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Intestinal Bacterial Metabolism of Rutin and its Relation to Mutagenesis

Dong-Hyun Kim[1], Sang-Bum Han[1], Eun-Ah Bae[2] and Myung Joo Han[2]

[1]College of Pharmacy and [2]Department of Food Sciences and Nutrition, Kyung-Hee University, #1Hoegi, Dongdaemun-ku, Seoul 130-701 Korea

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After rutin (50-1500 mg/kg) was administered orally to rats, the relationship between its metabolites and mutagenicity was investigated. Quercetin conjugates were detected in the urine of rats treated with more than 150 mg/kg. Administration of rutin less than 100 mg/kg resulted in phenolic acid-like metabolites. However, intact rutin was not detected in the urine of rats treated with different amounts. When rutin was cultured with human intestinal bacteria, the amount of quercetin was increased gradually with a corresponding decrease in the level of rutin and then quercetin was decreased gradually with a corresponding increase in the level of unidentified compounds. The ring fission bacterium of quercetin was Pediococcus Q-05. These results suggest that rutin could be metabolized and transformed from mutagenic to non-mutagenic by intestinal bacteria in human intestine.

Key words : Rutin, Guercetin, Metabolism, Intestinal bacteria, Mutagenesis

INTRODUCTION

Flavonoid glycosides are polyphenolic compounds produced by most fruits, vegetables and herbal medicines. These compounds are resistant to boiling and fermentation and ingested daily more than 1g by human.

After ingestion of flavonoid glycosides, most of them are not easily absorbed in mammalian gut and meet intestinal microflora in the intestine. Therefore, these compounds are metabolized by intestinal bacteria.

Flavonoid glycosides are ignored until Brown and Dietrich in 1979 noted that free flavonoid and aglycones possessed potent mutagenic properties towards bacteria used in Ames test. MacDonald *et al.* (1983) and Tamura *et al.* (1980) reported that the non-mutagenic flavonoid glycosides, such as rutin and quercitrin, are hydrolyzed by fecalase (cell-free extract of human feces) to mutagenic product, quercetin, detectable in Ames test.

In addition, a study on the carcinogenecity of quercetin in rats presented that quercetin showed intestinal and bladder tumors in Norweigen rats (Pamukcu *et al.*, 1980). However, negative results were also shown for mice (Saito *et al.*, 1980) and golden hamster (Morino *et al.*, 1982). Until now, it is not clear whether these flavonoids and aglycones are carcinogens and cocarcinogens or not.

Correspondence to: Dong-Hyun Kim, Hoegi, Dongdaemum-ku, Seoul 130-701 Korea

Aglycones related to mutagenesis are formed continuously in mammalian gut by bacterial hydrolysis of ingested flavonoid glycosides. Evidence for the involvement of the intestinal microflora in the metabolism of flavonoid compounds in vivo has been presented by Griffiths (1964) and Das and Griffiths (1968). Particularly, it was reported that the formation of ring fission products from orally administered flavonoids was significantly decreased by the coadministration of oral antibiotics (Griffith & Barrow, 1972; Nakagawa *et al.*, 1965).

It is important to know whether these components are metabolized by intestinal bacteria or not before these components are delivered to target site such as liver and kidney. In the present paper, we reported on the change of mutagenesis during metabolizing rutin by intestinal bacteria and the isolation of human fecal bacteria capable of metabolizing rutin.

MATERISLS AND METHODS

Rutin, quercetin, NADP, biotin, histidine, glucose 6-phosphate, sodium azide, 1-nitropyrene, 3,4-dihydroxyphenylacetic acid and 2,4,5-trihydroxyphenylacetic acid were purchased from Sigma Chem. Co.. General anaerobic medium (GAM) was purchased from Nissui Pharmaceutical Co., Ltd. The other media were purchased from Difco Co.. Tester strains, *Salmonella typimurium* TA98 and TA100, were donated from Dr. B. N. Ames, Berkely, USA.

Determination of the metabolite, quercetin, in the urine of rats treated with rutin

Male rats (SDD Wister, body weight 230-300 g) were maintained on the synthetic diet (44.1% wheat starch, 20% casein, 5% mineral mixture, 0.8% vitamin mixture and 0.1% choline chloride) and tap water *ad lib.*. Three to five rats were used for each group. The 25, 50, 100, 150, 250, 500, 1000, 1250 or 1500 mg of flavonoids were orally administered to rats. Urine samples were collected at 24, 48 and 72h after administration of flavonoids. Each urine was diluted 2-fold. Half of the urine was adjusted to pH2 with HCl and then extracted with ethylacetate. The other half of the urine was adjusted to 2N-HCl with 5N-HCl and the mixture was refluxed for 2hs at the boiling water bath. The resultant hydrolysate was then extracted with ethylacetate. These extracts were analyzed by TLC (Developing solvents, $CHCl_3$: MeOH=3 : 1).

Incubation of flavonoids with human intestinal bacteria

Human intestinal bacteria were inoculated into 500 ml GAM broth containing 50 mg flavonoids, which was then incubated anaerobically at 37°C for 5 days. Fifty ml of the cultured broth was septically taken out at 12, 18, 24, 48, 72, 96 and 120h and extracted with EtOAc. This extract was assayed by TLC and Ames test.

Ames test

The 100 μl extract containing the metabolites of rutin or its HCl-hydrolysate was subjected to the Ames assay for mutagenicity (Ames *et al.*, 1973) using tester strains *S. typhimurium* TA98 and TA100. Each test was done in triplicate.

Isolation and identification of the intestinal bacteria which degrade flavonoids

The intestinal bacteria were isolated from Korean according to our previous method (Kim *et al.*, 1994). The isolated bacteria were inoculated and incubated in GAM containing flavonoids. Flavonoids-transforming bacteria were judged by TLC analysis of the cultured media. The transformant-positive bacteria were identified according to Bergey's manual (Kreig & Holt, 1984).

Assay of antibacterial activity

The anti-*S. typhimurium* effect of quercetin was analyzed by the standard two-fold agar dilution method. The inhibitory percent of the quercetin was determined by measuring turbidity in the series of nutrient agar plates containing 0.5M histidine/biotin.

RESULTS

Metabolites and mutagenicity on the urine of rats which orally treated with rutin

Various concentrations of rutin were orally administered to rats which were given the synthetic diet over a period of 1 week. The urine was collected at 24h intervals and analyzed by TLC (Fig. 1). From the urine of rats administered more than 1250 mg rutin / kg body weight of rat, free quercetin was detected but rutin was not (Fig. 2). In the case of being adminstered 150-1000 mg rutin/kg rat, the free quercetin was not detected but by hydrolyzing the urine with 2N-HCl, the free quercetin was detected. However, in the case of administering less than 100 mg/kg, the hydrolysate of the urine was not detected and phenolic acid-like compounds were only detected.

The mutagenicity of the urine was investigated (Data were not shown). The urine of rats administered more than 1250 mg/kg and the hydrolysate of the urine of rats adiminstered 150-1000 mg/kg

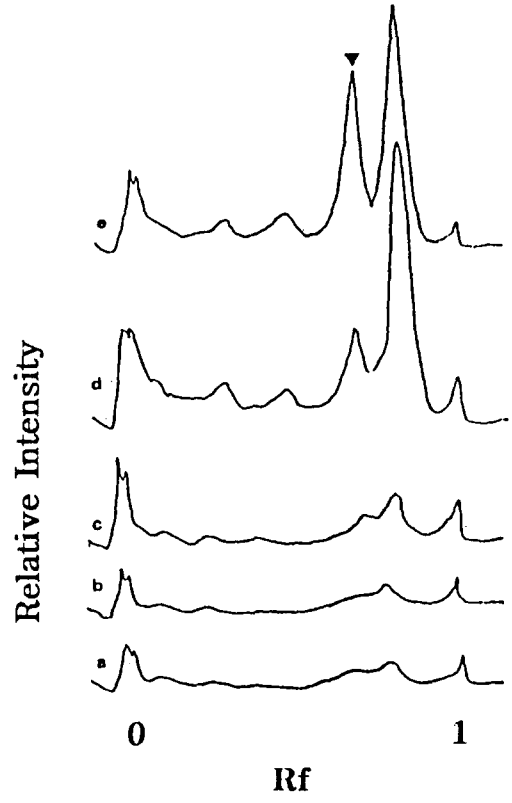

Fig. 1. TLC chromatogram of the urine of rats orally treated with rutin: a, control; b, treated with 100 mg/kg; c, 250 mg/kg; d, 500 mg/kg; e, 1000 mg/kg. Arrow indicates quercetin. Developed TLC plate was applied to TLC scanner (Shimazu systems Model CS-9000 : Wavelength 254 mn).

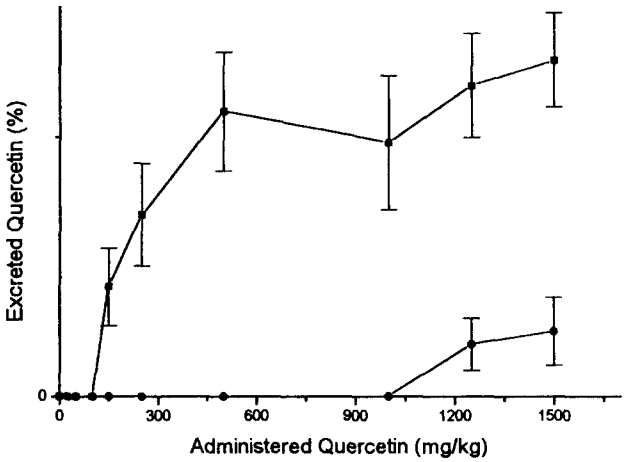

Fig. 2. Excreted quercetin and quercetin conjugate(s) into the urine, after rutin was administered into rats: ■, quercetin conjugates (urine treated with 2N HCl was extracted with ethylacetate); ●, free quercetin (untreated urine was extracted with ethylacetate).

were strongly mutagenic. However, the urine and its hydrolysate of rat treated with 100 mg/kg were not mutagenic.

Metabolites of rutin by human intestinal bacteria and their mutagenicity

To investigate metabolites of rutin by human intestinal bacteria and their mutagenicity, human intestinal bacteria were inoculated and cultured in GAM containing rutin. Then, metabolites of rutin by human intestinal bacteria were analyzed by TLC. As shown in Fig. 3, the amount of quercetin was increased gradually with a corresponding decrease in the level of rutin. The quercetin was increased for 12hs and then decreased gradually with a corresponding increase in the level of unidentified compounds. Each collected sample was extracted with ethylacetate and its mutagenicity was investigated. The revertants per plate of *Salmonella typimurium* TA 98 were increased until 12 h and decreased thereafter. The sample cultured for 48hs was not mutagenic. This pattern was similar to that of the formation and degradation of quercetin.

Mutagenicity and antimicrobial activity of quercetin

Quercetin was mutagenic on TA98 but not on TA 100 (Table 1). The revertants per plate of *S. typimurium* TA98 were increased on 0.1-0.5 mg/ml of top agar in a concentration-dependent manner. However, on the concentration of more than 0.5 mg/ml of top agar, the revertants per plate were not increased due to antimicrobial activity of quercetin.

The 50% inhibitory concentration of quercetin for *S. typhimurium* TA98 was 0.15 mg/ml of top agar. Minimal inhibitory concentration was 1 mg quercetin/ml of top agar.

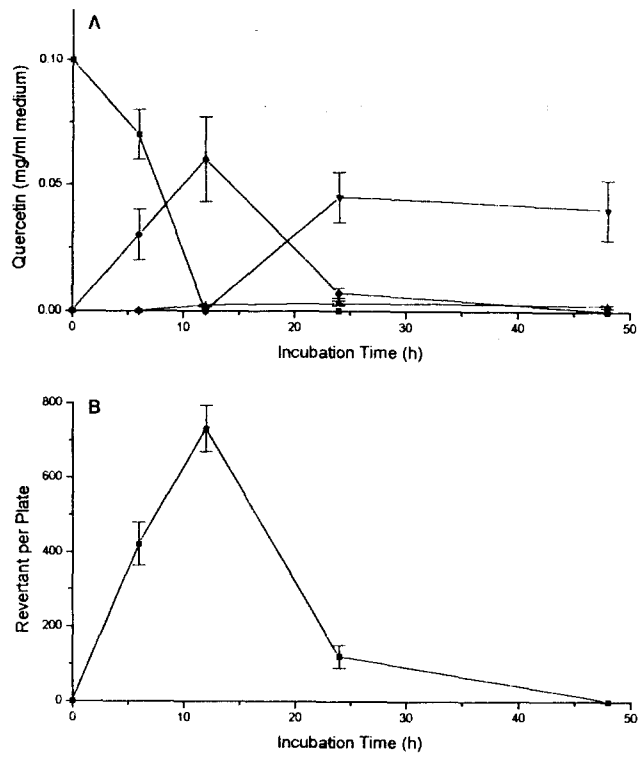

Fig. 3. Metabolism of rutin by intestinal bacteria and its relation to mutagenicity : A, Metabolites of rutin by human intestinal bacteria according to incubation time (■, rutin; ●, quercetin; ▲ & ◆, unidentified compounds) ; B, Mutagenicity of the extract of rutin metabolites.

Table 1. Antibacterial activity and mutagenesis of quercetin in *Salmonella*/mammalian microsome assay

Compound	Concentration	His+ revertant/plate		Antibacterial Activity (inhibition %)
		TA98	TA100	
DMSO		7	35	100
Quercetin	1	106	64	100
	0.5	295	66	98
	0.1	287	60	45
	0.05	179	56	16
	0.01	185	56	0
1-Nitropyrene		318	*	

Isolation and identification of the bacteria which degrade quercetin to phenolic acids

Rutin was not detected from the urine of rats orally treated with rutin. Quercetin was detected from the urine of rats orally administered less than 100 mg/kg rutin. From the urine of rats, which was previously treated with streptomycin and neomycin, the amount of detected quercetin was dramatically decreased. Therefore, we isolated the bacterium fissuring the B-ring of quercetin or rutin from human feces. The isolated bacterium Q-05 was *Pediococcus* species, but

Table 2. Biological characteristics of *Pediococcus* Q-05

	Pediococcus Q-05	*P. dextrinicus*	*P. acidilactici*
Colony diameter	1.0-2.5 mm	1.0-2.5 nm	1.0-2.5 nm
Gram stain	+	+	+
Shape	Coccus	Coccus	Coccus
VP test	-	-	-
MR test	+	+	+
Catalase	-	-	+
Simmon's test	-	-	-
Nitrate reduction	-	-	-
Indole production	-	-	-
β-Glucosidase	+	+	+
Urease	-	-	-
Gas production	-	-	-
Alkaline phosphatase	-	-	-
H_2S production	-	-	-
Growth at 35°C	+	+	+
40°C	+	+	+
Growth in 4.0% NaCl	+	+	+
6.5% NaCl	+	-	+
Growth at pH 4.2	+	-	+
pH 7.0	+	+	+

slightly different from *P. dextrinicus* and *P. acidilactici* (Table 2). *Pediococcus* Q-05 transformed quercetin to 3,4-dihydroxyphenylacetic acid. The metabolite was identified by comparing to authentic compounds

DISCUSSION

MacDonald *et al.* (1983) and Tamura *et al.* (1980) reported that rutin could be metabolized to quercetin by intestinal bacteria, which have β-glucosidase and/or β-rhamnosidase, and the formed quercetin was strong mutagenic. Bokkenheuser *et al.* (1987) reported that *Bacteroides distasonis* transformed rutin to quercetin among intestinal bacteria. In addition, Booth *et al.* (1956) reported that phenolic acids were detected from the urine of the rat orally treated with rutin and quercetin, either free or conjugated, was not.

We could also detected free and conjugated quercetin from the urine of rats treated with more than 1250 mg rutin/kg rat. However, in the urine of rats treated with less than 100 mg/kg rat, conjugated quercetin could not be detected and, in those of rats treated with less than 1000 mg rutin/kg rat, only conjugated quercetin was detected. In the case of rats treated with less than 100 mg/kg, we thought, rutin was metabolized by intestinal bacteria: rutin was transformed to quercetin and then was to phenolic acids.

This result was supported by the previous work of Booth *et al* (1956) that phenolic acids, such as 3,4-dihydroxyphenylacetic acid and 3-methoxy 4-hydroxyphenylacetic acid, were detected from the urine of rabbit and rat treated with quercetin or rutin.

Two factors may reduce the mutagenicity and carcinogenicity of rutin and quercetin in humans. First, quercetin and rutin does not seem to be absorbed in the case of being treated with less than 100 mg/kg (6 g/60 kg). In addition to our study, Gugler *et al* (1975) found that after oral dosage, free quercetin was present in the intestine but no quercetin, either free or conjugated, was detected in the bloodstream. The authors concluded that less than 1% of dose was absorbed. Second, microbial degradation of flavonoids is extensive. In our study, quercetin and rutin were easily transformed to phenolic acids by intestinal bacteria. This result was supported by the above Gugler *et al* (1975) who reported that less than 1% of the quercetin was absorbed by the intestine and only half of an oral dose of 70 mg/kg of body weight was recovered from the feces, indicating extensive microbial degradation.

Furthermore, we isolated a quercetin fissuring bacterium, *Pediococcus* Q-05, which was different to *P. dextrinicus* and *P. acidilactici* (Kreig & Holt, 1984).

These results suggest that the flavonoids, major components of heral medicines, could be metabolized by intestinal bacteria and transformed to the metabolite having novel biologial activity, such as from mutagenic to non-mutagenic by intestinal bacteria in human intestine.

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