



PERGAMON



The Pharmacokinetics and Metabolism of Sucralose in the Dog

S. G. WOOD, B. A. JOHN and D. R. HAWKINS

Huntingdon Research Centre Ltd, Huntingdon, Cambs PE18 6ES, UK

Abstract—The pharmacokinetics and metabolism of sucralose were investigated in dogs following intravenous or oral administration. Oral doses of ^{14}C -sucralose were rapidly absorbed, although there was some variation in the extent of absorption (range 18–48% of the dose). After intravenous or oral administration, radioactivity excreted in the urine was associated mainly with unchanged sucralose. One urinary metabolite was detected after both intravenous and oral doses and was identified by mass spectrometry as a glucuronic acid conjugate of sucralose. This metabolite accounted for about 15–20% of the intravenous dose but for only about 2–8% of the oral dose. © 2000 Elsevier Science Ltd. All rights reserved

Keywords: sucralose; metabolism; artificial sweetener; pharmacokinetics; dog.

Abbreviations: ACE = alternate chemical ionization/electron impact; EI = electron impact; FAB = fast atom bombardment; GC–MS = gas chromatography–mass spectrometry; TLC = thin-layer chromatography; TMS = trimethylsilyl.

INTRODUCTION

Sucralose is a high-intensity sweetener derived from sucrose by selective replacement of three hydroxyl groups by chlorine atoms. Toxicological evaluation of this compound has been carried out in the mouse, rat, rabbit, man and dog. In order to assist in the evaluation of results from toxicology studies and to extrapolate the findings to man, this study was carried out to investigate the metabolism and pharmacokinetics of sucralose in dogs.

MATERIALS AND METHODS

Materials

Uniformly labelled ^{14}C -sucralose [batch no. CFQ 4360; sp. act. 68 $\mu\text{Ci}/\text{mg}$; radiochemical purity >97% by thin-layer chromatography (TLC)] was obtained from Amersham International plc (UK). Non-radioactive sucralose (batch no. KL/5/31) and sucralose-6-carboxylate were supplied by Tate & Lyle Research and Development (Whiteknights, Reading, Berks, UK). Oral doses of sucralose were given dissolved in distilled water and iv doses were given in sterile isotonic saline (Travenol Laboratories Ltd, Thetford, Norfolk, UK). Pre-layered silica gel F_{254} plates, layer thickness 0.25 mm, were obtained from Merck (Darmstadt, Germany). Scintillation system M1-31 for liquid

scintillation analysis was obtained from Packard Instruments Ltd (Caversham, UK). β -Glucuronidase Type H1 from *Helix pomatia*, also containing aryl sulfatase activity and β -glucuronidase Type B3 from bovine liver were obtained from Sigma Chemical Co. Ltd Trisil-Z, (trimethylsilylimidazole in pyridine) and BSTFA/TMCS (*N,O*-bis(trimethylsilyl)-trifluoroacetamide containing trimethylchlorosilane) were supplied by Pierce UK Ltd (Cambridge, UK). All other chemicals and reagents were of analytical grade and were obtained from commercial sources.

Animals

Two male and two female beagle dogs, body weight 10–13 kg and age approximately 6 months, were obtained from Earlsbrook Stud (Solihull, UK). Animals were subjected to veterinary examination on arrival at Huntingdon Research Centre in order to establish their suitability for inclusion in the study, and were housed singly in stainless-steel cages with suspended wire-mesh floors, for the separate collection of urine and faeces. The dogs were fed normal laboratory diet (SPC Lab Diet A, Special Diet Services Ltd, Witham, Essex, UK) and were given tap water *ad lib*. Dogs were fasted for 16 hr prior to dosing and were offered food 4 hr after dosing.

Dosing and sample collection

After an acclimation period of 2 days in metabolism cages, ^{14}C -sucralose (2 mg/kg; 50 μCi) was administered iv into the cephalic vein. 15 days later, these dogs received single oral gavage doses of ^{14}C -sucralose (10 mg/kg; 50 μCi). Blood samples (approx. 6 ml) were taken from the jugular vein into heparinized tubes at pre-dose and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hr after an oral dose and at these times plus 5, 15 and 45 min after an iv dose. Plasma was separated after centrifugation and stored at about -20°C until analysed. Urine was collected into containers cooled in dry ice, at pre-dose, 0–3, 3–6, 6–12, 12–24 hr and then at 24-hr intervals up to 120 hr. Faeces were collected at 24-hr intervals up to 120 hr after an oral dose and at 2-hr intervals up to 8 hr, then at 12, 24, 48, 72, 96 and 120 hr after an iv dose. Faecal samples collected during 24 hr after the iv dose were pooled by animal to create a single 24-hr sample per animal. The cages were washed down with water (approx. 1000 ml) at 24-hr intervals after dosing and the washings retained for measurement of radioactivity. All samples were stored at about -20°C until analysed.

Measurement of radioactivity

Radioactivity in all samples was measured by liquid scintillation analysis using a Philips PW 4700 or LKB Wallac 1219 RackBeta automatic liquid scintillation counters. Faeces were weighed and then homogenized to a paste in water and the new weight of the homogenate was noted. Samples of faecal homogenate (approx. 0.2 g) were burned in oxygen using a Packard 306 Mk.2 automatic sample oxidizer. The combustion products were absorbed in Optisorb[®] and mixed with Optisorb S[®] scintillator system (Fisons). Recovery of radioactivity from carbon-14 standards (Amersham), containing known amounts of radioactivity and burned in the oxidizer, exceeded 97%. Radioactivity in all other samples was measured by mixing appropriate aliquots of the sample with scintillation fluid.

Where possible, radioactivity measurements were performed in duplicate. After selection of the optimal channel settings on the scintillation counters, quench correction curves were generated from radiochemical standards (^{14}C -hexadecane; Amersham) by the sample channels ratio and external standard channels ratio methods.

Data processing was performed using a North Star Horizon data processing system. The coefficients of a quadratic quench curve function were calculated by computer and entered in the analyser data processors, which automatically calculated disintegration rates.

Investigation of metabolites

Accurately weighed samples of faecal homogenate, prepared from faeces of orally dosed dogs,

were freeze-dried overnight using an Edwards Modulyo Freeze-Dryer. The freeze-dried faecal residues were resuspended in methanol (10 ml) and sonicated for 5 min. The suspensions were centrifuged to obtain a clear extract and aliquots (100 μl) of the methanol extracts were removed for the determination of carbon-14 content by liquid scintillation counting as described previously. Mean recovery of radioactivity from faeces spiked with ^{14}C -sucralose was $89\% \pm 3 \text{ SD}$ ($n = 5$) and from test samples was $91\% \pm 3 \text{ SD}$ ($n = 6$).

The nature of the carbon-14 material in urine and methanolic extracts of faeces was examined by TLC using silica gel F₂₅₄ plates of layer thickness 0.25 mm and developed in at least two of the following solvent systems:

Solvent system	Rf value of sucralose
A Diethyl ether:ethyl methyl ketone: water, 25:25:1, by vol.	0.13
B Ethyl acetate:methanol:water: ammonia (sp. gr. 0.88) 60:20:10:2, by vol.	0.55
C Ethyl acetate:methanol:water 6:2:1, by vol.	0.82

Urine and faecal standards for TLC were prepared by spiking ^{14}C -sucralose into pre-dose urine or methanol extracts of pre-dose faecal homogenates.

Radioactivity on the chromatograms was detected and quantified by a Berthold LB 2821 or LB 2842 Automatic TLC-Linear Analyser. Confirmation of the assignment of separated radioactive components was obtained by autoradiography of the plates using Singul-XRP X-ray plates (J. Blishen & Co. Ltd, London, UK) and subsequent photographic development of the X-ray plates.

Co-chromatography of dog and human urine

Human urine samples from normal healthy male volunteers given a single oral dose of ^{14}C -sucralose (1 mg/kg; 100 μCi) were obtained from Southampton Clinical Pharmacology Group (Roberts *et al.*, 2000). The urine samples used were a 3–6 hr sample from Subject 5 and a 0–3 hr sample from Subject 6. The urine samples were chromatographed separately and combined on the same TLC plate.

Enzyme hydrolysis studies

Aliquots of centrifuged 3–6 hr urine samples collected from one male and one female dog after iv dosing were incubated for about 18 hr at about 37°C in stoppered tubes with either buffer (incubation control) or β -glucuronidase (Type H1 from

Helix pomatia or Type B3 from bovine liver). Solutions were prepared by dissolving enzymes in 0.1 M acetate buffer (pH 5). Equal volumes of urine and β -glucuronidase in 0.1 M acetate buffer (about 6000 Fishman units/ml) were incubated. After about 18 hr, subsamples were taken and were further incubated (1 hr) with phenolphthalein glucuronide or *p*-nitrophenyl sulphate to confirm the presence of appropriate enzyme activity. The incubated samples were examined by TLC using solvent system B.

Isolation and identification of the major metabolite in dog urine

Samples of unchanged sucralose and the metabolite were isolated from dog urine (3–6 hr urine sample from an iv dosed dog). HPLC was performed in a reversed phase mode using a stainless-steel column (300 mm \times 3.9 mm) packed with μ Bondapak C₁₈ (mean particle diameter 10 μ m, Waters Associates Ltd, Cheshire, UK). The mobile phase, methanol–water (15:85, v/v), was delivered to the column at a flow rate of 2 ml/min using a Waters 6000A pump. The eluate was monitored using a Pye Unicam LC3 UV detector (Pye Unicam, Cambs, UK) operating at a wavelength of 205–220 nm and a Berthold on-line HPLC radioactivity monitor (Model LB 505) coupled to a Berthold chromatography data system (Model LB 510). The column eluate was collected as 1-min fractions and aliquots were prepared for liquid scintillation analysis. Sucralose and the major metabolite had retention times of about 15 min and 3 min, respectively. Eluted fractions associated with sucralose and the metabolite were pooled separately and taken to dryness using rotary evaporation under reduced pressure at about 40°C. The residues were redissolved in small volumes of methanol.

Further purification was carried out by preparative TLC using solvent system B. In each case, the area of silica corresponding to radioactivity associated with sucralose or the metabolite detected by autoradiography was removed by scraping and the radioactivity eluted by sonication in methanol. The methanol extracts were reduced to dryness under nitrogen at about 37°C and the residues triturated with a small volume of methanol. This procedure was repeated using solvent system C.

The isolated sample of metabolite was finally purified by HPLC using the mobile phase of methanol–water (10:90, v/v). Radioactivity eluted as a single peak with a retention time of about 4.5 min.

Collected fractions corresponding to the metabolite were pooled and the solvent removed by evaporation under reduced pressure at about 40°C and finally by freeze-drying. The sample of sucralose was also finally purified by HPLC using a mobile phase of methanol–water (25:75, v/v). Co-chromatographic TLC analysis of these samples with urine

confirmed that they had been unaffected during isolation and purification.

Mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) samples were analysed as trimethylsilyl (TMS) derivatives. Derivatization was performed by sonicating the sample with Trisil-Z (10–20 μ l) at room temperature for 0.5–1 hr.

Mass spectra were obtained using a VG7070E double-focusing mass spectrometer (VG Analytical, Manchester, UK) linked to a VG 11-250 data system (VG Analytical Ltd) for control and data processing. Samples were introduced into the mass spectrometer either using the direct insertion probe which was slowly heated to 430°C, or using an HP5792A gas chromatograph (Hewlett Packard Ltd, Wokingham, Berks, UK). The gas chromatograph was fitted with a 12.5 m \times 0.2 mm i.d. fused silica column coated with dimethylsilicone bonded phase at a film thickness of 0.33 μ m. The outlet end of the column was passed directly into the ion source of the mass spectrometer. Samples were injected using the purged splitless mode with the injector at 250°C, the oven at 100°C and the split valve closed for 0.75 min. After 1 min the oven was heated at 35°C/min to 200°C and then at 15°C/min to 300°C where it was held for up to 15 min.

Mass spectra were recorded under either electron impact (EI) or alternate chemical ionisation/electron impact (ACE) conditions with a source temperature of 200°C in each case. Electron impact spectra were obtained with an electron energy of 70 eV and a trap current of 100–300 μ A, while chemical ionisation spectra were obtained with an electron energy of 50 eV, an emission current of 500 μ A and ammonia as the reactant gas.

Fast atom bombardment (FAB) mass spectra were obtained using an Ion Tech fast atom gun (Ion Tech Ltd, Teddington, Middlesex, UK) attached to the VG7070E mass spectrometer, which was fitted with a FAB ion source. The FAB gun was operated at a voltage of 8 kV which gave a discharge current of 1.4 mA when using xenon as the ionizing gas at a source pressure of 3×10^{-6} mbar. Samples were prepared as solutions in aqueous sodium chloride (1 M) and an aliquot (1 μ l) containing the sample (15–30 g) was thoroughly mixed with thioglycerol (1–2 μ l) already smeared on a tungsten target. The target was introduced into the mass spectrometer source using the FAB insertion probe and mass spectra were recorded every 6 sec.

RESULTS

Excretion

After intravenous administration of ¹⁴C-sucralose (2 mg/kg) to dogs, radioactivity was rapidly excreted, mainly in the urine. During 3, 6 and 12 hr

after dosing, means of 29.3%, 63.9% and 74.1% of the dose, respectively, were excreted in the urine. Urinary excretion accounted for a mean of 80.9% of the administered dose after 5 days. Excretion of radioactivity in the faeces accounted for a mean of 10.4% of the dose after 24 hr, increasing to 11.9% after 5 days (Table 1). During 5 days, a mean total of 95.8% of the dose was recovered from urine, faeces and cage washings.

After oral administration of ^{14}C -sucralose (10 mg/kg) to dogs, radioactivity was excreted mainly in the faeces. Faecal excretion accounted for a mean of 65.9% of the dose during 24 hr increasing to 68.4% after 5 days. Excretion of radioactivity in the urine accounted for means of 13.8%, 22.3% and 26.5% of the dose during 6, 12 and 24 hr after dosing, respectively, increasing to 27.6% of the dose after 5 days. During 5 days, a mean total of 97.6% of the dose was recovered from urine, faeces and cage washings (Table 2).

After both iv and oral doses of ^{14}C -sucralose, there were no apparent sex-related differences in the rates, or extent, of excretion of radioactivity in the urine and faeces. Comparison of the urinary excretion of an oral dose (81%) with that of an iv dose (28%), considered equivalent to 100% absorption, indicated that about 35% of the oral dose was absorbed. However, there was some inter-animal variation in the apparent extent of absorption, with 40%, 33%, 18% and 48% absorption for dogs 1 and 2 male, 3 and 4 female, respectively.

Plasma concentrations

Concentrations of radioactivity in plasma following intravenous administration declined in an

apparently multi-exponential fashion, from a mean value of $8.46 \mu\text{g}$ equivalents/ml after 5 min to $0.057 \mu\text{g}$ equivalents/ml after 12 hr. Concentrations declined slowly thereafter and radioactivity was detectable in plasma of all animals after 120 hr (Fig. 1). After single oral doses of ^{14}C -sucralose to dogs at a dose level of 10 mg/kg, peak plasma concentrations were achieved between 0.5 and 1.5 hr after dosing. At the time of maximal concentrations (mean $2.80 \mu\text{g}$ equivalents/ml at 1.5 hr), they declined in an apparently multi-exponential fashion to a mean of $0.24 \mu\text{g}$ equivalents/ml at 12 hr and at 96 hr were near or below the limit of accurate determination ($0.05 \mu\text{g}$ equivalents/ml) in all animals (Fig. 1).

Comparison of the mean areas under the plasma concentration-time curves after oral or iv administration (AUC_{iv} per mg/kg dose AUC_{po} per mg/kg dose) confirmed that about 35% of the oral dose was absorbed. Again, some inter-animal variability was apparent with 41%, 37%, 25% and 50% absorption indicated for dogs 1 and 2 male, 3 and 4 female, respectively.

Chromatographic analysis of metabolites

Chromatographic analysis of radioactivity in urine indicated that unchanged sucralose was the major component after either oral or iv dosing, based on the essentially identical TLC R_{F} values of ^{14}C -sucralose and the major urine component, in three different solvent systems (Fig. 2). After oral dosing, unchanged sucralose accounted for an average of 66% of 0–24 hr urine sample radioactivity (solvent system B, Table 3). The remaining radioactivity in these samples was mainly associated with

Table 1. Excretion of radioactivity in urine and faeces during 5 days after single iv doses of ^{14}C -sucralose to dogs (2 mg/kg). Results are expressed as % dose

Time interval (hr)	Animal no.				Mean \pm SD
	1 Male	2 Male	3 Female	4 Female	
(i) Urine					
0–3	24.0	49.0	9.6	34.7	29.3 \pm 16.7
3–6	39.8	21.2	52.3	25.1	34.6 \pm 14.3
6–12	10.2	10.8	9.8	9.8	10.2 \pm 0.5
12–24	6.2	2.5	6.0	3.5	4.6 \pm 1.8
24–48	1.6	1.2	1.5	1.4	1.4 \pm 0.2
48–72	0.4	0.4	0.4	0.5	0.4 \pm 0.1
72–96	0.2	0.2	0.2	0.3	0.2 \pm 0.1
96–120	0.1	0.1	0.2	0.2	0.2 \pm 0.1
Total 0–120	82.5	85.4	80.0	75.57	80.9 \pm 4.2
(ii) Faeces					
0–24	10.8	9.1	10.9	10.8	10.4 \pm 0.9
24–48	1.8	0.6	0.7	1.7	1.2 \pm 0.6
48–72	0.2	0.1	0.1	0.3	0.2 \pm 0.1
72–96	0.1	ND	ND	0.1	NC
96–120	ND	0.1	ND	0.1	NC
Total 0–120	12.9	9.9	11.7	13.0	11.9 \pm 1.4
Total excreted*					
0–120	97.6	96.5	95.8	93.2	95.8 \pm 1.9

SD = Standard deviation; ND = Below the limit of detection ($<0.1\%$ dose); NC = Not calculated (at least one value below the limit of detection). *Including radioactivity in urine, faeces and cage washings.

Table 2. Excretion of radioactivity in urine and faeces during 5 days after single oral doses of ¹⁴C-sucralose to dogs (10 mg/kg). Results are expressed as % dose

Time interval (hr)	Animal no.				Mean ± SD
	1 Male	2 Male	3 Female	4 Female	
(i) Urine					
0-3	3.0	4.9	3.6	6.4	4.5 ± 1.5
3-6	12.1	10.2	3.2	11.8	9.3 ± 4.2
6-12	10.5	8.7	4.8	9.8	8.5 ± 2.5
12-24	5.3	3.2	1.8	6.3	4.2 ± 2.0
24-48	1.3	0.4	0.3	1.0	0.8 ± 0.5
48-72	0.3	0.2	0.2	0.4	0.3 ± 0.1
72-96	0.1	0.1	0.1	0.2	0.1 ± 0.1
96-120	0.1	0.1	0.1	0.1	0.1 ± 0.0
Total 0-120	32.7	27.8	14.1	36.0	27.6 ± 9.6
(ii) Faeces					
0-24	58.3	70.1	79.3	55.8	65 ± 10.9
24-48	5.8	0.3	0.8	2.3	2.3 ± 2.5
48-72	ND	0.1	0.1	0.3	NC
72-96	ND	ND	0.1	0.1	NC
96-120	ND	ND	ND	0.1	NC
Total 0-120	64.1	70.5	80.4	58.4	68.4 ± 9.4
Total excreted*					
0-120	97.9	99.4	95.6	97.6	97.6 ± 1.6

SD = Standard deviation; ND = Below the limit of detection (<0.1% dose); NC Not calculated (at least one value below the limit of detection). *Including radioactivity in urine, faeces and cage washings.

one component (M2) in all three solvent systems, which accounted for about 14% of 0-3 hr urine sample radioactivity increasing to about 25% of 3-6 and 6-12 hr urine sample radioactivity. After iv dosing, about 90% of 0-3 hr urine sample radioactivity was associated with unchanged sucralose decreasing to 62% and 59% of 3-6 and 6-12 hr urine sample radioactivity, respectively. Component M2 was the only notable metabolite in urine from iv-dosed animals, accounting for about 7% of 0-3 hr urine sample radioactivity and about 34% of 3-6 and 6-12 hr urine sample radioactivity. As a percentage of the administered dose, component M2 accounted for a mean of 18% of the iv dose and a mean of 6% of the oral dose.

Treatment of selected urine samples with β-glucuronidase/sulfatase preparations did not result in

any change in the profile of urinary radioactivity compared with untreated samples. The reference compound sucralose-6-carboxylic acid, a potential metabolite of sucralose, did not correspond to component M2, using TLC. TLC of methanolic faecal extracts from orally dosed dogs indicated that unchanged ¹⁴C-sucralose was the only component present. Co-chromatography of human urine along with urine from Dog 1 indicated the presence of three components (Fig. 3). The major component corresponded to unchanged sucralose; one of the two minor components corresponded to dog urine

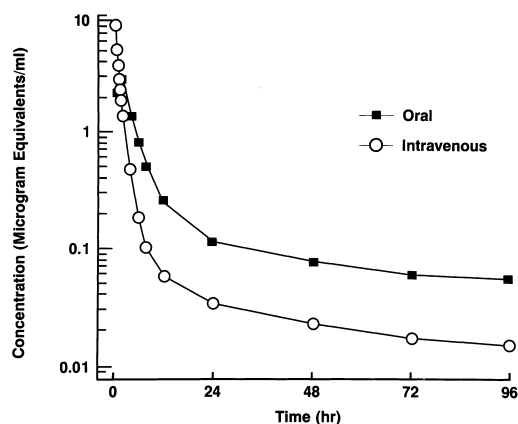


Fig. 1. Mean concentrations of radioactivity in plasma after oral (10 mg/kg) or iv (2 mg/kg) administration of ¹⁴C-sucralose.

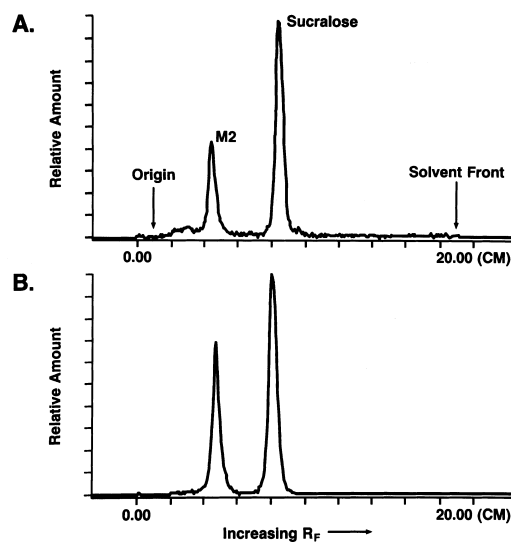


Fig. 2. Thin-layer radiochromatogram of dog 1: 3-6 hr urine after administration of ¹⁴C-sucralose developed in solvent system B. (A) 10 mg/kg oral dose; (B) 2 mg/kg iv dose.

Table 3. Proportions of sucralose and component M2 in dog urine after single iv (2 mg/kg) and oral doses (10 mg/kg) of ^{14}C -sucralose. Results are expressed as % total dose administered (mean \pm SD) (quantitated in solvent system B)

Time (hr)	iv dose		Oral dose	
	Sucralose	Component M2	Sucralose 0	Component M2
0-3	25.7 \pm 13.9	2.7 \pm 2.4	3.2 \pm 1.2	0.6 \pm 0.2
3-6	22.2 \pm 12.1	11.0 \pm 2.9	6.0 \pm 2.9	2.4 \pm 1.1
6-12	5.9 \pm 0.6	3.2 \pm 0.6	5.4 \pm 1.9	2.1 \pm 0.8
12-24	3.3 \pm 1.6	0.7 \pm 0.3	3.1 \pm 1.6	0.8 \pm 0.4
0-24	57.1 \pm 4.0	17.6 \pm 2.5	17.7 \pm 7.0	5.8 \pm 2.4

component M2 and the other (chromatographically more polar and designated component M1) was present in only trace amounts in dog urine but was more prominent in human urine.

Identification of the major dog urine metabolite

The identity of sucralose as a component in urine was confirmed by GC-MS and direct probe mass spectrometry. The GC retention time of the TMS-derivatized urine component was identical to that of TMS-derivatized authentic sucralose and the electron impact mass spectra of the isolated and authentic sucralose were essentially identical.

The theoretical ratios of the M:M + 2:M + 4 peaks for the pseudomolecular ions of the TMS derivative of sucralose are approximately 3:4:2. Regarding the mass spectra of such compounds, the m/z value of the peak of the lowest mass in the cluster is always quoted. For derivatized sucralose, the electron impact (probe) mass spectrum was essentially the same as that obtained under GC-MS conditions. Although the electron impact spectrum contained fragments derived from the galactopyranosyl (m/z 397) and fructofuranosyl (m/z 343) moieties, additional information was contained in the chemical ionisation spectrum. A pseudomolecular ion was indicated at m/z 774 corresponding to M + NH + 4, and another important ion (m/z 702) was derived by loss of a TMS group. An intense ion at m/z 360 corresponded to the fructofuranosyl fragment with addition of ammonia.

As expected, the electron impact and chemical ionisation spectra of TMS-derivatized sucralose iso-

lated from dog urine were essentially identical to the corresponding spectra of authentic sucralose, further confirming the identity of this isolated component.

In the chemical ionisation spectrum the TMS derivative of M2, (Fig. 4, the ion at m/z 702), corresponded to the same ion in the spectrum of sucralose but was more intense. The presence of this ion would indicate that the basic sucralose structure was intact and was indicative of component M2 being a conjugate of sucralose. The related peaks at m/z 360 (chemical ionisation) and m/z 343 (electron impact), attributable to the fructofuranosyl moiety of sucralose, also featured in the spectra for component M2, whereas the ions at m/z 397 and m/z 361, which are associated with the galactopyranosyl moiety of sucralose, did not. Such information suggested that component M2 was a conjugate of sucralose (Fig. 5), wherein the site of conjugation was the 4-chlorogalacto-pyranosyl moiety of sucralose.

Although no pseudomolecular ion was observed, information indicating that component M2 was a glucuronic acid conjugate was contained in the electron impact spectrum. The fragmentations m/z 465, 375 and 217 were a feature of this spectrum and are characteristic of glucuronic acid derivatives (Billets, 1973; Feng *et al.*, 1983; Hornke *et al.*, 1980).

Confirmation of dog urine component M2 as a glucuronic acid conjugate of sucralose was provided by fast atom bombardment mass spectrometry of the underivatized sample. Reference spectra were also obtained in this mode using authentic sucralose. The FAB spectrum of sucralose showed an ion cluster at m/z 419 [M + Na]⁺ and also sodium chloride adducts at m/z 477 [M + Na + NaCl]⁺, m/z 535 [M + Na + 2NaCl]⁺ and m/z 593 [M + Na + 3NaCl]⁺.

In the FAB spectrum of component M2 (Fig. 4), the ion cluster at m/z 617 corresponded to [M-H + 2Na]⁺ for a glucuronic acid conjugate of sucralose. Similarly, sodium chloride adducts were found at m/z 675 [M-H + 2Na + NaCl]⁺, m/z 733 [M-H + 2Na + 2NaCl]⁺ and m/z 791 [M-H + 2Na + 3NaCl]⁺. A similar type of spectrum was obtained for authentic sucralose-6-carboxylate with an ion cluster at m/z 455 [M-H + 2Na]⁺ and sodium chloride adducts at m/z 513 [M-

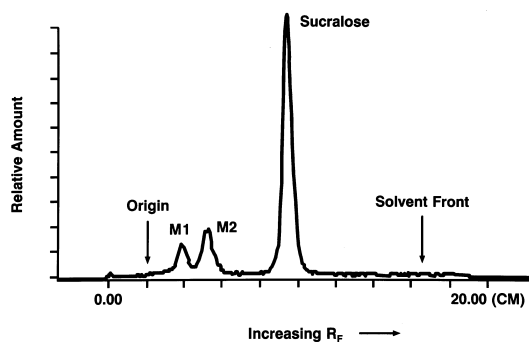


Fig. 3. Thin-layer radiochromatogram of Dog 1 urine (iv dose, 3-6 hr) co-chromatographed with human urine (oral dose 3-6 hr) developed in solvent system B.

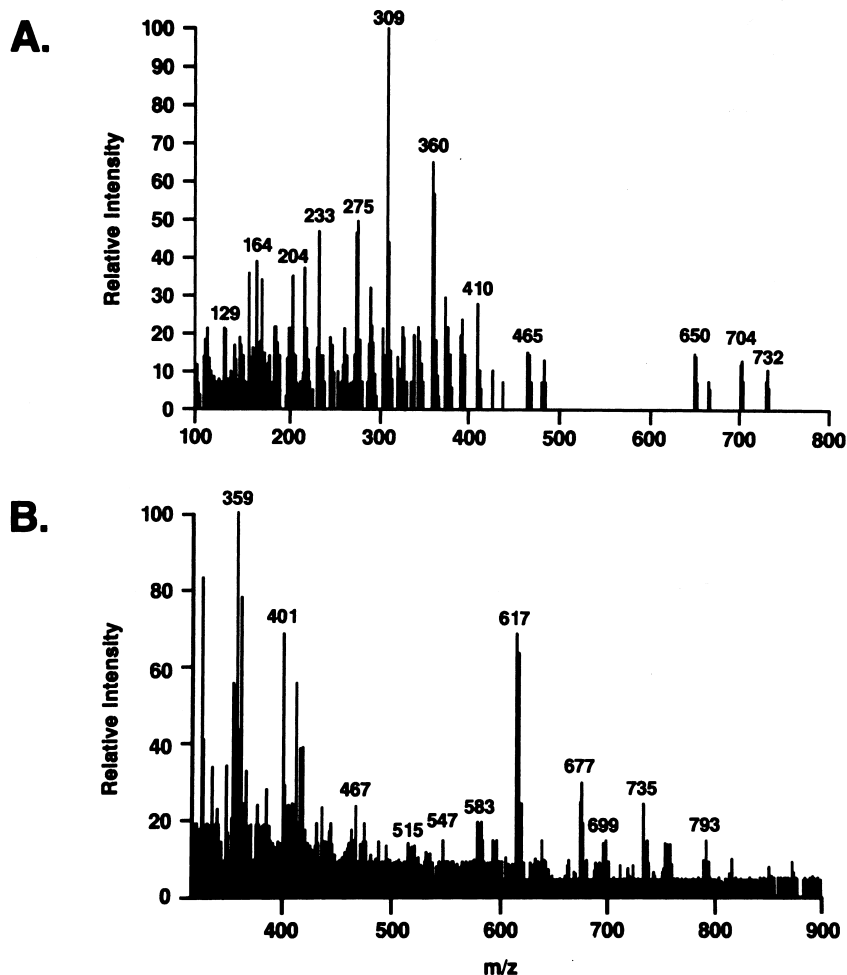


Fig. 4. Mass spectra of sucralose metabolite isolated from dog urine: (A) CI spectrum of TMS derivative; (B) FAB spectrum of underivatized metabolite.

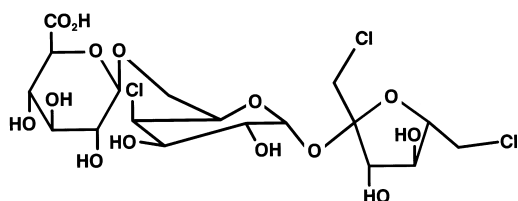


Fig. 5. Postulated structure of metabolite M2 showing glucuronic acid conjugated at position 6 of the 4-chlorogalactopyranosyl moiety of sucralose.

$H + 2Na + NaCl]^+$, m/z 571, m/z 629 and m/z 687.

DISCUSSION

¹⁴C-Sucralose was rapidly absorbed after oral administration although there was some variation in the extent of absorption estimated by comparison of urine excretion and plasma levels after intravenous doses. After intravenous administration, radio-

activity excreted in the urine was associated mainly with unchanged sucralose. One urinary metabolite was detected which was chromatographically more polar than sucralose and accounted for about 15–20% of the administered dose. This urinary metabolite of ¹⁴C-sucralose was identified as a glucuronic acid conjugate of sucralose by mass spectrometry. Similarly, after oral administration, the absorbed dose that was excreted in the urine was excreted mainly as unchanged sucralose. The glucuronic acid conjugate of sucralose observed in urine after iv dosing was also present after oral dosing, and in similar proportions, with respect to percentage of total urine sample radioactivity, but accounted for only about 2–8% of the oral dose.

The mass spectral evidence for the glucuronic acid conjugate of sucralose showed that the glucuronic acid was attached to the 4-chlorogalactopyranosyl moiety of sucralose but the actual position of conjugation remains ambiguous. It is possible that sucralose could be conjugated at the 2-, 3- or 6-carbon position, and for representative

purposes the conjugate is shown in Fig. 5 as the sucralose-6-glucuronic acid. An interesting feature of this glucuronic acid conjugate is that it is apparently resistant to hydrolysis by classical enzymatic techniques. Use of acid hydrolysis was precluded due to the lability of sucralose at low pH. The amount of conjugate eliminated in the urine after an oral dose was directly related to the extent of absorption and thus it is evident that it is formed systemically.

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