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Absorption of N^4-D-glucopyranosylsulphamethazine by rat everted intestinal sacs

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Abstract—Absorption of the N^4-D-glucose conjugate of sulphamethazine (glucose-SMZ, 0.5 mM) by isolated everted sacs of the rat small intestine was studied at 37° and pH 6.6. Phlorizin (0.5–2.0 mM) significantly reduced ($P < 0.05$) both mucosal and serosal transfer of glucose-SMZ and inhibition of mucosal transfer appeared to be concentration-dependent. Phloretin (0.5 mM) and removal of Na^+ from the incubation medium also diminished absorption of glucose-SMZ. Furthermore, D-glucose (0.5 and 5.0 mM) inhibited mucosal and serosal transfer of the glycoside. The results suggest the D-glucose/Na^+ cotransporter mediates absorption of glucose-SMZ from the small intestine of the rat. Thus, glucose-SMZ might be bioavailable from ingested tissues in which it is present.

The antibacterial drug sulphamethazine (SMZ) is extensively employed for prophylactic and therapeutic purposes in animal husbandry; administered either as a feed additive or by injection. Tissue residues of this drug and its metabolites after prophylactic use are minimized by withdrawing SMZ from animal feed for an appropriate period before slaughter. In pigs, where SMZ is widely used, the edible tissues of a significant number of animals slaughtered for human consumption are nevertheless found to contain SMZ residues above the statutory or advisory residue limits [1]. There is concern about the safety of such residues [2, 3], but rigorous toxicological evaluation of these is difficult because of uncertainties about the chemical nature of SMZ residues and their bioavailability [4].

N^4-D-Glycopyranosylsulphamethazine has been detected in edible tissues from pigs, though the origin of this "metabolite" is uncertain [5, 6]. This compound is hydrophilic and because of this it might be anticipated that its bioavailability from the gastro-intestinal tract is poor. However, it is possible that this glucose conjugate is a substrate for the D-glucose/Na^+ cotransporter in the small intestine and might, therefore, be absorbed from the human gut via this mechanism and be bioavailable in consumers of pig tissues containing the glucose conjugate. To investigate this possibility, the absorption of N^4-D-glycopyranosylsulphamethazine (glucose-SMZ) by rat isolated everted intestinal sacs has been studied.

Materials and Methods

Materials. α,β-D-Glucose was obtained from May and Baker (Dagenham, U.K.) and phloretin as well as phlorizin were purchased from the Sigma Chemical Co. (Poole, U.K.). SMZ (lot no. 08616, 99% stated purity) was bought from the Aldrich Chemical Co. (Gillingham, U.K.). All other reagents were of analytical grade.

Synthesis of glucose-SMZ. This compound was synthesized using the method described by Paulson *et al.* [6] and the crude product was purified on a silica column (3.5 × 50 cm) using ethyl acetate:methanol (85:15 v/v) as the mobile phase. The product was a white solid which decomposed above 120° and the reaction yield was about 47%. TLC (silica gel plates, 0.2 mm thick; mobile phase—ethyl acetate/methanol, 85:15 v/v) revealed a single species, by quenching of background fluorescence, with an R_f of 0.39. The product did not react with fluorescamine, a test for primary amines, and micro-analysis gave the following percentage composition (theoretical composition in parenthesis): carbon—47.2 (49.1); hydrogen—5.72 (5.49); nitrogen—11.7 (12.7) and sulphur—6.61 (7.28). Electron impact mass spectrometry gave a molecular ion of 440 Da for the product which is identical to the calculated molecular weight of glucose-SMZ. In addition, proton nuclear magnetic resonance gave a spectrum consistent with the product being glucose-SMZ. This spectrum also indicated the presence of both the α- and β-anomers of the glucose conjugate. The ratio of α- to β-form was about 1:3 by HPLC as described below.

Absorption experiments. Adult male albino Wistar rats (200–250 g body weight) were fasted for 18 hr and allowed free access to water. Animals were killed by cervical dislocation and the small intestine from 8 cm below the stomach to the ileocaecal junction isolated and stripped of adherent tissue. The intestine was removed and cut into three lengths of about 6 cm. Each section was weighed, everted and ligated to form a sac into which 0.5 mL of buffer, pH 6.6, was injected. The buffer was pre-gassed with 95% O_2–5% CO_2 and had the following composition (mM): NaH_2PO_4 (20), NaCl (123), KCl (4.93) and $CaCl_2$ (0.85). Sacs were weighed and incubated (80 oscillations/min) for 1 hr at 37° in 25 mL of pre-gassed buffer. After incubation, sacs were blotted, weighed, emptied of their contents and reweighed.

Sacs were randomly assigned to experimental groups with a minimum of six sacs per group. In all groups, the initial concentration of glucose-SMZ in the mucosal fluid was 0.5 mM. In some experiments, D-glucose (0.5 and 5 mM), phloretin (0.5 mM) or phlorizin (0.5–2.0 mM) was also added to the mucosal fluid. These concentrations were selected following preliminary experiments which confirmed that 0.5 mM phlorizin and phloretin significantly inhibited mucosal and serosal transport of D-glucose.

Determination of glucose-SMZ. The concentration of glycoside was determined in both the mucosal and serosal fluid by HPLC. Briefly, the HPLC system consisted of a Waters model 510 pump and U6K injector coupled to a C_{18} reverse phase column (Hichrom 5 μm, 25 cm × 4.6 mm i.d.). The mobile phase was 50 mM potassium phosphate buffer, pH 6.6, and acetonitrile (9:1 v/v) delivered at 1 mL/min. The eluent was monitored at 270 nm with a Waters 481 spectrophotometer and 740 integrator. Chromatography was carried out at room temperature (21°) and the volume of sample injected was 10 μL. The HPLC method used to assay glucose-SMZ resolved this compound into its α- and β-anomers (retention times 8.3 and 11.5 min, respectively). The peak areas of both anomers were summed for construction of the calibration curve and also for assay of the total concentration of glucose conjugate in incubation media. SMZ was used as the internal standard. The limit of assay sensitivity was 0.017 ± 0.002 μmol/mL (±SEM, N = 7) in mucosal/serosal fluid and intra/interassay coefficients of variation of the calibration curve slope were 1.0 and 3.4% (N = 6), respectively.

Analysis of results. Changes in sac weight and glucose-SMZ concentration were used to calculate net transfer of glucose-SMZ (μmol/g wet tissue weight). Data are given

Table 1. Effect of phloretin, removal of Na^+ and D-glucose on mucosal and serosal transfer of glucose-SMZ (0.5 mM) by rat isolated everted intestinal sacs incubated for 1 hr at 37° and pH 6.6

Group	Mucosal transfer (μmol/g wet wt)	Serosal transfer (μmol/g wet wt)
Control	18.1 ± 0.8 (12)*	0.78 ± 0.06 (12)
Phloretin (mM)		
0.5	6.05 ± 0.75 (6)†	0.43 ± 0.04 (6)†
Na^+-free medium	5.78 ± 0.86 (6)†	0.66 ± 0.07 (6)
D-Glucose (mM)		
0.5	3.94 ± 0.49 (6)†	0.33 ± 0.04 (6)†
5.0	6.07 ± 0.72 (6)†‡	0.39 ± 0.03 (6)†

* Values are given as mean ± SEM with number of sacs in parenthesis.

† $P < 0.001$ compared to control (Student's *t*-test or ANOVA).

‡ $P < 0.05$ relative to 0.5 mM D-glucose (ANOVA).

as mean ± SEM and statistical comparisons were made with non-paired Student's *t*-test or where appropriate, by one way analysis of variance (ANOVA) with means compared by Scheffe's test.

Results and Discussion

The mean recovery of glucose-SMZ incubated at 37° for 1 hr in the buffer alone was 93 ± 2% (N = 6). With everted sacs present, no SMZ could be detected in either the mucosal or serosal fluid at the end of the incubation period. Table 1 shows that for controls, loss of glucose-SMZ from the mucosal fluid was about 20-fold greater than gain of this compound by serosal fluid. As a proportion of the total amount of glucose-SMZ present, 37 ± 2% (N = 12) was transferred across the mucosal surface whereas only 1.3 ± 0.1% (N = 12) appeared in the serosal fluid. This suggests the bulk of absorbed glucose conjugate was retained within the tissue.

The effect of phlorizin (0.5–2.0 mM) on the mucosal and serosal transfer of glucose-SMZ is illustrated in Fig. 1 (A and B). Phlorizin, a β-glycoside and specific inhibitor of D-glucose transport across the luminal surface of the epithelial cell [7], decreased ($P < 0.05$) transfer of the glucose conjugate from the mucosal fluid and reduced its appearance in serosal fluid. Inhibition of transport across the serosal surface is probably a result of reduced mucosal transfer which would diminish the amount of glucose-SMZ available for serosal transport. The effect of phlorizin on mucosal transfer appeared concentration-dependent, since 1.0 and 2.0 mM phlorizin decreased transfer to a greater extent than 0.5 mM phlorizin (Fig. 1A). The residual transfer of glucose-SMZ in the presence of 1.0 and 2.0 mM phlorizin, may represent diffusion of glucose-SMZ across the tissue boundaries. Phloretin is the aglycone of phlorizin and an inhibitor of D-glucose transport across the contraluminal surface of the epithelial cell membrane [7]. This compound also diminished ($P < 0.001$) mucosal and serosal transfer of glucose-SMZ (Table 1), but concentrations of phloretin greater than 0.5 mM could not be tested because of its limited solubility in the incubation medium.

Uptake of D-glucose across the luminal surface of gut epithelial cells is Na^+-dependent [7]. Replacement of Na^+ with equimolar K^+ in the incubation medium, decreased mucosal and serosal transfer of glucose-SMZ; but this reduction was only statistically significant for mucosal transfer (Table 1). Table 1 also shows the effect of D-glucose (0.5 and 5 mM) on the absorption of glucose-SMZ. In the presence of D-glucose, both mucosal and serosal transfer were significantly inhibited ($P < 0.001$). However,

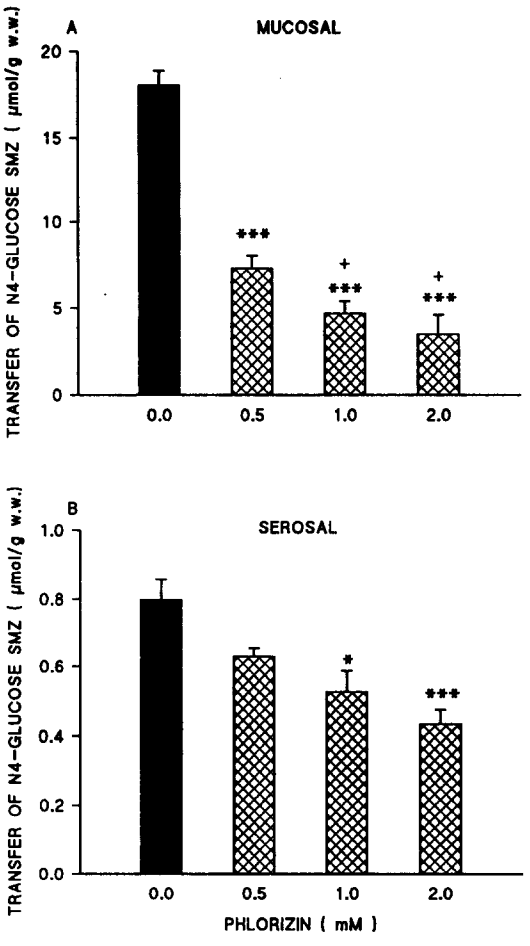

Fig. 1. Effect of phlorizin (0.0–2.0 mM) on net mucosal and serosal transfer of glucose-SMZ (0.5 mM) by rat isolated everted intestinal sacs incubated for 1 hr at 37° and pH 6.6. Net mucosal transfer is the amount of conjugate removed from the mucosal fluid and net serosal transfer is the amount of conjugate that appeared in serosal fluid at the end of the incubation time. Results are given as mean of either 12 (in the absence of phlorizin) or six sacs (in the presence of phlorizin) and vertical lines show SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ relative to control group (ANOVA); $^+P < 0.05$ relative to group with 0.5 mM phlorizin present (ANOVA).

0.5 mM D-glucose attenuated mucosal transport of glucose-SMZ to a greater extent ($P < 0.05$) than 5.0 mM glucose (Table 1). The reason for this difference in inhibitory effect is unclear.

The results are consistent with transport of glucose-SMZ across the epithelial cells of rat intestine by the Na^+-dependent D-glucose cotransport system. It is of interest that Mizuma *et al.* [8] found the glucose and galactose conjugates of *p*-nitrophenol were absorbed by everted sacs from the rat small intestine via the glucose carrier. These workers suggested conjugation with either D-glucose or D-galactose might provide a mechanism to improve the bioavailability of poorly absorbable drugs. Absorption of glucose-SMZ by intestinal sacs might mean that this metabolite of SMZ is bioavailable from ingested tissues in which it is present. Glucose-SMZ may not, however, be stable in gastric acid [9] and Fischer *et al.* [9] have shown

up to 70% of the N^4-glucose metabolite in pork is degraded to SMZ during cooking. Furthermore, it is likely the conjugate will be hydrolysed by host enzymes or by gut microbial flora to release SMZ. Hence any glucose-SMZ in food might be destroyed before it can be absorbed.

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