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# Fluorescence determination of D- and L-tryptophan concentrations in rat plasma following administration of tryptophan enantiomers using HPLC with pre-column derivatization☆

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ABSTRACT

Similar to L-tryptophan (L-Trp), D-Trp can be converted to unique metabolites in the mammalian body. In the present study, the difference in the plasma half-life ($t_{1/2}$) between Trp enantiomers was investigated by following the alterations in the plasma concentration of D- or L-Trp after intraperitoneal (*i.p.*) administration of each enantiomer to male Sprague–Dawley rats (100 mg/kg). The investigation was performed using reversed-phase high-performance liquid chromatography (HPLC) and pre-column fluorescence derivatization with a chiral fluorescent labeling reagent, *R*(−)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (*R*(−)-DBD-PyNCS). The $t_{1/2}$ value of D-Trp was significantly smaller than that of L-Trp, suggesting that D-Trp was eliminated from the plasma more rapidly than L-Trp. In addition, a significant increase in the plasma concentration of L-Trp was observed following administration of D-Trp, whereas no D-Trp was detected after L-Trp administration. Furthermore, the increase in the plasma concentration of L-Trp was significantly suppressed by pretreatment with an inhibitor of D-amino acid oxidase (DAAO), 3-methylpyrazole-5-carboxylic acid, which suggests that DAAO was involved in the conversion of D-Trp to L-Trp *in vivo*.

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## 1. Introduction

In the past two decades, there has been considerable progress in the physiological, pharmacological, and nutritional research on the D-amino acids, which had previously been considered to be unnatural amino acids [1,2]. The research conducted on these amino acids has been accompanied by biochemical and molecular biological studies on their metabolizing enzymes, D-amino acid oxidase (DAAO) [3,4] and D-aspartate oxidase [5]. With regard to D-tryptophan (D-Trp), its several unique features have been reported. For example, D-Trp can be converted enzymatically to the corresponding α-keto acid, indole-3-pyruvic acid (I3P) by DAAO [6], and the generated I3P is dimerized by nonenzymatic condensation to produce 1,3-di(1*H*-indol-3-yl)propan-2-one and 1-(1*H*-indol-3-yl)-3-(3*H*-indol-3-ylidene)propan-2-one, which are able to function as an agonist for aryl hydrocarbon receptor [7]. D-Trp is also metabolized to D-kynurenine (D-KYN) [8–10], which is further converted to kynurenic acid (KYNA) by DAAO [11,12]. KYNA is a known endogenous antagonist of *N*-methyl-D-aspartate and $\alpha_7$ nicotinic acetylcholine receptors [13,14], and it has recently been reported that KYNA was a ligand to act on the G protein-coupled receptor GPR 35 [15]. In addition, it has been previously reported that the body weight gain and food intake of rats fed with a niacin-free diet including 0.1% D-Trp or L-Trp were almost equal [16], and it was therefore concluded that D-Trp may possess the same nutritive value as L-Trp in rats [16,17]. On the basis of the results of the aforementioned studies of D-Trp metabolism, this study attempted to clarify the *in vivo* pharmacokinetic parameters of D-Trp itself following its administration as compared to that of its enantiomer, L-Trp. Recently, we reported an HPLC method for enantiomeric separation of D,L-Trp and D,L-KYN [18] by pre-column derivatization with *R*(−)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (*R*(−)-DBD-PyNCS) [19].

We report herein the changes in the plasma concentration of D- and L-Trp, following *i.p.* administration of each enantiomer to Sprague–Dawley rats, using the previously reported HPLC method with minor modification.

## 2. Experimental

### 2.1. Chemicals

*R*(−)-DBD-PyNCS [19] and 4-*N*, *N*-dimethylaminopyridine (DMAP) were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan).

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Acetic acid (AcOH) of HPLC grade was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Water was used after purification with a Milli-Q Labo system (Nihon Millipore Co. Ltd., Tokyo, Japan). 8-Aminocaprylic acid, phosphate buffered saline (PBS), and 3-methylpyrazole-5-carboxylic acid (MPC) [20] were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile ($CH_3CN$) and methanol (MeOH) of HPLC grade were obtained from Kanto Kagaku Co. Ltd. (Tokyo, Japan) and Wako Pure Chemical Industries Ltd. (Osaka, Japan), respectively.

### 2.2. Animal experiments

All animal experiments were approved (No. 10-54-17) by the Committee of Animal Care, Toho University. Male Sprague–Dawley rats were purchased from Charles River Japan (Kanagawa, Japan) and were housed in an environmentally controlled room for at least 1 week before use. D-Trp and L-Trp were dissolved in PBS ($10\,mg\,mL^{-1}$) and administered *i.p.* to 8-week-old rats ($100\,mg\,kg^{-1}$; $n = 3$). A heparinized syringe with a 25-gauge needle was used to draw blood (approximately 0.20 mL) from the left jugular vein 0.5, 1, 1.5, 2, 3, 4, 5, and 6 h after the administration of D- and L-Trp. Before the administration of D- or L-Trp, blood (approximately 0.20 mL) was obtained from each rat and used as the control. The blood was centrifuged at 3000 *g* for 10 min at 4 °C (Kubota 3500, Kubota Co. Ltd., Tokyo, Japan); rat plasma was obtained, transferred to another tube, and stored at −80 °C until the time of analysis.

### 2.3. Preadministration of MPC

A solution of MPC (1.0 mg/mL) was prepared in PBS/EtOH/PEG (8/1/1), and was administered *i.p.* (50 mg/kg) to the rats 30 min before the administration of D-Trp ($n = 3$). Subsequently, D-Trp was administered, and blood was drawn as described earlier. The plasma samples were treated as described above.

### 2.4. Pretreatment procedure of rat plasma

Pretreatment of rat plasma sample was carried out according to previous paper with minor modification [18,19]. Briefly, rat plasma (10 μL) was added to 10 μL of 200-μM 8-aminocaprylic acid (internal standard, I.S.) in $H_2O/CH_3CN$ (50/50), 10 μL of $H_2O/CH_3CN$ (50/50), and vigorously mixed with 120 μL of $CH_3CN$. After centrifugation at 1300 × *g* for 20 min at 4 °C, 10 μL of the supernatant was sampled and mixed with 10 μL of a 10-mM solution of *R*(−)-DBD-PyNCS in $CH_3CN$, and 10 μL of a 30-mM solution of DMAP in $CH_3CN$. The resulting solution was heated at a temperature of 55 °C for 20 min, and then, 20 μL of a 1.1% AcOH solution in 39% $CH_3CN$–$H_2O$ was added in order to dilute the reacted solution. A 5.0 μL aliquot of the final solution was injected into the HPLC system for fluorescence detection.

### 2.5. Precision and accuracy

First, 10 μL of rat plasma was spiked with 10 μL of 25-, 50-, 100-, and 250-μM Trp and KYN enantiomers dissolved in $H_2O/CH_3CN$ (50/50) and 10 μL of 200-μM I.S. solution and vigorously mixed with 120 μL of $CH_3CN$. After centrifugation at 1300 × *g* for 20 min at 4 °C, 10 μL of the supernatant was sampled, and was mixed with 10 μL of a 10-mM solution of *R*(−)-DBD-PyNCS in $CH_3CN$, and 10 μL of a 30-mM solution of DMAP in $CH_3CN$. Following fluorescence derivatization and the HPLC separation were carried out in a manner similar to that described above. The precision was expressed as relative standard deviation (RSD, %), and the accuracy was expressed as relative mean error (RME, %). RSD and RME were calculated by the following Eqs. (1) and (2), respectively.

$$\mathrm{RSD}\,(\%) = \left(\frac{\text{standard deviation}}{\text{mean}}\right) \times 100 \quad (1)$$

$$\mathrm{RME}\,(\%) = \left[\frac{(\text{mean of measured concentration} - \text{added (theoretical) concentration})}{\text{added (theoretical) concentration}}\right] \times 100 \quad (2)$$

In the case of L-Trp, (mean of measured concentration − mean of endogenous L-Trp concentration) was used instead of (mean of measured concentration) in Eq. (2).

### 2.6. HPLC conditions

The HPLC system consists of a Shimadzu LC10AD pump, a Shimadzu CTO-10Avp column oven, a Shimadzu RF-10A$_{XL}$ fluorescence detector (Shimadzu Corporation, Kyoto, Japan), and PC software, CDS ver.5 (LAsoft Ltd., Chiba, Japan). The separation column used in this study was an Inertsil ODS-3 column (250 mm × 2.0 mm i.d., 3 μm) (GL Sciences Co. Ltd., Tokyo, Japan). The mobile phase was 1.1% AcOH in 39% $CH_3CN$–$H_2O$, eluted isocratically at 0.2 mL/min. The mobile phase was prepared using a 500 mL measurement flask. Column temperature was maintained at 40 °C. Fluorescence detection was carried out by setting the emission wavelength at 565 nm and the excitation wavelength at 440 nm.

### 2.7. Pharmacokinetic parameters and statistical analysis

Pharmacokinetic parameters obtained in the present study were determined by a moment analysis. Statistical analyses between two groups were performed using Student's *t*-test. Significant differences among three groups were analyzed by a one-way ANOVA followed by Bonferroni's multiple comparison test. A $p$ value below 0.05 was judged as a significant difference.

## 3. Results and discussion

### 3.1. Optimization of mobile phase condition

Previously, we reported the use of *R*(−)-DBD-PyNCS as a pre-column diastereomer derivatization reagent (Fig. 1) for the separation of the enantiomers of Trp and KYN on an ODS column and that simultaneous separation was achieved under the mobile phase condition of 1.5% acetic acid in $H_2O/CH_3CN$ (60/40) [18]. The separation factors ($\alpha$) and resolution (*R*s) of D,L-Trp and D,L-KYN derivatized with *R*(−)-DBD-PyNCS were 1.22 and 7.02, and 1.19 and 5.56, respectively. A stock solution of 5-aminoindole dissolved in $CH_3CN$ was used as an I.S.; however, 5-aminoindole in $CH_3CN$ is rather labile at 4 °C, and therefore, preparation of the I.S. solution had to be carried out prior to each experiment. In the present study, a more stable I.S. was sought, and 8-aminocaprylic acid tagged with *R*(−)-DBD-PyNCS was found to be suitable for use as an I.S., because it was eluted at retention time of 38 min, that there were no interfering peaks (Fig. 2(a) and (b)). 8-Aminocaprylic acid was stable in $CH_3CN$ solution at 4 °C for at least 2 months.

Consequently, the mobile phase condition was also reexamined in the present study. Fig. 2(c) shows a representative chromatogram of blank rat plasma spiked with 100 μM D,L-Trp and D,L-KYN, respectively. Simultaneous separation of D,L-Trp and D,L-KYN including the I.S. was achieved under the mobile phase condition

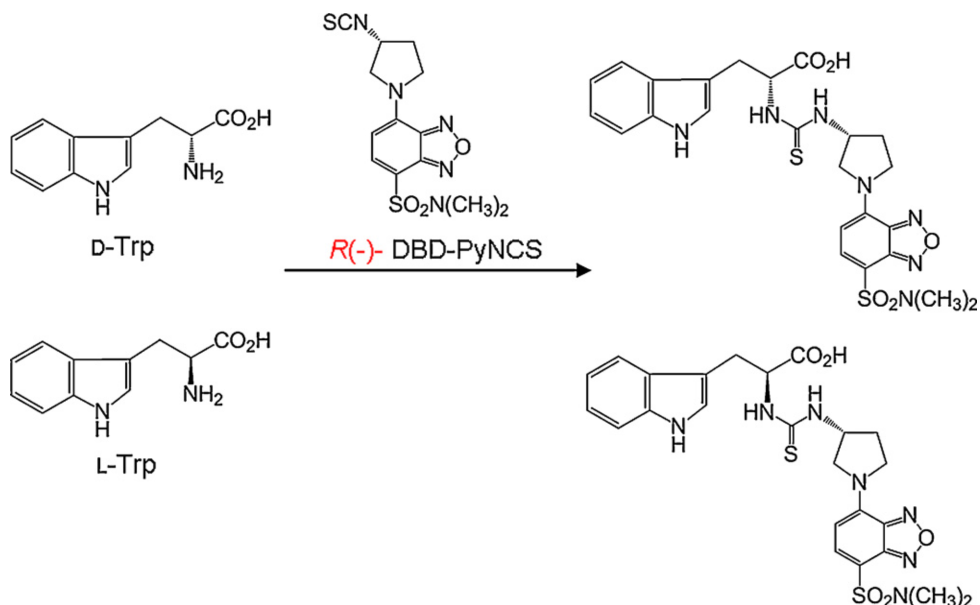


**Fig. 1.** Diastereomeric and fluorescence derivatization of D- or L-Trp with *R*(−)-DBD-PyNCS.

Table 1

Validation data on the determination of D-KYN, D-Trp, L-KYN, and L-Trp in rat plasma by the proposed HPLC method ($n = 4$, mean ± S.D.).

| | Added D-KYN concentration (μM) | | | | |
|---|---|---|---|---|---|
| | 0 | 25 | 50 | 100 | 250 |
| Intra-day | | | | | |
| Mean ± S.D. | ND | 23.2 ± 0.814 | 48.3 ± 2.72 | 97.7 ± 5.84 | 231 ± 13.8 |
| RSD (%) | – | 3.51 | 5.63 | 5.98 | 5.97 |
| RME (%) | – | −7.20 | −3.40 | −2.30 | −7.60 |
| Inter-day | | | | | |
| Mean ± S.D. | ND | 22.6 ± 1.15 | 46.3 ± 2.52 | 97.5 ± 7.13 | 230 ± 13.6 |
| RSD (%) | – | 5.09 | 5.44 | 7.31 | 5.91 |
| RME (%) | – | −9.60 | −7.40 | −2.50 | −8.00 |
| | Added D-Trp concentration (μM) | | | | |
| | 0 | 25 | 50 | 100 | 250 |
| Intra-day | | | | | |
| Mean ± S.D. | ND | 24.4 ± 1.24 | 46.8 ± 1.62 | 97.5 ± 5.64 | 232 ± 14.8 |
| RSD (%) | – | 5.08 | 3.46 | 5.78 | 6.38 |
| RME (%) | – | −2.40 | −6.40 | −2.50 | −7.20 |
| Inter-day | | | | | |
| Mean ± S.D. | ND | 24.0 ± 0.810 | 45.4 ± 3.23 | 96.3 ± 4.22 | 229 ± 20.7 |
| RSD (%) | – | 3.38 | 7.11 | 4.38 | 9.04 |
| RME (%) | – | −4.00 | −9.20 | −3.70 | −8.40 |
| | Added L-KYN concentration (μM) | | | | |
| | 0 | 25 | 50 | 100 | 250 |
| Intra-day | | | | | |
| Mean ± S.D. | ND | 23.4 ± 0.70 | 46.1 ± 5.58 | 97.8 ± 5.58 | 245 ± 13.1 |
| RSD (%) | – | 2.99 | 4.34 | 5.71 | 5.35 |
| RME (%) | – | −6.40 | −7.80 | −2.20 | −2.00 |
| Inter-day | | | | | |
| Mean ± S.D. | ND | 23.1 ± 0.79 | 45.7 ± 1.71 | 98.0 ± 2.88 | 232 ± 18.0 |
| RSD (%) | – | 3.42 | 3.74 | 2.94 | 7.76 |
| RME (%) | – | −7.60 | −8.60 | −2.00 | −7.20 |
| | Added L-Trp concentration (μM) | | | | |
| | 0 | 25 | 50 | 100 | 250 |
| Intra-day | | | | | |
| Mean ± S.D. | 78.3 ± 2.84 | 101 ± 1.10 | 126 ± 3.78 | 177 ± 2.38 | 312 ± 12.0 |
| RSD (%) | 3.63 | 1.09 | 3.00 | 1.34 | 3.85 |
| RME (%) | – | −9.20 | −4.60 | −1.30 | −6.52 |
| Inter-day | | | | | |
| Mean ± S.D. | 80.2 ± 3.13 | 104 ± 1.66 | 127 ± 4.65 | 173 ± 8.10 | 307 ± 22.7 |
| RSD (%) | 3.90 | 1.60 | 3.66 | 4.68 | 7.39 |
| RME (%) | – | −4.80 | −6.40 | −7.20 | −9.28 |

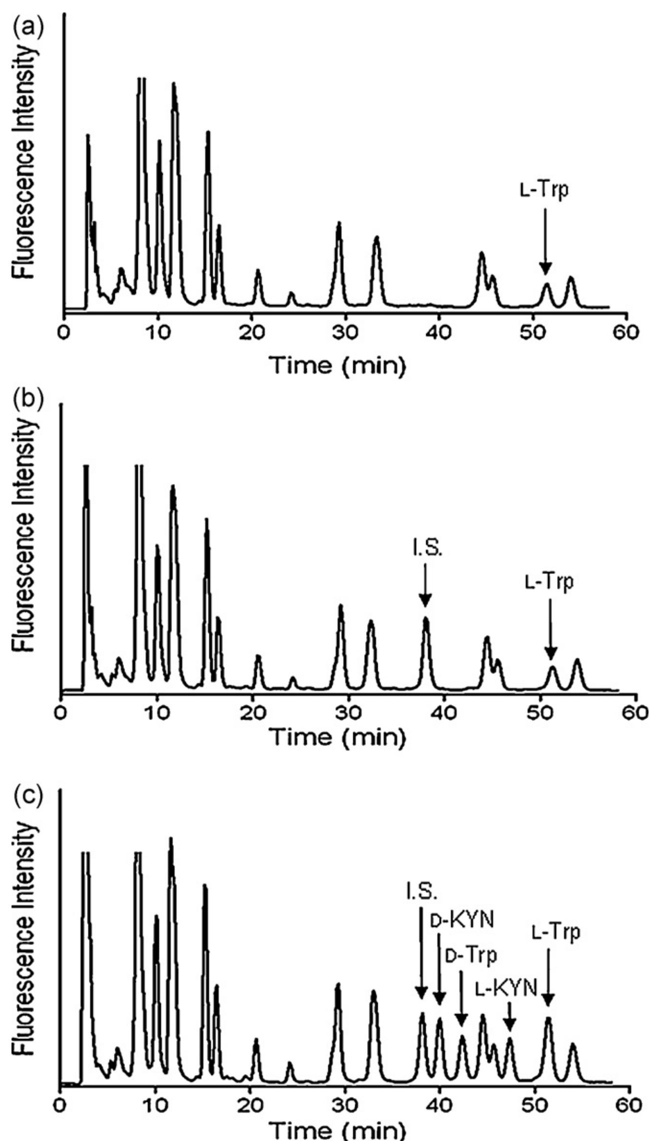


**Fig. 2.** Typical chromatograms of D-Trp, L-Trp, D-KYN, and L-KYN derivatized with *R*(−)-DBD-PyNCS on the Inertsil ODS-3 column. The mobile phase condition was 1.1% AcOH in 39% $CH_3CN–H_2O$. Blank rat plasma (a), blank rat plasma spiked with I.S. (200 μM) (b), and blank rat plasma spiked with standards of 100 μM D-Trp, L-Trp, D-KYN, and L-KYN (c).

of 1.1% AcOH in 39% $CH_3CN$-$H_2O$, and the $\alpha$ and *Rs* of D,L-Trp and D,L-KYN derivatized with *R*(−)-DBD-PyNCS were 1.22 and 6.71 and 1.19 and 5.98, respectively. The *Rs* values were changed, while no changes of $\alpha$ values of D,L-Trp and D,L-KYN were observed by slight change of the mobile phase condition from 1.5% AcOH in $H_2O/CH_3CN$ (60/40) to 1.1% AcOH in 39% $CH_3CN–H_2O$. Although the *Rs* value of D,L-Trp was slightly lower than that obtained under the previously employed HPLC conditions, these values of $\alpha$ and *Rs* were acceptable for the simultaneous separation of the Trp and KYN enantiomers.

Table 1 lists the validation data, precision and accuracy for the determination of the plasma concentrations of D- and L-Trp, and D- and L-KYN by the present HPLC method. Intra- and inter-day precisions (RSD, $n = 4$) were in the ranges of 1.09–6.38 and 1.60–9.04 (%), and intra- and inter-day accuracies (RME, $n = 4$) were in the ranges of −1.30 to −9.20 and −2.00 to −9.60 (%), respectively. These validation data were sufficient for the determination of the plasma concentrations of D- and L-Trp, and D- and L-KYN. Therefore, the present HPLC conditions were used in the determination of the plasma concentration of D-Trp or L-Trp after *i.p.* administration of each Trp enantiomer.

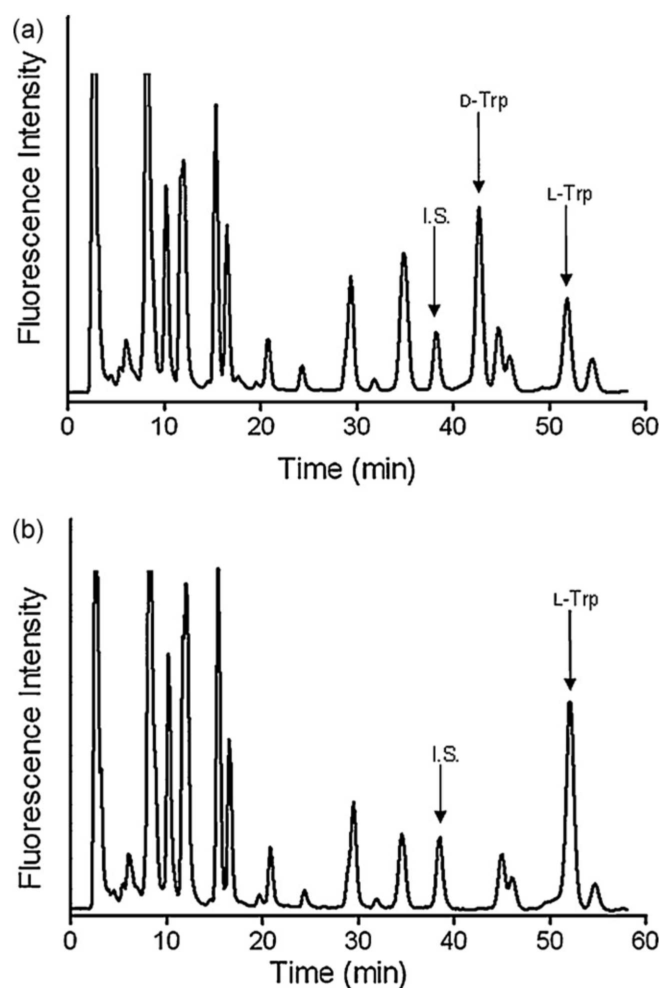


**Fig. 3.** Typical chromatograms of rat plasma sample 30 min after D-Trp administration (a), and rat plasma sample 30 min after L-Trp administration (b).

### 3.2. Determination of plasma D-Trp or L-Trp

In the present study, the purpose was to investigate differences of the pharmacokinetic parameters between Trp enantiomers in rats, thus, we adopted an *i.p.* administration, because of its ease of operation.

Fig. 3(a) and (b) shows chromatograms of rat plasma at 30 min after *i.p.* administration of D-Trp or L-Trp (100 mg/kg), respectively. Both D-Trp and L-Trp peaks were well detected under the present HPLC conditions; however, the peaks of D-KYN and L-KYN could not be detected in the chromatogram of rat plasma for 0–6 h after the administration under the present HPLC conditions. It was reported that endogenous L-KYN concentration in rat plasma (ca. 1–2 μM) [21] was considerably lower than L-Trp, suggesting that L-KYN was hardly accumulated in the plasma. Therefore, no observation of D-KYN or L-KYN peaks in the rat plasma might be due to below the detection limit (approximately 1.0 pmol on column) by the present HPLC condition.

Fig. 4 shows time-course profiles of the plasma concentrations of D-Trp and L-Trp in rats after administration of each enantiomer. The maximal concentration values of both D-Trp and L-Trp were obtained 30 min after the administration, and the concentrations were shown to decrease with the progression of time. Table 2

**Table 2**
Pharmacokinetic parameters, half-life ($t_{1/2}$), area under the curve (AUC), total clearance ($CL_{tot}$), and distribution volume ($V_d$) of plasma concentration of D-Trp and L-Trp following D-Trp or L-Trp administration to rats (100 mg/kg) ($n$ = 3, mean ± S.E.).

| | $t_{1/2}$ (h) | AUC (μmol h/L) | $CL_{tot}$ (L/h/kg) | $V_d$ (L/kg) |
|---|---|---|---|---|
| D-Trp | 0.92 ± 0.09 | 702 ± 32 | 0.70 ± 0.03 | 0.92 ± 0.04 |
| L-Trp | 1.55 ± 0.05* | 1691 ± 89* | 0.29 ± 0.02* | 0.65 ± 0.05 |
| D-Trp[a] | 1.00 ± 0.01 | 2080 ± 133* | 0.24 ± 0.02* | 0.34 ± 0.03 |

* $p < 0.01$ *versus* D-Trp (Student's *t*-test).
[a] Plasma D-Trp of rats pretreated with MPC (50 mg/kg).

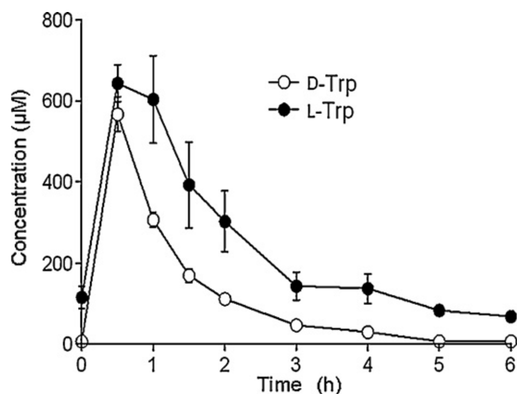


**Fig. 4.** Time-course profiles of plasma concentrations of D-Trp and L-Trp after *i.p.* administration of D-Trp (open circles) or L-Trp (closed circles) ($n$ = 3, mean ± S.E.). The open and closed circles represent D-Trp and L-Trp concentrations, respectively. Experimental details are as described in the text.

lists the pharmacokinetic parameters, half-life ($t_{1/2}$), area under the curve (AUC), total clearance ($CL_{tot}$), and distribution volume ($V_d$) of plasma concentrations of D-Trp and L-Trp in rats. Significant differences ($p < 0.01$) of $t_{1/2}$, AUC, and $CL_{tot}$ were observed between the enantiomers. A smaller $t_{1/2}$ value of plasma D-Trp suggested that D-Trp was more rapidly eliminated from plasma than L-Trp.

The two main enzymes responsible for the cleavage of the indole ring in the metabolism of L-Trp are tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) [22,23]. After the oxidative cleavage of the indole ring, L-Trp is mainly metabolized in the kynurenine pathway to produce L-KYN, KYNA, 3-hydroxy-L-KYN, anthranilic acid, etc. [24,25]. In addition, it was reported that L-Trp is reversibly converted to indole-3-pyruvic acid (I3P) by aspartate aminotransferase (AST) (EC 2.6.1.1) [26].

In contrast, only IDO can act to cleave the indole ring of D-Trp [27], and the $K_m$ value (ca. 5 mM) was approximately 25-fold larger than that of L-Trp (ca. 20 μM) [27,28]. Considering that the $t_{1/2}$ value of D-Trp was significantly smaller than that of L-Trp in spite of the small $K_m$ value of IDO to D-Trp, it was postulated that DAAO might be active in the metabolism of D-Trp in addition to IDO. It was reported that the $K_m$ value of pig kidney DAAO to D-Trp, D-Ala, and D-Ser was 845.0 [29], 3.1 mM, and 41 mM [30], respectively, indicating that DAAO possesses a particularly high affinity to D-Trp. Interestingly, it was reported that a derivative of D-Trp––1-methyl-D-Trp––which is reported to be an inhibitor of IDO, was hardly metabolized in rats [31], but it seems to be likely in the present case that D-Trp was metabolized by both DAAO and IDO.

On the other hand, a significant increase in the plasma concentration of L-Trp was found in rats administered with D-Trp (Fig. 5(b)), whereas few D-Trp was detected in the rat plasma during the 0–6 h period after L-Trp administration (data not shown). It has been previously suggested that D-Trp and L-Trp may have the same nutritive value in rats as a result of the chiral inversion of D-Trp to L-Trp through the α-keto acid, I3P, *in vivo* [16,17,32]. Other than D-Trp, D-methionine (Met) and D-leucine (Leu) were converted to their enantiomers, L-Met and L-Leu, respectively [33–35]. In the metabolism of D-Met and D-Leu, the action of DAAO first gave rise to the corresponding α-keto acid. L-Met and L-Leu were then produced stereoselectively from the α-keto acid by aminotransferase *in vivo*. In a manner similar to that proposed for D-Met and D-Leu, the increased L-Trp in the rat plasma following administration of D-Trp may arise from the conversion of D-Trp to L-Trp through the proposed metabolic pathway shown in Fig. 6; this can be said considering the fact that DAAO, which is responsible for the production of I3P by the oxidative deamination of D-Trp [6], possesses a high affinity to D-Trp [29]. In order to verify the involvement of DAAO in the conversion of D-Trp to L-Trp, the changes in the plasma concentration of D- or L-Trp were investigated following the preadministration of 3-methylpyrazole-5-carboxylic acid (MPC), an inhibitor of DAAO [20], which serves to suppress the activity of DAAO.

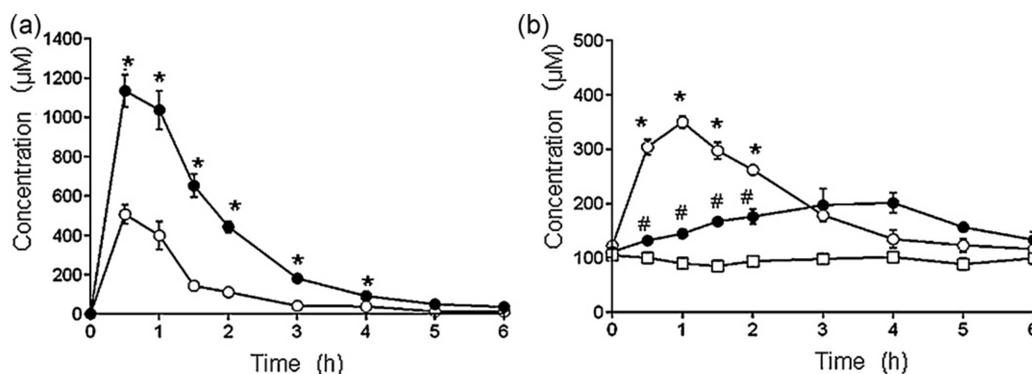


**Fig. 5.** (a) Time-course profiles of plasma D-Trp concentrations after *i.p.* administration of D-Trp (100 mg/kg) to normal rats (open circles) and rats pretreated with MPC (50 mg/kg) (closed circles) ($n$ = 3, mean ± S.E.). * $p < 0.05$ *versus* normal rats (Student's *t*-test). (b) Time-course profiles of plasma concentration of L-Trp after D-Trp was administered *i.p.* (100 mg/kg) to normal rats (open circles) and rats pretreated with MPC (50 mg/kg) (closed circles) ($n$ = 3, mean ± S.E.). The open squares represent plasma concentrations of L-Trp after saline (1.0 mL/kg) was administered *i.p.* to normal rats. *$p < 0.05$ *versus* normal rats administered with saline, #$p < 0.05$ *versus* normal rats (Bonferroni test).

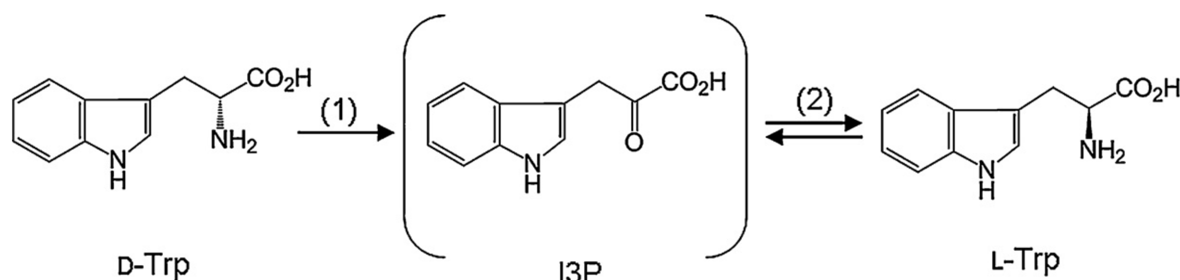


**Fig. 6.** Proposed metabolic pathway of D-Trp. (1) DAAO, (2) AST.

### 3.3. Effect of MPC on the plasma concentration of D- or L-Trp

Fig. 5(a) and (b) show time-course profiles of the plasma concentration of D- or L-Trp in rats pretreated with or without the DAAO inhibitor, i.e., MPC [20]. As shown in Fig. 5(a), the plasma concentration of D-Trp increased remarkably by pretreatment with MPC. The area under the curve (AUC) of plasma concentration of D-Trp showed a ca. threefold increase in the case of rats pretreated with MPC (50 mg/kg) (Table 2). These data indicated that MPC administration (50 mg/kg) might indeed act to suppress the DAAO activity in rats. As shown in Fig. 5(b), the plasma concentration of L-Trp in MPC-pretreated rats was significantly lower than that of normal rats during the 0.5–2 h period, indicating that pretreatment with MPC (50 mg/kg) acts to suppress the increase in the L-Trp concentration after D-Trp administration. These observations suggest that the significant increase in L-Trp concentration following D-Trp administration was a result of the involvement of DAAO activity *in vivo*. After 3 h, the plasma concentration of L-Trp in MPC-pretreated rats increased and became higher than that of normal rats. The $t_{1/2}$ value of MPC in the plasma of rats has been reported to be 5.59 h after intravenous administration [20]. However, it seems probable that the efficiency of MPC in the inhibition of DAAO activity may last up to 3 h. From these results, it can be concluded that DAAO may metabolize D-Trp to the corresponding α-keto acid, I3P [6], which might further be transaminated to form L-Trp by an aminotransferase, AST. Similar to D-Trp, I3P is produced enzymatically from L-Trp by AST [26]; however, there was no detection of D-Trp after L-Trp administration. The lack of detection of D-Trp after L-Trp administration indicates that AST may aminate I3P stereospecifically to produce only L-Trp.

## 4. Conclusion

The plasma concentrations of D-Trp and L-Trp were determined following *i.p.* administration of D-Trp and L-Trp to male Sprague–Dawley rats, by HPLC with fluorescence detection using *R*(−)-DBD-PyNCS as a pre-column diastereomer derivatization reagent, and the pharmacokinetic parameters of plasma D-Trp and L-Trp were calculated. It was found that D-Trp was eliminated from the plasma more rapidly than L-Trp in rats. In addition, the data suggested that administered D-Trp was in part converted to L-Trp *in vivo* by the action of DAAO and subsequently aminotransferase.


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