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Studies on Antitumor Agents. III.[1] Metabolites of 5(4)-Amino-4(5)-imidazolethiocarboxamide Derivatives in Urine

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5(4)-Formamido-4(5)-imidazolethiocarboxamide and 1-β-D-ribofuranosyl-5-amino-4-imidazolethiocarboxamide were active against various tumors. Their metabolites in urine of mice were determiend with thin-layer chromatography.

5(4)-Amino-4(5)-imidazolethiocarboxamide, which was inactive against Nakahara–Fukuoka sarcoma, was excreted intact and 6-mercaptopurine was not found in urine after the intraperitoneal injection. On the other hand, the large quantity of 6-mercaptopurine was found in urine after the administration of 5(4)-Formamido-4(5)-imidazolethiocarboxamide. 1-β-D-Ribofuranosyl-5-amino-4-imidazolethiocarboxamide was mainly excreted intact, and a small quantity of 5(4)-amino-4(5)-imidazolethiocarboxamide and a trace amount of 6-mercaptopurine were found in urine.

5(4)-Formamide-4(5)-imidazolethiocarboxamide and 1-β-D-ribofuranosyl-5-amino-4-imidazolethiocarboxamide were demonstrated to be active against various tumors,[1,3] but not against a 6-mercaptopurine resistant leukemia.[1] These facts suggested the similarity between these compounds and 6-mercaptopurine in both antitumor activity and chemical structure. 6-Mercaptopurine was reported to be excreted intact as one of the main metabolites in urine of mice.[4]

In the present experiment, the ability of metabolic changes of these two compounds to 6-mercaptopurine was examined through determination of metabolites in urine.

Materials and Methods

Animals——Female mice of ddN strain weighing 20±2 g were used. The commercial chow diet (CA-1; CLEA Japan, Inc., Tokyo) and water were offered ad libitum).

Materials——The following five compounds were used: 5(4)-amino-4(5)-imidazolethiocarboxamide (TAICA),[5] 1-β-D-ribofuranosyl-5-amino-4-imidazolethiocarboxamide (TAICA riboside),[5] 5(4)-formamido-4(5)-imidazolethiocarboxamide (FTAICA),[5] 6-mercaptopurine (6MP) (Takeda), and 9-β-D-ribofuranosyl-6-mercaptopurine (6MPR).[6]

Detection of Metabolites in Urine with Thin-Layer Chromatography——Five mice per each group were intraperitoneally injected with the compound to be tested at the dose of 0.33 mmole/10 ml/kg corresponding to 50 mg/kg of 6MP and the urine of each group was collected in a metabolism cage for 48 hours.

TLC plates of Silicagel F_{254} precorted "Merck" were used and the following three solvent systems (acidic, alkaline and neutral) were applied; I. *n*-BuOH–AcOH–H_2O (60:20:20),[7] II. $CHCl_3$–MeOH (3:1),[8] III. $CHCl_3$–MeOH–28% NH_4OH (240:80:1). The sample of 5 or 10 μl was spotted on the plate and developed

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ascendingly with one of these solvent systems. Reference compounds on the plate were detected by UV absorption or iodine adsorption for the measurement of *Rf* values. Metabolites were detected by iodine adsorption. To obtain quantitative results, the spot area and the color intensity of unknown substance were compared with spots obtained from the same volume of a known concentration of the same substance developed on the same chromatogram. This method was more sensitive than the elution metod.[9] TAICA and 6MP were only separated on the solvent system III, which was, therefore, mainly used throughout this experiment. A trace of 6MP in urine was detected by the following procedures: Urine (2 ml) was acidified to pH 1—2 with 1N HCl. Then the precipitate was collected by centrifugation, washed with distilled water (1 ml), dissolved in a small quantity of 1N NaOH and chromatographed.

Results and Discussion

Rf values of some possible metabolites are shown in Table I. Table II shows the quantity of metabolites in urine.

Table I. *Rf* Values of Reference Compounds

Compound[a]	Solvent system[b] I	II	III
5(4)-Amino-4(5)-imidazolethiocarboxamide (TAICA)[5]	.60	.44	.36
TAICA riboside[5]	.57	.33	.26
5(4)-Formamido-4(5)-imidazolethiocarboxamide (FTAICA)[5]	.60[c]	.55	.28[d], .50
5(4)-Amino-4(5)-imidazolecarboxamide (AICA)[10]	.49	.22	.22
AICA riboside[11]	.48	.15	.13
5(4)-Formamido-4(5)-imidazolecarnoxamide (FAICA)[12]	.58, .49[e]	.33	.33
6-Mercaptopurine (6MP)	.64	.45	.28
6-Mercaptopurine riboside (6MPR)[6]	.53	.25	.10
6-Thiouric acid[f]	.47	.05	.04

a) suffix showing reference for preparation
b) I: *n*-BuOH–AcOH–H_2O (60:20:20); II: $CHCl_3$–MeOH (3:1); III: $CHCl_3$–MeOH–28% NH_4OH (240:80:1)
c) Changed to TAICA.
d) Changed to 6MP in part.
e) Changed to AICA in part.
f) oxidized product of 6MP with xanthine oxidase

TAICA, which was inactive against Nakahara-Fukuoka sarcoma,[3] was excreted intact in urine, and other metabolites were not detected.

The large quantity of 6MP and a small amount of TAICA were found in urine after administration of FTAICA, but the intact FTAICA was not detected. This compound is labile under both sides of pH, and changed to TAICA in acidic condition and to 6MP in alkaline. The urine was, therefore, collected into the test tube containing hydrochloric acid, but no quantitative changes in both metabolites were found. This fact indicates that intact FTAICA is not excreted in urine and 6MP itself is excreted as the main metabolite. TAICA riboside was mainly excreted intact, but a small quantity of TAICA and a trace of 6MP were found. Further, 6MP was found in urine after the administration of 6MPR. These findings show that the low antitumor activity of TAICA riboside might be due to low metabolic change either to 6MP or to 6MPR.

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Table II. Metabolites in Urine and Antitumor Activity

Administered compound[a]	Metabolites in urine[a]	Integrated excretion ratio (%)[b] 0—4 (hr)	0—24 (hr)	0—48 (hr)	Antitumor activity[c] ED_{50}(mg/kg/day)
6MP	6MP	18	36	38	3.6
6MPR	6MP	22	30	30	5.1
TAICA	TAICA	19	26	27	inactive
	6MP[d]	0	0	0	
FTAICA	FTAICA	0	0	0	4.8
	TAICA	5	9	9	
	6MP	20	38	38	
TAICA riboside	TAICA riboside	34	46	51	21.0
	TAICA	2	6	7	
	6MP[d]	<1	1	2	

a) Administered 0.33 mmol/kg of the compound, corresponding to 50 mg/kg of 6MP. Abbreviation was shown in Table I.
b) maen value (3 specimens)
c) Nakahara-Fukuoka sarcoma was used.
d) Determined after concentration.

The positive correlation was found between the antitumor activity of these compounds and the amounts of 6MP in urine. As a result, the greater part of the antitumor activity of FTAICA and TAICA riboside is considered to be owing to the metabolite, that is 6MP.

The possible main metabolic pathways of these compounds are summarized in Fig. 1.

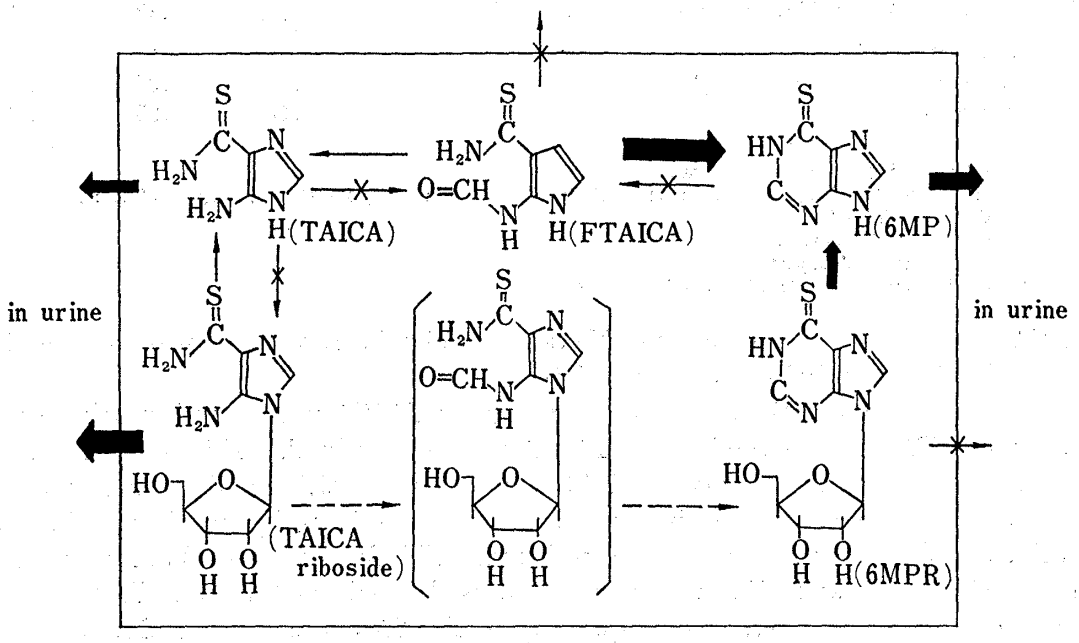

Fig. 1. Main Metabolic Pathways

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