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The Pharmacokinetics and Metabolism of Sucralose in the Mouse

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

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

Abstract—The excretion and metabolism of ^{14}C-sucralose has been investigated in mice following intravenous and oral administration. A 20 mg/kg intravenous dose was rapidly excreted mainly in the urine (80% in 5 days). After 100, 1500 and 3000 mg/kg oral doses of ^{14}C-sucralose, means of 23%, 15% and 16% of the dose, respectively, were excreted in the urine during 5 days. Comparison with the intravenous dose indicated that 20–30% of the oral doses was absorbed. Sucralose was excreted almost entirely unchanged and represented more than 80–90% of the radioactivity in all urine and faeces samples. Only two minor metabolites were detected, representing 2–8% of urine radioactivity. *© 2000 Elsevier Science Ltd. All rights reserved*

Keywords: sucralose; metabolism; artificial sweetener; pharmacokenetics; mouse.

Abbreviations: GC = gas chromatography; LC = liquid chromatography; MS = mass spectrometry; TLC = thin-layer chromatography.

INTRODUCTION

Sucralose is a high-intensity sweetener derived from sucrose by selective replacement of three hydroxyl groups by chlorine atoms. This paper is one of a series concerned with investigations on the metabolism and pharmacokinetics of sucralose in laboratory animals in order to assist in the evaluation of results from toxicology studies and the extrapolation to man. This study in mice was designed to establish the extent of absorption of oral doses of sucralose and the influence of dose level on this parameter and to investigate the nature of excreted material.

MATERIALS AND METHODS

Materials

[U-^{14}C]Sucralose (batch no. CFQ 4643; sp. act. 8.5 mCi/mmol, radiochemical purity >99% by HPLC) was obtained from Amersham International plc (UK). Non-radioactive sucralose (batch no. KL/5/15), ^{14}C-1,6-dichlorofructose and ^{14}C-4-chlorogalactose (the hydrolysis products of sucralose) were supplied by Tate & Lyle Group Research & Development (Whiteknights, Reading, Berks, UK). All other chemicals and reagents were of analytical grade and were obtained from commercial sources.

Animals

Male and female mice (CD-1 strain) of body weight range 26–29 g on the day of dosing were obtained from Charles River (UK) Ltd (Margate, Kent, UK). All animals received water and LAD 1 standard diet (Labsure, Croydon, Surrey, UK) *ad lib*. throughout the study.

Dosing and sample collection

Animals were randomly assigned to one of four groups. An iv dose of ^{14}C-sucralose (20 mg/kg) in sterile isotonic saline (5 μl/g body weight) was administered by tail vein injection to four males and four females. Oral doses of ^{14}C-sucralose were administered by gavage in isotonic saline (20 μl/g body weight) to three groups of animals receiving nominal doses of 100 mg/kg (four males, four females), 1500 mg/kg (two males, two females) and 3000 mg/kg (two males, two females), respectively. The specific activity of the dose material was adjusted so that all animals received about 10 to 12 μCi ^{14}C-sucralose. After dosing, animals from each group were housed in pairs (two males or two females) in glass metabowls (Jencons Ltd, Leighton Buzzard, Beds, UK) to facilitate separate collection of urine and faeces. Urine was collected into containers, cooled by dry ice, at 0–12 and 12–24 hr and then at 24-hr intervals for up to 120 hr after dosing. Faeces were collected at 24-hr intervals for 120 hr.

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Expired air from animals receiving the 100 mg/kg oral dose was trapped in a mixture of ethanolamine–2-ethoxyethanol (1:4, v/v) contained in two traps in series. Cages were rinsed with water to remove residual urine. 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 counter. Faeces were weighed and then homogenized to a paste in water and the new weight of 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 I™ and mixed with Optisorb S® scintillator system (Fisons plc, Loughborough, UK). 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.

Radioactivity measurements were performed in duplicate. After selecting 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. 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

Analysis of samples was performed by thin-layer chromatography (TLC) using silica gel plates of layer thickness 0.25 mm (E. Merck A.G., Darmstadt, Germany). The developing solvent was ethyl acetate–methanol–water–ammonia (sp. gr. 0.88) 60:20:10:2 (by vol), which was shown in other studies to provide the optimum separation of metabolites (Wood *et al*., 2000). Aliquots (25 μl) of urine samples were applied directly to TLC plates. Faeces samples were homogenized with methanol, centrifuged and aliquots (25 μl) of the separated methanol extracts applied directly to TLC plates. Samples of urine were also co-chromatographed with reference standards and with a 3–6-hr human urine sample obtained from the human metabolism study (Roberts *et al*., 2000). Radioactivity on chromatograms was detected and quantified by a Berthold LB 2832 or LB 2842 Automatic TLC-Linear Analyser. Confirmation of the assignment of separated radioactive components was obtained by autoradiography of the developed TLC plates using Singul-XRP X-ray film (J. Blishen & Co. Ltd, London, UK)

RESULTS

Excretion

An iv dose of sucralose was rapidly excreted mainly in the urine (80% in 5 days) with about 70% excreted during the first 12 hr (Table 1). Faecal excretion accounted for a mean of 22% dose (12% in first 24 hr) indicating that there was some excretion of material in bile or other secretion into the intestinal tract by another mechanism.

Similar proportions of oral doses were excreted in urine with corresponding greater amounts in faeces (Table 1). Following a 100 mg/kg oral dose the total urinary excretion was 23% of the dose, 12% occurring during the first 24 hr. Comparison of the urinary excretion of an oral dose (23%) with that of an iv dose (80%), which is considered equivalent to 100% absorption, indicated that about 29% of the oral dose was absorbed. Only 0.3–0.4%

Table 1. Mean rate of excretion of radioactivity by mice after intravenous and oral administration of ^{14}C-sucralose expressed as percentage administered dose

	iv dose		Oral dose	
Sample collection (hr)	20 mg/kg*	100 mg/kg*	1500 mg/kg†	3000 mg/kg†
Urine				
0–24	69.5	11.8	6.8	6.5
12–24	5.7	5.9	5.3	3.7
24–48	4.1	4.6	2.4	4.4
48–120	0.8	0.9	0.6	1.6
Total urine	80.1	23.2	15.1	16.2
Faeces				
0–24	12.2	41.0	30.4	41.7
24–48	8.6	27.2	35.6	24.1
48–120	1.5	1.5	7.5	6.6
Total faeces	22.3	69.7	73.5	72.4
Cage wash/debris	1.9	2.8	3.3	5.0
Total recovery	104.3	95.7	91.9	93.6

*Mean values for four males and four females. †Mean values for two males and two females.

Table 2. Mean amounts of sucralose and its metabolites in urine (0–72 hr) after iv and oral administration of ^{14}C-sucralose to mice expressed as percentage administered dose

	iv dose		Oral dose	
Component	20 mg/kg*	100 mg/kg*	1500 mg/kg†	3000 mg/kg†
Sucralose	71.2	19.7	13.0	13.1
Component M_2	3.1	0.8	0.6	0.5
Component M_3	2.5	1.2	0.6	0.8
Others	2.8	1.1	0.5	0.9

*Mean values for four males and four females. †Mean values for two males and two females.

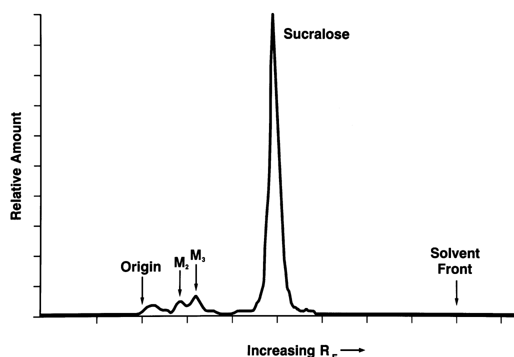

Fig. 1. Thin-layer radiochromatogram of 0–12-hr male mouse urine after a 100 mg/kg oral dose of ^{14}C-sucralose.

of the dose was eliminated in the expired air, indicating that the radiolabel was placed in metabolically stable positions in the molecule. This level of excretion in expired air is within the level of impurities in the dosed ^{14}C-sucralose and may not be considered representative of sucralose catabolism. The proportions of the dose excreted in urine after the 1500 mg/kg and 3000 mg/kg oral doses were very similar, 15% and 16%, respectively. Thus, the extent of absorption was very similar at about 20% of the administered dose calculated by comparison with the urinary excretion of an intravenous dose as described above. In all cases, excretion of the dose was essentially complete after 72 hr, and the total recoveries over 5 days were greater than 92%. This represents essentially a quantitative recovery once the small losses during sample collection are taken into account.

Investigation of metabolites

Chromatographic analysis of urine samples showed that unchanged sucralose was the single major radiolabelled component in all samples (Fig. 1, Table 2) representing about 80–90% of the sample radioactivity. There were no appreciable differences in the sample profiles from male and female animals or in urine collected at different times. Similarly, neither the route of administration nor the dose level had any systematic effect on the proportion of sucralose in urine samples. Some minor radiolabelled components were detected on most radiochromatograms, the most quantitatively important being two components designated M2 and M3, both chromatographically more polar than sucralose (Fig. 1). Neither of these components corresponded to 4-chlorogalactose or 1,6-dichlorofructose, the hydrolysis products of sucralose. The proportions of these components were similar and in general represented 2–8% of the sample radioactivity. Most of the remaining radioactivity was associated with polar material near the baseline of the chromatograms and contained no quantifiable peaks.

Radioactivity in methanolic extracts of the faeces of both sexes was essentially completely associated with unchanged sucralose (94–99% of sample radioactivity) (Table 3). The majority of non-sucralose material was made up of small amounts of components M2 and M3. The levels of these minor components ranged from below the level of reliable quantitation at the 100 mg/kg dose up to a maximum 3% of sample radioactivity (in one pair only) at 3000 mg/kg.

Table 3. Mean amounts of sucralose and its metabolites in faeces after iv and oral administration of 14C-sucralose to mice expressed as percentage administered dose

	iv dose		Oral dose	
Component	20 mg/kg*	100 mg/kg*	1500 mg/kg†	3000 mg/kg†
Sucralose	20.0	67.3	70.2	69.2
Component M_2	0.3	<0.6	1.1	0.7
Component M_3	0.3	<0.6	<0.4	0.2
Others	0.6	1.5	1.3	1.9

*Mean values for four males and four females. †Mean values for two males and two females.

Some information on the identity of the minor urine metabolites was obtained by comparison with a urine sample from the human metabolism study (Roberts *et al.*, 2000 subject 5, 3–6 hr). Chromatographic data visualized by autoradiography indicated that in addition to sucralose, the major urinary component, the two minor radioactive components (M1 and M2) present in human urine were also present in mouse urine. The minor component M2 co-chromatographed with the minor metabolite in human urine that has been identified as the glucuronic acid conjugate of sucralose originally identified in the dog (Wood *et al.*, 2000).

The most chromatographically polar radioactive component in human urine (M1) co-chromatographed with a trace radioactive component in mouse urine. This trace component, visualised by autoradiography, was not apparent at the sensitivity of radiochromatogram scanning. Mouse urine component M3 did not co-chromatograph with any radioactive component present in human urine.

DISCUSSION

Although urinary excretion was the major route of elimination of an iv dose, overall the data indicated that about 22% of the dose was excreted via the bile or some other secretion, in the faeces.

After oral administration, the profiles of excretion in urine and faeces were similar across the dose range studies (100–3000 mg/kg). After oral administration of ^{14}C-sucralose, the dose was mainly excreted in the faeces. Profiles of excretion were similar in both sexes with 70–74% of the dose excreted in the faeces and 15–23% in the urine, in 5 days. Less than 0.5% of the dose was excreted in expired air, which was within the impurity level of the dosed material. When the faecal excretion of an iv dose is taken into account, it is estimated that approximately 20–30% of an oral dose of sucralose was absorbed.

As may be expected for a very hydrophilic xenolustic compound such as sucralose, it is mainly excreted unchanged. After the iv dose, sucralose represented more than 90% of the radioactivity in urine and faeces. Thus, sucralose is either excreted into the intestinal tract unchanged or as a conjugate which is subsequently hydrolysed. Similar results were obtained after the oral dose, throughout the dose range used. The minor radiolabelled components detected in urine represented a similar low proportion irrespective of the route of administration or dose level.

Co-chromatographic and autoradiographic analyses of both mouse and human urine samples were conducted to compare the urinary components of the two species. Studies in humans have demonstrated the presence of two urinary components, in addition to unchanged sucralose. Unchanged sucralose accounts for the majority of urinary material. Mouse urine component M2 is chromatographically equivalent to the human urinary component conclusively identified by LC and MS as a glucuronide conjugate of sucralose and also found in the dog. The other human metabolite, M1, probably also a glucuronide conjugate (Roberts *et al.*, 2000), co-chromatographed with a trace component in mouse urine, detectable only by radiochromatogram scanning. Mouse urine component M3 did not co-chromatograph with any human urinary component.

Further investigations would be necessary to confirm the identity of the mouse metabolites, M1 and M3, but formation of a glucuronide(s) may explain the excretion of material in bile. Overall, the metabolism of oral doses of sucralose in the mouse is similar to that in man following oral administration of ^{14}C-sucralose.

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