〈原　　著〉

Urinary Excretion of N^1-Methyl-2-pyridone-5-carboxylic Acid, Trigonelline (N^1-Methylnicotinic Acid) and N^1-methylnicotinamide in Human Subjects after Oral Administration of Nicotinic Acid, Trigonelline and N^1-Methyl-2-pyridone-5-carboxylic Acid

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S. YUYAMA *Urinary excretion of N^1-methyl-2-pyridone-5-carboxylic acid, trigonelline (N^1-methylnicotinic acid) and N^1-methylnicotinamide in human subjects after oral administration of nicotinic acid, trigonelline and N^1-methy-2-pyridone-5-carboxylic acid.*
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The urine of human subjects given a single administration of nicotinic acid (NiA), trigonelline (Tg), and N^1-methyl-2-pyridone-5-carboxylic acid (Tg-2Py) was examined for thc excretion of Tg-2Py, Tg and N^1-methylnicotinamide (MNA). The excretion of Tg-2Py was determined by cellulose high performance thin-layer chromatography.

In human subjects given the ordinary diet, Tg was excreted at the same level of MNA excretion in 24 hr urine. The level of Tg-2Py excretion was about 1.5 times of Tg or MNA excretion.

In subjects receiving 50 mg (406 μmole) of NiA, the levels of Tg-2Py and Tg excretion were increased significantly, and that of MNA also incrdased.

In subjects receiving 50 mg (288 μmole) of Tg, the excretion of Tg-2py and non-metabolized Tg were increased, but the recovery of unchanged Tg was very low, and no increase of MNA excretion was observed.

In subjects receiving 50 mg (326 μmole) of Tg-2Py, the excretion of unchanged Tg-2Py incrdased and no increase of Tgand MNA excretion were observed.

NiA tius is converted into Tg and Tg-2Py, and MNA. Tg is not converted into MNA, and Tg-2Py is not converted into Tg and MNA. Therefore it was indicated that both reactions of amidation of Tg to MNA and demethylation of Tg to NiA do not occur and that Tg was oxidized to a pyridone (Tg-2Py). From these results, it is concluded that when a considerable amount of Tg (50-100 mg, 288-576 μmole) is administered to humans, the niacin allowance does not have to be changed.

Key Words trigonelline, N^1-methyl-2-pyridone-5-carboxylic acid, N^1-methylnicotinamide, urinary excretion, high performance thin-layer chromatography.

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INTRODUCTION

Many investigators have already shown that the human body is capable of converting nicotinamide (Nam) to nicotinic acid (NiA), NiA to Nam via NAD coenzymes, Nam to N^1-methylnicotioamide

(MNA), MNA to N¹-mehtyl-2-pyridone-5-carboxamide (MNA-2Py) or N¹-methyl-2-pyridone-3-carboxamide (MNA-4Py). These changes do not appear to be reversible.

Perlzweig et al.[1] and Ellinger et al.[2] have failed to demonstrate that in human subjects, trigonelline (Tg) is a metabolite of NiA. Holman et al.[3] showed that NiA is not methylated directly by human body, but methylated-NiA (Tg) can be oxidized to pyridone. Lindenblad et al.[4] found that the pyridone is N¹-methyl-2-pyridone-5-carboxylic acid (Tg-2Py). But Holman et al.[3] were unable to detect it in human urine. Huff[5] showed that the origine of Tg in the urine can be traced to coffee and legumious food in the ordinary diet. Since Taguchi et al.[6] reported that Tg was localized in many kinds of foods and extremely high contess (up to 1 %) in coffee beans and very high contens in marine shellfishes, it was thought that humans ingest a considerable amount of Tg.

Furthermore, Taguchi et al.[7,8] reported that Tg-demethylating enzyme activity was found in animals, plants and microorganisms, and Tg-syntiesizing enzyme activity was found in marine shellfishes and plants but not detceted in land animals (including mammals, bird etc.).

If the demethylation of Tg occur in human body, it would affect on the niacin allowance.

In our previons report,[9,10] we described that NiA is converted into Tg and MNA, but Tg is not converted into MNA. These results confkict with other findings that suggest NiA is not converted into Tg in humans and rats. Although of Tg-demethylating enzyme activity was detected in mammals, it was suggested that Tg is not circuitously converted into MNA via NiA (demethylatdon, Tg → NiA → NAD coenzymes → Nam → MNA). Further, it was suggested that Tg is not directly converted into MNA (amidation). This suggestion is in agreement with the results of Shibata et al.[11] Also when a large amount of Tg was administered to human subjects (100 mg, 576 μmole)[9] or rats (5 mg 28.8 μmole),[10] about 20 % of the dose was recovered. However, Shibata et al.[11] showed that all of Tg ingestion was excreted unchanged to rat urine.

In the present paper, to reassess whether NiA is converted into Tg and Tg is converted into MNA, and to invesigate whether Tg-2Py is excreted or not in normal Japanese human urine and Tg is converted into Tg-2Py and Tg-2Py is also converted into Tg and MNA via NiA, the levels of Tg-2Py, Tg and MNA excretions were examined after oral administration of NiA, Tg and Tg-2Py.

MATERIALS and METHODS

1) Materials

N¹-Methyl-2-pyridone-5-carboxylic acid was synthesized from nicotinic acid by the methods of Holman et al.[12]. Other materials were mentioned in the previous report[9,10].

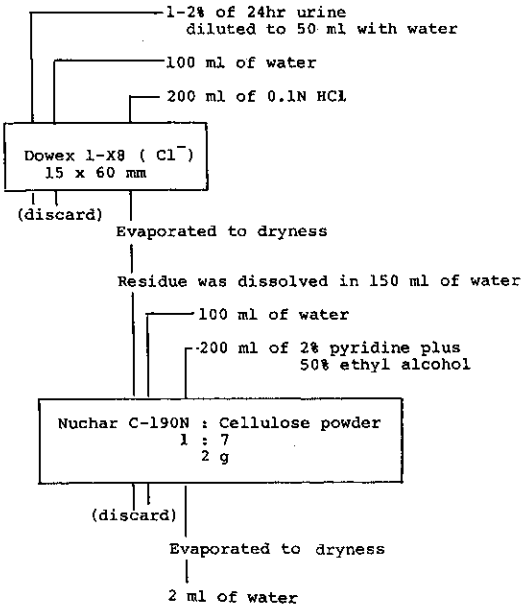
2) Subjects and diets

Nine female volunteers, aged 20 to 21 years, who had no history of serious renal, gastrointestinal or metabolic diseases were the subjects for studies. They were kept at an ordinary diet for 5 days before and 4 days during the experimental period. Urine samples were collected in bottles containing 2 ml of acetic acid. After collecting one 24 hr urine sample (before oral administration of NiA), 50 mg (406 μmole) of NiA was given to each of 9 subjects and 24 hr urine was collected for 1 day. Further after 2 days, 50 mg (288 μmole) of Tg was administered orally to 5 subjects, and 50 mg (326 μmole) of Tg-2Py was administered orally to 4 subjects. Aliquots of all samples were stored at −20 ℃ until analyzed.

3) Analytical methods

a) Extraction of Tg-2Py from urine

One to two percent of the urine within 24 hr were diluted to 50 ml with water. The extraction procedure of Tg-2Py is shown in Scheme 1. The extraction and determination of Tg and MNA from



Scheme 1. Extraction of Tg-2Py from human urine.

human urine has been described previously[9].

b) Determination of Tg-2Py

Five to twenty μl portions of the Tg-2Py extract were applied in a small band at the origin of a HPTLC cellulose plate using a Linomat 111 sample applicator (Camag Co., Muttenz, Switzerland). Plates were developed in n-butyl alcohol : water : acetic acid (60 ; 20 : 3, by volume) and were then scanned in the reflectance mode at 254 nm using an HPTLC/TLC Scanner (Camag Co.). Tg-2Py was quantified by comparing the peak areas with authentic Tg-2Py standard. The recovery of Tg-2Py added to urine was 96±2(SD)%.

RESULTS

1) Excretion of Tg-2Py, Tg and MNA by 9 subjects on the ordinary diet.

The daily excretion of Tg-2py, Tg and MNA in the urine is shown in Table 1. The level of Tg-2Py excretion was about 1.5 times of Tg or MNA excretion. Therefore it became clear that Tg-2Py was excreted in Japanese human urine.

Table 1. Urinary excretion of N^1-methyl-2-pyridone-5-carboxylic acid, trigonelline and N^1-methylnicotinamide by 9 healthy subjects on the ordinary diet.

	N^1-Methyl-2-pyridone-5-carboxylic acid(Tg-2Py)	Trigonelline (Tg)	N^1-Methyl nicotinamide (MNA)
		(μmole/day)	
Range (9)	28.9−41.6	20.6−28.3	19.8−26.5
Average±SD	36.0± 4.6	25.1± 2.6	24.0± 2.8

Mean values±SD are given for 9 subjects.

2) Excretion of Tg-2Py, Tg and MNA by 9 subjects after oral administration of 50 mg (406 μmole) of NiA.

Table 2 shows the results of studies on the relationship of Tg-2Py, Tg and MNA to the metabolic fate of the administered NiA. The excretion of Tg-2Py, Tg and MNA significantly were increased on the first day. Values ranging between 43.6 and 53.7 μmole/day were obtained for Tg-2Py. The excretion of Tg-2Py returned almost to the value of the ordinary diet group after 2 days. The actual conversion NiA to Tg-2Py was about 3.3 % of the dose, while NiA conversion to MNA and Tg was 12.7 and 5.5 % of the dose, respectively. Thus NiA is a source of MNA or Tg and Tg-2Py.

Table 2. Urinary excretion of various metabolites of nicotinic acid before and after oral administration of 50 mg (406 μmole) of nicotinic acid

Derivatives of nicotinic acid	Subject	Urinary excretion (μmole/day) Before	After	% of dose excreted in the urine
Tg-2Py	(9)	36.0±4.7	*49.8±3.4	3.3±0.7
Tg	(9)	25.1±2.6	*47.3±4.1	5.5±0.5
MNA	(9)	24.0±2.8	*75.9±6.8	12.7±1.7

Mean values±SD are given for 9 subjects.

* Significantly different from corresponding ordinary diet (Student's t test) ; $p<0.01$.

Percent of daily dose was calculated as follows :

$$\frac{\text{After value} - \text{Before value}}{\text{Daily dose}} \times 100$$

3) Excretion of Tg-2Py, Tg and MNA in 5 healthy subjects after oral administration of 50 mg (288 μmole) of Tg.

The data describing the excretion of Tg-2Py, Tg and MNA by subjects after the administration of Tg are smmarized in Table 3. The daily excretion of Tg-2Py in this group for 1 day after the administration of Tg increased on the first day and decreased on the second day. The excretion of Tg-2Py in the Tg group returned almost to the value of the ordinary diet after 2 days. About 10 % of the dose of Tg was excreted in the urine as Tg-2Py within 24hr. The daily excretion of Tg in this group was between 82.4 and 90.3 μmole, and about 21 % of Tg dose was recovered in the 24 hr urine. No increase of MNA excretion in the urine was observed.

Table 3. Urinary excretion of various metabolites of nicotinic acid before and after oral administration of 50 mg (288 μmole) of trigonelline

Derivatives of nicotinic acid	Subject	Urinary excretion (μmole/day)		% of dose excreted in the urine
		Before	After	
Tg-2Py	(5)	35.7±4.2	*64.5±6.1	10.0±0.8
Tg	(5)	26.8±1.9	*86.4±3.4	20.7±1.2
MNA	(5)	24.9±2.7	24.6±3.1	——

Mean values±SD are given for 5 subjects.

* Significantly different from corresponding ordinary diet (Student's t test); $p<0.01$.

Percent of daily dose was calculated as follows:

$$\frac{\text{After value}-\text{Before value}}{\text{Daily dose}}\times 100$$

4) Excretion of Tg-2Py, Tg and MNA in 4 healthy subjects after oral administration of 50 mg (326 μmole) of Tg-2Py.

When Tg-2Py was administred, the daily excretion of Tg-2Py ranged between 195.3 and 210.1 μmole (in Table 4). The excretion of Tg-2Py returned almost to the value of the ordinary diet after 2 days. about 51 % of the dose of Tg-2Py was recovered in the urine within 24 hr as unchanged Tg-2Py. No increase of Tg and MNA excretion in the urine was observe.

Table 4. Urinary excretion of various metabolites of nicotinic acid before and after oral administration of 50 mg (326 μmole) of N¹-methol-2 pyridone-5-carboxylic acid.

Derivatives of nicotinic acid	Subject	Urinary excretion (μmole/day)		% of dose excreted in the urine
		Before	After	
Tg-2Py	(4)	36.4±5.9	*203.7±6.6	51.3±1.7
Tg	(4)	23.0±1.7	23.0±1.7	——
MNA	(4)	23.0±2.8	22.5±2.4	——

Mean values±SD are given for 4 subjects.

* Significantly different from corresponding ordinary diet (Student's t test); $p<0.01$.

Percent of daily dose was calculated as follows:

$$\frac{\text{After value-Before value}}{\text{Daily dose}}\times 100$$

DISCUSSION

The outline of the discussion about the niacin metabolites is shown in Figure 1. The daily excretion of Tg-2Py in 9 healthy subjects given the ordinary diet was 36.0 μmole. The range of values for Tg-2Py in this group of subjects is of the same order of magnitude as that found by Lindenblad et al.[3] in

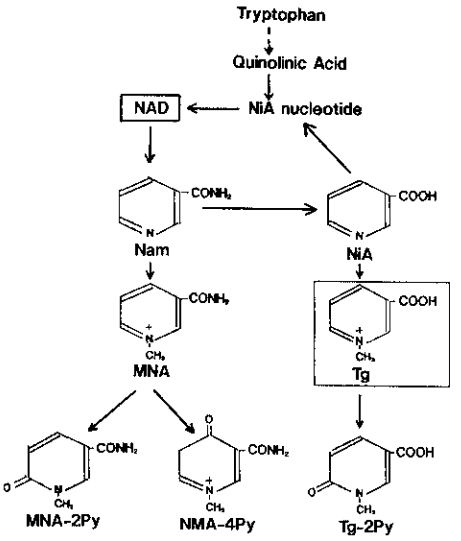


Figure 1. Urinary metabolites of nicotinic acid and nicotinamide

3 male subjects (3-6 mg, 19.6-39.2 μmole). The present value is the first report on the excretion of Tg-2Py in Japasese human subjects. The excretion of Tg in 9 healthy subjects given the ordinary diet was 25.1 μmole and the ratio of Tg and MNA excretion is almost identical to that described in the previou report.[9]

After the administration of NiA, the actual conversion of NiA into MNA, Tg and Tg-2Pay was 12.7, 5.5 and 3.3 % of the dose, respectively. With regard to the possible conversion of various compounds into Tg in the body, Perlzweig et al.[13] have already shown that neither Nam nor MNA yields any appreciable amount of Tg in the urine and also Holman et al.[3] have tested in 3 male subjects after ingestion of NiA and found no evidence of conversion into Tg. Further, Taguchi et al.[8] have shown that Tg-synthesizing enzyme activity was not detected in land animals (including mammals, bird etc.). However, it is a fact that the conversion of NiA into Tg occur in human body and it is further interesting to note that NiA can be converted into Tg-2Py via Tg.

After the administration of Tg, the excretion of Tg-2Py and Tg were increased and no increase of MNA excretion was observed. The actual conversion of Tg into Tg-2Py was about 10 % of the dose and only 21 % of the dose was recovered in the urine within 24 hr as unchanged Tg. Regardless of Tg-demethylating enzyme activity was detected in mammals, it was suggested that Tg is neither directly converted into MNA (amidation) nor circuitously enverted into MNA (demethylation, Tg → NiA → NAD coenpymes → NAD → Nam → MNA). This suggestion is in agreement with the results of Shibata et al.[11] (i.e. when a large amount of Tg or MNA was administered to rats, the excretion pattern of the niacin meatbolites in the urine did not increase.). The results which only 21 % of the dose was excreted as unchanged Tg in human[9] and rat[10] urine conflict with the result of Shibata et al. (i.e. when a large amount of Tg was administered to rats, all of the dose was excreted unchanged into urine.)

When Tg-2Py was administered to human subjects, the recovery of Tg-2Py in the urine was 51 % of the dose. although Lindenblad at al.[4] and Holman et al.[3] showed that about 22-35 % of the dose could be accounted for in the urine. It is not evident that Tg-2Py is an end product, in fact, further metabolic pathway of this compound in the body is likely to be present. Also Tg-2Py is not converted into Tg or MNA and thus these changes do not appear to be revesible. MNA-2Py or MNA-4Py was already known for the end product.

The combined results show that the body in capable of converting NiA into MNA via NAD, NiA can be converted into Tg and Tg-2Py, and Tg into Tg-2Py. These changes do not appear to be reversible, and there is no evidence that any other intercovertion occurs. Therefore, it is concluded that when a considerable amount of Tg (50-100 mg[9], 288-576 μmole) was administered to human subjects, the niacin allowance does not have to be changed. It in interesting to note that NiA is methylated directly by human body, and that can be oxidized to a pyridone (Tg-2Py).

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