggested that a transnasal drug delivery system using PLA and PVP may be a promising novel method for transporting acetaminophen to the brain.

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Figure captions

Figure 1. Acetaminophen concentrations in the brain after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration in rats.

The brain regions: I (olfactory bulb), II (cerebral cortex) and III (hypothalamus).

Each data column represents the mean ± S.E. (n=3-4).

*: $p < 0.05$, **: $p < 0.01$ compared with *i.n.* administration (Student's *t*-test).

Figure 2. Percentage antipyretic effect-time curves after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration of acetaminophen in rats.

Open symbol: control (PBS alone), Closed symbol: acetaminophen-PBS.

Each data point represents the mean ± S.E. (n=3-4).

*: $p < 0.05$, **: $p < 0.01$ compared with administration of PBS alone (Student's *t*-test).

Figure 3. Acetaminophen concentrations in the brain after *i.n.* administration with PVP and/or PLA in rats.

The brain regions: I (olfactory bulb), II (cerebral cortex) and III (hypothalamus).

Each data column represents the mean ± S.E. (n=3-4).

*: $p < 0.05$, **: $p < 0.01$ compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).

Figure 4. Percentage of the antipyretic effect-time curves after *i.n.* administration of acetaminophen with PVP and/or PLA in rats.

□: PBS alone, ■: acetaminophen-PBS, ○: with 5.0% PVP, ▲: with 0.5% PLA, ◇: with 5.0% PVP and 0.5% PLA.

Each data point represents the mean ± S.E. (n=3-4).

a : $p < 0.05$, acetaminophen-PBS with 5.0% PVP (○) was compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).

b : $p < 0.05$, acetaminophen-PBS with 0.5% PLA (▲) was compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).

c : $p < 0.05$, d : $p < 0.01$, acetaminophen-PBS with 5.0% PVP and 0.5% PLA (◇) was compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).

Credit Author Statement

Kaho Ogawa : Investigation, Writing – Original

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Mitsutoshi Kimura : Validation, Writing – Review & Editing

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Hiroyuki Uchida : Formal analysis, Writing – Review & Editing

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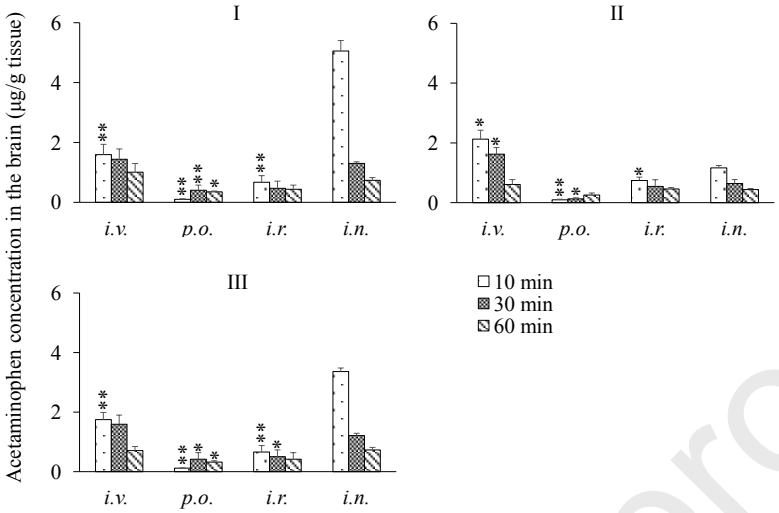
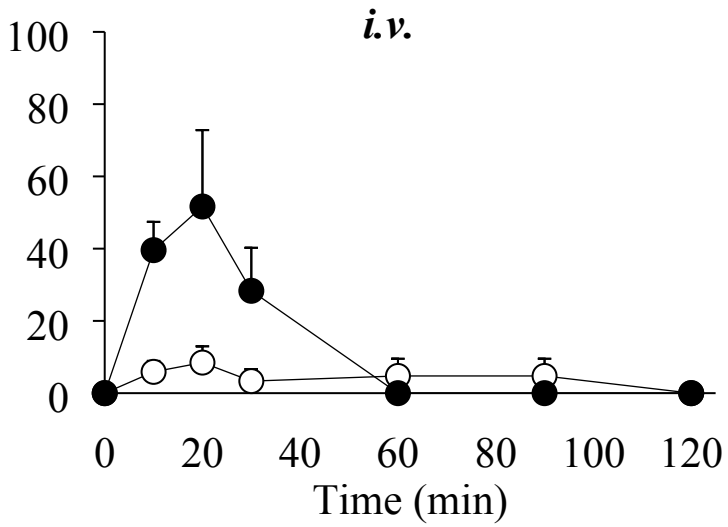
Declaration of interests

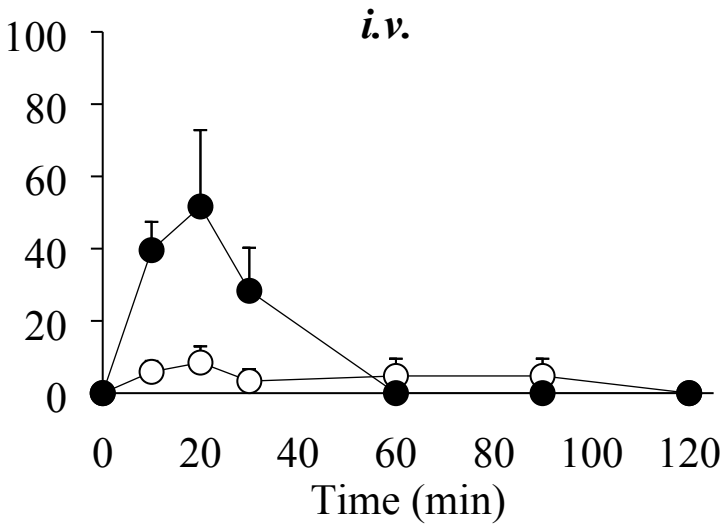
☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure 1







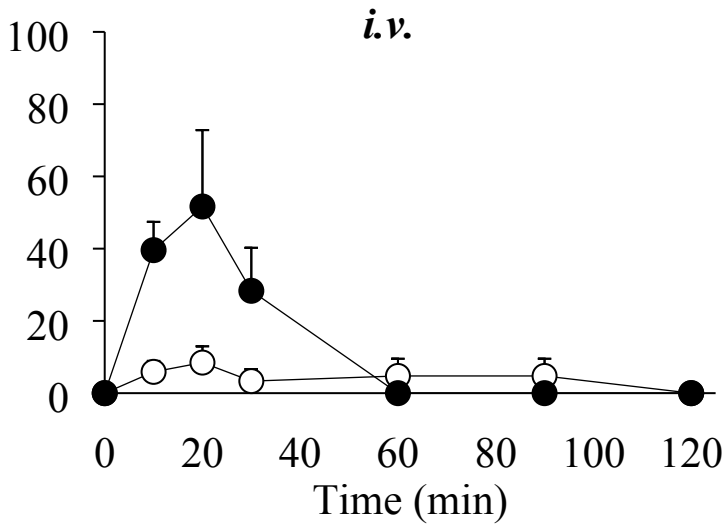


Table 1. Pharmacokinetic parameters of acetaminophen calculated from plasma data after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration in rats.

Route	$C_{max, plasma}$ (μg/mL)	$T_{max, plasma}$ (min)	$AUC_{plasma\ 0\text{-}120}$ (μg·min/mL)	$F_{0\text{-}120}$ (%)
i.v.	—	—	144 ± 2.45	—
p.o.	2.18 ± 0.624	16.7 ± 6.67	104 ± 8.00	72.3
i.r.	3.56 ± 0.166	10.0 ± 0.00	128 ± 4.97	88.7
i.n.	5.05 ± 0.421	5.00 ± 0.00	124 ± 7.06	86.3

Each data represents the mean or mean ± S.E. (n=3-4).

*: $p < 0.05$ compared with *i.n.* administration (Student's *t*-test).

Table 2. Pharmacokinetic parameters of acetaminophen in the brain after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration in rats.

Route	Brain region	$C_{max, brain}$ (μg/g tissue)	$T_{max, brain}$ (min)	$AUC_{brain\ 0\text{-}60}$ (μg·min/g tissue)
i.v.	I	1.59 ± 0.344	< 10	74.9
	II	2.13 ± 0.295	< 10	81.8
	III	1.75 ± 0.243	< 10	76.6
p.o.	I	0.403 ± 0.173	30	16.8
	II	0.255 ± 0.0708	60	8.43
	III	0.420 ± 0.221	30	17.1
i.r.	I	0.669 ± 0.222	< 10	28.3
	II	0.745 ± 0.111	< 10	31.7
	III	0.662 ± 0.216	< 10	28.9
i.n.	I	5.05 ± 0.351	< 10	119

II	1.16 ± 0.0790	< 10	40.1
III	3.36 ± 0.121	< 10	91.7

The brain regions: I (olfactory bulb), II (cerebral cortex) and III (hypothalamus).

Each data represents the mean or mean ± S.E. (n=3-4).

*: $p < 0.05$, **: $p < 0.01$ compared with *i.n.* administration (Student's *t*-test).

Table 3. Pharmacodynamic parameters of acetaminophen after *i.v.*, *p.o.*, *i.r.* or *i.n.* administration in rats.

Route	Dosage solution	$E_{max,\ effect}$ (%)	$T_{max,\ effect}$ (min)	$AUC_{effect\ 0-}$ (%.min)
i.v.	PBS (control)	8.46 ± 4.51	20	496.0 ± 18
	Acetaminophen-PBS	51.7 ± 21.1	20	1479 ± 36
p.o.	PBS (control)	4.17 ± 4.17	20	154.2 ± 10
	Acetaminophen-PBS	29.6 ± 10.9	20	1163 ± 47
i.r.	PBS (control)	5.00 ± 5.00	60	233.3 ± 14
	Acetaminophen-PBS	35.2 ± 10.6	< 10	738.3 ± 19
i.n.	PBS (control)	11.7 ± 3.97	< 10	371.2 ± 10
	Acetaminophen-PBS	82.8 ± 3.25	< 10	1657 ± 32

Each data represents the mean or mean ± S.E. (n=3-4).

*: $p < 0.05$, **: $p < 0.01$ compared with *i.n.* administration (Student's *t*-test).

Table 4. Pharmacokinetic parameters of acetaminophen calculated from the plasma data after *i.n.* administration with PVP and/or PLA in rats.

Route	Additive agent	$C_{max, plasma}$ (μg/mL)	$T_{max, plasma}$ (min)	AUC (μg.
i.v.	—	—	—	14
i.n.	—	5.05 ± 0.421	5.00 ± 0.00	12
	5.0% PVP	3.77 ± 0.369	5.00 ± 0.00	95.
	0.5% PLA	3.42 ± 0.340	6.67 ± 1.67	98.
	5.0% PVP and 0.5% PLA	3.68 ± 0.252	5.00 ± 0.00	87.

Each data represents the mean or mean ± S.E. (n=3-4).

*: $p < 0.05$ compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).

Table 5. Pharmacokinetic parameters of acetaminophen in the brain after *i.n.* administration with PVP and/or PLA in rats.

Route	Additive agent	Brain region	$C_{max, brain}$ (μg/g tissue)	$T_{max, brain}$ (min)	A (μg·
i.n.	—	I	5.05 ± 0.351	< 10	
		II	1.16 ± 0.0790	< 10	
		III	3.36 ± 0.121	< 10	
i.n.	5.0% PVP	I	4.96 ± 0.254	< 10	
		II	1.28 ± 0.0807	< 10	
		III	3.40 ± 0.289	< 10	
i.n.	0.5% PLA	I	7.28 ± 0.653	< 10	
		II	1.56 ± 0.0871	< 10	
		III	2.18 ± 0.581	< 10	

i.n.	5.0% PVP and 0.5% PLA	I	7.77 ± 1.19	< 10
		II	1.67 ± 0.145	< 10
		III	2.44 ± 0.0103	30

The brain regions: I (olfactory bulb), II (cerebral cortex) and III (hypothalamus).

Each data represents the mean or mean ± S.E. (n=3-4).

*: $p < 0.05$ compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).

Table 6. Pharmacodynamic parameters of acetaminophen after *i.n.* administration with PVP and/or PLA in rats.

Route	Dosage solution	$E_{max, effect}$ (%)	$T_{max, effect}$ (min)	A
i.n.	PBS (control)	11.7 ± 3.97	< 10	3
	Acetaminophen-PBS	82.8 ± 3.25	< 10	1
	with 5.0% PVP	74.3 ± 2.28	20	2
	with 0.5% PLA	113 ± 8.28	< 10	3
	with 5.0% PVP and 0.5% PLA	107 ± 7.41	< 10	4

Each data represents the mean or mean ± S.E. (n=3-4).
*: $p < 0.05$, **: $p < 0.01$ compared with *i.n.* administration of acetaminophen-PBS alone (Student's *t*-test).