Large Amounts of Picolinic Acid Are Lethal but Small Amounts Increase the Conversion of Tryptophan-Nicotinamide in Rats

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Summary
Picolinic acid (PiA) is an endogenous metabolite of tryptophan that has been reported to possess a wide range of physiological actions. We investigated the effects of dietary PiA on the metabolism of tryptophan to nicotinamide in growing rats. Feeding an ordinary diet containing 1% PiA to growing rats (6 wk) caused death within a few days. Toxicity of PiA was higher than that of analogs such as nicotinic acid and quinolinic acid. Feeding an ordinary diet containing 0.05% and 0.1% PiA did not elicit decreased intake of food or loss in body weight. PiA did not affect the in vitro liver activities of quinolinic acid phosphoribosyltransferase or $\cdot$-amino-$\cdot$-carboxymuconate-$\cdot$-semialdehyde decarboxylase (ACMSDase, a Zn-dependent enzyme). Concentrations of NAD and NADP in the liver and blood were not affected by PiA. PiA administration did not affect tryptophan metabolites such as anthranilic acid, kynurenic acid, and xanthurenic acid. However, quinolinic acid and subsequent metabolites such as nicotinamide and its catabolites were increased by administration of a diet containing 0.05% PiA but not by a 0.1% PiA diet. These results suggest that the in vivo activity of ACMSDase is controlled by the Zn level. Therefore, a small amount of PiA has a beneficial effect for conversion of tryptophan to nicotinamide, but an excessive amount of PiA can be very toxic.

Key Words
picolinic acid, tryptophan, nicotinamide, toxicity, rat

Picolinic acid (PiA) is a metabolite of the complete degradation pathway of tryptophan (Trp) (see, Fig. 1), which has been reported to possess a wide range of physiological actions (e.g., neuroprotective (1, 2) and immunological (3) effects) and zinc (Zn) metabolism (4).

PiA formation is controlled by the activities of two enzymes: $\cdot$-amino-$\cdot$-carboxymuconate-$\cdot$-semialdehyde decarboxylase (ACMSDase) and $\cdot$-aminomuconate-$\cdot$-semialdehyde dehydrogenase (AMSDHase). ACMSDase catalyzes the reaction of $\cdot$-amino-$\cdot$-carboxymuconate-$\cdot$-semialdehyde (ACMS) to $\cdot$-aminomuconate-$\cdot$-semialdehyde (AMS) and AMSDHase the reaction of AMS to 2-aminoacidic acid in the presence of NAD(P)H.

PiA is created by non-enzymatic means from AMS. Quinolnic acid (QA) is produced non-enzymatically from ACMS, which is metabolized to nicotinic acid mononucleotide by QA phosphoribosyltransferase (QPRTase) in the presence of 5-phosphoribosyl 1-pyrophosphate (PRPP). QA is the direct precursor of NAD in the tryptophan (Trp)-nicotinamide (Nam) biosynthetic pathway, but dietary QA is not incorporated efficiently into NAD molecules (5). The low replacement efficiency of QA for NAD is considered to result from inefficient penetration of QA into cells (6–9).

The toxicity of QA is reported to be low. For example, feeding a diet containing 0.5% QA to weaning rats affects food intake and body-weight gain slightly compared with those of rats fed a non-QA control diet (10). As in the case of QA, excessive amounts of nicotinic acid, Nam, $N^3$-methylnicotinic acid, and $N^3$-methylnicotinamide (MNA) affect food intake and body-weight gain only slightly (10). Administration of nicotinic acid and Nam elicits a much greater increase in Nam catabolites such as MNA, $N^3$-methyl-2-pyridone-5-carboxamide (2-Py) and $N^3$-methyl-4-pyridone-3-carboxamide (4-Py). However, administration of MNA and $N^3$-methylnicotinic acid do not produce increases in Nam catabolites (10). Whether dietary PiA affects the metabolism of Trp to Nam is unknown. We investigated the effects of dietary PiA on the growth, food intake, and metabolism of Trp to Nam in growing rats.

MATERIALS AND METHODS

Chemicals. Vitamin-free milk casein, sucrose, and L-methionine were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, a mineral mixture (11), and a vitamin mixture (11) were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). PiA, anthranilic acid (AnA), QA and Nam were obtained from Wako Pure Chemical Industries. MNA chloride, xanthurenic acid (XA), kynurenic acid (KA), and 3-hydroxyanthranilic acid (3-HA) were purchased from Tokyo Chemical Industries (Tokyo, Japan). Compounds 2-Py and 4-Py were synthesized according to the method of Pullman and Colowick (12) and that of Shibata et al. (13), respectively. All other chemicals were of the highest purity available from commercial sources.

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Animals and treatment. The care and treatment of experimental animals conformed to guidelines set by The University of Shiga Prefecture (Shiga, Japan). The animal room was maintained at temperature of \(\sim 22^\circ C\) and with \(\sim 60\%\) humidity. A 12-h light–dark cycle was also in operation (18:00–06:00).

Six-week-old male Wistar rats obtained from CLEA Japan, Inc. (Tokyo, Japan) were housed individually in metabolic cages (CT-10; CLEA Japan). Rats were divided into four groups at 09:00, which was designated as the “zero time” of the experiment elapsed time. One group \((n=5)\) was fed a conventional 20% casein diet and allowed to drink tap water ad libitum for 21 d; this group was the control group (Table 1). The other groups \((n=5)\) were fed the same diets with PiA addition (Table 1) and allowed to drink tap water ad libitum for 21 d. The concentration of dietary PiA was selected from the data about the toxicity of quinolinic acid \((10)\) and nicotinic acid \((10, 14)\). Urine samples (24 h; 09:00–09:00) were collected at day 21 in amber bottles containing 1 mL of 1 mol/L HCl. Acidified urine samples were stored at \(-25^\circ C\) until needed. After the last urine samples had been collected, the rats were killed. Blood from the carotid artery and the livers were removed, and were

![Fig. 1. Metabolic pathway of Trp. ACMS, \(\alpha\)-amino-\(\beta\)-carboxymuconate-\(\epsilon\)-semialdehyde; AMS, \(\alpha\)-aminomuconate-\(\epsilon\)-semialdehyde; NMN, nicotinamide mononucleotide.](image)

Table 1. Compositions of diets.

<table>
<thead>
<tr>
<th></th>
<th>20% Casein diet</th>
<th>+0.05% PiA</th>
<th>+0.10% PiA</th>
<th>+1.0% PiA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin-free casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Gelatinized-cornstarch</td>
<td>464</td>
<td>463.5</td>
<td>463</td>
<td>454</td>
</tr>
<tr>
<td>Sucrose</td>
<td>224</td>
<td>224</td>
<td>224</td>
<td>224</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture (^1)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture (NiA-free) (^1)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>PiA</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

used for measurements of NAD and NADP as well as the enzyme activities of QPRTase and ACMSDase.

Enzyme assays. The liver was dissected, and one portion homogenized immediately with a Teflon-glass homogenizer in 5 volumes of cold 50 mmol/L KH$_2$PO$_4$ – K$_2$HPO$_4$ buffer at pH 7.0. The resulting homogenate was used as an enzyme source.

The methods of measuring of QPRTase (nicotinate nucleotide: pyrophosphate phosphoribosyltransferase, EC 2.4.2.19) (15) and ACMSDase (EC 4.1.1.45) (16) have been reported previously.

Measurement of Trp metabolites. Amounts of Trp metabolites such as AnA (17), KA (18), XA (19), 3-HA (19), QA (20), Nam (13), MNA (21), 2-Py (13), and 4-Py (13) in urine were measured by high-performance liquid chromatography.

Measurement of levels of NAD and NADP. Concentrations of NAD (22) and NADP (23) in the livers and blood were measured by enzyme cycling methods.

Statistical analyses. Values are the mean±SE. Significance was determined by one-way ANOVA, followed by Tukey’s multiple-comparison test; p<0.05 was considered significant. Prism version 5.0 (Graph Pad, San Diego, CA) was used for all analyses.

RESULTS

Effects of a high-PiA diet on body weight and food intake

Body weights of rats fed the 1% PiA diet decreased by 1.9 g on day 1, and by 20 g on day 3. Three of the five rats died on day 6, one rat on day 8, and one rat on day 9. Rats ate 10 g of the 1% PiA diet on day 1, and 3 g/d during days 2 to 5, but ate little after day 5.

Effects of low PiA on the metabolism of Trp to Nam

Feeding a diet containing low amounts of PiA (0.05% and 0.1%) to rats did not affect food intake or body-weight gain (Fig. 2 and Table 2). Liver weights were also not affected by administration of low amounts of PiA (Table 2). Concentrations of NAD and NADP in the liv-
that decreased synthesis of PiA leads to Zn deficiency and then to decreased levels of urocanic acid (a natural sunscreen compound in skin (25) and increased levels of the hem precursor 5-aminolevulinic acid and photoactive porphyrins. It is probable that PiA affects the metabolism of divalent minerals because PiA can chelate with divalent cations. However, we did not investigate the effects of PiA on the metabolism of divalent cations. We investigated the scale of toxicity of dietary PiA as well as the effects on the metabolism of the Trp to Nam pathway, which is very important for supplying Nam (26).

The toxicity of dietary PiA is very high compared with that of QA (10). Administration of a diet containing 1% PiA to rats will kill them within a few days. Nicotinic acid has been reported to be non-toxic (10). It is very unlikely that mammals (including humans) would consume large amounts of PiA, but the present study is important because some people take chromium(III) picolinate for health reasons. We have no data on the extent of endogenous formation of PiA in rats. Formation of QA is ≈6% of Trp intake in mice (27).

Administration of a small amount of PiA (0.05%) strengthened QA formation, and subsequent formation of Nam and its metabolites. Thus, PiA increased the conversion percentage of Trp to Nam. The reaction of PiA to QA has not been reported previously. Increased formation of QA by PiA administration cannot be explained clearly, but PiA might inhibit ACMSDase activity because the enzyme is a Zn-dependent amido-
hydrolase (28). Therefore, a moderate concentration of PiA, for example a diet containing 0.05% PiA, increases the efficiency of Zn availability, whereas an excessive concentration of PiA, for example a diet containing over 0.1% PiA, might decrease the availability of Zn. A lower activity of ACMSDase leads to accumulation of ACMS (which is produced from 3-HA by 3-HADOase), QA is made spontaneously from ACMS.
CONCLUSION

A high concentration of dietary PiA was lethal, but a low concentration of dietary PiA increased the conversion of Trp to Nam by changing the bioavailability of Zn, which leads to control of Zn-dependent ACMSDase activity in vivo.

Author contributions
K.S. designed the study. K.S. drafted the manuscript. T.F. assisted in these tasks.

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Author disclosure
K. Shibata and T. Fukuwatari have no conflicts of interest.

REFERENCES


