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Metabolic Fate of Plant Guazatine Residues in Male Rats

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INTRODUCTION

We previously reported the metabolic fate of the fungicide guazatine triacetate (TA), 1,1'-iminodi(octamethylene)diguanidine triacetate, in dwarf apple trees.¹⁾ Because of the low permeability, guazatine stayed on the surface of leaves and fruit of dwarf apple for a long time and an appreciable part of it was photodecomposed to produce several photoproducts, their major one (Pm) being tentatively identified as 4 (or 6)-methyl-5-oxo-9-azaheptadecane-1,17-diguanidine.²⁾ Thus, the photoproducts of guazatine as well as guazatine itself can be ingested by mammals during the consumption of treated plants.

From the viewpoint of environmental safety of pesticides it is important to clarify the metabolic fate of plant pesticide residues in mammals, and there have been several studies concerning this problem.³⁻¹⁰⁾ This paper describes the fate in rats of (1) the crude mixture of photoproducts of ¹⁴C-guazatine and (2) all apple fruit residues of ¹⁴C-guazatine, which may reflect real residual nature of guazatine in plants.

MATERIALS AND METHODS

1. Radiochemicals

Radioactive guazatine triacetate (TA), 1,1'-iminodi(octamethylene)diguanidine triacetate, used was labeled at guanidino carbons with a specific radioactivity of 24.3 mCi/mmol. The stock solution (1000 ppm) was prepared by dissolving it in distilled water or physiological saline. The details were reported previously.¹¹⁾

2. Oral Administration of Photoproducts of ¹⁴C-Guazatine

To the bottom of thirty 100-ml beakers, 0.1-ml aliquots of the stock solution (distilled water) of

¹⁴C-guazatine-TA were applied and diluted with 5 ml of methanol. The solvent was allowed to air-dry to make thin film of ¹⁴C-guazatine-TA. The beakers were placed in a controlled growth chamber and continuously irradiated by artificial sunlight lamps (λ_{max} ; 515 nm) for 7 weeks as previously described.²⁾ Photoproducts in the beakers were recovered by washing with 10% acetic acid, and after vacuum evaporation of the solvent they were redissolved in distilled water (0.5 mg eq. of ¹⁴C-guazatine-TA/ml) to make a photoproduct solution. TLC analysis showed that the photoproducts consisted of 39% of unchanged guazatine, 35% of Pm, and 26% of eight or more minor photoproducts, when calculated on a radioactivity basis. The composition of the photoproducts was identical with the one by the previous experiments.²⁾

Animals used in the study were male rats of Wistar-Imamichi strain (7 weeks old, SPF, body weight; 220.0 ± 0.8 g). Three rats were given a single oral dose of the above solution so that each rat received 3 mg eq. of ¹⁴C-guazatine-TA/kg (Expt.-1).

3. Oral Administration of Apple Fruit Treated with ¹⁴C-Guazatine-TA

In Expt.-2, two rats were orally intubated with the homogenate of apple fruit which had been treated with ¹⁴C-guazatine-TA for 12 weeks. The fruit samples were the same ones used for the study on the metabolism of ¹⁴C-guazatine in dwarf apple trees.¹⁾ Fruit of dwarf apple trees (*Malus pumila* sp. cv. "Inu-apple") were surface-drenched to run-off with 1000 ppm aqueous solution of ¹⁴C-guazatine-TA *in situ*, and grown normally in a controlled growth chamber for 12 weeks. Five fruit were harvested (22 g) and homogenized with an equal amount (v/w) of distilled water. The specific radioactivity of the homogenate was 0.132 μ Ci/g. Radioactivity in this homogenate consisted of approximately 81% of unchanged guazatine, 4% of Pm, 7% of minor photoproducts, and 8% of radioactive materials that were recovered in aqueous and unextractable fractions as reported previously.¹⁾ Two rats were orally intubated five times with the homogenate at the intervals of 2 hr under slight ether anesthesia. The total dose level of ¹⁴C was 0.2 mg eq. of ¹⁴C-guazatine-TA and that of the homogenate approximately 50 g/kg.

As a reference of Expt.-2, an apple fruit homogenate was prepared by homogenizing untreated dwarf apple fruit (10 g) with an equal amount (v/w) of the stock solution (physiological saline) of ^{14}C -guazatine-TA. Three rats were given a single oral dose of the homogenate under slight ether anesthesia. The dose level of ^{14}C -guazatine-TA was 3 mg/kg and that of the homogenate 5.3 g/kg (Expt.-3).

4. Collection and Analysis of Samples

All animals dosed were individually housed in metabolic cages, and the urine and feces were collected separately at 24-hr intervals. The animals were killed 7 days after the dosing and their tissues and organs were removed as previously reported.¹²⁾ The body weight of the rats sacrificed was 276.4 ± 11.0 g. Radioactivity in the urine, feces, tissues, and apple fruit homogenates were assayed by the liquid scintillation counting (lsc) or the combustion/lsc procedure. Urine samples (0–96 hr) were combined, lyophilized, and the residues were extracted with methanol. After filtration and concentration, radioactive materials were analysed by TLC.¹²⁾

RESULTS AND DISCUSSION

1. Metabolic Fate of Photoproducts of ^{14}C -Guazatine in Rats (Expt.-1)

Since it was difficult to prepare a sufficient amount of individual photoproducts of ^{14}C -guazatine, rats were given a single oral dose (3 mg eq. of ^{14}C -guazatine-TA/kg) of a mixture of the photoproducts of ^{14}C -guazatine, which consisted of unchanged guazatine (39%), Pm (35%), and other minor photoproducts (26%). The metabolic fate of the photoproducts was estimated by subtracting the contribution of the metabolic fate of ^{14}C -guazatine itself after a single oral dose (3 mg/kg) that was reported previously (control group).¹²⁾

Fig. 1-a illustrates the cumulative excretion of radioactivity in the urine and feces of three rats dosed with a crude mixture of the photoproducts of ^{14}C -guazatine. The fecal and urinary excretion in 7 days accounted for 78.0% and 17.9% of the dosed radioactivity, respectively. Since the dosed mixture contained 39% of ^{14}C -guazatine and the cumulative urinary excretion of ^{14}C in the control group was 4.6%, approximately 10% of radioactivity in the urine (1.79% of dose) was estimated to come from unchanged guazatine ($4.6\% \times 0.39$) and thus remaining 90% from the photoproducts. This suggests that when only the photoproducts were orally intubated to rats at 3 mg eq. of ^{14}C -guazatine-TA/kg, approximately

Fig. 1 Cumulative daily excretion of radioactivity from rats after oral dosing of photoproducts of ^{14}C -guazatine (Expt.-1) or homogenates of apple fruit treated with ^{14}C -guazatine (Expts.-2 and -3).

a: Expt.-1, b: Expt.-2, c: Expt.-3. Each point represents the mean of duplicate (Expt.-2) or triplicate (Expts.-1 and -3) samples.

26% of the dose radioactivity would appear in the urine in 7 days. Although the exact value is unknown, the bioavailability of the photoproducts, thus, seems to be significantly higher than that of guazatine.

The concentrations of radioactivity (total ^{14}C -levels) in the representative tissues 7 days after oral dosing are presented in Table 1. We previously reported that the kidney was a principal target organ for ^{14}C -retention in rats given a single oral dose of ^{14}C -guazatine-TA (3 mg/kg, control group). However it was found that the total ^{14}C -level in the kidney of Expt.-1 group was approximately one-third of that of the control group, while in the liver it was approximately two times higher than that of the control group. In other words, the greatest quantity of radioactivity was found in the liver which retained 0.29% of the dosed radioactivity, while the kidney kept only 0.11%. Considering the data of the control group, the total ^{14}C -level in the kidney was assumed to be mostly attributable to guazatine ($0.28\% \times 0.39 = 0.11\%$), but in the liver it was presumed to be mainly due to the photoproducts since the contribution of unchanged guazatine can be estimated to be minute ($0.13\% \times 0.39 = 0.05\%$). Thus, it was concluded that the photoproducts were principally retained in the liver and their affinity to the kidney was negligible.

The radioactivity excreted in the 0–96 hr urine consisted of six components, and TLC cochromatography with the dosed solution showed that none of the photoproducts were excreted in their

Table 1 Total ^{14}C -levels in tissues of male rats 7 days after oral dosing of photoproducts of ^{14}C -guazatine-TA (Expt.-1) or homogenates of apple fruit treated with ^{14}C -guazatine-TA (Expts.-2 and -3).

Tissues	Expt.-1	Expt.-2	Expt.-3	Control ^{a)}
	Dose level (mg eq. of ^{14}C -guazatine-TA/kg)			
	3.0	0.2	3.0	3.0
Blood	0.001 ^{b)}	<0.001	0.001	0.002
Plasma	<0.001	0.001	<0.001	<0.001
Liver	0.080 (0.29) ^{c)} [25.9] ^{d)}	0.001 (0.04) [7.72]	0.026 (0.10) [11.7]	0.043 (0.13) [13.3]
Kidney	0.183 (0.11) [10.1]	0.011 (0.10) [16.7]	0.367 (0.24) [28.7]	0.523 (0.28) [28.6]
Spleen	0.037	<0.001	0.047	0.060
Testis	0.002	<0.001	0.003	0.005
Adipose tissue	0.001	<0.001	0.002	0.005
Skeletal muscle	0.006	<0.001	0.003	0.006
Gut and its contents	— ^{e)} (0.04)	— (0.09)	— (0.03)	— (0.05)
Remaining carcass	0.012 (0.71)	0.001 (0.45)	0.009 (0.49)	0.012 (0.53)
Whole body (except gut and its contents)	(1.12)	(0.59)	(0.84)	(0.98)

^{a)} Data from the rats given a single oral dose of guanidino- ^{14}C labeled guazatine-TA (3 mg/kg) reported previously.¹²⁾

^{b)} Values presented are the mean of duplicate (Expt.-2) or triplicate (Expts.-1 and -3) samples and expressed as μg eq. of ^{14}C -guazatine/g fresh tissues.

^{c)} % of the dosed radioactivity.

^{d)} % of the radioactivity retained in the whole body (except gut and its contents).

^{e)} Not determined.

Fresh weight of the liver at sacrifice was 14.4 ± 1.5 g and that of the kidney 2.41 ± 0.22 g ($N=8$).

intact forms. Among the urinary metabolites, three components appeared to be specific to the photoproducts, but the sum of the radioactivity of these metabolites comprised only 22.1% of the urine- ^{14}C . More than five metabolites (77.9% of the urine- ^{14}C) were common to the urinary metabolites of the control group, including a major urinary metabolite (UGm) formed by deamidation of the guanidine moiety.¹²⁾ Since the contribution of unchanged guazatine to the urine- ^{14}C was only 10%, it is probable that 67.9% of urine- ^{14}C consisted of the metabolites that are common to ^{14}C -guazatine. In other words, 25% of the urinary metabolites of the photoproducts were specific and 75% common to ^{14}C -guazatine.

2. Metabolic Fate in Rats of ^{14}C -Guazatine Residues in Dwarf Apple Fruit (Expts.-2 and -3)

To simulate the metabolic fate of plant residues of guazatine, rats were orally dosed with the

homogenate of dwarf apple fruit treated with ^{14}C -guazatine-TA for 12 weeks (Expt.-2). Radioactive residues in the homogenate may reflect the real residual nature of guazatine in natural environments. As a reference of Expt.-2, rats were orally administered ^{14}C -guazatine itself (3 mg/kg) together with untreated dwarf apple fruit (Expt.-3).

Figures 1-b and 1-c illustrate the cumulative excretion of radioactivity in the urine and feces of rats in Expts.-2 and -3, respectively. In Expt.-3, the urinary excretion reached 13.1% of the dosed radioactivity in 7 days. We reported that after a single oral administration of ^{14}C -guazatine (3 mg/kg, control group), 4.6% of the dosed radioactivity was excreted in the urine in 7 days and the bioavailability of ^{14}C -guazatine after oral administration was estimated to be 8–14% by comparing the urinary output data and blood- ^{14}C pharmacokinetic data with those of the intra-

venously treated rats.¹²⁾ Thus, the urinary excretion in Expt.-3 was approximately three times greater than that of the control group, suggesting that the uptake of the compound with dwarf apple fruit increased the bioavailability of ¹⁴C-guazatine in rats.

The urinary excretion of Expt.-2 group accounted for only 4.6% of the dose. This might owe to a decrease in the bioavailability of ¹⁴C-guazatine apple residues caused by some stress during administration, since the rats were given the residues in the apple fruit homogenate five times under slight ether anesthesia in 8 hr.

The total ¹⁴C-levels in representative tissues 7 days after dosing are also shown in Table 1. The results of Expt.-3 were quite similar to those of the control group; the kidney was also found to be the principal target organ for ¹⁴C-retention where approximately 29% of the radioactivity in the whole body (except gut and its content) was retained. In contrast, the total-¹⁴C levels in the representative tissues of Expt.-2 were extremely low. This is probably due to the lower dose level of ¹⁴C-guazatine-TA (0.16 mg/kg) and the extremely lower dose level of the photoproducts (less than 0.04 mg eq. of ¹⁴C-guazatine-TA/kg). However it should be noted that even in this Expt.-2 group a significant level of ¹⁴C was detected in the kidney.

The amount of radioactivity in the urine of Expt.-2 group was not sufficient for TLC analysis of urinary metabolites. The data thus obtained, however, suggested that when rats were orally given ¹⁴C-guazatine residue in apple fruit, the general fate of radiocarbon was similar to that of the orally administered ¹⁴C-guazatine. This was probably due to the fact that the residues mainly consisted of unchanged guazatine (more than 80%).

3. Conclusion

The fate of the photoproducts of ¹⁴C-guazatine orally dosed to rats was found to be apparently different from that of ¹⁴C-guazatine-TA, characterized by the higher bioavailability (more than 26%), the higher retention in the liver, and the lower or negligible affinity to the kidney than guazatine. Approximately 25% of the urinary metabolites of the photoproducts were found to be specific, while 75% were common to ¹⁴C-guazatine.

Since the residue of dwarf apple fruit treated with ¹⁴C-guazatine chiefly consisted of unchanged guazatine (more than 80%), the metabolic fate of the residue in rats seemed to be similar to that of ¹⁴C-guazatine itself. The effect of the

photoproducts was below the observable level probably due to their low contents in the residue. The results can well reflect the metabolic fate of real plant residue of guazatine ingested by mammals.

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要 約

グアザチンの植物体残留物の雄ラットにおける代謝運命

佐藤 清, 加藤保博, 牧 伸一, 俣野修身, 後藤真康
グアザチンの植物中での主分解生成物である光分解物の雄ラットにおける代謝運命を, ¹⁴C-グアザチンの光分解混合物を1回経口投与することにより調べた。本光分解混合物の組成は、未変化物(39%)と主光分解物(35%)および約8種の微量光分解物であった。光分解物に由来する¹⁴Cの動態は、吸収率がグアザチンにくらべ高い点(26%以上)と、¹⁴Cの主貯留臓器が肝臓であり腎臓への貯留性が認められない点で、グアザチンの体内動態とは明らかに異なっていた。また、光分解物に由来する尿中代謝物の約25%は光分解物に固有であり、そのほかはグアザチンと共通であった。一方、植物体残留物の摂取実験に近い試験として、イヌリンゴ果実(¹⁴C-グアザチン処理12週間後)の磨砕物を雄ラットに2時間間隔で5回経口投与した。果実中の放射性残留物の80%以上が未変化のグアザチンであったため、¹⁴Cの体内動態は¹⁴C-グアザチンの単独投与時と差がなかった。