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Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans

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Abstract

Stevia mixture, sweeteners extracted from the leaves of *Stevia rebaudiana* Bertoni, consists mainly of the glycosides of the diterpene derivative steviol. The aims of this study were to investigate the absorption (in rats) and the hepatic metabolism (in rats and humans) of both stevia mixture and steviol. Absorption was investigated both in vivo and ex vivo. In ex vivo experiments using the rat everted sac method, no absorption of stevia mixture was observed, but significant absorption of steviol was noted (equivalent to approximately 70% of the absorption reference- salicylic acid- value). In the in vivo experiment, rats received a single oral administration of either steviol or stevia mixture; a peak steviol concentration in plasma was observed 15 min after its oral administration, demonstrating rapid absorption. However, after oral administration of stevia mixture, the steviol concentration in plasma increased steadily over 8 h, suggesting that stevia mixture components are first degraded and then absorbed as steviol in the rat intestine. Steviol metabolism in humans and rats was examined by incubating steviol with liver microsomes from the two species. Oxidative (monohydroxy and dihydroxy) metabolites of steviol were observed by LC-ESI/MS after incubation with both human and rat liver microsomes. The intrinsic clearance of steviol in human liver microsomes was 4-times lower than that found in rat liver microsomes. In conclusion, this study suggests that there are no major species differences in steviol hepatic metabolism between rats and humans. Absorption from the human intestine can be predicted to occur in an analogous manner to that from the rat intestine.

Keywords: Stevia mixture; Steviol; Rat everted sac method; In vitro absorption; Hepatic metabolism

1. Introduction

Stevia rebaudiana Bertoni, belonging to the Compositae family, is a sweet herb native to South America. The plant has also been cultivated in China and Southeast Asia. Stevia sweeteners, crude extracts from its leaves, have been used for a few decades to sweeten beverages and foods in Japan, Korea and Brazil. Stevia rebaudiana products are used in USA as dietary supplements. Stevia sweeteners are glycosides of the diterpene derivative steviol (*ent*-13-hydroxykaur- 16-en-19-oic acid), consisting mainly of stevioside (triglucosylated steviol) and rebaudioside A (tetraglucosylated steviol), together with the other components rebaudioside C and dulcoside A (Fig. 1). These chemicals together account for approximately 5–10% of the dry leaf weight. Only stevioside and rebaudioside A, but not dulcoside A and rebaudioside C have sweetening potentials 200–300 times that of sucrose (Soejarto et al., 1982; Hanson and De Oliveira, 1993). In a comprehensive study, stevioside was incubated for up to 3 months at pH values ranging from 2 to 8 and temperatures ranging from 5 to 90 °C: no discernable breakdown in steviol was generated, indicating the stability of stevioside to cooking, storing or processing (Pezzuto et al., 1985).

The toxicity of stevioside has been investigated in a range of study types by various workers (Toskulkao et

Abbreviations: HPLC, high performance liquid chromatography; LC/MS/ES1, liquid chromatography mass spectrometry electrospray ionization; Rt, retention time

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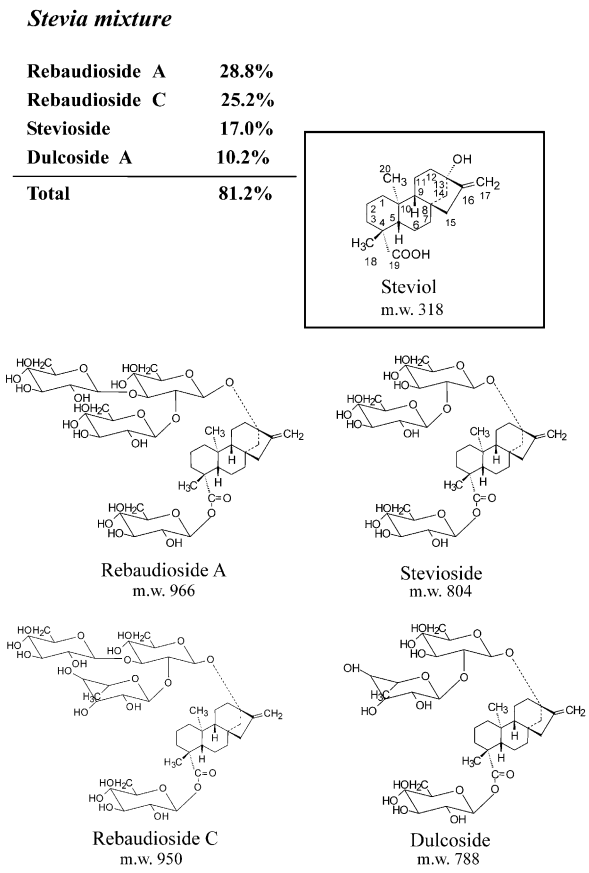

Fig. 1. Chemical structures of the major sweet-tasting glycosides of stevia mixture.

al., 1997; Aze et al., 1991; Xili et al., 1992; Toyoda et al., 1995, 1997; Matsui et al., 1996a,b; Mori et al., 1981) and the results of short- and long-term toxicity, fertility, carcinogenicity and genotoxicity studies suggest a favourable toxicological profile in mammalian species. The pharmacokinetics of stevioside have been studied in rats (Nakayama et al., 1986; Cardoso et al., 1996). In vivo studies indicate that stevioside is hydrolyzed to steviol (the aglycone) in the rat caecum and then absorbed. In the same manner, stevioside and rebaudioside A are ultimately degraded to steviol by rat intestinal microflora under anaerobic conditions (Wingard et al., 1980). However, the absorption pattern of steviol alone, and of stevia mixture (including stevioside, rebaudioside A, rebaudioside C and dulcoside A), which is marketed and used as a food additive, are not well known.

The pharmacokinetics of stevia-related compounds have not been fully investigated in humans. In a previous study with human fecal homogenates, we concluded that the human intestinal metabolism of stevioside, rebaudioside A and stevia mixture may be analogous to their metabolism in rats, and that stevia related compounds are finally metabolized to steviol by human gut flora (Koyama et al., 2003). Based on these findings, if steviol is absorbed as the final degradation product of stevia related compounds in the human intestine, a further study of the hepatic metabolism of steviol was considered appropriate.

The aims of this study were to investigate the absorption of steviol and stevia mixture using rat ex vivo and in vivo experiments, and to examine the species difference in hepatic metabolism of steviol between rats and humans, using liver microsomes.

2. Materials and methods

2.1. Chemicals

Stevia mixture (main components: rebaudioside A, stevioside, rebaudioside C, dulcoside A, Fig. 1), were kindly provided by the Japan Stevia Industrial Association (Tokyo, Japan). Abietic acid and salicylic acid were purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents were of the highest purity commercially available or of HPLC grade.

2.2. Animals

Male Sprague–Dawley rats (for the ex vivo study: 7–8-weeks old, 248–315 g., For the in vivo study: 8–9-weeks old, 312–390 g) were obtained from Japan Charles River (Kanagawa, Japan). The animals were housed for an acclimation period of more than seven days in an air-conditioned room (temperature 22±3 °C, relative humidity 55±15%, 12-h light/dark cycle). Food (CRF-MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given ad libitum.

2.3. Liver microsomes

Rat liver microsomes were purchased from *In Vitro* Technologies, Inc. (Baltimore, Maryland, USA). Human liver microsomes (pooled from 10 healthy donors, 5 male and 5 female, HHM-0273) were purchased from the International Institute for the Advancement of Medicine (Scranton, Pennsylvania, USA).

2.4. Everted gastrointestinal sacs: preparation and measurements

The methodology used was as described by Wilson and Wiseman (1954). The duodenum (a 10-cm segment sampled 10 cm beyond the pylorus) and caecum of each rat were quickly removed under diethyl ether anesthesia, everted, washed with 0.9% NaCl, filled with Krebs–Ringer buffer (pH 7.0) and ligated. After preincubation with the buffer solutions for 20 min under a 95% O_2/5% CO_2 gas mixture, the everted sacs were dosed as descri-

bed below, then incubated under gentle agitation (70–90 cycles/min) for 30 min at 37 °C in Krebs-Ringer incubation buffer (approximately 0.2 ml/cm intestine) with the same O_2/CO_2 gas mixture being bubbled outside the sacs (i.e. on the mucosal side).

The incubation buffer was dosed to give test concentrations of 5 mg/ml stevia mixture, 0.1 mg/ml steviol or 10 μg/ml salicylic acid (containing 1 v/v% DMSO and incubated as described above, using the everted digestive sections of four rats per treatment. Samples (0.2 ml) of the mucosal fluids were collected after both the preincubation and the incubation periods. After incubation, the digestive mucosa were washed with water, and the serosal fluids were collected from the mucosa by 24-gauge needle injection. The exterior of the sac was designated the *mucosal* side; the interior of the sac was designated the *serosal* side.

Both the mucosal and serosal fluid mixtures were directly injected onto the HPLC systems as described below. All assays were performed in duplicate.

The samples were separated on a YMC-Pack Pro C_{18} column (250×4.6 mm, 5 μm; YMC, Tokyo, Japan) by a gradient solvent system consisting of acetonitrile and 0.1 v/v% phosphoric acid. The percentage of acetonitrile was increased from 30 to 85% over 30 min, with the solvent flow rate set at 0.8 ml/min. After 30 min, the column was re-equilibrated with the initial mobile phase for 10 min. The UV detector was set at 210 nm and the column temperature was 40 °C. The Hitachi D-7000 series HPLC system (Hitachi, Tokyo, Japan) consists of a L-7100 type pump, a L-7455 type UV detector, a L-7300 type column oven, a L-7200 type autosampler and a L-7610 type degasser.

2.5. Drug administration, blood sampling and measurements

Rats were given a single oral dose (dose volume 5 ml/kg) of 45 mg/kg steviol in corn oil or 125 mg/kg stevia mixture in 2 w/v% Gum Arabic (dosage approximately equimolar to that of steviol). Blood samples were collected in heparinized tubes following puncture of the portal vein prior to dosing and thereafter at 0.25, 0.5, 0.75, 1, 2, 4 and 8 h post-dose from four rats per time point under diethyl ether anesthesia. Blood samples were centrifuged at 1620 g for 10 min and plasma samples were frozen and stored at −80 °C until analysis.

Plasma samples were thawed at room temperature, separated into 50 μl aliquots and added to 800 μl of acetonitrile, 250 μl of water and 50 μl of 4 μg/ml abietic acid in acetonitrile as an internal standard. The mixtures were centrifuged at 1620 g for 10 min at 10 °C and the supernatants were filtered through 0.45 μm chromatodisk filters (GL Sciences, Tokyo, Japan). Ten μl of each sample was injected onto the LC-MS systems as described below.

The samples were separated on a Mightysil RP-18 column (150×2.0 mm, 5 μm; Kanto Chemical, Tokyo, Japan) by a gradient solvent system consisting of acetonitrile and 10 mM aqueous ammonium acetate. The percentage of acetonitrile was increased from 50 to 80% over 25 min, with the solvent flow rate set at 0.2 ml/min. After 25 min, the column was re-equilibrated with the initial mobile phase for 10 min. LC-MS was carried out by coupling a Hewlett Packard HPLC system (HP-1100) to a API-2000 ion trap mass spectrometer (PE Biosystems, San Jose, CA). LC-ESI/MS was performed on the mass spectrometer operated in the negative-ion detection mode. The samples were detected by operating the mass spectrometer by selected ion monitoring at m/z 317 (steviol) and m/z 301 (abietic acid, IS). Recovery of steviol and variability of the determinations were 90% and less than 10%, respectively. The detection limit for steviol was 0.1 μg/ml.

2.6. Liver microsomes incubation, extraction and measurements

Microsomal protein (0.4 or 0.5 mg/ml) was added to 0.33 mM Tris/ 0.167 mM EDTA buffer and 50 μM or 1 mM steviol (DMSO solution) to give final volumes of 1 ml with final DMSO concentrations of 1% v/v. After preincubation at 37 °C for 5 min, the reaction was started by the addition of a NADPH generating system (0.5 mM $NADP^+$, 1 unit/ml G-6-PDH, and 5 mM G-6-P). The reaction mixture was incubated for 60 or 120 min at 37 °C and the reaction was then terminated by the addition of 4 ml of acetonitrile. Abietic acid (50 μL of 800 μg/ml) was then added to the mixtures as an internal standard and they were centrifuged at 1620 g for 10 min at 10 °C. Supernatants were evaporated at room temperature under nitrogen flow and 200 μL of 50% v/v acetonitrile in water was added to dissolve the residue. The samples were then injected onto the HPLC or the LC-MS systems for analysis of steviol and/or its metabolites as described below.

The samples were separated on a Mightysil RP-18 GP column (250×4.6 mm, 5 μm; Kanto Chemical, Tokyo, Japan) by a gradient solvent system consisting of acetonitrile and 10 mM ammonium acetate. The percentage of acetonitrile was increased from 30 to 85% over 33 min. After 33 min, the column was re-equilibrated with the initial mobile phase for 10 min. The solvent flow rate was set at 0.8 ml/min. The UV detector was set at 210 nm and the column temperature was set at 40 °C. LC-MS was carried out by coupling a Hewlett Packard HPLC system (HP-1050, Hewlett Packard, CA) to a Finnigan TSQ-7000 ion trap mass spectrometer (Thermo Finnigan, CA). LC-ESI/MS was performed on the mass spectrometer operated in the negative-ion detection mode, because our previous study (Koyama et al., 2003) showed that steviol is

detected in the negative ion mode, but not the positive ion mode. The samples were analyzed by operating the mass spectrometer either in the full scan mode or by selected ion monitoring of the pseudo-molecular ions at m/z 317, 331, 333 and 351.

2.7. Analysis of the intrinsic clearance of steviol

Preliminary experiments indicated that the decline of steviol concentration after incubation for 60 min was linear up to 0.5 mg protein/ml at a substrate concentration of 25 μM and was also linear up to a substrate concentration of 200 μM at 0.4 mg protein/ml, in both pooled human and rat liver microsomes. Therefore, incubation with pooled human or rat liver microsomes was conducted at 50 μM steviol and 0.4 mg protein/ml.

Pseudo-first order rate constants for steviol elimination were calculated from the slopes of the linear regression lines (k_e) from plots of the logarithm of steviol concentration against the incubation time. The intrinsic clearance was calculated as the k_e value divided by protein concentration and results were presented as means ± S.D. Student's t test was used to compare intrinsic clearance between pooled human and rat liver microsomes. Data were considered to be significantly different at $P < 0.05$.

3. Results

3.1. Ex vivo absorption of stevia mixture and steviol

Table 1 shows concentrations of the serosal and mucosal sides in rat everted sacs incubated with stevia mixture components and steviol.

When salicylic acid was incubated for 30 min with rat everted sacs, the serosal to mucosal ratios (transport indices) for salicylic acid were 0.411 and 0.364 in the duodenum-jejunum and the ileum, respectively (Table 1). This confirmed that the everted sac preparations were functional and thus suitable for the study of intestinal absorption.

Steviol was rapidly transported from the mucosal side to the serosal side, giving transport indices of 0.311 and 0.345 in the duodenum-jejunum and the ileum, respectively (approximately 76 and 95% of the corresponding salicylic acid indices). However, extremely low levels of stevia mixture components were detected in the serosal fluids, with more than 93% of the total quantity remaining in the mucosal fluids: this led to transport indices of less than 0.021 (less than 0.5% of the salicylic acid indices) in both the duodenum-jejunum and the ileum. No preferential absorption site for steviol (from duodenum to ileum) was observed.

Table 1
Concentration of stevia mixture, steviol and salycilic acid (positive control) in the serosal and mucosal incubation mediums of rat everted intestinal sac

Compound	Component	Site	Serosal side[a] (mg/ml or μg/ml)	Mucosal side[a] (mg/ml or μg/ml)	The serosal/mucosal concentration ratio
			Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Stevia mixture	Rebaudioside A	Duodenum-Jejunum	0.10 ± 0.07	4.91 ± 0.10	0.021 ± 0.014
		Ileum	0.8 ± 0.03	4.92 ± 0.08	0.016 ± 0.007
	Stevioside	Duodenum-Jejunum	0.10 ± 0.07	4.98 ± 0.11	0.020 ± 0.014
		Ileum	0.08 ± 0.04	4.99 ± 0.11	0.015 ± 0.008
	Rebaudioside C	Duodenum-Jejunum	0.11 ± 0.08	4.93 ± 0.18	0.021 ± 0.017
		Ileum	0.08 ± 0.04	4.83 ± 0.09	0.017 ± 0.008
	Dulcoside A	Duodenum-Jejunum	N.D.	4.93 ± 0.06	N.C.
		Ileum	0.05 ± 0.06	4.92 ± 0.04	0.011 ± 0.012
Steviol		Duodenum-Jejunum	0.0265 ± 0.0096	0.0845 ± 0.0058	0.311 ± 0.072
		Ileum	0.0298 ± 0.0076	0.0856 ± 0.0049	0.345 ± 0.072
Salycilic acid		Duodenum-Jejunum	0.0036 ± 0.0013	0.0088 ± 0.0001	0.411 ± 0.0142
		Ileum	0.0033 ± 0.0003	0.0090 ± 0.0001	0.364 ± 0.030

Each concentration of stevia mixture component was calculated as stevia equivalent.
Stevia solution 5 mg/ml, steviol solution 0.1 mg/ml or salycilic acid (10 μg/ml) were incubated with rat everted intestinal. sac for 30 min after pre-incubation for 20 min at 37°C.
N.D.: > 0.02 mg/ml for rebaudioside A, stevioside and rebaudioside C; > 0.0007 mg/ml for steviol; 0.08 μg/ml for salycilic acid.
N.C.: Not calculated.

3.2. In vivo rat absorption study

The time-dependent portal plasma concentration profiles of steviol after single oral administration to male rats of steviol (45 mg/kg) or stevia mixture (125 mg/kg: almost equimolar to steviol) are shown in Fig. 2. A peak plasma concentration of steviol, 18.31 μg/ml, was observed 15 min after its administration, indicating rapid absorption from the rat gastrointestinal tract. However, the time-dependent plasma concentration profiles of steviol after administration of stevia mixture differed from those after steviol administration. Steviol concentration was below detection limit (less than 0.1 μg/ml) after 1 h, but appeared in plasma at 2 h, and thereafter increased in a time-dependent manner up to 8 h after dosing. The initial delay before steviol was first detected at 2 h and the subsequent increase in its concentration for 6 h are most probably consequences of the time taken for the degradation from stevia mixture to steviol in the rat gastrointestinal tract.

3.3. Steviol metabolism by rat and pooled human liver microsomes: LC/ESI/MS analysis

HPLC-UV determination was not used in this analysis since no metabolite peaks except for steviol were found on the HPLC-UV chromatogram (210 nm) despite the decline in the peak area of steviol (data not shown). Instead, mass spectral analysis in the negative ESI ion mode was used for steviol metabolite detection. Fig. 4 shows typical LC/ESI-MS chromatograms of steviol incubated with pooled human liver microsomes. The full scan mass spectrum shows deprotonated molecules (M−) of m/z 333 and 351. Peaks of M-1 (Rt; 7.3 min) and M-2 (Rt; 18.0 min) were detected at m/z 351, whereas peaks of M-3 (Rt; 5.1 min), M-4 (Rt; 8.0 min), M-5 (Rt; 9.8 min), M-7 (Rt; 15.1 min) and M-8 (Rt; 18.1 min) were found at m/z 333 on the chromatogram. Representative LC/ESI-MS spectra of steviol incubated with rat liver microsomes are shown in Fig. 5. The full scan mass spectrum shows deprotonated molecules (M^-) of m/z 333 and 351. Rts and monitor ions of peaks obtained from rat liver microsomes were consistent with those obtained from pooled human liver microsomes except for the M-6 peak at m/z 333 (Rt; 12.8 min). The prominent peaks at m/z 333 and m/z 351 point to oxidative metabolites, hydroxy and dihyroxymetabolites, respectively.

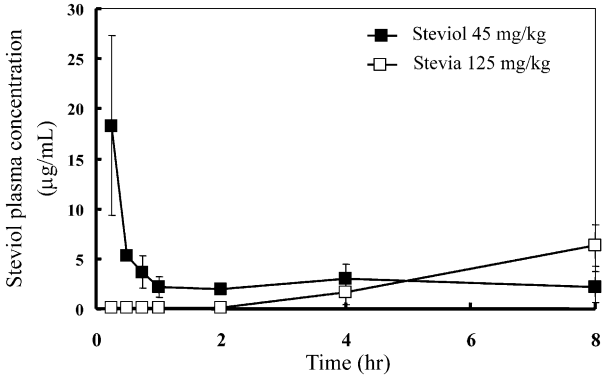

Fig. 2. Time-portal plasma concentration profiles of steviol in rats after a single oral administration of approximately equimolar doses of steviol and stevia mixture. Each point represents the mean of 4 animals.

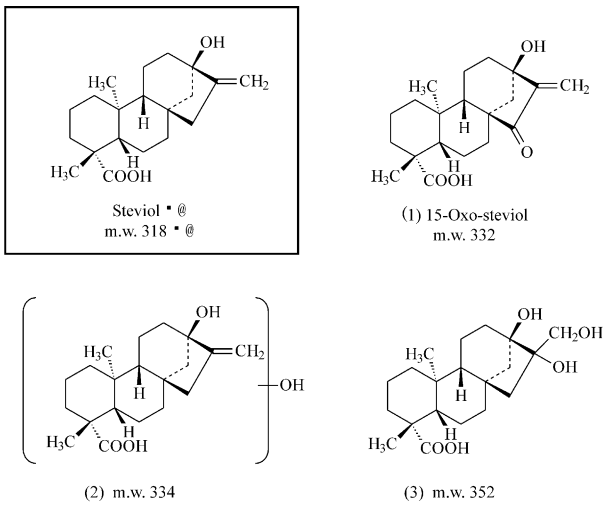

Fig. 3. Chemical structures of steviol and its metabolites as reported in the literature.

3.4. The intrinsic clearance of steviol

Fig. 6 shows time–concentration profiles of steviol in rat and pooled human liver microsomes and the inset shows the intrinsic clearance of steviol obtained from both liver microsomes. The intrinsic clearance of steviol obtained from pooled human liver microsomes was approximately 4-times lower than that obtained from rat liver microsomes (significantly different: $P < 0.01$).

4. Discussion

The purpose of the present study was 2-fold: in the first part we examined the absorption of steviol and stevia mixture in both ex vivo and in vivo rat experiments. The second part of the study was concerned with evaluating the species difference between human and rat in vitro hepatic metabolism. Together, these two sets of investigations provide initial evidence of the validity of extrapolating toxicity findings for stevia related compounds from rats to humans.

In the rat everted sac assay, no significant absorption of stevia mixture was found, whereas steviol was highly absorbed (giving transport indices greater than 70% of the salicylic acid reference values). No preferential site for the absorption of steviol (between duodenum and

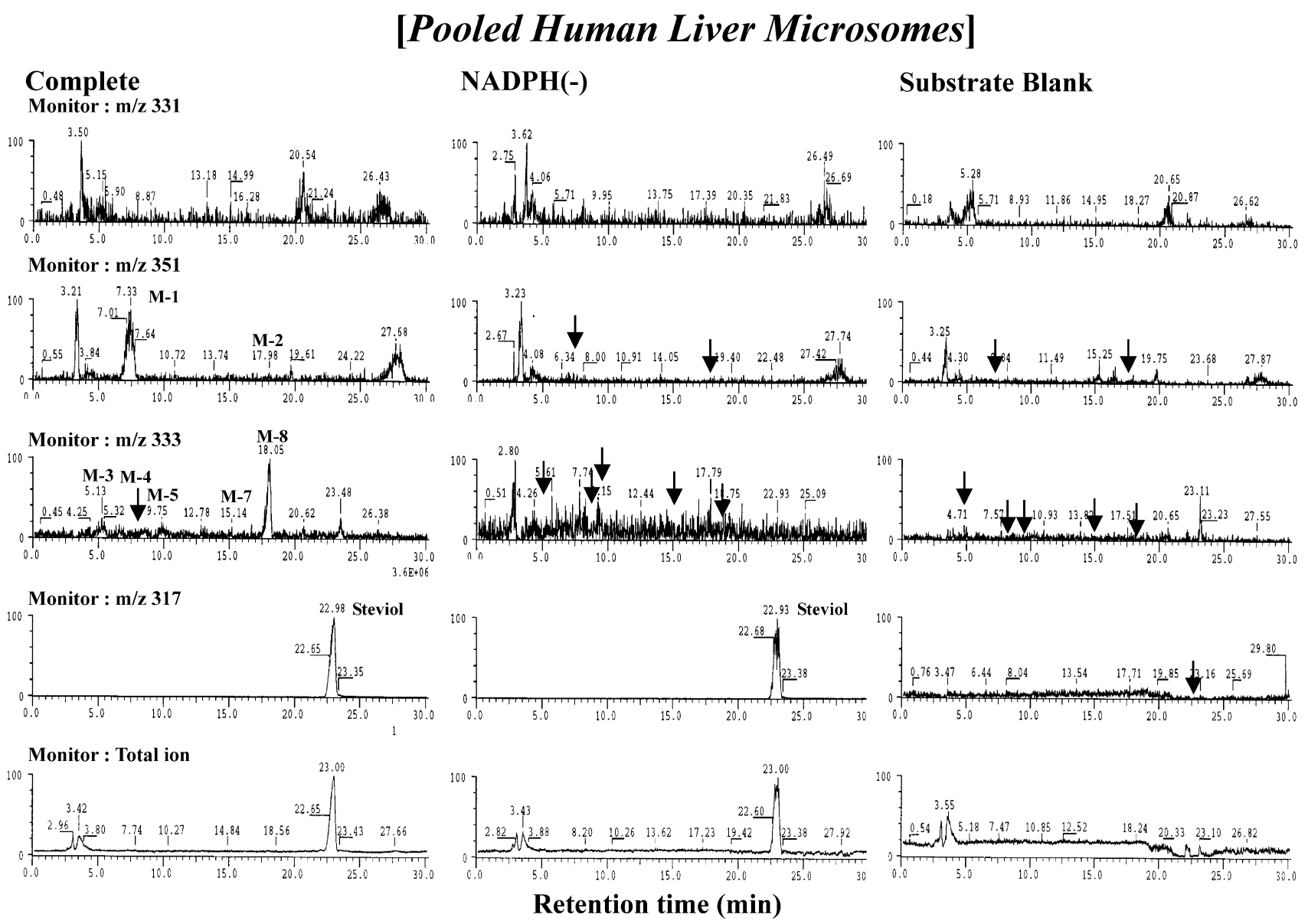

Fig. 4. Typical chromatograms of steviol incubated with rat liver microsomes. 1 mM steviol was incubated with rat liver microsomes (0.5 mg/ml) at 37 °C or 120 min in the presence of a NADPH generating system (Complete). The reaction mixture was incubated in the absence of a NADPH generating system (NADPH(-)), and without steviol (Substrate blank). The analysis by LC-ESI/MS was performed in the full scan mode, and at the selected ion monitoring of *m*/*z* 331, *m*/*z* 351, *m*/*z* 333 and *m*/*z* 317, which were detectable 15-oxo-steviol, dihydroxymetabolite(s), monohydroxy-metabolite(s) and steviol, respectively, as shown in Fig. 3.

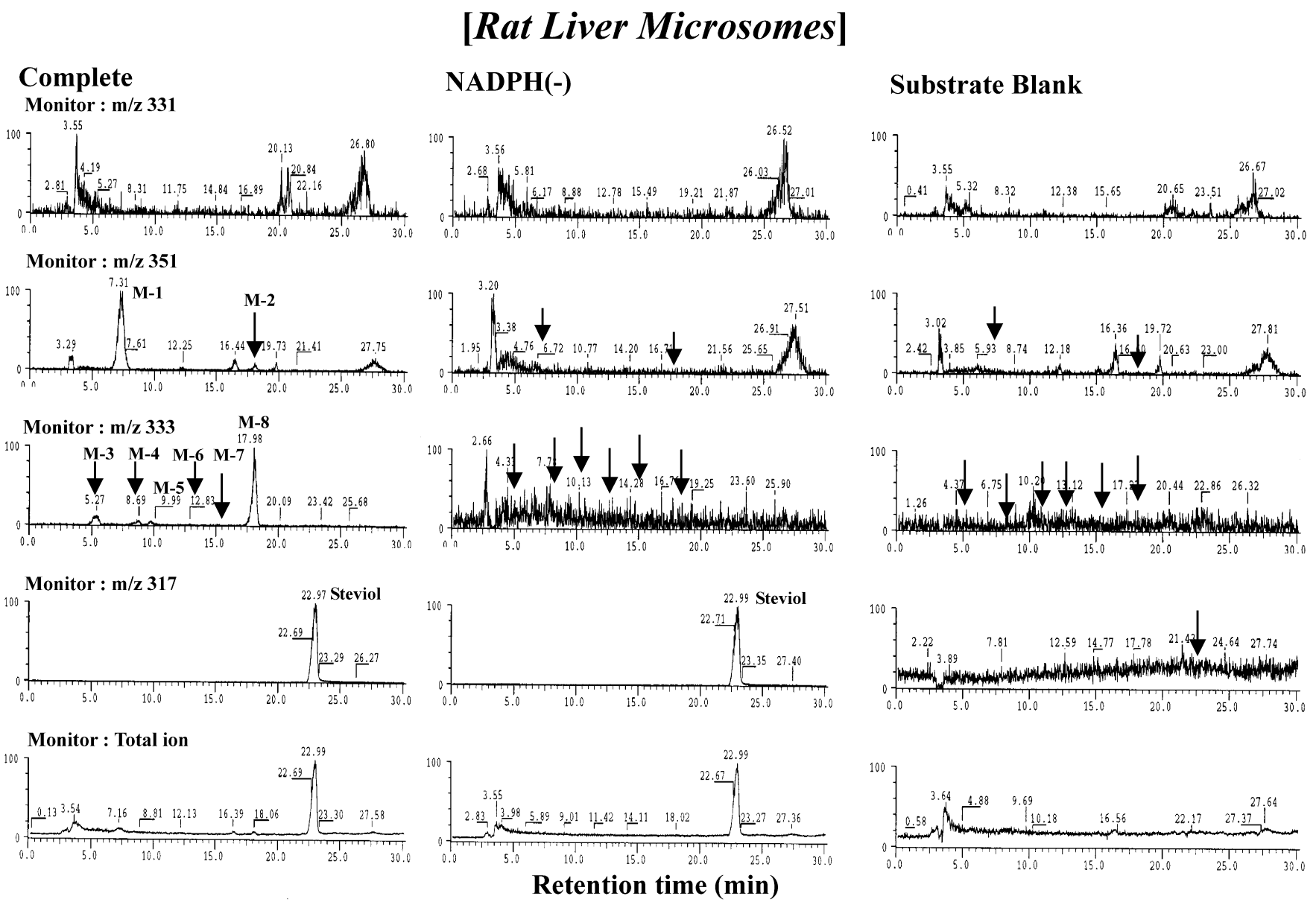

Fig. 5. Typical chromatograms of steviol incubated with pooled human liver microsomes. 1 mM steviol was incubated with pooled human liver microsomes (0.5 mg/ml) at 37 °C for 120 min in the presence of a NADPH generating system (Complete). The reaction mixture was incubated in the absence of a NADPH generating system (NADPH(-)), and without steviol (Substrate blank). The analysis by LC-ESI/MS was performed in the full scan mode, and at the selected ion monitoring of *m*/*z* 331, *m*/*z* 351, *m*/*z* 333 and *m*/*z* 317, which were detectable 15-oxo-steviol, dihydroxymetabolite(s), monohydroxymetabolite(s) and steviol, respectively, as shown in Fig. 3.

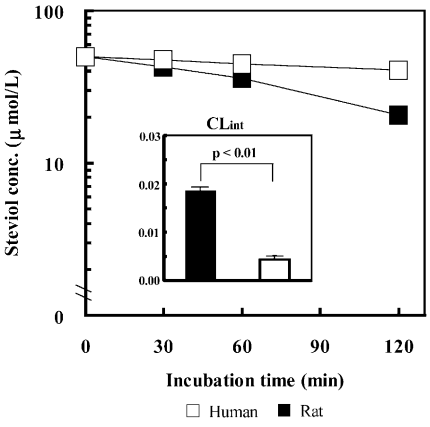

Fig. 6. Steviol concentration–time profiles and intrinsic clearances in rat and pooled human liver microsomes 50 μM steviol was incubated with rat or pooled human liver microsomes (0.4 mg protein/ml) at 37 °C. Each point represents the mean of triplicate determinations.

ileum) was observed. The major process for the absorption of xenobiotics is passive diffusion, which is directly proportional to the concentration gradient, the lipid-water partitioning and the molecular weights of xenobiotics (Chhabra, 1979; Gobath et al., 1993). The hydrophilic nature of stevia mixture components, since they contain more than one or two glucose and/or rhamnose units, may explain the poor absorption observed in this study. In addition to passive diffusion, carrier mediated transport contributes to absorption through the intestinal membrane (Amidon et al., 1988, 1995). It is thought that the mechanism of absorption of the reference substance (salicylic acid) may consist of passive diffusion and active transport via the monocarboxylic acid transporter in the rat intestine (Yamamoto et al., 1984). As the membrane permeability of steviol containing the monocarboxylic moiety proved to be analogous to that of salicylic acid, it is possible that steviol absorption may occur by the same mechanism. However, in vitro absorption of steviol using rat intestine is shown by our experiment to be very limited. Additional experiments are required to confirm and further characterize the mechanism of the observed steviol absorption.

The findings of the in vivo absorption experiment support those of the ex vivo experiment and our previous study (Koyama et al., 2003). As shown in Fig. 2, the maximum concentration of steviol in rat portal plasma was reached 15 min after its oral administration, reflecting rapid absorption from the stomach and upper intestine. The portal plasma concentration of steviol was maintained at 2–3 μg/ml after 2 h: This finding suggests the possibility that steviol was absorbed at the lower part of intestine, and that steviol was reabsorbed by the intestine-liver circulation (Nakayama et al., 1986).

The time- portal plasma concentration profile of steviol after administration of stevia mixture appeared different from that seen after administration of steviol (Fig. 2). Steviol concentrations were below the detection limit (less than 0.1 μg/ml) up to 1 h, indicating no degradation of stevia components to steviol in the stomach. Steviol was detected at 2 h and thereafter increased in a time-dependent manner up to 8 h. Wingard et al. (1980) showed that stevioside is degradaded ultimately to steviol by rat intestinal microbial cells. Together with the results of our ex vivo experiment, these findings suggest that stevia mixture (stevioside, rebaudioside A, rebaudioside C and dulcoside A) is degraded to steviol by rat intestinal microflora, then absorbed in the upper small intestine, in a similar manner to stevioside alone.

On the basis of literature data (Fig. 3, Comapadre et al., 1988; Pezzuto et al., 1985), the in vitro hepatic metabolism of steviol was investigated using rat and pooled human liver microsomes and LC/MS/ESI and HPLC-UV analyses. The metabolic profile obtained from pooled human liver microsomes was similar to that obtained from rat liver microsomes, except for metabolite M-6 (Figs. 4 and 5), indicating that there may be no apparent species difference in the metabolic profile of steviol between humans and rats. All metabolites required a NADPH generating system, and were estimated to be hydroxy (*m*/*z* 333) or dihydroxy metabolites (*m*/*z* 351). This finding suggests that cytochrome P450 may be involved in steviol oxidation in both human and rat liver microsomes. Compadre et al. (1988) reported that eight peaks corresponding to steviol metabolites were identified in rat liver S-9 by GC-MS analysis, which is consistent with our findings using rat liver microsomes and LC-MS analysis (except that we did not detect 15-oxo-steviol). However, 15-oxo-steviol formation may occur by a two step process involving oxidation by cytochrome P450 in the microsomal fraction and reduction by reductase in the cytosol fraction at the 15-position. The in vivo study of Nakayama et al. (1986) suggested that two unknown steviol conjugates are found in rat bile. Similarly, we predict that hydroxy or dihydroxy steviol may be further metabolized in humans. Additional study is required to further characterize the metabolism of oxidated steviol in humans.

The intrinsic clearance of steviol obtained from pooled human liver microsomes was approximately 4-times lower than that obtained from rat liver microsomes (Fig. 6). This may be due to differences in enzyme content per unit of microsomal protein or to differing enzyme(s) or affinity(ies) of enzyme(s) involved in steviol metabolism.

In conclusion, steviol is rapidly absorbed in the stomach or the upper small intestine, whereas stevia mixture is degradaded to steviol by intestinal microflora and then absorbed (probably in the lower large intestine) in rats. No preferential site for steviol absorption (between duodenum and ileum) has been identified.

From our previous study (Koyama et al., 2003), absorption of steviol from the human intestine can be predicted to occur in an analogous manner to its absorption from the rat intestine. We have found no major species difference in steviol oxidative metabolism between humans and rats; extrapolation of the accumulated toxicity data in rats to the human exposure scenario may therefore be valid.

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