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# Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men

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ABSTRACT

This randomized, double-blind, cross-over study assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside in healthy adult male subjects. Steviol glucuronide appeared in the plasma of all subjects after administration of rebaudioside A or stevioside, with median $t_{max}$ values of 12.0 and 8.00 h post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 h for both compounds. Administration of rebaudioside A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean $C_{max}$ value (1472 ng/mL) than administration of stevioside (1886 ng/mL). The geometric mean $AUC_{0-t}$ value for steviol glucuronide after administration of rebaudioside A (30,788 ng h/mL) was approximately 10% lower than after administration of stevioside (34,090 ng h/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72 h collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety or vital signs.



## 1. Introduction

Steviol glycosides are a group of intensely sweet compounds that have been extracted and purified from *Stevia rebaudiana* (Bertoni) for use as a sweetener in food and beverages. Of the glycosides that are responsible for sweetness, stevioside and rebaudioside A are the most abundant in the Stevia plant (JECFA, 2005). Both compounds are diterpenoid glycosides that differ only by the presence of a single glucose moiety (Fig. 1).

Human digestive enzymes of the mouth, stomach, and small intestine do not hydrolyze steviol glycosides (Koyama et al., 2003a; Hutapea et al., 1997), whereas *in vitro* and *in vivo* studies have shown that both rebaudioside A and stevioside are hydrolyzed to the aglycone steviol by microflora in the colon *via* the successive removal of glucose units (Gardana et al., 2003; Geuns et al., 2003, 2007) and that this hydrolysis is required before absorption can occur. Hydrolysis has also been demonstrated following incubation with intestinal microflora from the rat cecum (Wingard et al., 1980), and human fecal bacteria (Gardana et al., 2003; Koyama et al., 2003a). Rebaudioside A has been shown to undergo *in vitro* hydrolysis to the aglycone more slowly than stevioside (Gardana et al., 2003). This is thought to be due to the additional glucose moiety on rebaudioside A and suggests that systemic exposure to its aglycone steviol may be lower following ingestion of rebaudioside A than stevioside. These authors also reported that

*Abbreviations:* Ae, amount excreted into urine or feces; AE, adverse event; ALT, alanine aminotransferase; ANOVA, analysis of variance; APCI, atmospheric pressure chemical ionization; AST, aspartate aminotransferase; $AUC_{0-t}$, area under the plasma concentration–time curve to the time of the last quantifiable sample; bpm, beats per minute; CFR, Code of Federal Regulations; $CL_R$, renal clearance; $C_{max}$, peak concentration; d, day; ECG, electrocardiogram; FDA, Food and Drug Administration; GCP, Good Clinical Practices; gamma GT, gamma glutamyl transpeptidase; Hb, hemoglobin; Hct, hematocrit; Hg, mercury; HPLC, high performance liquid chromatography; ICH, International Conference of Harmonisation; IRB, Institutional Review Board; IS, internal standard; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LC, liquid chromatography; LLOQ, lower limit of quantitation; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; $\lambda_z$, terminal elimination rate constant; PK, pharmacokinetic; psi, pounds per square inch; RBC, red blood cells; SAS, statistical analysis software; $t_{1/2}$, half-life; $t_{max}$, time to reach maximum concentration; WBC, white blood cells.

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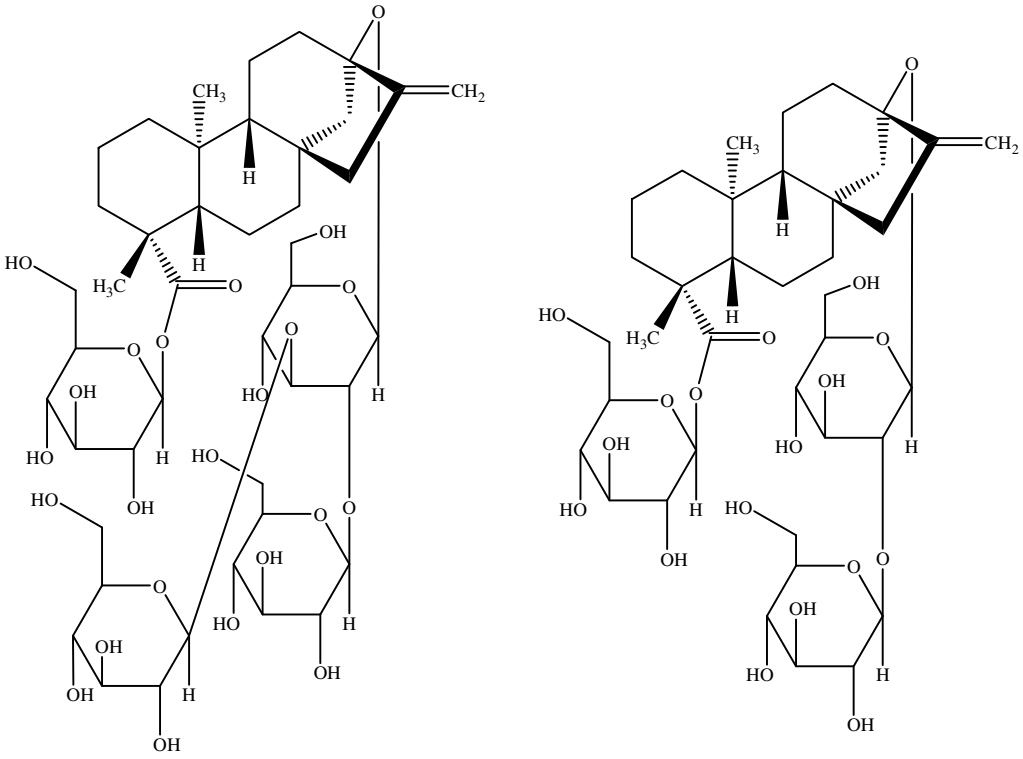



**Fig. 1.** Comparison of the chemical structures of rebaudioside A and stevioside.

among the human fecal cultures tested, *bacteroides* species were most efficient in metabolizing these compounds. Characteristics of intestinal absorption and transport of steviol glycosides and the aglycone steviol have been investigated in a Caco-2 cell monolayer model (Geuns et al., 2003) and in rat everted gut sacs (Koyama et al., 2003b). In Caco-2 cells, transport of stevioside and rebaudioside A was very low, with apparent permeability coefficients, $0.16 \times 10^{-6}$ and $0.11 \times 10^{-6}$ cm/s, respectively. In contrast, transport of the aglycone steviol was more effective, demonstrating a higher apparent permeability coefficient for absorptive transport of $38.6 \times 10^{-6}$ cm/s. A similar observation was made in rat everted gut sacs by Koyama et al. (2003b). In this *ex vivo* model, no significant absorption of a stevia mixture (steviol glycosides) occurred, while steviol was readily absorbed.

Steviol glucuronide has been identified in both plasma and urine of human subjects following administration of stevioside (Geuns et al., 2006, 2007). In rats, rebaudioside A and stevioside are metabolized to steviol glucuronide (Fig. 2) and excreted in the bile and returned to the intestine where it undergoes deconjugation prior to elimination in the feces (Roberts and Renwick, 2008).

The present study was designed to examine the comparative pharmacokinetics and metabolism of rebaudioside A and stevioside in healthy adults in order to establish whether the two steviol glycosides are handled similarly in humans.

## 2. Methods

### 2.1. Study design

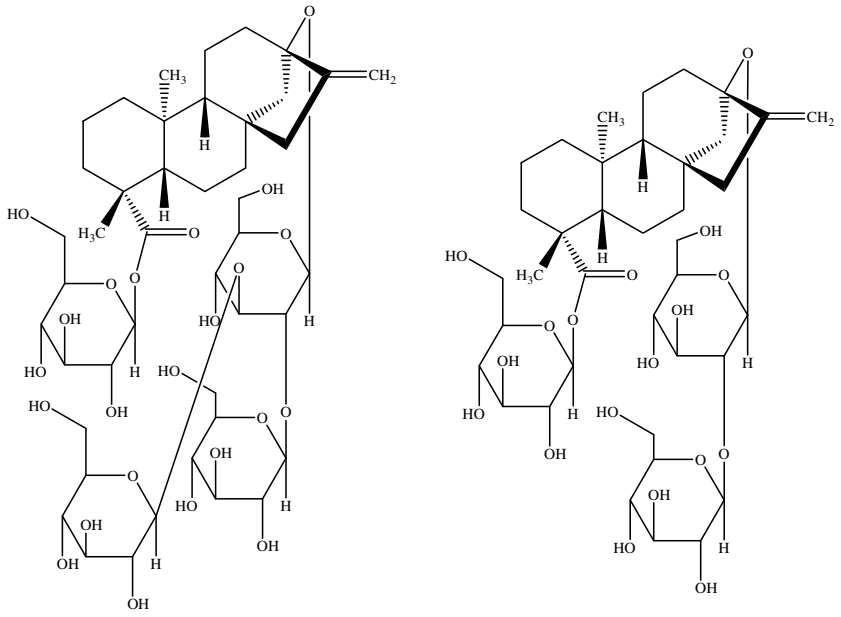
This was a randomized, double-blind, two-way cross-over study assessing the effects of single doses of stevioside and rebaudioside A in healthy adult male subjects ($n = 8$). The study was conducted according to US Title 21 CFR, along with the applicable International Conference on Harmonisation (ICH) Guidelines, commonly known as Good Clinical Practices (GCPs), which are consistent with the Declaration of Helsinki. An Institutional Review Board (IRB) reviewed and approved the protocol and informed consent documentation prior to study initiation. Signed informed consent was obtained from each subject prior to any study procedures, and all subjects were informed of their right to withdraw from the study without prejudice at any time.

### 2.2. Subjects

In order to be eligible for the study, subjects were required to be healthy males between the ages of 18 and 45 with a body mass index in the range of 19.0–29.0 kg/m$^2$. Subjects were also required to have sufficient understanding of the implications of the study and to be able to provide written informed consent. Subjects with clinically relevant medical history, physical findings, ECG or laboratory values at the pre-trial screening assessment, acute or chronic illness or history of chronic illness, impaired endocrine, thyroid, hepatic, respiratory, or renal function, diabetes mellitus, coronary heart disease, or history of any psychotic mental illness were excluded from the study. The study also excluded subjects with a history of severe adverse reaction to any drug or sensitivity to artificial sweeteners, pre-trial use of prescription medications (within 28 days) or over-the-counter medications, herbal remedies or dietary supplements (within 14 days), use of *Stevia* leaves or extracts within one month of the study, and those who had participated in a clinical trial of a prescription medication or new chemical entity within three months of the study. In addition, all subjects with current or previous substance abuse, intake of more than 21 units of alcohol or use of tobacco products within six months, seated systolic blood pressure outside the range 90–140 mmHg, seated diastolic blood pressure outside the range 40–90 mmHg or heart rate outside the range 40–100 bpm, or evidence of drug abuse, positive tests for hepatitis or HIV, or loss of blood exceeding 400 mL within three months, were also excluded.

### 2.3. Study product and procedures

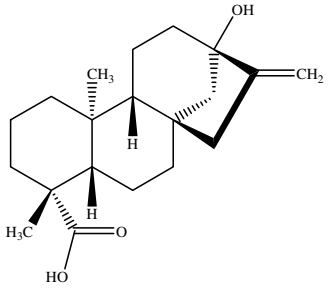
Unlabeled rebaudioside A (rebiana, the common name for high purity rebaudioside A; purity 98.7% by HPLC) and unlabeled stevioside (purity 96.6% by HPLC) were supplied by Cargill, Incorporated. Moisture analysis was conducted prior to dose preparation to assure accurate preparation of aqueous solutions containing the test substances. Subjects received single oral doses of 5 mg/kg rebaudioside A and 4.2 mg/kg stevioside, providing exposure of approximately 1.6 mg/kg of steviol equivalents. The test articles were constituted, diluted, packaged, and labeled at the clinic.



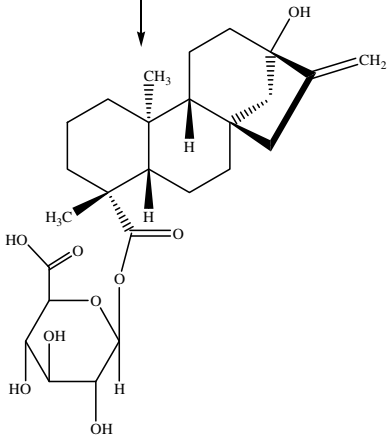
**Rebaudioside A**



**Stevioside**



**Steviol**



**Steviol glucuronide**

**Fig. 2.** Metabolism of rebaudioside A and stevioside.

All subjects underwent a screening visit within three weeks of entering the study. After meeting all entry criteria, each subject received a single dose of rebaudioside A and stevioside as an aqueous solution in a randomized sequence with at least 14 days between treatments. Subjects were resident in the clinic from the morning prior to dosing, until 72 h post-dose for each treatment. Subjects had a follow-up visit 5–10 d after the second treatment. Subjects were fasted overnight from 23:00 on the evening prior to dosing until lunchtime on the day of dosing. Standard meals and drinks were provided at 4, 10 and 24 h post-dose. No food or drink containing grapefruit was allowed from 7 days pre-dose until the end of the trial. No alcoholic or caffeinated drinks were permitted during the period from 24 h pre-dose until the end of each period of residence. Subjects refrained from exercising from 24 h pre-dose until discharge from the facility.

### 2.4. Study assessments

Medical history was documented at screening, while physical examinations were conducted at both screening and prior to discharge from the study. Body weight and height were recorded at entry into the study, and weight again at the end of the study. Vital signs were recorded at regular intervals throughout the study both at rest and supine. A 12 lead ECG was recorded at screening, and on each day while resident in the clinic. Tests for drugs of abuse (amphetamines, cocaine, methamphetamine, cannabis, barbiturates, methadone, benzodiazepines, opiates, phencyclidines and methylenedioxymethamphetamine) as well as blood alcohol and breath carbon monoxide content were conducted at screening, and on each admission day.

Blood samples were collected at screening, pre-dose, and daily throughout the admission period, and at follow-up for hematology and clinical chemistry. Hematology analyses included hemoglobin (Hb), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit, white blood cells (WBC) and differential, and platelets. Clinical chemistry included urea, creatinine, uric acid, total bilirubin, total protein, albumin, globulin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma GT, glucose, phosphate, cholesterol, triglycerides, potassium, sodium, calcium, chloride, bicarbonate and urinalysis (dipstick test: protein, blood, ketones, glucose, bilirubin, urobilinogen, leukocyte esterase, specific gravity, nitrites, pH) and were conducted at the same time as hematology. Adverse events were assessed throughout the study. Adverse events were defined as any untoward medical occurrence regardless of relationship to study product.

### 2.5. Pharmacokinetic endpoints

Samples were collected for analysis of steviol and steviol glucuronide in plasma prior to dosing, and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36 and 72 h after dosing. Urinary output was collected during the following periods: −12 to 0 h pre-dose, and at 0–3, 3–6, 6–12, 12–24, 24–48 and 48–72 h post-dose. Fecal collections were made as individual evacuations, from 12 h pre-dose through 72 h post-dose.

### 2.6. Sample analysis

#### 2.6.1. Plasma and urine sample preparation

Plasma and urine samples were stored at −70 °C until the samples were removed for extraction and sample preparation. Samples were allowed to thaw completely at room temperature prior to the sample preparation procedure. The samples were prepared by solid-phase extraction. A 96-well HLB μElution plate (Waters, Millford, MA) was used for the plasma and urine sample extractions. The plates were prepared for use by rinsing each well with 200 μL methanol followed by 200 μL 1% formic acid. Samples were prepared by transferring 200 μL of sample to a 2-mL polypropylene microcentrifuge tube before adding the internal standard (IS), isosteviol. After the addition of the IS and, 200 μL of 1% formic acid, the samples were vortexed and microcentrifuged at 13,000 rpm for 10 min. After centrifugation, the supernatant was transferred to the μElution plate and a slight vacuum was applied to draw the samples through the well at an approximate flow rate of 1 mL/min. They were then rinsed with 200 μL of 1% formic acid and dried under maximum vacuum for 5 min. The target analytes were eluted from the well by adding 200 μL of methanol to the well and carefully applying a light vacuum to start the elution. After elution, the sample extracts were taken to dryness in a Zipvap concentrator with nitrogen and reconstituted in 100 μL methanol.

#### 2.6.2. Preparation of fecal samples for HPLC/MS/MS analysis

Fecal samples were stored at −70 °C until the samples were removed for extraction. Samples were allowed to thaw at room temperature prior to the sample preparation procedure. Fecal samples (total) were prepared by adding two volumes of water to one volume of sample on a weight to volume basis (mL water to grams of feces) and homogenized until a consistent suspension was obtained. Internal standard (isosteviol) was added to the fecal homogenate, acidified with formic acid and extracted with a 0.75 g aliquot of *tert*-butyl ether (MTBE) following shaking. The samples were then centrifuged and the MTBE decanted. The extracts were concentrated to dryness using a Turbovap II (Zymark, Hopkinton, MA) with a bath temperature of 40 °C and a nitrogen pressure of 20 psi. The dried sample extracts were reconstituted in 500 μL methanol.

#### 2.6.3. Bioanalytical methods

Steviol (purity 99.9% by HPLC), isosteviol (purity 99.88% by HPLC) and steviol glucuronide (purity 96.4% by HPLC) reference standards were supplied by The Coca-Cola Company.

The concentrations of steviol and steviol glucuronide in plasma, urine and feces were measured using an LC/MS/MS method that was validated according to the current FDA guidelines (May 2001). The analyses were performed using an Applied Biosystems QTrap 4000 hybrid mass spectrometer equipped with a LEAP HTC PAL autosampler and twin Shimadzu LC20AD pumps. A Sunfire, 2.5 μm, 50 × 4.6 mm (Waters, Milford, MA) column was used and separation was achieved using a gradient elution using 0.1% formic acid (A) and 0.1% formic acid in acetonitrile (B) changing from 95% A to 95% B over 10 min at a flow rate of 1 mL/min. Separation was performed at ambient temperature, resulting in retention times of: steviol glucuronide, 5.95 min; steviol, 7.42 min and isosteviol, 8.27 min. The sample extracts were kept at 4 °C while in the autosampler drawer and stored at −70 °C after analysis.

The extracts were analyzed using atmospheric pressure chemical ionization (APCI) with the following general parameters (compound specific information is provided below): curtain gas 20 psi; collision gas 8; probe temperature 600 °C; GS1 35 L/min; GS2 30 L/min.

Two transitions were used for the analysis. The transition that provided the best sensitivity was used for quantitation, and the second transition was used for confirmation. The following compound specific conditions were used:

*Steviol glucuronide*: negative ion APCI, primary transition: 493–317 amu; secondary transition: 493–113 amu; Declustering potential −75 V.

*Steviol:* positive ion APCI, primary transition: 301–255 amu; secondary transition: 301–91 amu; declustering potential 56 V.

*Isosteviol*: negative ion APCI in the MS mode measuring the ion at 317 amu. No MS/MS fragmentation could be achieved for this compound.

For urine, the steviol lower limit of quantitation (LLOQ) was 50 ng/mL with a linear response from 50 ng/mL to 7500 ng/mL, and the steviol glucuronide LLOQ was 250 ng/mL with a linear response from 250 ng/mL to 7500 ng/mL. For plasma, the steviol and steviol glucuronide LLOQ was 100 ng/mL with a linear response from 100 ng/mL to 7500 ng/mL. For feces, the steviol LLOQ was 167 ng/g with a linear response from 150 ng/g to 20,000 ng/g and the steviol glucuronide LLOQ was 5000 ng/g with a linear range from 1000 ng/g to 20,000 ng/g.

### 2.7. Pharmacokinetic analysis

Noncompartmental methods were used to determine the pharmacokinetics of steviol and steviol glucuronide in plasma, urine and feces. The following pharmacokinetic (PK) parameters were determined: $C_{max}$, $t_{max}$, $t_{½}$, terminal rate constant ($\lambda_z$), $AUC_{0-t}$, and $AUC_{0-inf}$ of steviol and steviol glucuronide in plasma; amounts excreted in urine and feces (Ae) and $CL_R$ of steviol and steviol glucuronide in urine; and Ae of steviol and steviol glucuronide in feces. PK parameters were calculated using WinNonlin (Pharsight Corporation, Version 5.2).

### 2.8. Statistical methodology

Data from all subjects who received a test article were included in the analysis of safety. Numerical data were summarized using means, medians, and other descriptive statistics according to the type and distribution of the data. Adverse events were listed and relationship to treatment assessed. Where data were available, treatment-related differences were examined between test (rebaudioside A) and reference (stevioside) for steviol and steviol glucuronide using a linear mixed-model approach with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect. An analysis of variance (ANOVA) was performed for $CL_R$, and natural log (ln) transformed $C_{max}$, ln-$AUC_{0-t}$, ln-$Ae_f$(0–72) and ln-$Ae_u$(0–72). The 90% confidence intervals for the test group mean relative to the reference group mean were obtained by taking the antilog of the corresponding 90% confidence intervals for the difference between the means on the log scale for ln-$C_{max}$, ln-$AUC_{0-t}$, ln-$Ae_f$(0–72) and ln-$Ae_u$(0–72). The 90% confidence interval for $CL_R$ was also presented. Statistical analysis of the PK parameters was performed by using SAS (SAS Institute Inc.; Cary, NC, USA).

## 3. Results

### 3.1. Subject characteristics

All subjects enrolled in the study met all entry criteria and completed observations until day 28 of the study, and therefore, were included in the safety and pharmacokinetic populations. Mean subject age was 28 years and mean body mass index was 23.8 kg/m$^2$.

### 3.2. Pharmacokinetic analyses

Plasma analysis indicated that steviol was detected in plasma at levels above the lower limit of quantitation in only 1 out of 8 subjects following the administration of both compounds. Following administration of rebaudioside A, 227 ng/mL steviol was detected in the plasma of a single subject at the 72 h time point. Following administration of stevioside, 121 ng/mL steviol was detected in a single plasma sample 6 h following consumption of the dose (Table 1). Steviol was not detected above the lower limit of quantitation in any other plasma samples.

In contrast, steviol glucuronide appeared in plasma of all subjects after administration of rebaudioside A and stevioside. Steviol glucuronide was detected as early as 2 h post-dose (rebaudioside A, $n = 1$; stevioside, $n = 2$) and increased in concentration to peak after 12.0 and 8.00 h post-dose, respectively (Table 2 and Fig. 3). By 5 h post-administration of rebaudioside A and stevioside, steviol glucuronide was detected at levels above the lower limit of quantitation in 5 of 8 subjects and 7 of 8 subjects, respectively. Steviol glucuronide was then eliminated from the plasma, with mean $t_{1/2}$ values of 14.8 and 14.0 h after administration of rebaudioside A and stevioside, respectively (Table 2). Administration of rebaudioside A resulted in a significantly lower (22%) steviol glucuronide geometric mean $C_{max}$ value (1472 ng/mL) than administration of stevioside (1886 ng/mL). The geometric mean $AUC_{0-t}$ value for steviol glucuronide after administration of rebaudioside A (30,788 ng h/mL) was lower (9.7%) than after administration of stevioside (34,090 ng h/mL).

Summary tables of the pharmacokinetic parameters for steviol and steviol glucuronide are presented in Tables 1 and 2, respectively. A summary of the statistical analysis of pharmacokinetic data for steviol and steviol glucuronide (rebaudioside A relative to stevioside) is presented in Table 3. In the case of steviol, the entire profile for most subjects was below the lower limit of quantitation, therefore, only fecal excretion could be analyzed. The ratio of parameter means (ln-parameter) for fecal steviol excretion following a single oral dose of rebaudioside A and stevioside was 115% suggesting similarity between the two compounds. However, the 90% confidence interval (CI) for the ratio of parameter geometric means was 44–299% indicating large variability in the measurement due to the small number of fecal samples. Statistical analysis of steviol glucuronide in blood and urine also supported similarity between rebaudioside A and stevioside.

Small amounts of steviol were eliminated in urine after administration of rebaudioside A ($Ae_u(0–72) = 0.0510$ mg) and stevioside ($Ae_u(0–72) = 0.0238$ mg), while larger amounts were excreted in feces. The mean $Ae_f(0–72)$ values for steviol after administration of rebaudioside A (5.88 mg) and stevioside (6.50 mg) were similar. In contrast, steviol glucuronide was primarily excreted in the urine. Administration of rebaudioside A resulted in a similar steviol glucuronide mean $Ae_u(0–72)$ value (106 mg) to administration of stevioside (112 mg). In addition, mean renal clearance of steviol glucuronide after treatment with rebaudioside A (3.73 L/h) was comparable after administration of stevioside (3.36 L/h). Following oral administration of rebaudioside A and stevioside, no detectable amount of steviol glucuronide was found in the feces.

**Table 1**
Summary of the mean (SD) pharmacokinetic data for steviol

| Parameter (units) | Treatment | | | |
|---|---|---|---|---|
| | *N* | Rebaudioside A | *N* | Stevioside |
| $C_{max}$ (ng/mL) | 1 | 227 (NA) | 1 | 121 (NA) |
| $t_{max}$ (h) | 1 | 72.0 (NA) | 1 | 6.00 (NA) |
| $AUC_{0-t}$ (ng h/mL) | 0 | NA (NA) | 0 | NA (NA) |
| $AUC_{0-inf}$ (ng h/mL) | 0 | NA (NA) | 0 | NA (NA) |
| $t_{1/2}$ (h) | 0 | NA (NA) | 0 | NA (NA) |
| $\lambda_z$ (1/h) | 0 | NA (NA) | 0 | NA (NA) |
| $Ae_u(0–72)$ (mg) | 8 | 0.0510 (0.0877) | 8 | 0.0238 (0.0675) |
| $CL_R$ (L/h) | 0 | NA (NA) | 0 | NA (NA) |
| $Ae_f(0–72)$ (mg) | 6 | 5.88 (6.95) | 7 | 6.50 (7.08) |

NA = not applicable.

**Table 2**
Summary of the mean (SD) pharmacokinetic data for steviol glucuronide

| Parameter (units) | Treatment | | | |
|---|---|---|---|---|
| | *N* | Rebaudioside A | *N* | Stevioside |
| $C_{max}$ (ng/mL) | 8 | 1588 (700) | 8 | 2222 (1078) |
| $t_{max}$ (h) | 8 | 12.0 (6.02, 24.0) | 8 | 8.00 (6.00, 12.0) |
| $AUC_{0-t}$ (ng h/mL) | 8 | 33904 (15139) | 8 | 39928 (20129) |
| $AUC_{0-inf}$ (ng h/mL) | 4 | 46197 (18604) | 4 | 53211 (23782) |
| $t_{1/2}$ (h) | 4 | 14.8 (3.32) | 4 | 14.0 (5.61) |
| $\lambda_z$ (1/h) | 4 | 0.0483 (0.00908) | 4 | 0.0551 (0.0221) |
| $Ae_u(0–72)$ (mg) | 8 | 106 (24.0) | 8 | 112 (36.8)7) |
| $CL_R$ (L/h) | 8 | 3.73 (2.01) | 8 | 3.36 (2.51) |
| $Ae_f(0–72)$ (mg) | 6 | 0 (0) | 7 | 0 (0) |

$t_{max}$ is presented as Median (Min, Max).

### 3.3. Metabolism of rebaudioside A and stevioside

The fractional conversion and elimination of both steviol and steviol glucuronide in urine and feces are shown in Table 4. The results show that more than 64% of the dose was accounted for following both treatments. The primary route of elimination following oral administration of both rebaudioside A and stevioside was in urine as steviol glucuronide, accounting for approximately 59% and 62% of the dose, respectively. Feces accounted for approximately 5% of the dose in both cases with only a trace recovered in urine as steviol.

### 3.4. Safety results

The only adverse event reported during this study was an ecchymosis of the left antecubital fossa, the site of venipuncture, reported after stevioside treatment. The event was mild and unlikely to be related to study product; no action was taken, and the event resolved.

Sporadic out-of-range values occurred in several subjects for various biochemistry, hematology, and urinalysis measurements. None of these values were clinically significant, and there were no apparent treatment-related trends. Neutrophil values for 2 subjects shifted from low to normal, and lymphocyte values for 2 subjects shifted from high to normal (stevioside dosing). However, no apparent trends were noted in shift data (from baseline to follow-up) for laboratory parameters.

There were no post-dose vital sign measurements or changes reported as adverse events. There were some minor fluctuations in heart rate over time; however, there were no apparent treatment-related trends. There were no treatment related effects on ECG parameters at any time in the study.

## 5. Discussion

Similar metabolism and elimination pathways were observed after dosing with either rebaudioside A or stevioside. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide indicating rapid first-pass conjugation prior to urinary excretion (rebaudioside A: 59%; stevioside: 62%). Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). Administration of rebaudioside A

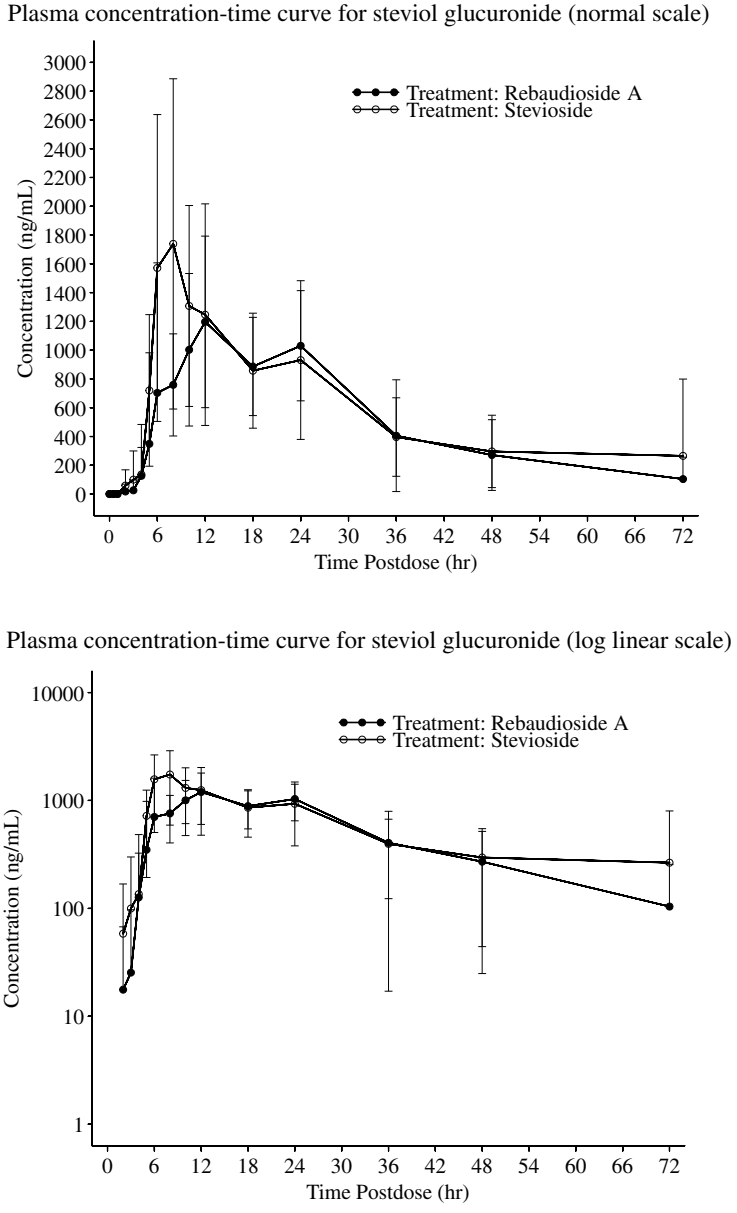



**Fig. 3.** (a) Plasma concentration-time curve for steviol glucuronide (normal scale). (b) Plasma concentration-time curve for steviol glucuronide (log linear scale).

**Table 3**
Summary of the statistical analysis of pharmacokinetic data for steviol and steviol glucuronide: rebaudioside A relative to stevioside

| Parameter (units) | *N* Test/*N* reference | Test mean[a] (rebaudioside A) | Reference mean[a] (stevioside) | Test/reference[b] | 90% Confidence interval[c] |
|---|---|---|---|---|---|
| *Steviol* | | | | | |
| $Ae_f$(0–72) (mg) | 6/7 | 6.23 | 5.42 | 114.96 | (44.22, 298.82) |
| *Steviol glucuronide* | | | | | |
| $C_{max}$ (ng/mL) | 8/8 | 1472 | 1886 | 78.05 | (50.64, 120.30) |
| $AUC_{0-t}$ (ng h/mL) | 8/8 | 30,788 | 34,090 | 90.31 | (57.25, 142.48) |
| $CL_R$ (L/h) | 8/8 | 3.73 | 3.36 | 0.37 | (−1.52, 2.26) |
| $Ae_u$(0–72) (mg) | 8/8 | 103 | 108 | 95.81 | (77.26, 118.82) |

[a] Least squares mean from ANOVA. Natural log (ln) parameter means calculated by transforming the natural log means back to linear scale (i.e., geometric means).
[b] Ratio of parameter means for ln-parameter (expressed as a percent). Natural log transformed ratios transformed back to linear scale. For $CL_R$, difference of means.
[c] 90% Confidence interval for ratio of parameter means of ln-transformed parameter (expressed as a percent). Natural log transformed confidence limits transformed back to linear scale. For $CL_R$, 90% confidence limits for difference of means.

resulted in significantly lower $C_{max}$ (approximately 22%) and longer $t_{max}$ values than stevioside for steviol glucuronide (geometric mean of 1472 ng/mL for rebaudioside A compared with 1886 ng/mL for stevioside). Rebaudioside A has one additional glucose moiety that must be removed prior to absorption as steviol in the colon, and therefore, it is not unexpected that the formation of steviol from stevioside would be more rapid than that of rebaudioside A. These observations support the earlier *in vitro* and *in vivo* findings

**Table 4**
Metabolism of a 5 mg/kg body weight dose of rebaudioside A and 4.2 mg/kg body weight dose of stevioside

| | | Amount (mg) | % of Dose[a] |
|---|---|---|---|
| *Rebaudioside A* | | | |
| Steviol | $Ae_u(0–72)$ | 0.051 | 0.04 |
| Steviol | $Ae_f(0–72)$ | 5.88 | 4.8 |
| Steviol glucuronide | $Ae_u(0–72)$ | 106 | 59 |
| Steviol glucuronide | $Ae_f(0–72)$ | 0 | 0 |
| *Stevioside* | | | |
| Steviol | $Ae_u(0–72)$ | 0.0238 | 0.02 |
| Steviol | $Ae_f(0–72)$ | 6.5 | 5.2 |
| Steviol glucuronide | $Ae_u(0–72)$ | 112 | 62 |
| Steviol glucuronide | $Ae_f(0–72)$ | 0 | 0 |

[a] Percentage represents molecular equivalents.

of Koyama et al. (2003a,b), which demonstrated hydrolysis of stevioside and rebaudioside A to the aglycone steviol within 10 and 24 h, respectively following incubation with human microflora. Following 72 h of incubation, steviol remained unchanged, suggesting that human microflora are not capable of hydrolyzing the steviol structure further.

The requirement for a plant-derived dietary constituent to undergo deglycosylation prior to intestinal absorption is not unique to steviol glycosides. For example, this requirement has also been described for soybean isoflavones (Setchell et al., 2002). The glycosides genistin and daidzin found in most soy foods, must undergo hydrolysis to their aglyone forms before they can be absorbed from the gastrointestinal tract. In a human pharmacokinetic study, the plasma appearance of isoflavones was significantly more rapid ($t_{max}$ = 1 h) following consumption of the aglycone forms as compared to consumption of the glycoside forms ($t_{max}$ = 6 h) (Kano et al., 2006). In contrast to steviol glycosides which have been shown to resist hydrolysis in the small intestine, isoflavone glycosides have been shown to undergo deglycosylation in the small intestine (Walsh et al., 2007). Similar to steviol glycosides, aglycone isoflavones can undergo conjugation with glucuronide, although isoflavone compounds are known to circulate in human plasma in a variety of forms (Shelnutt et al., 2002).

There were no meaningful differences observed in urinary recovery after administration of either rebaudioside A or stevioside. Koyama et al. (2003b) studied *in vitro* hepatic metabolism of steviol in both rat and human liver microsomes. These investigators reported similar oxidative metabolites generated by both rat and human microsomes that required an NADPH generating system, suggesting that cytochrome P450 may be involved.

The results of the present study are consistent with observations made in a recent comparative pharmacokinetic and excretion/mass balance study of $^{14}$C-rebaudioside A, $^{14}$C-stevioside, and $^{14}$C-steviol conducted in rats (Roberts and Renwick, 2008). In this investigation, maximum plasma concentration of radioactivity was attained quickly following oral administration of the aglycone steviol, $t_{max}$ = 0.25 h, as compared to rebaudioside A and stevioside. Similar to the observations made in humans, the pharmacokinetics and excretion of both $^{14}$C-rebaudioside A and $^{14}$C-stevioside in rats were also comparable. However, in contrast to rats, rebaudioside A and stevioside are excreted mainly as steviol glucuronide in the urine, with lesser amounts of steviol in the feces following oral administration. This difference is the result of the excretion of steviol glucuronide primarily *via* the bile in rats because they have a lower molecular weight threshold for biliary excretion compared to humans (Kwon, 2002; Renwick, 2008).

Only one mild adverse event was reported that quickly resolved and no clinically significant changes or findings were noted from clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs. Overall, the changes in the clinical safety assessments were unremarkable and there was no evidence of any effects on any safety parameter.

In summary, administration of both rebaudioside A and stevioside to healthy human subjects results in substantial formation of steviol glucuronide systemically with very limited amounts of steviol observed. These data are consistent with those from metabolism studies in rats where administration of radiolabeled rebaudioside A and stevioside resulted in metabolism to steviol, followed by extensive glucuronidation to steviol glucuronide. On the basis of the similarity in human metabolism to the primary metabolite steviol glucuronide following administration of rebaudioside A or stevioside through the classical phase II detoxification mechanism, it can be concluded that previous human studies and rodent toxicological studies conducted with stevioside are relevant for assessing the human safety of rebaudioside A.

## Conflict of interest statement

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