Note

Effect of Lipid Peroxidation Products on the Catabolic Fate of Nicotinamide in Rats

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Some investigators have suggested that the ratio of urinary N^1 -methyl-2-pyridone-5-carboxamide (2-Py) to N^1 -methylnicotinamide (MNA) excretion can be used as a tool for assessing niacin status.^{1,2)} A low ratio has been interpreted to indicate a deficiency of niacin in the diet, body tissues or both. We have succeeded in developing high-performance liquid chromatographic measurements of nicotinamide³⁾ and its major catabolic metabolites such as MNA,⁴⁾ 2-Py,⁵⁾ and N^1 -methyl-4-pyridone-3-carboxamide (4-Py),⁶⁾ and have been investigating the effects of nutrients on the metabolic fate of nicotinamide in rats. However, our results did not indicate any evident relationship between the urinary excretion of (2-Py+4-Py)/MNA and niacin status. Under specified conditions, its excretion ratio reflected an adequacy of amino acid nutrition,^{7–12)} and even when the amino acid nutrition was adequate, an excess administration of tryptophan,¹³⁾ nicotinamide,¹⁴⁾ MNA,¹⁵⁾ or pyridoxal¹⁶⁾ and feeding of a riboflavin-deficient diet,¹⁷⁾ or a diet containing an excess oil,¹⁸⁾ caused a decrease in its excretion ratio. In the present report, we investigated the daily variation of the urinary excretion of (2-Py+4-Py)/MNA after rats were administered with linoleic acid, its hydroperoxides and secondary degradation products.

MNA chloride was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). 2-Py and 4-Py were synthesized from MNA chloride by the methods of Pullman and Colowick¹⁹⁾ and Shibata *et al.*,⁶⁾ respectively. The peroxidation products of linoleic acid were prepared and characterized by the method of Kanazawa *et al.*²⁰⁾ Linoleic acid obtained from Nacalai Tesque Inc. (Kyoto, Japan) was autooxidized at 37°C for 7 days. The autooxidized linoleic acid was purified to linoleic acid, its mono-hydroperoxides, and secondary products by silica-gel column and thin-layer chromatographies. The purities of the linoleic acid and hydroperoxides were about 97% and 90%, respectively, as checked by high-performance liquid chromatography.

The fraction of the secondary products consisted of a 36% mixture of polymers, 26% endoperoxides and epoxy-hydroperoxides, which were identified by gas chromatography,²¹⁾ 4.8% 9-oxononanoic acid, 3.7% hexanal, 2.5% nonanedioic acid, 2.4% short-chain carboxylic acids, 0.75% 8-oxooctanoic acid, 0.34% 12-oxadodecadienoic acid, and other smaller unidentified compounds. The hydroxy alkenals peak was also observed²²⁾ in an amount roughly estimated to be less than 1%. All other chemicals used were of the highest purity available from commercial sources.

Male rats of the Wistar strain (about 280 g in body weight) obtained from Clea Japan Inc. (Tokyo, Japan) were used in this experiment. The rats were placed individually in metabolic cages (CT-10 from Clea Japan) and fed with a 20% casein diet.¹¹⁾ To facilitate the administration of diet samples to the rats, the food intake of each rat on the day before the administration was reduced to about a half (6.5 g/day). The rats were administered by intubation with linoleic acid (750 mg/rat), linoleic acid hydroperoxides (400 mg/rat), secondary degradation products (750 mg/rat), or sterilized saline (750 mg/rat) on day 0 at 09:00 hr. Urine samples were collected in flasks containing 1 M HCl during day –2 to day –1, day –1 to day 0, day 0 to day 1, day 1 to day 2, day 2 to day 3, day 3 to day 4, day 4 to day 5, day 5 to day 7, and day 8 to day 9, which were stored at –25°C until needed. The nicotinamide,³⁾ MNA,⁴⁾ 2-Py,⁵⁾ and 4-Py⁶⁾ contents in the urine were measured as described in the literature.

The animal room was kept at 22±2°C and at 60% humidity, and a 12 hr light/dark cycle was maintained.

The administration of linoleic acid, linoleic acid hydroperoxides, or secondary degradation products did not affect the body weight gain and food intake when compared with the group administered with saline (control group).

On day 0 to day 1, the sum (control, 8–10 µmol/day) of the urinary excretion of nicotinamide (control, 0.3–0.5 µmol/day), MNA (control, 0.45–0.6 µmol/day), 2-Py (control, 0.4–0.5 µmol/day), and 4-Py (control, 7–8.5 µmol/day) decreased to about 70% due to the administration of secondary degradation products, hydroperoxides, or linoleic acid. The decreased excretion of the sum was mainly attributed to the decrease in 4-Py excretion [7 µmol/day (control group)→4.5 µmol/day (test groups)]. This phenomenon was probably due to the inactivation of 4-Py-forming MNA oxidase by the administration of these compounds, because the excretion ratio of (2-Py+4-Py)/MNA in the groups administered the secondary degradation products, hydroperoxides, and of linoleic acid decreased compared with the control group (Table I) during day 0 to day 1. The lower sum of the urinary excretion in the groups administered with secondary degradation products, hydroperoxides, or linoleic acid than in the group administered with saline (control) might have been attributable to the poorer absorption of such

Table 1. EFFECT OF ORALLY ADMINISTERING LINOLEIC ACID (LA), AND ITS HYDROPEROXIDES (HP) AND SECONDARY DEGRADATION PRODUCTS (SP) ON THE URINARY EXCRETION RATIO OF (2-PY + 4-PY)/MNA IN RATS

	Saline	LA	HP	SP
Day -2 to -1	14.0±0.6	14.2±1.1	13.4±0.7	13.7±0.6
Day -1 to 0	14.1±1.1	13.7±1.4	13.6±1.4	13.5±1.1
Day 0 to 1	14.3±0.6 ^a	9.9±1.4 ^b	7.7±1.2 ^b	9.8±1.9 ^b
Day 1 to 2	16.1±0.8 ^a	11.3±1.2 ^b	6.0±0.4 ^c	8.1±0.9 ^c
Day 2 to 3	14.5±1.1 ^a	10.6±1.6 ^b	7.9±1.6 ^b	7.2±0.5 ^b
Day 3 to 4	14.8±0.9 ^a	13.3±1.2 ^{a,b}	10.7±1.3 ^{b,c}	9.1±0.9 ^c
Day 4 to 5	15.4±0.9	14.4±1.1	12.8±2.4	11.4±1.4
Day 5 to 7	14.1±0.6	13.2±1.7	12.7±1.2	12.2±0.9
Day 8 to 9	15.0±0.9	14.7±1.5	15.6±1.6	15.4±1.3

Values are means±SEM for 4-5 rats; values with different superscript letters in the same row are statistically significantly different at $p < 0.05$ as determined by Duncan's new multiple range test.²⁵⁾

micro-nutrients as nicotinic acid in the test groups than in the control group, since the food intake among the four groups was almost the same (about 20 g/day). During day 1 to day 2 and thereafter, the sum of the urinary excretion of nicotinamide and its metabolites was almost the same in the four groups (8-10 µmol/day). However, the excretion ratio further decreased during day 1 to day 2, slowly recovered, and then had completely returned to within the normal range during day 5 to day 7 (Table I). The decrease in the excretion ratio was mainly due to the accumulation of MNA. This change was probably caused by the change in 4-Py-forming MNA oxidase activity. The order of decrease in the ratio was the secondary degradation product group = the hydroperoxide group > the linoleic acid group. The complete recovery means that the rats could excrete the secondary degradation products or hydroperoxides that were administered. This result agrees with our previous result that oxidative stress caused by the oral administration of peroxidation products had disappeared by 4 days after the administration.²³⁾

From these results, it was found that linoleic acid, hydroperoxides and its secondary degradation products decreased the excretion ratio of (2-Py+4-Py)/MNA. The change in the excretion ratio was similar to that when rats were fed with excess tryptophan,¹³⁾ nicotinamide,¹⁴⁾ MNA,¹⁵⁾ or pyridoxal.¹⁶⁾ The decrease in the ratio by administering the hydroperoxides and secondary products might have been due to the inactivation of 4-Py-forming MNA oxidase through oxidation of the active site of its enzyme, because its enzyme had been inactivated during catalysis, possibly by active oxygen radicals.²⁴⁾

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