Nuclear Medicine & Biology, Vol. 23, pp. 97–100, 1996.

ISSN 0969-8051/96/$15.00 + 0.00
SSDI 0969-80518051(95)02072-1

Pharmacokinetic Studies of ^{131}I-Stevioside and its Metabolites

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ABSTRACT. **^{131}I-STEVIOSIDE (1.10 MBq) was injected i.v in Wistar male rats, in distribution in the body and metabolism were studied. The highest concentration of radioactivity was observed in the liver and in the small intestine after 10 and 120 minutes, respectively. At 2 h after injection, the radioactivity eliminated in the bile was 52.0% of the original dose. The results of RP-HPLC analysis of the bile showed that stevioside was degraded *in vivo* and that steviol appeared as a major metabolite. However, in the urine; RP-HPLC analysis did not show the presence of steviol.** NUCL MED BIOL 23;1:99–102, 1996.

KEY WORDS. ^{131}I-Stevioside, Metabolites, Wistar rat

INTRODUCTION

Stevia rebaudiana is a shrub of the compositae family widely found in Paraguay and Brazil, (Bridel & Lavielle, 1931; Felippe, 1977). The leaves of *Stevia rebaudiana* contain large amounts of stevioside, which is composed of steviol, a diterpenic carboxylic alcohol, and three glucose molecules. Stevioside has two glucose molecules in β (1 − > 2) linkage attached to the hydroxyl group of steviol and other molecule attached to the carboxyl group. (Mosettig and Nes. 1955).

Stevioside is 300 times sweeter than sucrose and it has been employed as a non-caloric sweetener in Brazil, Japan and other countries (Ishii *et al.* 1987).

The toxicity of stevioside has been extensively investigated (Akashi and Yokoyama. 1975; Lee *et al.* 1979; Yamada *et al.* 1985). The results of these investigations suggest that stevioside presents low toxicity in mammals. *In vitro* studies have shown that rat intestinal microflora degrade stevioside to steviol (Wingard *et al.* 1980). Although stevioside demonstrates no mutagenic activity, steviol is mutagenic for *Salmonella typhimurium* TM677 (Pezzuto *et al.* 1985; Pezzuto *et al.* 1986).

Since the use of stevioside as a sugar substitute continues to increase, the purpose of this study was to investigate the biological distribution, its excretion and the possibility of stevioside being degraded to steviol *in vivo*, when injected i.v in rats.

MATERIALS AND METHODS

Stevioside and steviol were kindly provided by Dr. Mauro Alvarez (Maringá University, Brazil). They were labeled with ^{131}iodine (IPEN/CNEN, São Paulo, Brazil) using the method described by Mathor *et al* (1989). The radiochemical purity (97%) was determined by electrophoresis in Whatman No. 1 paper with sodium acetate buffer, pH 5.4 and 300 volts for 60 min. The specific activity was 3, 7 MBq/mg.

Samples of urine and bile were analyzed for labeled compounds by high performance liquid chromatography (HPLC), using a 25.0 × 0.4 cm Nucleosil C-18 column and elution with methanol: NaOH 5 mM (65:35), as described by Alvarez *et al* (1987). The flow rate was 1.5 mL/min.

^{131}I-Stevioside (1, 10 MBq) was injected i.v in Wistar male rats weighing about 300 g. Before the test, they were fasted for 16 h, sacrificed by decapitation at 1, 3, 5, 10, 30, 45, 60, 120, 240, and 1440 min after injection and the blood was collected in heparinized tubes. The tissues were removed, washed with water and the radioactivity was counted. Intestinal contents of some animals were removed for the determination of radioactivity.

In some animals the bile duct was cannulated with polyethylene tube under urethane anesthesia, as described by Atallah and Glenn (1982). ^{131}I-Stevioside (1.10 MBq) was injected i.v and the bile was collected for 4 h at intervals of 60 min.

Animals were housed individually in metabolic cages for 24 h. Urine and feces were collected during this period for the determination of the radioactivity.

Statistics

All data related to percentage of the injected dose present in the small intestine are expressed as the mean ± SD and represent six analyses. Statistical significance was evaluated using Student's test.

RESULTS

The plasma level and tissue concentrations of radioactivity, expressed as percentage of the ^{131}I-Stevioside injected dose, are shown in Table 1. As can be seen, the radioactivity present in the plasma decreased rapidly, showing a fast biological distribution.

The radioactivity level of tissues such as heart, lung, stomach, spleen, testes, and muscles was less than 1.80% of the injected dose during the whole experiment, but the level increased in the thyroid. In the Kidney, the highest level was observed three minutes after injection of ^{131}I-Stevioside. The highest concentration of radioactivity in the tissues was observed in the liver and in the small intestine after 10

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Accepted 25 April 1995.

TABLE 1. Tissue levels of radioactivity after injection i.v. of the ^{131}I-Stevioside in rats

Tissue	1 min	3 min	5 min	10 min	30 min
Heart	0.59 ± 0.01	0.56 ± 0.06	0.36 ± 0.007	0.20 ± 0.005	0.16 ± 0.01
Lung	1.78 ± 0.33	1.25 ± 0.24	0.79 ± 0.09	0.61 ± 0.13	0.36 ± 0.09
Stomach	0.42 ± 0.10	0.55 ± 0.06	0.38 ± 0.09	0.40 ± 0.08	0.34 ± 0.04
Spleen	0.24 ± 0.04	0.32 ± 0.05	0.21 ± 0.01	0.09 ± 0.02	0.06 ± 0.01
Testis	0.18 ± 0.03	0.20 ± 0.03	0.16 ± 0.02	0.17 ± 0.03	0.17 ± 0.01
Muscle	0.09 ± 0.01	0.12 ± 0.02	0.06 ± 0.01	0.09 ± 0.02	0.04 ± 0.009
Kidney	4.34 ± 0.82	5.84 ± 0.58	2.54 ± 0.31	2.35 ± 0.85	1.32 ± 0.30
Liver	28.20 ± 2.53	38.20 ± 3.68	42.60 ± 6.04	44.69 ± 4.75	30.50 ± 1.35
S. intest.	5.22 ± 0.39	6.70 ± 0.42	16.84 ± 1.06	18.35 ± 2.80	42.02 ± 6.23
L. intest.	0.33 ± 0.06	0.46 ± 0.11	0.56 ± 0.05	0.41 ± 0.08	0.42 ± 0.10
Thyroid	0.12 ± 0.01	0.14 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.11 ± 0.02
Plasma	51.14 ± 6.70	40.74 ± 4.10	20.10 ± 1.61	16.22 ± 2.74	6.16 ± 1.04

Tissue	45 min	60 min	120 min	240 min	1440 min
Heart	0.15 ± 0.02	0.13 ± 0.03	0.07 ± 0.008	0.10 ± 0.02	0.02 ± 0.06
Lung	0.28 ± 0.02	0.59 ± 0.20	0.17 ± 0.04	0.23 ± 0.03	0.06 ± 0.01
Stomach	0.42 ± 0.08	0.40 ± 0.04	0.61 ± 0.13	0.89 ± 0.22	0.08 ± 0.03
Spleen	0.09 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.02 ± 0.00
Testis	0.18 ± 0.01	0.10 ± 0.02	0.14 ± 0.02	0.24 ± 0.03	0.04 ± 0.01
Muscle	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.007 ± 0.01
Kidney	0.77 ± 0.15	0.84 ± 0.15	0.59 ± 0.06	0.48 ± 0.06	0.14 ± 0.02
Liver	14.35 ± 0.85	9.90 ± 0.92	4.77 ± 0.58	4.05 ± 0.38	0.51 ± 0.08
S. intest.	57.20 ± 11.0	59.30 ± 3.60	65.80 ± 6.29	16.30 ± 3.80	5.02 ± 0.57
L. intest.	0.46 ± 0.06	0.60 ± 0.12	1.10 ± 0.48	40.00 ± 6.23	10.70 ± 0.22
Thyroid	0.39 ± 0.07	0.26 ± 0.09	0.84 ± 0.33	2.21 ± 0.61	5.98 ± 1.42
Plasma	5.71 ± 0.70	5.60 ± 0.70	4.51 ± 0.29	4.02 ± 0.36	0.83 ± 0.14

(Expressed as % of the injected dose)
The values represent the means ± SD for groups of six animals, S. intest. = Small intestine; L. intest. = Large intestine.

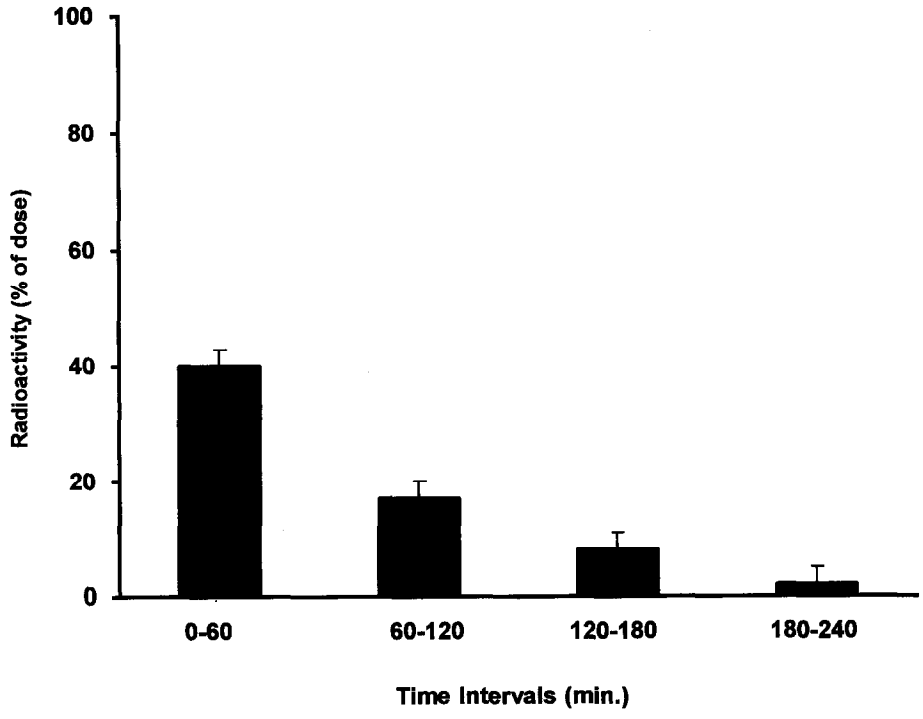

FIG. 1. Percentage of the injected dose excreted into the bile. Values are means ± SD (n = 3). After injection i.v of ^{131}I-Stevioside in rats, bile was collected for 4 h at intervals of 60 min.

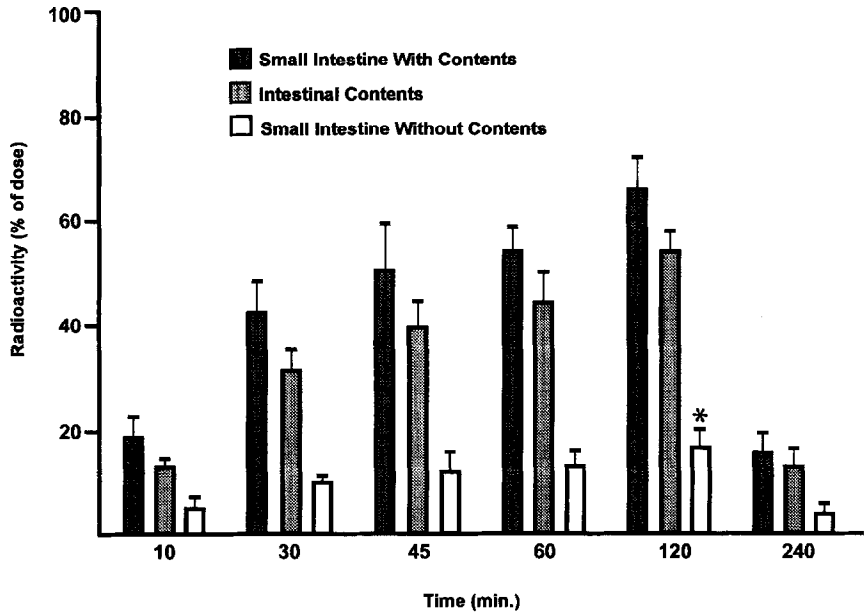

FIG. 2. Percentage of the injected dose present in the small intestine. Each column represents the means ± SD for groups of six animals *$p \leq 0, 05$ compared to 10, 30, 45, 60, and 240 min groups to small intestine without contents.

and 120 min, respectively. The radioactivity level in the large intestine increased 240 min after injection, while radioactivity in the small intestine decreased.

The radioactivity eliminated into the bile 120 min after injection was about 52.0% of the injected dose, as shown in Fig. 1.

Most of the radioactivity in the small intestine (52.0%) was present in the intestinal contents 120 min after injection, while about 17.0% was found in the intestine tissues as shown in Fig. 2.

Excretions of radioactivity into the urine and feces are shown in Table 2. Fecal and urinary excretions were determined only at 24 h after injection of the ^{131}I-Stevioside; the radioactivity excreted into the feces and urine were 35, 30% and 34, 70%, respectively.

RP-HPLC analysis of the bile and urine are shown in Fig. 3. Steviol appeared as a major metabolite (47.3% of radioactivity present in the bile, while stevioside represented 37.3% and the remaining 15.4% was due to an unidentified metabolite with a retention time of 8.0 min. In the urine, RP-HPLC analysis showed the presence of stevioside and an unknown metabolite with the same retention time as that observed in the bile.

DISCUSSION

The disposition and metabolism of ^{131}I-Stevioside after injection i.v in rats were studied. Plasma radioactivity decreased rapidly and reached 5.60% of the injected dose 60 min after administration, showing a fast distribution in the body.

The results of tissues distribution showed that radioactivity reached the small intestine through the liver, showing the highest dose 2 h after

TABLE 2. Excretion of radioactivity after injection i.v. of ^{131}I-Stevioside

Time (h)	Urine (% dose)	Feces (% dose)
24	34.70 ± 6.30	35.30 ± 3.55

(% of the injected dose)
The values represent the mean ± SD for groups of six animals.

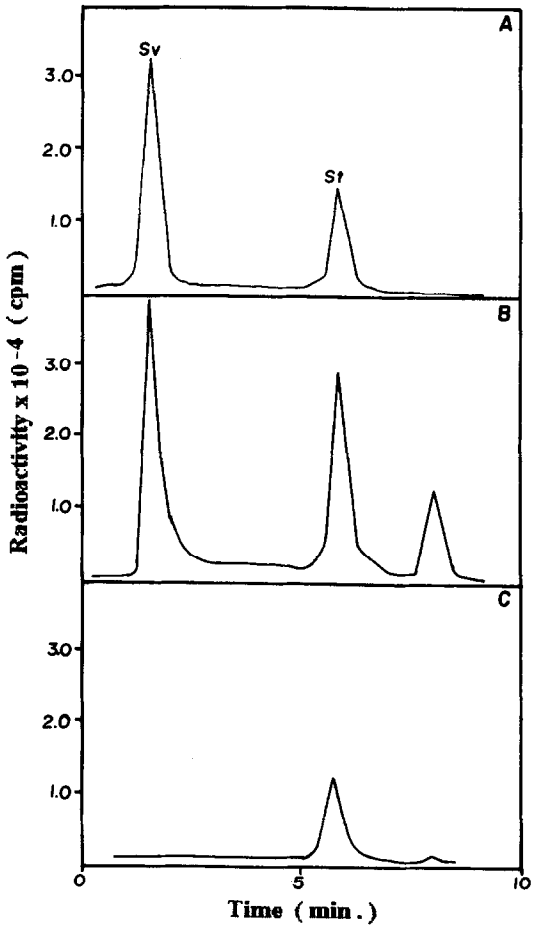

FIG. 3. High performance liquid chromatography profile of: A-Standards of steviol(sv) and stevioside(st) labelled with ^{131}I. B and C-Bile and urine samples, respectively, were collected for 90 minutes after injection of the ^{131}I-stevioside.

injection. The radioactivity distribution in the small and large intestine showed that most of the radioactivity was present in the intestinal contents and that a part of the radioactivity was absorbed from the bowel of the rat.

Wingard *et al.* (1980) have reported that stevioside is degraded *in vitro* by the rat fecal microflora to steviol and that the steviol was completely absorbed from lower bowel of the rat after oral administration.

The results obtained of bile HPLC-analysis showed that stevioside was degraded to steviol and an unidentified metabolite. The stevioside was probably metabolized in the liver, since the metabolites were detected in the bile of the cannulated duct. Thus, it seems that stevioside is metabolized in the intact rat to steviol, which is excreted through the bile.

An unknown compound was excreted in the bile and also appeared in the urine. Steviol was not detected in the urine collected 90 min after injection. Its excretion in this fluid, however, can not be ruled out, since the time elapsed after injection was not sufficiently long.

As the literature reports that steviol presents mutagenic activity (Pezzuto *et al.* 1985), the results presented in this communication, raise the question about the consequences of the degradation of stevioside to steviol *in vivo*. A similar degradation is supposed to occur in man.

The authors are grateful to Dr. Tasso M. Santos and Dr. J. L. Pesquero for helping in the preparation of this manuscript and to Alfredo Santos for technical assistance.

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