Tryptophan promoted β-defensin-2 expression via the mTOR pathway and its metabolites: kynurenine binding to aryl hydrocarbon receptor in rat intestine

Zhiru Tang, a Baoshi Shi,† a Weizhong Sun,† a Yulong Yin, b Qingju Chen, a Taha Mohamed, a Changwen Lu a and Zhihong Sun a*, a

In this study, we investigated the signalling pathways mediating tryptophan (Trp)-promoted β-defensin-2 (BD-2) expression in rat intestinal mucosa. Sprague Dawley rats were administered with L-Trp and treated with rapamycin (RAPA), 1-methyltryptophan (1-MT), or para-chlorophenyl-amine (PCPA) to inhibit mammalian target of rapamycin (mTOR), indoleamine-2,3-dioxygenase (IDO), or tryptophan hydroxylase (TPH), respectively. The mRNA and protein levels of BD-2 in the jejunal and ileal mucosa of rats increased with administration of L-Trp. Intraperitoneal injection of RAPA significantly decreased the mRNA level of BD-2 and the concentrations of p-mTORC1 and BD-2 in the jejunal and ileal mucosa of rats with administration of L-Trp (P < 0.05). Oral administration of 1-MT decreased the IDO activity and the mRNA and protein levels of BD-2, and increased the concentrations of tumour necrosis factor (TNF-α), interleukin (IL)-17, and IL-22 in the jejunal and ileal mucosa of rats with administration of L-Trp (P < 0.05). Intraperitoneal injection of PCPA decreased the TPH activity and increased the mRNA and protein levels of BD-2, but did not change the concentrations of TNF-α, IL-17, or IL-22 in the jejunal and ileal mucosa of rats with administration of L-Trp. The results indicate the Trp-promoted BD-2 expression in the jejunum and ileum via the mTOR pathway and its metabolites: kynurenine binding to aryl hydrocarbon receptor in rat intestine.

Introduction

Beta-defensin-2 (BD-2) plays a key role in the prevention of intestinal pathogenic microbial infection. The BD-2 expression is induced by infective stimuli, including Gram-negative and Gram-positive bacteria and their components or pro-inflammatory mediators, for example, tumour necrosis factor (TNF-α) and interleukin (IL)-1β.1–3

Amino acids (AAs) have been shown to play important roles in immune responses by regulating (1) the production of cytokines, antibodies and other cytotoxic substances;4,5 (2) gene expression, cellular redox state and lymphocyte proliferation; and (3) the activation of B and T lymphocytes, natural killer cells and macrophages. Studies have shown that dietary specific AA supplementation enhances the immune status of animals with malnutrition and infectious diseases, thereby reducing morbidity and mortality.6,7 Notably, tryptophan (Trp) is associated with many important immune functions. Dietary Trp supplementation improves the growth performance of piglets challenged by pathogenic Escherichia coli.8,9 Other studies have shown that Trp is useful in decreasing intestinal permeability and improving the intestinal barrier function.10 Hashimoto et al.11 reported that, Trp controls expression of small intestinal antimicrobial peptides (AMPs).

L-Trp plays important roles in protein synthesis and as a precursor of various bioactive compounds, such as kynurenine (Kyn) and 5-hydroxytryptamine (5-HT).12,13 Thus, L-Trp might promote the expression of BD-2 in the epithelial cells through the following pathways: (1) activating the mTOR pathway via its intracellular receptors thereby promoting the expression of BD-2; (2) promoting the expression of BD via Kyn, which is as a ligand of aryl hydrocarbon receptor (Ahr) can promote intestinal epithelial cell secretion of IL-22, IL-17, and other cytokines that enhance the expression of BD-2 in the intestinal epithelial cells via the NF-κB pathway;14–16 or (3) promoting the expression of BD via 5-HT. In this study, Sprague Dawley rats were used to investigate the signalling pathways mediating Trp-promoted BD-2 expression in the rat intestinal mucosa.
Materials and methods

Animal use and care

Specific pathogen-free SD rats were obtained from Chongqing Academy of Chinese Materia Medica (Chongqing, China) at 5 weeks of age. All experimental procedures were approved by the License of Experimental Animals (SYXK 2014-0002) of the Animal Experimentation Ethics Committee of Southwest University, Chongqing, China.

Experimental diets and design

The basal diet (Table 1) was formulated according to the AIN-93G requirements reported by the American Institute of Nutrition. The rats were kept individually in pens (0.5 m × 0.5 m × 0.5 m) in a temperature-controlled (25 ± 1.0 °C) and mechanically ventilated room. Feed and water were provided ad libitum.

**Experiment 1.** Forty male rats were randomly allotted to one of four treatments to investigate the role of mTOR in the expression of BD-2 promoted by Trp in the intestinal mucosa (n = 10 per treatment). The four treatments were as follows: (1) rats were orally administered with 0.5 mL saline and intraperitoneally injected with 0.5 mL saline daily (CON); (2) rats were orally administered with 0.5 mL saline and intraperitoneally injected with 0.5 mL saline containing rapamycin (1.5 mg kg⁻¹) daily (RAPA; Sigma-Aldrich, Oakville, ON, Canada); (3) rats were orally administered with 0.5 mL saline containing L-Trp (260 mg kg⁻¹) daily (TRP; Sigma-Aldrich); and (4) rats were orally administered 0.5 mL saline containing L-Trp (260 mg kg⁻¹) and intraperitoneally injected with 0.5 mL saline containing rapamycin (1.5 mg kg⁻¹) daily (RAPA + TRP). The experimental period lasted for 4 days. Daily doses for oral delivery and intraperitoneal injection were divided into two equal portions and were administered at 08:00 h and 20:00 h. Six rats were randomly selected from each group for slaughter on day 5. Rats were anaesthetized with a mixture (0.3 mL/100 g) of sterile water, midazolam (5 mg mL⁻¹) and hypnorm at a ratio of 2:1:1. After anaesthetization, the rats were exsanguinated by severing the jugular vein and carotid artery. The abdominal cavity was then opened, 1–2 g of jejunal and ileal mucosa were scraped with a glass slide, immediately placed in liquid nitrogen and stored at -80 °C.

**Experiment 2.** Forty male rats were randomly allotted to one of four treatments to investigate the role of indoleamine-2,3-dioxygenase (IDO) in the expression of BD-2 promoted by Trp in the intestinal mucosa (n = 10 per treatment). The four treatments were as follows: (1) rats were orally administered with 0.5 mL saline daily (CON); (2) rats were orally administered with 0.5 mL saline containing 1-methyltryptophan (1-MT) (50 mg kg⁻¹) daily (MT; Sigma-Aldrich); (3) rats were orally administered with 0.5 mL saline containing 1-MT (50 mg kg⁻¹) daily (MT; Sigma-Aldrich); and (4) rats were orally administered with 0.5 mL saline containing 1-MT (50 mg kg⁻¹) and L-Trp (260 mg kg⁻¹) daily (TRP + MT). The experimental period lasted for 4 days. Daily doses of 1-MT and L-Trp were divided into two equal portions, administered at 08:00 h and 20:00 h. Six rats were randomly selected from each group for slaughter on day 5. The anaesthetization procedures were the same as those in experiment 1. After anaesthetization, the abdominal cavity was opened, and 2 mL of blood was collected from the vena cava for Trp and Kyn analysis. The blood samples were diluted twice with 4% sulfosalicylic acid and centrifuged at 16 000 rpm for 2 min at 4 °C. The supernatant was filtered using 0.22 nm filter and the filtrates were harvested for Trp and Kyn analysis. The rats were then exsanguinated and mucosal samples from the jejunum and ileum were collected following the procedures used in experiment 1.

**Experiment 3.** Forty male rats were randomly allotted to one of four treatments to investigate the role of tryptophan hydroxylase (TPH) in the expression of BD-2 promoted by Trp in the intestinal mucosa (n = 10 per treatment). The four treatments were as follows: (1) rats were orally administered with 0.5 mL saline and intraperitoneally injected with 0.5 mL saline daily (CON); (2) rats were orally administered with 0.5 mL saline and intraperitoneally injected with 0.5 mL saline containing para-chlorphenyl-amine (200 mg kg⁻¹) daily (PCPA; Sigma-Aldrich); (3) rats were orally administered with 0.5 mL saline containing 1-methyltryptophan (1-MT) (50 mg kg⁻¹) and L-Trp (260 mg kg⁻¹) daily (TRP + MT). The experimental period lasted for 4 days. Daily doses of 1-MT and L-Trp were divided into two equal portions, administered at 08:00 h and 20:00 h. Six rats were randomly selected from each group for slaughter on day 5. The anaesthetization procedures were the same as those in experiment 1. After anaesthetization, the abdominal cavity was opened, and 2 mL of blood was collected from the vena cava for Trp and Kyn analysis. The blood samples were diluted twice with 4% sulfosalicylic acid and centrifuged at 16 000 rpm for 2 min at 4 °C. The supernatant was filtered using 0.22 nm filter and the filtrates were harvested for Trp and Kyn analysis. The rats were then exsanguinated and mucosal samples from the jejunum and ileum were collected following the procedures used in experiment 1.

### Table 1. Ingredients and composition of diets fed to Sprague Dawley rats (DM basis)

| Ingredients                          | (%)  |
|--------------------------------------|------|
| Corn starch                          | 74.36|
| Soy-bean oil                         | 6.00 |
| Sucrose                              | 13.67|
| Ferric citrate                       | 1.00 |
| Calcium hydrophosphate               | 2.00 |
| Vitamin premixª                      | 0.30 |
| Mineral mixtureª                     | 2.74 |
| Salt                                 | 0.03 |
| Total                                | 100.00|

ª Providing the following per kg diet: vitamin A, 14 000 IU; vitamin D, 1500 IU; vitamin E, 120 IU; vitamin K, 5 mg; vitamin B1, 13 mg; vitamin B2, 12 mg; vitamin B6, 12 mg; vitamin B12, 0.022 mg; nicotinic acid, 60 mg; biotin, 0.2 mg; pantothenic acid, 24 mg; folic acid, 6 mg.

### Table 2. The sequences of primers

| Gene      | Product length | Primer sequences (5’ → 3’) | Tm  |
|-----------|----------------|----------------------------|-----|
| GAPDH     | 149 bp         | F: GAAGTCCGGAGTGAACGGAT    | 65 °C|
|           |                | R: CATTGGTGAGAATCATACTGAACA |     |
| BD-2      | 150 bp         | F: ACATGGGGCTCGCTGCTCA     | 61 °C|
|           |                | R: CCTGGTGCTCCCTCGATATT    |     |

ª Abbreviations: GAPDH: glyceraldehyde-3-phosphate dehydrogenase; BD-2: β-defensin.
containing L-Trp (260 mg kg\(^{-1}\)) and intraperitoneally injected with 0.5 mL saline daily (TRP); and (4) rats were orally administered with 0.5 mL saline containing L-Trp (260 mg kg\(^{-1}\)) and intraperitoneally injected with 0.5 mL saline containing para-chlorophenyl-amine (200 mg kg\(^{-1}\)) daily (PCPA + TRP). The experimental period lasted for 4 days. Daily doses of L-Trp and PCPA were divided into two equal portions, administered at 08:00 h and 20:00 h. Six rats were randomly selected from each group for slaughter on day 5. The anaesthetization procedures followed those in experiment 1. After anaesthetization, samples of blood and jejunal and ileal mucosa were collected following the procedures used in experiment 2.

Chemical analysis

Trp, Kyn, and 5-HT were measured using liquid chromatography (Agilent Technologies, Santa Clara, CA, USA) on a biphenyl column (100 mm × 2.1 mm, Kinetex 2.6 µm).

### Table 3

|                | MT0 Trp0 | MT0 Trp1 | MT1 Trp0 | MT1 Trp1 | SEM  | P-value       | MT   | Trp  | MT × Trp |
|----------------|----------|----------|----------|----------|------|---------------|------|------|----------|
| Serum          |          |          |          |          |      |               |      |      |          |
| Trp (µg mL\(^{-1}\)) | 6.27     | 17.8     | 7.20     | 18.6     | 0.13 | <0.001 | 0.007 | <0.001 | 0.239   |
| Kyn (µg mL\(^{-1}\)) | 0.12     | 0.24     | 0.07     | 0.10     | 0.01 | <0.001 | <0.001 | <0.001 | <0.001  |
| Kyn/Trp (%)   | 1.79     | 1.29     | 1.24     | 0.54     | 0.04 | <0.001 | <0.001 | <0.001 | <0.001  |
| Jejunum mucosa (ng g\(^{-1}\) protein) |          |          |          |          |      |               |      |      |          |
| IDO           | 42.3     | 60.1     | 25.3     | 39.2     | 0.87 | <0.001 | <0.001 | <0.001 | <0.144  |
| IL-17         | 328      | 881      | 316      | 469      | 17.5 | <0.001 | <0.001 | <0.001 | <0.001  |
| IL-22         | 253      | 377      | 182      | 320      | 5.32 | <0.001 | <0.001 | 0.347   |         |
| TNF-α         | 259      | 461      | 147      | 358      | 13.5 | <0.001 | <0.001 | 0.813   |         |
| Ileum mucosa (ng g\(^{-1}\) protein) |          |          |          |          |      |               |      |      |          |
| IDO           | 41.3     | 74.6     | 30.6     | 60.2     | 2.70 | <0.001 | <0.001 | <0.001 | 0.622   |
| IL-17         | 701      | 1362     | 628      | 807      | 31.0 | <0.001 | <0.001 | <0.001 | <0.001  |
| IL-22         | 315      | 356      | 234      | 247      | 5.22 | 0.002  | <0.001 | 0.084   |         |
| TNF-α         | 242      | 432      | 139      | 323      | 8.87 | <0.001 | <0.001 | 0.802   |         |

Data are presented as mean ± SEM (n = 6). Abbreviations: MT0 or MT1, rats were orally administered with 0.5 mL saline containing or no containing 1-methyltryptophan (50 mg kg\(^{-1}\)) daily; Trp0 or Trp1, rats were orally administered with 0.5 mL saline containing or no containing L-Trp (260 mg kg\(^{-1}\)) daily; Kyn, kynurenine; IDO, indoleamine-2,3-dioxygenase; IL-17, interleukin 17; TNF-α, tumour necrosis factor.

### Table 4

|                | PCPA0 Trp0 | PCPA0 Trp1 | PCPA1 Trp0 | PCPA1 Trp1 | SEM  | P-value       | PCPA | Trp  | PCPA × Trp |
|----------------|------------|------------|------------|------------|------|---------------|------|------|------------|
| Serum          |            |            |            |            |      |               |      |      |            |
| Trp (µg mL\(^{-1}\)) | 6.28      | 17.4       | 6.94       | 19.1       | 0.19 | <0.001 | <0.001 | 0.312   |         |
| 5-HT (ng mL\(^{-1}\)) | 32.8      | 57.0       | 23.9       | 31.1       | 1.65 | <0.001 | <0.001 | <0.001  |         |
| Jejunum mucosa (ng g\(^{-1}\) protein) |            |            |            |            |      |               |      |      |            |
| TPH            | 48.8       | 96.3       | 40.9       | 68.2       | 1.27 | <0.001 | <0.001 | <0.001  |         |
| IL-17          | 298        | 842        | 313        | 844        | 19.72| 0.255  | <0.001 | 0.879   |         |
| IL-22          | 245        | 372        | 254        | 380        | 8.56 | 0.267  | <0.001 | 0.777   |         |
| TNF-α          | 266        | 468        | 287        | 472        | 15.32| 0.428  | <0.001 | 0.863   |         |
| Ileum mucosa (ng g\(^{-1}\) protein) |            |            |            |            |      |               |      |      |            |
| TPH            | 39.4       | 59.9       | 32.6       | 45.1       | 1.38 | <0.001 | <0.001 | 0.011   |         |
| IL-17          | 706        | 1367       | 717        | 1368       | 37.92| 0.275  | <0.001 | 0.805   |         |
| IL-22          | 255        | 386        | 264        | 397        | 5.85 | 0.076  | <0.001 | 0.750   |         |
| TNF-α          | 332        | 453        | 339        | 463        | 7.85 | 0.225  | <0.001 | 0.754   |         |

Data are presented as mean ± SEM (n = 6). Abbreviations: PCPA0 or PCPA1: rats were intraperitoneally injected with 0.5 mL saline containing or no containing para-chlorophenyl-amine (200 mg kg\(^{-1}\)) daily; Trp0 or Trp1, rats were orally administered with 0.5 mL saline containing or no containing L-Trp (260 mg kg\(^{-1}\)) daily; 5-HT, 5-hydroxytryptamine; IL-17, interleukin 17; TNF-α, tumour necrosis factor; TPH, tryptophan hydroxylase.
Table 5 Oral L-Trp affects the mRNA level of BD-2 in the jejunal and ileal mucosa of rats

|                | Jejunal mucosa | Ileal mucosa |
|----------------|----------------|--------------|
| MT0            | 0.62           | 1.68         |
| MT1            | 3.95           | 5.18         |
| MT0            | 0.30           | 0.85         |
| MT1            | 1.43           | 3.34         |
| Pooled SEM     | 0.20           | 0.26         |

\[ P\text{value} \]

|                | MT   | Trp  |
|----------------|------|------|
| MT0            | <0.001 | <0.001 |
| MT1            | <0.001 | <0.001 |
| MT × Trp       | <0.001 | 0.067 |

|                | MT0 | MT1 |
|----------------|-----|-----|
| PCPA0          | 3.12 | 4.03 |
| MT0            | 34.3 | 19.9 |
| MT1            | 1.90 | 2.86 |
| Pooled SEM     | 1.35 | 0.87 |
| Pvalue         |      |     |

|                | RAPA | PCPA |
|----------------|------|------|
| MT0            | >0.001 | >0.001 |
| MT1            | >0.001 | >0.001 |
| MT × Trp       | >0.001 | >0.001 |

|                | MT0 | MT1 |
|----------------|-----|-----|
| PCPA0          | 4.53 | 5.08 |
| MT0            | 20.4 | 20.2 |
| MT1            | 18.0 | 19.7 |
| Pooled SEM     | 0.88 | 0.80 |
| Pvalue         |      |     |

|                | PCPA | Trp |
|----------------|------|-----|
| MT0            | >0.001 | >0.001 |
| MT1            | >0.001 | >0.001 |
| MT × Trp       | >0.001 | >0.001 |

|                | PCPA | Trp |
|----------------|------|-----|
| MT0            | 0.160 | 0.714 |
| MT1            | >0.001 | >0.001 |
| MT × PCPA      | 0.225 | 0.821 |

\[ a \] Data are presented as mean ± SEM (n = 6). Abbreviations: BD-2, β-defensin 2; MT0 or MT1, rats were orally administered with 0.5 mL saline containing or no containing 1-methyltryptophan (50 mg kg\(^{-1}\)) daily; Trp0 or Trp1, rats were orally administered with 0.5 mL saline containing or no containing L-Trp (260 mg kg\(^{-1}\)) daily; RAPA0 or RAPA1: rats were intraperitoneally injected with 0.5 mL saline containing or no containing rapamycin (1.5 mg kg\(^{-1}\)) daily; PCPA0 or PCPA1: rats were intraperitoneally injected with 0.5 mL saline containing or no containing para-chlorophenyl-amine (200 mg kg\(^{-1}\)) daily.

Biphenyl, 100 Å, Phenomenex, CA, USA). The mobile phase comprised 0.2 M zinc acetate, 8.3 mM acetic acid and 2.8% acetonitrile; elution was performed at a flow rate of 1.5 mL min\(^{-1}\), using an elution gradient. The excitation and emission wave lengths for Trp, Kyn, and 5-HT were 254 nm and 404 nm, 365 nm and 480 nm, and 285 nm and 480 nm, respectively. The column temperature was 25 °C and the analysis period of each sample was 60 min. Results were expressed as ng of Trp and Kyn and μg of 5-HT per mL.

For ELISA, the mucosa were placed in chilled lysis buffer (1.5% Triton X-100, 1% Tris buffered saline, 0.5% deoxycholic acid sodium salt, 0.1% SDS, 1 mM PMSF, and protease inhibitor cocktail). The homogenate was placed on ice for 30 min, and then centrifuged at 10 000 × g at 4 °C for 30 min. The supernatant was harvested for ELISA. The concentrations of TPH (PPS039, R&D systems, MN, USA), TNF-α (ab100785; Abcam, Cambridge, MA, USA), IL-17 (ab214028; Abcam), and IL-22 (ab223857; Abcam) in the jejunal and ileal mucosa were determined by rat-specific ELISA kits.

For the western blotting analysis, 100 mg of the jejunal and ileal mucosa was homogenized in 1 mL RIPA buffer (50 mM Tris-base, 1.0 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 1 mM PMSF) and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to a PVDF membrane by the semi-dry transfer method. The PVDF membranes were blocked in a blocking buffer overnight at 4 °C, incubated in blocking buffer with rabbit-anti rat BD-2 (ab178728, 1 : 1000, Abcam, Cambridge, MA, USA), β-actin (5125S, 1 : 1000, CST, Danvers, USA), mTORC1 (ab32028; 1 : 1000, Abcam) or phospho S2448-mTORC1 (ab109268, 1 : 1000, Abcam), and incubated in blocking buffer with F(ab)\(_{2}\) of goat-anti rabbit Ig (1 : 2500) labelled with horseradish peroxidase diluted in PBSS. The PVDF membrane was soaked in a chemiluminescent liquid (Millipore). Pictures were taken using a Chemiluminescence Imaging System (Bio-Rad).

Statistical analysis

All statistical analyses were conducted using the general linear model procedure in SAS statistical software (SAS Institute Inc., Cary, NC, USA). Because the experiments were performed in a 2 \times 2 factorial design, a two-way ANOVA was used to test the effects of Trp concentration, MT, RAPA or PCPA concentration, and Trp × MT, Trp × RAPA and Trp × PCPA concentration interactions. Duncan’s multiple range test was performed to identify differences among groups. The significance was set at \( P < 0.05 \). All data are presented as means ± SEM.

Results

Oral administration of L-Trp increased the serum Trp and Kyn concentrations, the IDO activity (assessed as the kynurenine to tryptophan ratio) and the concentrations of IL-17, IL-22, and TNF-α in the jejunal and ileal mucosa (Table 3; \( P < 0.05 \)). Oral administration of 1-MT increased the serum L-Trp concentrations, while decrease the serum Kyn and Kyn/Trp concentration, the IDO activity and the jejunal and ileal mucosa of IL-17, IL-22, and TNF-α concentrations (Table 3; \( P < 0.05 \)). There were significant interactions in the serum Kyn concentration and the
IL-17 concentration in the jejunal and ileal mucosa of the rats between the MT and TRP treatments (Table 3; \( P < 0.05 \)).

As shown in Table 4, oral administration of L-Trp increased the serum Trp and 5-HT concentrations and the TPH activity and the concentrations of IL-17, IL-22, and TNF-\( \alpha \) in the jejunal and ileal mucosa (\( P < 0.05 \)). Intraperitoneal injection of PCPA increased the serum Trp concentration and the IL-17, IL-22, and TNF-\( \alpha \) concentration in the jejunal and ileal mucosa, while decreased the serum 5-HT concentration and the of TPH activity in the jejunal and ileal mucosa (Table 4; \( P < 0.05 \)). The interactions of the serum 5-HT concentration and the TPH activity in the jejunal and ileal mucosa of rats between PCPA and Trp treatments were observed (Table 4; \( P < 0.05 \)).

Oral administration of L-Trp increased the mRNA level of BD-2 in the jejunal and ileal mucosa of the rats (Table 5; \( P < 0.05 \)). Intraperitoneal injection of RAPA decreased the mRNA level of BD-2 in the jejunal and ileal mucosa (Table 5; \( P < 0.05 \)). Significant interactions were observed in the mRNA level of BD-2 in the jejunal mucosa between the MT and Trp treatments and between the PAPA and Trp treatments (Table 5; \( P < 0.05 \)).

Intraperitoneal injection of RAPA decreased the concentrations of BD-2 and \( p\)-mTORC1 in the jejunal and ileal mucosa of the rats (Fig. 1; \( P < 0.05 \)). Oral administration of L-Trp increased the concentrations of BD-2 and \( p\)-mTORC1 in the jejunal and ileal mucosa (Fig. 1; \( P < 0.05 \)). Intraperitoneal injection of RAPA or oral administration of L-Trp did not change the concentrations of total mTORC1 in the jejunal and ileal mucosa (Fig. 1; \( P > 0.05 \)). There were interactions in the concentrations of BD-2 and \( p\)-mTORC1 in the jejunal and ileal mucosa between RAPA and Trp treatments (Fig. 1; \( P > 0.05 \)). Oral administration of L-Trp increased the concentration of BD-2 in the jejunal and ileal mucosa (Fig. 2; \( P < 0.05 \)) and oral administration of L-Trp increased the concentration of BD-2 in the jejunal and ileal mucosa (Fig. 2; \( P < 0.05 \)). There were interactions in the

**Fig. 1** Oral L-Trp affects the concentrations of BD-2, \( p\)-mTOR, and mTOR in the jejunal and ileal mucosa of the rats. Data are presented as mean ± SEM (\( n = 6 \)). \( ^{a,b,c}\)Values with different letter superscripts within the same index mean significant difference (\( P < 0.05 \)). Abbreviations: CON, rats were orally administered with 0.5 mL saline and intraperitoneally injected with 0.5 mL saline daily; RAPA, rats were orally administered with 0.5 mL saline and intraperitoneally injected 0.5 mL saline containing rapamycin (1.5 mg kg\(^{-1}\)) daily; TRP, rats were orally administered with 0.5 mL saline containing L-Trp (260 mg kg\(^{-1}\)) and intraperitoneally injected with 0.5 mL saline daily; RAPA + TRP, rats were orally administered with 0.5 mL saline containing L-Trp (260 mg kg\(^{-1}\)) and intraperitoneally injected with 0.5 mL saline containing rapamycin (1.5 mg kg\(^{-1}\)) daily; BD-2, \( \beta\)-defensin-2; \( p\)-mTOR, phosphorylated mammalian target of rapamycin.
concentration of BD-2 in the jejunal and ileal mucosa between 1-MT and TRP treatments (Fig. 2; \( P < 0.05 \)). Intraperitoneal injection of PCPA increased the concentration of BD-2 in the jejunal and ileal mucosa of the rats administered with L-Trp (Fig. 3; \( P < 0.05 \)) and there were no significant interaction in the concentration of BD-2 in the jejunal and ileal mucosa between 1-MT and TRP treatments (Fig. 3; \( P > 0.05 \)).

**Discussion**

BD-2 is an inducible AMP that is present in various epithelia. AAs play important roles in epithelial BD expression. \(^{26,27}\) Dietary Trp controls the expression of small intestinal AMPs. \(^{11}\) However, information about the effects of Trp on the expression of BD-2 and its relative mechanisms is limited. In the present study, intraperitoneal injection with RAPA decreased the concentration of \( \rho \)-mTORC1 and the mRNA and protein level of BD-2 in the jejunum and ileum of rats. The results indicate that Trp-promoted BD-2 expression in the intestinal mucosa of rats is related to the mTOR signal pathway. Although AAs are the most potent activator of mTOR, \(^{28}\) the mechanism by which mTOR senses AAs signalling is not well understood. However, it is clear that the Rag guanosine triphosphatases (GTPases) and the Ras-homolog enriched in the brain have necessary but distinct roles. \(^{29-32}\) L-Trp is the precursor in two important metabolic pathways: 5-HT synthesis and Kyn synthesis. 5-HT is synthesized from L-Trp by TPH and aromatic AA decarboxylase. TPH exists in the gastrointestinal tract, \(^{33}\) and aromatic AA decarboxylase exists in the small intestine, \(^{34}\) appendix, \(^{34}\) and liver. \(^{35}\) Kyn is synthesized by IDO from L-Trp, which accounts for 90% of Trp catabolism. \(^{36}\) Theoretically, Trp exerts its effects on intestinal AMPs by Kyn or through 5-HT. The possible pathways in which it acts need to be explored.

This study demonstrated that the rats administered with L-Trp have a higher serum Kyn and Kyn/Trp ratio, which reflects IDO activity. \(^{37,38}\) The metabolites of Trp, mainly Kyn and kynurenic acid, all have AhR ligand activity. \(^{39}\) The process that produces these metabolites is initiated and controlled by IDO. \(^{38}\) AhR in combination with these ligands can regulate expression of downstream target genes. \(^{40}\) In this study, with the increase of dietary Trp, serum Kyn concentration, the mRNA and protein level of BD-2, the concentrations of IL-17, IL-22, and TNF-\( \alpha \) in the jejunal and ileal mucosa were increased. IL-17, IL-22, and TNF-\( \alpha \) are important pro-inflammatory factors. Pro-inflammatory cytokines, such as IL-17 and TNF-\( \alpha \), can induce the expression of BD-2 in epithelial cells. \(^{21,44}\) IL-17 mediates its
effect on BD-2 mostly primarily through a Janus kinase and nuclear factor kappa-B signalling events.\textsuperscript{14} We expected there to be two-way regulation of inflammatory cytokines and BD-2. On one hand, the increase of inflammatory factor levels stimulates BD-2 expression. On the other hand, the increase in BD-2 expression reduces the release of inflammatory cytokines. This provides a reasonable explanation for the decrease in the concentrations of IL-17, IL-22, and TNF-\(\alpha\) in the jejunal and ileal mucosa of rats administered with L-Trp with increasing BD-2.

Oral administration of 1-MT decreased the IDO activity in the jejunal and ileal mucosa, and serum Kyn concentration and consequently reduced the mRNA and protein level of BD-2 and decreased the concentrations of IL-17, IL-22, and TNF-\(\alpha\) in the jejunal and ileal mucosa of the rats. The results indicate that Kyn mediates the signalling pathways of Trp-promoted BD-2 expression in the intestinal mucosa of rats.

Intraperitoneal injection with PCPA decreased the serum 5-HT concentration and the TPH activity in the mucosa of jejenum and ileum. 5-HT is synthesized in enterochromaffin cells by the rate-limiting enzyme TPH1 and in the brainstem and myenteric plexus neurons by TPH-2.\textsuperscript{40-43} The decrease in concentration of 5-HT in the serum and the TPH activity suggests that intraperitoneal injection with PCPA was successful. In this study, intraperitoneal injection with PCPA increases the concentration of BD-2 in the jejunal and ileal mucosa of rats fed diets supplemented with \(\lambda\)-Trp. In addition, intraperitoneal injection with PCPA did not change the concentrations of IL-17, IL-22 and TNF-\(\alpha\) in the jejunal and ileum of the rats. These results indicate that Trp-promoted BD-2 expression in the intestinal mucosa of rats is not related to 5-HT.

In conclusion, rats administered \(\lambda\)-Trp showed a higher concentration of BD-2 in the mucosa of the jejunum and ileum. Intraperitoneal injection of PAPA decreased the concentrations of \(p\)-mTORC1 and BD-2 in the jejunal and ileal mucosa; oral administration of 1-MT decreased the mRNA and protein level of BD-2 in the mucosa of the jejunum and ileum; and intraperitoneal injection of PCPA increased the mRNA and protein level of BD-2 in the mucosa of the jejunum and ileum of the rats. Our study indicates the Trp-promoted BD-2 expression in the jejunal and ileum via the mTOR pathway and its metabolites: kynurenine banding to aryl hydrocarbon receptor in rat intestine.

\textbf{Conflicts of interest}

There are no conflicts to declare.
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