Regular Article

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Pharmacokinetics and Brain Distribution of Tetrahydropalmatine and Tetrahydroberberine after Oral Administration of DA-9701, a New Botanical Gastroprokinetic Agent, in Rats

Ji Won Jung, Yong Sam Kwon, Jin Seok Jeong, Miwon Son, and Hee Eun Kang

College of Pharmacy and Integrated Research Institute of Pharmaceutical Sciences, The Catholic University of Korea; Bucheon 420–743, Republic of Korea: and Research Center, Dong-A Pharmaceutical Co.; Yongin 446–905, Republic of Korea.

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DA-9701, a new botanical gastroprokinetic agent, has potential for the management of delayed gastric emptying in Parkinson’s disease if it has no central anti-dopaminergic activity. Therefore, we examined the pharmacokinetics of DA-9701 components having dopamine D2 receptor antagonizing activity, tetrahydropalmatine (THP) and tetrahydroberberine (THB), following various oral doses (80–328 mg/kg) of DA-9701. The distribution of THP and THB to the brain and/or other tissues was also evaluated after single or multiple oral administrations of DA-9701. Oral administration of DA-9701 yielded dose-proportional area under the plasma concentration–time curve (AUC_{\text{0-\text{inf}}}) and maximum plasma concentration (C_{\text{max}}) values for THP and THB, indicating linear pharmacokinetics (except for THB at the lowest dose). THP and THB’s large tissue-to-plasma concentration ratios indicated considerable tissue distribution. High concentrations of THP and THB in the stomach and small intestine suggest an explanation for DA-9701’s potent gastroprokinetic activity. The maximum concentrations of THP and THB in brain following multiple oral DA-9701 for 7 d (150 mg/kg/d) was observed at 30 min after the last oral DA-9701 treatment: 131±67.7 ng/g for THP and 6.97±4.03 ng/g for THB. Although both THP and THB pass through the blood–brain barrier, as indicated by brain-to-plasma concentration ratios greater than unity (approximately 2–4), oral administration of DA-9701 at the effective dose in humans is not expected to lead to sufficient brain concentrations to exert central dopamine D2 receptor antagonism.

Key words

DA-9701; tetrahydropalmatine; tetrahydroberberine; pharmacokinetics; tissue distribution; rat
Impaired gastric emptying is commonly reported in both early and advanced Parkinson's disease (PD) and is related to motor function impairment and motor fluctuations, but the basis of this association is not fully understood. Moreover, levodopa treatment in PD causes gastric stasis for 1–2 h, which delays responses to orally administered levodopa because its main site of absorption is the small intestine. The use of prokinetic agents has been suggested for the management of delayed gastric emptying in PD. To date, only oral domperidone has been shown effective and safe in PD. Domperidone prevents levodopa-induced delayed gastric emptying without impairing its central therapeutic effects because domperidone in normal doses does not cross the blood-brain barrier. DA-9701 could be used in PD to manage delayed gastric emptying, if it has no central dopaminergic activity.

To explore this possibility, we examined the pharmacokinetics of THP and THB after oral administration of various doses of DA-9701. The distribution of THP and THB to the brain and/or other tissues was also evaluated after single or multiple oral administrations.

**MATERIALS AND METHODS**

**Materials**  THP, THB, and DA-9701 (Lot No. CP2401, containing 0.45% THP and 0.12% THB; Lot No. CP3001, containing 0.51% THP and 0.14% THB) were supplied by Dong-A Pharmaceutical Co. (Yongin, Republic of Korea). THP (purity 99.1%) and THB (purity 97.2%) were obtained from ChromaDex (Irvine, CA, U.S.A.). Verapamil [internal standard (IS)] for THP and THB analysis], polysorbate (Tween-80), dimethyl sulfoxide (DMSO), and ammonium acetate were purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). Other chemicals were of reagent or HPLC grade.

**Animals**  Animal study protocols were approved by the Department of Laboratory Animals, Institutional Animal Care and Use Committee on the Sungsim Campus of the Catholic University of Korea (Approval No. 2011-015; Bucheon, Republic of Korea). Male Sprague-Dawley (SD) rats, 8–9 weeks old and weighing 265–335 g, were purchased from Orient Bio Inc. (Seongnam, Republic of Korea). Rats were housed and handled as previously reported.

**Pharmacokinetic Study Following Various Oral Doses of DA-9701**  The carotid artery of each rat was cannulated for blood sampling. DA-9701 [dissolved in 1:2:12 (v/v/v) DMSO:Tween 80:distilled water] at doses of 80, 164, and 328 mg/kg (equivalent to 0.36, 0.74, and 1.5 mg/kg THP or 0.10, 0.20, and 0.39 mg/kg THB; n=6, 5, and 6, respectively) was administered orally using a gastric gavage tube. A blood sample of ca. 50 µL was collected via the carotid artery at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, and 480 min after oral administration of DA-9701. Blood samples were centrifuged, and 20 µL of each plasma sample was stored at –70°C until used for LC-MS/MS analysis. Urine and gastrointestinal (contents and feces) samples were collected over 24 h or at the 24-h time point, respectively, and handled as previously reported.

**Tissue Distribution Study Following Single or Multiple Oral Dose of DA-9701**  Plasma and tissue samples were collected at 30, 120, and 360 min following a single dose (328 mg/kg; equivalent to 1.5 mg/kg THP and 0.39 mg/kg THB) or after the final dose of multiple administrations of DA-9701 (150 mg/kg/d for 7d; equivalent to 0.77 mg/kg/d THP and 0.21 mg/kg/d THB). At each time point, as much blood as possible was collected via the carotid artery from each rat (n=3–4 at each time point). At the same time, each rat was euthanized by carbon dioxide asphyxiation, and approximately 0.3 g each of the whole brain and/or the other tissues (liver, stomach, small intestine, large intestine, mesentery, heart, lung, kidney, and fat) was excised and blotted onto tissue paper. Each tissue sample was homogenized (Minilys®; Bertin Technologies, Montigny le Bretonneux, France) with three volumes (3 mL/g tissue) of 0.9% NaCl-injectable solution and centrifuged (9000×g, 4°C) for 10 min. Two 20 µL aliquots of the supernatant and plasma samples were collected and stored at –70°C until LC-MS/MS analysis.

**LC-MS/MS Analysis of THP and THB**  The LC-MS/MS system was comprised of an Agilent 6460 triple quadrupole with an Agilent 1260 LC system (Agilent, Waldbronn, Germany). Instrument control and data acquisition were performed using Agilent MassHunter Workstation software (Version B. 04. 01).

Concentrations of THP and THB were determined using a method developed in our laboratory. In brief, 20 µL of 5 mM formic acid and 50 µL of acetonitrile containing 10 mg/mL of verapamil as the IS were added to 20 µL of each biological sample. After mixing and centrifugation (16000×g, 5 min), the supernatant was collected and 3 µL injected directly onto a reverse-phase HPLC column (Kinetex® C18; 4.6 mm i.d.×50 mm l; particle size, 2.6 µm; Phenomenex, Torrance, CA, U.S.A.). The mobile phase, 2 mM ammonium acetate buffer–acetonitrile [40:60 (v/v)], was run through the column at a flow rate
of 0.4 mL/min. The temperature of the column was maintained at 35°C and the autosampler at 4°C. The eluent was monitored using a triple quadrupole tandem mass spectrometer equipped with an electrospray ionisation (ESI) source and operated in positive ion (ESI⁺; 2–10 min) mode with multiple reaction monitoring. The instrument parameters were set as follows: gas temperature of 210°C, sheath gas temperature of 400°C, gas flow of 5 L/min, sheath gas flow of 12 L/min, nebuliser of 55 psi, and electrospray voltage of 3.5 kV. The fragmentor voltage was set at 150 V for both THP and THB, and the IS was 23, 22, and 20 eV, respectively. The precursor to product ion transitions for THP, THB, and IS were ([M+H]⁺→192.1, m/z 340.2 ([M+H]⁺→176.1, and m/z 455.3 ([M+H]⁺→165.1, respectively. The retention times of THP, THB, and the IS were approximately 3.3, 4.2, and 8.3 min, respectively.

The calibration ranges of THP and THB in rat plasma samples were 0.5–250 ng/mL and 0.4–200 ng/mL, respectively. The equations for the concentrations of THP and THB (YTHP’, YTHB’, and YTHB’), where ‘YTHP’ and ‘YTHB’ are the peak area ratios of THP and THB to IS, were:

\[
Y_{\text{THP}} = 0.04320 \times C_{\text{THP}} - 0.002311
\]

(weighting factor of \(1/C\), \(r^2 = 0.998\))

\[
Y_{\text{THB}} = 0.04004 \times C_{\text{THB}} - 0.005446
\]

(weighting factor of \(1/C\), \(r^2 = 1.000\))

The calibration ranges of THP and THB in rat brain homogenates were all 5–250 ng/g tissue, and the equations for brain concentrations were:

\[
Y_{\text{THP}} = 0.002965 \times C_{\text{THP}} - 0.008328
\]

(weighting factor of \(1/C\), \(r^2 = 0.998\))

\[
Y_{\text{THB}} = 0.002162 \times C_{\text{THB}} - 0.006819
\]

(weighting factor of \(1/C^2\), \(r^2 = 0.972\))

The calibration ranges for urine and GI samples were all 2.5–250 ng/mL, and those for the other tissue samples were 10–250 ng/mL for both THP and THB. The mean intra- and inter-day coefficients of variation (CV) were below 5.76% for THP and 3.61% for THB and the assay accuracies ranged within 82.6–112% for THP and 84.2–113% for THB.

**Pharmacokinetic Analysis** The total area under the plasma concentration–time curve from time zero to the last measured time (t) in plasma (\(AUC_{0-\text{\(\infty\)}}\)) was calculated by the trapezoidal method\(^1\) using WinNonlin® software (Pharsight Corporation, Mountain View, CA, U.S.A.). The maximum plasma concentration (\(C_{\text{max}}\)) and time to reach \(C_{\text{max}}\) (\(T_{\text{max}}\)) were directly read from experimental data.

**Statistical Analysis** A \(p\) value of <0.05, determined by Tukey (in case of equal variance) or Dunnett’s T3 (in case of unequal variance) test posteriori ANOVA among the unpaired data using the Statistical Package for Social Sciences (SPSS) program, was deemed to indicate statistical significance. All data were expressed as mean±standard deviation (S.D.), except \(T_{\text{max}}\), which was expressed as median (range).

**RESULTS**

**Pharmacokinetics of THP after Oral Administration of DA-9701** Figure 2 presents the mean arterial plasma concentration–time profiles of THP after oral administration of DA-9701 at doses equivalent to 0.36, 0.74, and 1.5 mg/kg THP to rats. Table 1 lists the relevant pharmacokinetic parameters. After oral administration of DA-9701, THP was detected in plasma from the first blood sampling time point (5 min) and reached \(C_{\text{max}}\) rapidly (30–75 min), suggesting that absorption of THP in the gastrointestinal tract is rapid. Dose-normalized \(AUC_{0-\text{\(\infty\)}}\) and \(C_{\text{max}}\) values of THP remained constant. The percentage of the dose excreted in the urine over 24 h (\(Ae_{0-24h}\)) and that recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h (\(GI_{24h}\)) were also independent of DA-9701 doses, and the values were both

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral doses as THP</th>
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<tbody>
<tr>
<td>(AUC_{0-\text{(\infty)}}) ((\mu)g·min/mL)(^a)</td>
<td>0.36 mg/kg (n=6)</td>
</tr>
<tr>
<td>(C_{\text{max}}) (ng/mL)(^a)</td>
<td>6.10±1.47</td>
</tr>
<tr>
<td>(T_{\text{max}}) (min)(^a)</td>
<td>26.8±5.46</td>
</tr>
<tr>
<td>(Ae_{0-24h}) (% of dose) (\times) (1/\text{weighting factor of 1/}, 1/0.998)</td>
<td>0.0688±0.0461</td>
</tr>
<tr>
<td>(GI_{24h}) (% of dose) (\times) (1/\text{weighting factor of 1/}, 0.998)</td>
<td>0.670±0.499</td>
</tr>
</tbody>
</table>

Vertical bars represent S.D.

Data are expressed as mean±S.D. \(a\) Dose-normalized values were compared when statistical analysis was performed. \(b\) \(T_{\text{max}}\) is expressed as median (range).
negligible. The above data suggest that the pharmacokinetics of THP following oral administration of DA-9701 are linear.

**Pharmacokinetics of THB after Oral Administration of DA-9701**  
Figure 3 presents the mean arterial plasma concentration–time profiles of THB after oral administration of DA-9701 at doses equivalent to 0.10, 0.20, and 0.39 mg/kg THB to rats. Table 2 lists the relevant pharmacokinetic parameters. After oral administration of DA-9701, THB was detected in plasma from the first blood sampling time point (5 min) and reached $C_{\text{max}}$ rapidly (15–75 min), suggesting that absorption of THB in the gastrointestinal tract is also rapid. Dose-normalized $AUC_{0-8}$ and $C_{\text{max}}$ values of THB remained constant following oral administration of DA-9701 at doses equivalent to 0.20 and 0.39 mg/kg THB. However, dose-normalized $AUC_{0-8}$ value of the lowest dose (equivalent to 0.1 mg/kg THB) was significantly smaller (by 59.1%) than that of the middle dose. The $Ae_{0-24h}$ and $GI_{24h}$ values could not be determined because the concentrations of THB in urine and GI samples were below the lower limit of quantification (2.5 ng/mL).

**Brain Distribution of THP and THB after Single Oral Administration of DA-9701**  
Figure 4 and Table 3 present the concentrations of THP and THB recovered from brain and plasma, as well as brain-to-plasma concentration (Br/P) ratios, following a single oral administration of 328 mg/kg DA-9701 (equivalent to 1.5 mg/kg THP and 0.39 mg/kg THB). Br/P ratios of both THP and THB were greater than unity (approximately 2–4) at all time points. The maximum concentrations of THP and THB in the brain ($376 \pm 51.9$ ng/g for THP and $36.3 \pm 6.43$ ng/g for THB) were observed at 30 min post-administration.

**Distribution of THP and THB to Other Tissues after Single Oral Administration of DA-9701**  
Table 3 also lists the concentrations of THP and THB recovered from other tissues, as well as tissue-to-plasma concentration (T/P) ratios,
following a single oral administration of 328 mg/kg DA-9701 (equivalent to 1.5 mg/kg THP and 0.39 mg/kg THB). The T/P ratios of both THP and THB were greater than unity in all tissues studied at 30, 120, and 360 min post-administration. High concentrations of THP and THB were observed in the liver, stomach, small intestine, and mesentery. Concentrations and T/P ratios of THP and THB in fat gradually increased over time, suggesting that both THP and THB distributed to fat tissue slowly, but considerably.

**Brain Distribution of THP and THB after Multiple Oral Administration of DA-9701**

Figure 5 presents the concentrations of THP and THB recovered from brain and plasma, as well as Br/P ratios following multiple oral administrations of DA-9701 (150 mg/kg/d for 7 d; equivalent to 0.77 mg/kg/d as THP and 0.21 mg/kg/d as THB; n=4 at Each Time Point) following multiple oral administration of DA-9701 (150 mg/kg/d of DA-9701 for 7 d; equivalent to 0.77 mg/kg/d as THP and 0.21 mg/kg/d as THB; n=4 at Each Time Point)

**DISCUSSION**

The effective oral dose of DA-9701 is 0.3–3 mg/kg in rats, and the recommended human dose of DA-9701 is 30 mg three times daily.
times per day. However, the pharmacokinetics of DA-9701 at the effective dose could not be investigated because DA-9701 contains a small proportion (<1%) of THP and THB. The doses of DA-9701 (80–328 mg/kg) in this study were based on the maximum tolerated dose of DA-9701 and assay sensitivity.6)

Linear pharmacokinetics of THP were observed following oral administration of DA-9701 at doses of 80, 164, and 328 mg/kg (equivalent to 0.36, 0.74, and 1.5 mg/kg THP). We inferred linearity from the constant dose-normalized $AUC_{0-8h}$ and $C_{max}$ values for THP (Table 1). Dose-normalized $AUC_{0-8h}$ values of THP were similar to reported values following oral administration of Corydalis rhizoma extract equivalent to ca. 3–4 mg/kg THP.12) THB also exhibited linear pharmacokinetics following oral administration of DA-9701 at doses of 164 and 328 mg/kg (equivalent to 0.20 and 0.39 mg/kg THB), as indicated by dose-proportional $AUC_{0-8h}$ and $C_{max}$ values (Table 2). Dose-normalized $AUC_{0-8h}$ values of THB at doses of DA-9701 eq to 0.20 and 0.39 mg/kg THB were also similar to reported values following oral administration of 50 mg/kg THB.13) Dose-normalized $AUC_{0-8h}$ value of THB became smaller at the lowest DA-9701 dose (80 mg/kg). Possible reasons for this smaller dose-normalized $AUC_{0}$ of THB at lower oral dose could be reduced absorption of THB from the gastrointestinal tract, increased first-pass extraction, and/or faster systemic clearance (mainly non-renal clearance based on negligible recovery of THB in urine). Since THB was not metabolized by human intestinal bacteria,14) reduced absorption from the gastrointestinal tract at lower dose due to degradation of THB by gut microflora is not likely to occur. Therefore, at lower oral dose, greater first-pass extraction and/or faster non-renal clearance of THB could possibly result in smaller dose-normalized $AUC_{0}$ of THB.

Absorption of THP and THB from the GI tract seems almost complete, as $G_{t,GI}$ values were negligible (Tables 1, 2). The negligible $Ae_{0,2h}$ values of THP and THB after oral administration of DA-9701 suggest that most of the administered dose of THP and THB is eliminated via non-renal clearance. A previous pharmacokinetic study of THP13) also reported negligible recovery of unchanged THP in urine and bile (1.48% and 0.4% of the administered THP dose) following oral administration of 40 mg/kg THP, and identified three O-desmethyl metabolites. THP is reportedly metabolized predominantly by CYP3A1/2 and CYP1A2 in rat liver microsomes.14) The $F$ values of THP and THB are reportedly low: 2.5–17.8% for THP following oral administration of various formulations of Corydalis decumbens rhizome extracts at doses containing ca. 12 mg/kg THP17) and 25.6% for THB following oral administration of 50 mg/kg THB.13)

$Br/P$ ratios of both THP and THB were greater than unity (approximately 2–4) at 30, 120, and 360 min after single and multiple oral administrations of DA-9701. The maximum concentrations of THP and THB in brain were observed at 30 min after administration (Figs. 4, 5). These results suggest that both THP and THB pass through the blood-brain barrier. A previous study examining the distribution of THP following oral administration of a 40 mg/kg dose also demonstrated that $Br/P$ ratios reach a maximum of approximately 5.0 after 5–30 min in all brain regions, except the hippocampus, where the ratio reached a maximum of 3.2 at 2 h, confirming that THP crosses the blood–brain barrier rapidly.19) Intravenous administration of THP (1–10 mg/kg) caused central nervous dopamine D2 receptor antagonism to induce hypotension and bradycardia.15) Intravenous THB also caused dopamine D2 receptor antagonism in nigral dopaminergic neurons.20) Therefore, whether THP and/or THB in DA-9701 induce antagonism of central dopamine D2 receptors, which may worsen Parkinsonism, deserves examination. Because the $F$ values of THP and THB are both low, central dopamine D2 receptor antagonism following oral administration would likely require higher doses than those inducing antagonism via intravenous administration.19,20) Multiple oral administrations of DA-9701 (150 mg/kg/d for 7 d; equivalent to 0.77 mg/kg/d as THP and 0.21 mg/kg/d as THB) did not result in accumulation of THP or THB in plasma or brain. The maximum concentrations of THP and THB in brain observed at 30 min after the last oral DA-9701 treatment, 131 ng/g for THP and 6.97 ng/g for THB, were much lower than the IC50 values for antagonism of GTP7S binding to dopamine D2S receptor by THP and THB, 1.32 μM (469 ng/mL) and 0.622 μM (211 ng/mL), respectively (preliminary report from Dong-A Pharmaceutical Co., Ltd.). The maximum brain concentrations of marker compounds of DA-9701, chlorogenic acid and corydaline, were also observed at 30 min after the last dose of multiple oral DA-9701 treatment; 4.61±1.32 mg/g (0.0130 μmol) for chlorogenic acid and 245±202 ng/g (0.664 μmol) for corydaline (our unpublished data). Note that the dose of DA-9701 used in this study is 50 times higher than the effective oral dose of DA-9701 (0.3–3 mg/kg in rats). Because human equivalent dose (0.486 mg/kg or 34 mg/70 kg; using scale-up with body surface area) converted from the effective dose of in rats (3 mg/kg) is similar to recommended dose in humans (30 mg) and both THP and THB have linear pharmacokinetics (or smaller dose-normalized exposure at lower dose for THB), DA-9701 would not likely exert central dopamine D2 receptor antagonism.

The distribution of THP and THB to other tissues was considerable, based on their high tissue concentrations and large T/P ratios (Table 3). The reported T/P ratios of THP at 15 min following oral administration of 40 mg/kg THP were also greater than unity in order of liver > kidney > lung > spleen.20) The extensive tissue distribution of THB is consistent with previously reported large volume of distribution values of THB (47.08 and 21.80 L/kg for (+)- and (-)-THB, respectively). The high concentrations of THP and THB observed in stomach and small intestine could account for DA-9701’s potent gastrokinetic effects (apparent at very low oral effective doses, 0.3–3 mg/kg, in rats).19)

CONCLUSION

Linear pharmacokinetics of THP and THB were observed following oral administration of DA-9701 (except for THB at the lowest dose). Tissue distribution of THP and THB was considerable, as evidenced by high tissue concentrations and large T/P ratios. High concentrations of THP and THB observed in the stomach and small intestine could explain the potent gastrokinetic activity of DA-9701. Although both THP and THB pass through the blood–brain barrier, as indicated by Br/P ratios greater than unity, the effective oral dose of DA-9701 is likely too low to exert central dopamine D2 receptor antagonism.
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Conflict of Interest The authors declare no conflict of interest.

REFERENCES