Effects of Excess Pantothenic Acid Administration on the Other Water-Soluble Vitamin Metabolisms in Rats

Katsumi SHIBATA, Chisato TAKAHASHI, Tsutomu FUKUWATARI and Ryuzo SASAKI

Laboratories of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522–8533, Japan
(Received May 19, 2005)

Summary To acquire the data concerning the tolerable upper intake level which prevents health problems from an excessive intake of pantothenic acid, an animal experiment was done. Rats of the Wistar strain (male, 3 wk old) were fed on a diet which contains 0%, 0.0016% (control group), 1%, or 3% calcium pantothenate for 29 d. The amount of weight increase, the food intake, and the organ weights were measured, as well as the pantothenic acid contents in urine, the liver and blood. Moreover, to learn the influence of excessive pantothenic acid on other water-soluble vitamin metabolism, thiamin, riboflavin, a vitamin B6 catabolite, the niacin catabolites, and ascorbic acid in urine were measured. As for the 3% addition group, enlargement of the testis, diarrhea, and hair damage were observed, and the amount of weight increase and the food intake were less than those of the control group. However, abnormality was not seen in the 1% addition group. The amount of pantothenic acid in urine, the liver, and blood showed a high correlation with intake level of pantothenic acid. It was only for 4-pyridoxic acid, a vitamin B6 catabolite, in urine that a remarkable difference was observed against the control group. Moreover, the (2-Py+4-Py)/MNA excretion ratio for these metabolites of the nicotinamide also indicated a low value in the 3% pantothenic acid group. As for the calcium pantothenate, it was found that the 3% level in the diet was the lowest-observed-adverse-effect-level (LOAEL) and the 1% level was the no-observed-adverse-effect-level (NOAEL).

Key Words pantothenic acid, excess administration, rat, urine, vitamin

Pantothenic acid (PaA), a vitamin, is essential for humans and animals for growth and normal physiological functions. It is an integral part of the acylation carriers, CoA and acyl carrier proteins, which are involved in more than 100 different metabolic pathways including energy metabolism of carbohydrates, proteins and lipids, and the synthesis of lipids, neurotransmitters, and steroid hormones (1, 2).

It was reported that PaA deficiency induced in experimental animals when fed on a diet without PaA led to growth retardation with reduced food intake (3, 4) and functional impairments in all systems (5–13). PaA deficiency has also been induced in humans by use of a metabolic antagonist, α-methyl PaA along with a PaA-deficient diet (14): Signs and symptoms reported include depression, personality changes, cardiac instability, frequent infection, fatigue, abdominal pains, sleep disturbances and neurological disorders including numbness, paresthesia (abnormal sensation such as “burning feet” syndrome), muscle weakness and cramps. Naturally occurring PaA deficiency in humans is very rare and has been observed only in cases of severe malnutrition: World War II prisoners in the Philippines, Burma, and Japan experienced numbness and painful burning and tingling in their feet (“burning feet” syndrome), which was relieved specifically by PaA administration (15). The cause of this syndrome must originate from stresses because PaA involves the formation of adrenocortical hormones (16). Therefore, the specific PaA deficiency in humans would be “burning feet” syndrome. There is an interesting hypothesis that the formation of ketone bodies under fasting conditions induces a deficiency of cellular PaA (17). Supplementation of this vitamin would facilitate complete catabolism of fatty acids and thus the formation of ketone bodies could be circumvented. Oral contraceptives (birth control pills) containing estrogen and progestin may increase the requirement for PaA (18). Recently, a report that PaA protects cells and organs against peroxidative damage by increasing the content of cell glutathione was published (19).

The reports (17–19) that pantothenic acid prevents stresses, peroxidation reactions and the formation of the ketone bodies predict a possibility that the intake of pantothenic acid will increase more and more in the future.

PaA is not known to be toxic in humans. The only adverse effect noted was diarrhea resulting from very high intakes of 10 to 20 g/d of calcium D-pantothenate (18). However, there is one case report of life-threatening eosinophilic pleuropneumonic effusion in an elderly woman who took a combination of 10 mg/d of biotin
and 300 mg/d of PaA for 2 mo (20). In rats, there are two reports on the effect of excess PaA: one details changes in hepatocellular lipid production because of an excess of PaA (21) and the other is a report on adrenocortical alterations induced by deficiency and excess of PaA (22). In the present paper, we report the effects of administering excess PaA to rats to acquire data concerning the tolerable upper intake level to prevent health problems especially the effects on the other water-soluble vitamin metabolisms.

**MATERIALS AND METHODS**

**Chemicals.** Vitamin-free milk casein, sucrose, and l-methionine were purchased from Wako Pure Chemical Industries (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, the mineral mixture (AIN-93M) (23) and the vitamin mixture (AIN-93-VX containing 25% choline bitartrate) (23) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Thiamin hydrochloride (C_{12}H_{17}CIN_{4}0-337.27), riboflavin (C_{17}H_{20}N_{4}0_{6}=376.37), nicotinamide (C_{6}H_{6}N_{2}0=122.13), calcium pantothenate (PaA-Ca, C_{18}H_{32}N_{2}O_{10}-Ca=476.54), and l(+)-ascorbic acid (C_{6}H_{8}0_{6}=176.13) were purchased from Wako Pure Chemical Industries, Ltd. 4-Pyridoxic acid (4-PIC, C_{8}H_{9}NO_{4}=183.16) was made by ICN Pharmaceuticals (Costa Mesa, California, USA) and obtained through Wako. 3'-Methylthioinosinamide (MNA) chloride (C_{6}H_{9}NOCl=159.61) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N'-Methyl-2-pyridone-5-carboxamide (2-Py, C_{7}H_{8}N_{2}O_{2}=152.15) and N'-methyl-4-pyridone-3-carboxamide (4-Py, C_{7}H_{8}N_{2}O_{2}=152.15) were synthesized by the methods of Pullman and Colowick (24) and Shibata et al. (25), respectively.

All other chemicals used were of the highest purity available from commercial sources.

**Animals and diets.** The care and treatment of the experimental animals conformed with the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Male rats of the Wistar strain (3 wk old with a body weight of around 40 g) were obtained from CLEA Japan, Inc. (Tokyo, Japan) and immediately placed in individual metabolic cages (CT-10; CLEA Japan, Inc.). They were then divided into four groups and fed ad libitum for 29 d, one group with a PaA-free, 20% casein diet, and the others with the same diet+0.0016% PaA-Ca (used as the control group), +1.0% PaA-Ca, or +3.0% PaA-Ca (Table 1).

The room temperature was maintained at around 22°C and about 60% humidity, and a 12-h light (06:00-18:00)/12-h dark (18:00-06:00) cycle was maintained. Body weight and food intake were measured every 2 d at around 10:00. Urine samples (24-h; 10:00-10:00) were collected in amber bottles containing 1 mL of 1 mol/L HCl on the last day of the experiment, and were stored at -25°C until needed. The rats were killed by decapitation at around 10:00 on the last day (day 29) after the collection of the urine sample had been completed, and the various tissues were removed and measured. The liver of each animal was removed, and a portion (approximately 0.2 g) was immediately treated as described in the literature to measure PaA (26) and CoA (27).

**Analyses.**

Vitamin B_{1} (thiamin): The determination of vitamin B_{1} in urine was measured by the HPLC-post labeled fluorescence method of Kimura et al. (28).

Vitamin B_{2} (riboflavin): Urinary concentration of riboflavin was analyzed according to the method of Ohkawa et al. (29).

4-PIC: Urinary excretion of 4-PIC, which is a catabolite of vitamin B_{6}, was determined according to the method described by Gregory and Kirk (30).

Niacin: The quantities of Nam, 2-Py and 4-Py in urine were measured simultaneously by the HPLC method of Shibata et al. (25). The content of MNA was measured by the method of Shibata (31).

Pantothenic acid: The content of free pantothenic acid in urine was directly measured by using *Lactobacillus plantarum* ATCC 8014 (26).

Ascorbic acid: The contents of the reduced and oxidized ascorbic acids and 2, 3-diketoglutaric acid were measured by the method of Kishida et al. (32).

CoA: The content of CoA in liver was measured by the methods of Alled and Guy (33).

**Statistical analysis.** For the statistical evaluation, the significance of the differences in the mean concentrations among groups was treated with ANOVA and when the analysis of ANOVA was significant, the Tukey-Kramer multiple comparisons test was performed. Dif-

<table>
<thead>
<tr>
<th>Table 1. The composition of the diets.</th>
<th>% PaA-Ca diet</th>
<th>0.0016% PaA-Ca diet</th>
<th>1% PaA-Ca diet</th>
<th>3% PaA-Ca diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin-free milk casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Gelatinized-cornstarch</td>
<td>45.9</td>
<td>45.9</td>
<td>45.4</td>
<td>44.4</td>
</tr>
<tr>
<td>Sucrose</td>
<td>22.9</td>
<td>22.9</td>
<td>22.4</td>
<td>21.4</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mixture (AIN-93M)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mixture (PaA-Ca free) (AIN-93-VX containing 25% choline bitartrate)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PaA-Ca</td>
<td>0</td>
<td>0.0016</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
Effects of Excess Pantothenic Acid on the Vitamin Metabolisms in Rats

Diff erences of p<0.05 were considered to be statistically significant. Instat software (version 2.00; obtained from GraphPad Software, Inc., San Diego, CA, USA) was used for all analyses.

RESULTS

Effects of excessive PaA administration on the body weight gain and food intake in rats

The diet containing 0.0016% PaA-Ca was considered as the control. The body weight gain and food intake were almost the same between the control and the 0% PaA-Ca groups in the first 5–7 d; however, these signifi cantly decreased in the 0% group compared with the control after that day as shown in Fig. 1. The result means that some days are needed for appearance of PaA-deficiency. On the other hand, the body weight gain and food intake of the rats fed on the 3% PaA-Ca diet were signifi cantly lower than those in the control group, especially in the initial 5 d of the experiment with the food intake (Fig. 1). The food intakes in the 3% group became similar in amount with those of the control group from around day 20 of the experiment. The body weight gains in the control and the 3% PaA groups were almost the same from day 7. This finding complies with the possibility that the rats acquire a detoxification process of PaA by exposure to an excess amount of PaA. No adverse effects were observed in the rats fed on the 1% PaA-Ca diet.

Effects of excessive PaA administration on the tissue weights of rats

Table 2 shows the tissue weights of the rats fed on the diets supplemented with various amounts of PaA-Ca. The values were expressed as g/100 g of body weight of rat. The weights of heart, liver, and kidneys revealed no signifi cant difference among the four groups. In the present experiment, the group fed on the 0.0016% PaA-Ca diet is the control group. The brain and testis weights were signifi cantly higher in the PaA-deficient group than in the other PaA-containing groups. In the comparison between the control and 1% PaA-Ca groups, all of the measured tissue weights were almost the same. But the weights of lung and spleen were signifi cantly higher in the 3% group than in the control group.

Effects of excessive PaA administration on the PaA contents in urine and liver and the CoA content in liver

The urinary excretion of PaA increased with the intake of PaA as shown in Fig. 2A. The PaA content in the liver also increased with the intake of PaA as shown in Fig. 2B; however, the degree of the increase was not so dramatic compared with the result of the urine samples, while the CoA content in the liver did not increase with the intake of PaA as shown in Fig. 2C. The saturation of CoA in the liver of rats was attained by feeding the diet containing 0.0016% PaA-Ca, namely by feeding the control diet. The excessive administration to the rats did not yield any signifi cant increase in CoA level in the liver although the PaA level in the liver increased with the excessive administration of PaA.

Effects of excessive PaA administration on the other B-group vitamins concerned with energy metabolism

The coenzymes such as TDP (thiamin diphosphate), FAD (flavin adenine dinucleotide), PLP (pyridoxal phos-
Fig. 2. Effects of PaA-Ca administration on the urinary excretion of PaA (A), the content of PaA in liver (B), and the content of CoA in liver (C). A: Twenty-four hour urine samples were collected on the last day of the experiment. The PaA content in urine samples were measured. B, C: The rats were killed after urine samples had been collected and the livers removed. The livers were treated as described in "Materials and Methods," and total PaA and CoA contents in the liver measured. Values are means±SE for five rats; different superscript letters indicate significant differences at p<0.05 in the Tukey-Kramer multiple comparisons test.

Fig. 3. Effects of PaA-Ca administration on the urinary excretion of sum of Nam and its metabolites such as MNA, 2-Py, and 4-Py (A) and the excretory ratio of the (2-Py+4-Py)/MNA (B). Twenty-four hour urine samples were collected on the last day of the experiment. A: The contents of Nam, MNA, 2-Py, and 4-Py were measured. B: The excretory ratio of the (2-Py+4-Py)/MNA was calculated. Nam, nicotinamide; MNA, N1-methylnicotinamide; 2-Py, N1-methyl-2-pyridone-5-carboxamide; 4-Py, N1-methyl-4-pyridone-3-carboxamide. Values are means±SE for five rats; different superscript letters indicate significant differences at p<0.05 in the Tukey-Kramer multiple comparisons test.

phate) and NAD (nicotinamide adenine dinucleotide) were concerned in the metabolism of glucides, amino acids, and fatty acids as well as CoA. Thus, the excessive PaA administration had effects on the metabolism of vitamin B1, vitamin B2, vitamin B6, and niacin (vitamin B3).

Niacin and PaA are especially concerned with energy metabolism. The effects of excessive administration of PaA on the metabolism of the de novo nicotinamide synthetic pathway (tryptophan-quinolinic acid pathway) were investigated. As results, the respective urinary excretion of kynurenic acid, anthranilic acid, xanthurenic acid, 3-hydroxyanthranilic acid, and quinolinic acid was not changed by the administration of excessive PaA (data not shown). The excessive PaA administration did not affect the total urinary excretion of nicotinamide, MNA, 2-Py, and 4-Py (Fig. 3A), though it did affect the urinary excretory ratio of (2-Py+4-Py)/MNA (Fig. 3B), in comparison with the control group. The decreased ratio means that the excessive PaA induced some adverse effects in the rats, because the low value of the ratio of (2-Py+4-Py)/MNA indicates retrogression of the metabolism of nicotinamide.

Furthermore, the present data (Fig. 3) show that the deficiency of PaA affects the nicotinamide metabolism. The decreased total urinary excretion in the 0% PaA group compared with the control means that PaA deficiency increases the demand for niacin requirement.

Figure 4 shows the effects of PaA administration on the urinary excretion of vitamin B1, vitamin B2, and 4-PIC (a catabolite of vitamin B6). The urinary excretion of vitamin B1 and 4-PIC decreased according to the increase in the intake of PaA, while that of vitamin B2 did not.

Effects of excessive PaA administration on the urinary excretion of ascorbic acid

In rats, ascorbic acid can be made from glucose, so ascorbic acid is not a vitamin. Therefore, many reports
have been published that the production of ascorbic acid is changeable by many factors. In the present experiment, the urinary excretion of ascorbic acid was measured. Values are means±SE for five rats.

**DISCUSSION**

PaA is an integral part of CoA, which is an essential coenzyme in a variety of reactions that sustain life. CoA is required for biochemical reactions that generate energy from food (fats, carbohydrates, and proteins). The synthesis of essential fats, cholesterol, and steroid hormones requires CoA, as does the synthesis of the neurotransmitter, acetylcholine, and the hormone, melatonin (2). Metabolism of a number of drugs and toxins by the liver requires CoA (34). Most acetylated proteins in the body have been modified by the addition of an acetate group that was donated by CoA. Protein acetylation affects the 3-dimensional structure of proteins, potentially altering their function, the activity of peptide hormones, and appears to play a role in cell division and DNA replication. Protein acetylation also affects gene expression by facilitating the transcription of mRNA. A number of proteins are also modified by the attachment of long-chain fatty acids donated by CoA. These modifications are known as protein acylation, and appear to play a central role in cell signaling (1, 2). As mentioned in the introduction, supplementation of PaA would facilitate complete catabolism of fatty acids (17) and oral contraceptives (birth control pills) containing estrogen and progestin may increase the requirement for PaA (18). Furthermore, PaA protects cells and organs against peroxidative damage by increasing the content of cellular glutathione (19). These findings induce use of excessive PaA for prevention against stresses, peroxidation, the formation of ketone bodies and so on.

The present experiments in rats clearly indicated that excessive intake of PaA had an adverse effect on body weight gain (Fig. 1A), food intake (Fig. 1B), the excretory ratio of (2-Py+4-Py)/MNA (Fig. 3B), the urinary excretion of vitamin B₁ (Fig. 4A), and the urinary excretion of 4-PIC (Fig. 4C) as well as PaA deficiency. The level causing the no-observed-adverse-effect level (NOAEL) in the rats was 1% in the diet and that causing the lowest-observed-adverse-effect-level (LOAEL) was 3% in the diet. The rats in the 1% PaA group consumed around 16 g/d of their diet during day 20 to day 29 and the mean body weight during the days was about
160 g. So, the PaA intake was calculated as 3,000 mg/kg body weight a day. The rats in the 3% PaA group consumed around 16 g/d during day 20 to day 29 and the mean body weight during the days was about 180 g. So, the PaA intake was calculated as 1,000 mg/kg body weight a day, which is a proposed acceptable daily intake of PaA-Ca for rats. Although the data were not shown in the present experiments, we also done an experiment in which rats were fed on a 5% PaA diet. But, four of the five rats died with sever diarrhea within 2 d of starting the diet. The surviving rat, although maintaining the same diet, was restored to vigor after 1 wk and gained as much weight as the control. This phenomenon looked like the results in the 3% PaA group (Fig. 1A).

The excessive intake of PaA affected the metabolism of other B-group vitamins. The decreased excretory ratio of (2-Py+4-Py)/MNA, but no effect on the sum of the nicotinamide catabolites, MNA+2-Py+4-Py, means that excessive PaA inhibits the activity of MNA oxidase, which catalyzes the reactions of MNA → 2-Py and 4-Py (35), although the conversion pathway of tryptophan to nicotinamide was not affected. The excess intake of PaA also decreased the urinary excretion of 4-PIC, a catabolite of pyridoxal. The reaction of pyridoxal → 4-PIC is catalyzed by a similar enzyme, aldehyde oxidase (35). So, the excess intake of PaA might influence the oxidases. The urinary excretion of vitamin B1 decreased in the 3% PaA group compared with that in the control and 1% groups. The decreased excretion indicated that the rats fed on the 3% PaA diet required a higher amount of vitamin B1 compared with those fed on the control and 1% diets. The mechanism is not clear.

It is known that the ascobic acid formation increases when the rats are exposed to xenobiotics to metabolize and excrete into urine (36). A large amount of PaA administration to the rats did not induce the formation of ascobic acid (Fig. 5). So, excess amount of PaA is not recognized as xenobiotic in the rats.

In conclusion, the NOAEL of PaA, an essential part of CoA that sustains life was 1% in the dietary level and the LOAEL was 3% in the dietary level. In a daily intake per kg of body weight of rat, the NOAEL was around 1,000 mg, while the LOAEL was around 3,000 mg. If a safety factor would be 100 (species difference 10, individual difference 10) (37), the tolerable upper intake level is around 10 mg/kg body weight a day. We would propose 10 mg of PaA-Ca/kg body weight a day as a tentative tolerable upper intake level.

Acknowledgments

This report is a part of the studies on the Japanese Dietary Reference Intakes (Principle investigator, Katsumi Shibata) and the investigation was supported by a grant from the Ministry of Health, Labor and Welfare (Comprehensive Research on Cardiovascular Diseases).

REFERENCES


Williams & Wilkins, Baltimore.


23) Reeves PG. 1997. Components of the AIN-93 diets as
improvements in the AIN-76A diet. 


