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Site-dependence and structure-effect relationships for alkylglycosides as transmucosal absorption promoters for insulin

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Abstract

Alkylglycoside surfactants have recently been identified as a new type of membrane permeation enhancer. The purpose of this study was to compare the effects of alkylglycosides on buccal, nasal, and rectal absorption, and to examine structure/effect relationships for buccal permeation enhancement. Insulin was used as a model, poorly bioavailable, protein drug, and pharmacologic effect was used as an index of absorption. Octylglucoside and dodecylmaltoside at 5% concentrations had greater enhancing effects on buccal absorption than on nasal or rectal insulin absorption. Dodecylmaltoside increased buccal insulin absorption to some extent at concentrations as low as 1%. There was no consistent relationship between the alkyl chain lengths of the alkylglycosides and their absorption promoting effects, nor between the critical micelle concentrations of the enhancers and their absorption promoting effects. A thioglucoside and an alkylglucamide increased insulin absorption, but sorbitan monolaurate, an ester, did not.

Key words: Enhancer; Absorption; Buccal absorption; Insulin; Permeation; Surfactant

1. Introduction

One approach to improve the bioavailability of drugs that are otherwise poorly absorbed is to coadminister the drug and a membrane permeation enhancer by a transmucosal route. This would be most useful for protein and peptide drugs, since these typically have poor membrane permeation and low bioavailability. Many types of compounds are known to have membrane permeation enhancing effects. The most commonly studied enhancers are nonionic and ionic surfactants and steroidal detergents. However, there is still an unfulfilled need for safe and effective mucosal membrane permeation enhancers (Lee et al., 1991), and evaluation of new enhancers could help to fulfill this need. In this study a relatively new category of membrane permeation enhancers, alkylglycosides, were examined.

Alkylglycosides are nonionic surfactants with sugar substituents as the polar head groups. Octylglucoside and other similar alkylglycosides are effective solubilizers of lipids and proteins below their critical micelle concentrations (CMC), and because of these properties they are commonly used as tools in various biochemical techniques and in membrane research (Yanagita and Kagawa, 1986). They are often used to reconsiti-

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tute active enzymes or other proteins from crude biological preparations. For such biochemical uses they have the advantages of not denaturing the proteins being isolated, and they are more easily removed by dialysis than are other surfactants, because of their higher CMC values (Womack et al., 1983).

Several alkylglycosides have recently been shown to have absorption promoting effects, altering the permeability of the rectal mucosal membrane and increasing the absorption of carboxyfluorescein (Murakami et al., 1992). We have evaluated the effects of alkylglycosides on the transmucosal absorption of insulin, a poorly absorbed protein drug. Our previous work showed that mucosal membranes vary in their susceptibility to permeation enhancement (Aungst and Rogers, 1988). One aspect of the present study was to compare the effects of alkylglycosides on rectal, nasal, and buccal absorption. The greatest promoting effects were on buccal insulin absorption. The effects of a more extended series of alkylglycosides and related compounds were then examined for their effects on buccal insulin absorption. The results of this study provide more detailed information on the use of alkylglycosides as a new category of mucosal membrane absorption promoter.

2. Materials and methods

2.1. Materials

Bovine insulin (activity 22–24 U/mg) was obtained from Sigma Chemical Co., and was used for all studies. Suppliers of other experimental compounds were as follows. Octylglucoside (*n*-octyl-β-D-glucopyranoside) and dodecylglucoside (*n*-dodecyl-β-D-glucopyranoside) were from Sigma Chemical Co. Hexylglucoside (*n*-hexyl-β-D-glucopyranoside), nonylglucoside (nonyl-β-D-glucopyranoside), octylthioglucoside (octyl-β-D-thioglucopyranoside), decylmaltoside (decyl-β-D-maltoside), dodecylmaltoside (dodecyl-β-D-maltoside), and decanoyl-*N*-methylglucoside (MEGA-10) were obtained from Calbiochem. Sorbitan monolaurate (Span 20) was supplied by ICI Americas Inc., and methyl gluceth-10 (Glucam E-10) was supplied by Amerchol.

2.2. Methods

All dosing solutions were prepared by dissolving the adjuvant in 0.1 M phosphate buffer, adjusting the pH to 7.4 if necessary, and then adding the insulin, mixing the solution at approx. 40°C. Male Lewis rats (Charles River) were fasted at least 16 h before the study. The rats weighed 275–350 g. Insulin was administered buccally, nasally, and rectally. Doses ranged from 2 to 50 U/kg. In each case the dosing volume was 0.2 ml/kg. The methods for isolating the dosing site, sample analysis, and data treatment were described previously (Aungst et al., 1988). Briefly, either surgical or physical methods were used to maintain the dosing solution at the buccal, nasal, and rectal dosing sites. Rats were maintained under urethane anesthesia. Blood samples were collected by tail bleeding before, and at various times after insulin dosing. Plasma glucose concentrations were measured using an autoanalyzer, and post-dose concentrations were expressed as a percentage of the pre-dose concentration. Insulin decreased the plasma glucose concentrations over the 4 h experiment, and the area of the decrease in the concentration vs time curve was measured for each rat. This represents the response value. Similar data were also obtained after i.m. insulin dosing, and a dose/response curve was constructed. The potency of transmucosally administered insulin was calculated relative to the i.m. route using methods described previously (Aungst et al., 1988). After obtaining a response value for a transmucosally administered insulin dose, the i.m. dose providing an equivalent response was calculated from the i.m. dose/response curve. From a comparison of the i.m. and transmucosal doses producing equivalent responses, the relative potency of transmucosal insulin was obtained. This is indicative of, but not necessarily equivalent to, bioavailability. Various reports have shown that the changes in plasma glucose concentrations are proportional to plasma insulin concentrations, including when insulin was administered rectally (Van Hoogdalem et al., 1990)

and nasally (Mishima et al., 1989). All data are reported as mean ± S.E.

3. Results

Transmucosal insulin absorption was evaluated by assessing the potency of buccal, nasal, and rectal insulin, relative to i.m. insulin. This required administering insulin doses which produced a pharmacologic response within the linear portion of the i.m. dose/response curve. Therefore, various insulin doses and adjuvant concentrations were used. Control (no adjuvant) groups and groups coadministered adjuvants were not necessarily administered equal insulin doses. Rectally administered insulin produced measurable responses at 2–50 U/kg doses, as shown in Fig. 1. The potency of rectal insulin, relative to i.m. insulin, averaged 9.5 ± 2.0% over these dose ranges. This can be seen in the 10-fold difference in the dose/response curves shown in Fig. 1. When insulin was administered without adjuvants by the buccal and nasal routes, 50 U/kg doses were required to produce consistent decreases in plasma glucose concentrations. There was therefore only the 50 U/kg insulin dose in the buccal and nasal control groups, but the data for the rectal route are given as both the 50 U/kg insulin dose and as the average from the 2–50 U/kg dose range (Table 1). These results with the various control groups have been previously reported (Aungst and Rogers, 1988; Aungst et al., 1988).

Octylglucoside and dodecylmaltoside were initially evaluated using 10 U/kg insulin doses and

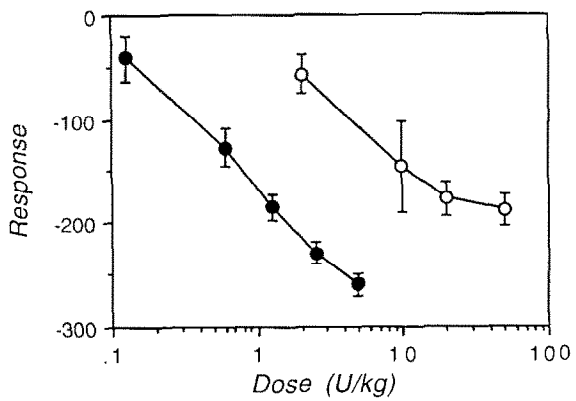

Fig. 1. Dose/response curves for intramuscularly (●) and rectally (○) administered insulin in rats. The measured response was the cumulative percentage change of plasma glucose concentrations over a 4 h period. There were six or more rats in each group.

5% adjuvant concentrations. Typical results are illustrated in Fig. 2, which shows the effects of dodecylmaltoside on the decrease of plasma glucose concentrations after buccal insulin. All results are summarized in Table 1. The absorption promoting effects of octylglucoside and dodecylmaltoside depended on the absorption site, with buccal > nasal > rectal.

Dodecylmaltoside was further evaluated in studies in which the insulin dose and the dodecylmaltoside concentration were varied. As shown in Table 2, buccal insulin doses as low as 2 U/kg produced quantifiable pharmacologic responses when administered with 5% dodecylmaltoside. The 50 U/kg dose produced an average response near the maximum response on the i.m. dose/response curve. In such instances when the mea-

Table 1
Potency of insulin administered via the buccal, nasal, or rectal mucosa, relative to intramuscular injection, and the effects of octylglucoside and dodecylmaltoside as vehicle additives

Group	Insulin Dose (U/kg)	Potency relative to i.m. (%) [a]		
		Buccal	Nasal	Rectal
Control	50	0.8 ± 0.3 (9)	2.0 ± 0.5 (6)	3.2 ± 0.6 (6)
	2–50			9.5 ± 2.0 (24)
5% octylglucoside	10	20.4 ± 5.0 (8) [b]	11.7 ± 4.1 (8)	5.5 ± 3.0 (6)
5% dodecylmaltoside	10	30.0 ± 8.7 (8) [b]	8.8 ± 2.7 (6) [b]	7.6 ± 2.3 (6)

[a] Values in parentheses denote the numbers of animals in the group.
[b] Significantly different ($p < 0.05$) from the control group; for rectal doses the 2–50 U/kg dose range was used as controls.

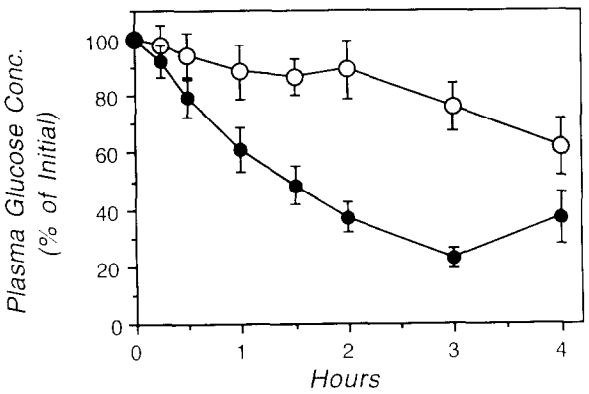

Fig. 2. Plasma glucose concentration vs time curves in rats administered insulin buccally at 50 U/kg with no adjuvant (○) or at 10 U/kg with 5% dodecylmaltoside (●). Data are mean ± S.E. of 9 and 8 rats, respectively.

sured response approaches the maximum response, relative pharmacologic potency would underestimate relative bioavailability. The effect of varying the dodecylmaltoside concentration, using 10 U/kg insulin doses, is summarized in Table 3. All concentrations tested above 0.5% increased the average value of potency of buccal insulin, but because there was much inter-animal variabil-

Table 2
Pharmacologic potency, relative to i.m., of various doses of buccally administered insulin in 5% dodecylmaltoside vehicles

Insulin dose (U/kg)	N [a]	Potency relative to i.m. (%)
2	6	17.6 ± 4.7
5	6	10.2 ± 4.0
10	8	30.0 ± 8.7
20	7	17.8 ± 6.3
50 [b]	6	8.7 ± 1.1

[a] Number of animals.
[b] Insulin was not completely in solution.

Table 3
Effect of various concentrations of dodecylmaltoside on buccal insulin absorption (10 U/kg doses), as indicated by its pharmacologic potency relative to i.m. doses

Dodecylmaltoside concentration (%)	N [a]	Potency relative to i.m. (%)
5	8	30.0 ± 8.7 [b]
2.5	6	8.1 ± 2.9
1	7	15.4 ± 5.6
0.5	6	1.2 ± 0.7

[a] Number of animals.
[b] Significantly different from control, 0.5%, and 2.5% groups at $p < 0.05$ using analysis of variance and Duncan's multiple range test. Other differences were not significant.

Table 4
Effects of various adjuvants on buccal insulin absorption from pH 7.4 aqueous vehicles in rats

Adjuvant	Molar concentration [a]	CMC (mM) [b]	Insulin dose (U/kg)	Potency relative to i.m. (%)	N [c]
Control	–		50	0.8 ± 0.3	9
Hexylglucoside	0.19	250	10	8.2 ± 3.9	6
Octylglucoside	0.17	20–25	10	20.4 ± 5.0 [d]	8
Nonylglucoside	0.16	6.5	10	2.4 ± 1.2	6
Decylglucoside [e]	0.16	2–3	10	18.2 ± 5.1 [d]	6
Dodecylglucoside [e]	0.14	0.13	10	6.7 ± 3.1	6
Octylthioglucoside	0.16	9	10	12.9 ± 4.5	6
Decylmaltoside	0.10	1.6	10	6.4 ± 3.9	7
Dodecylmaltoside	0.10	0.1–0.6	10	30.0 ± 8.7 [d]	8
Decanoyl-*N*-methylglucamide	0.14	6–7	10	26.6 ± 6.4 [d]	6
Sorbitan monolaurate [e]			50	1.3 ± 0.5	6
Methyl gluceth 10			50	0.7 ± 0.2	6

[a] All solutions were prepared at 5% concentrations.
[b] Critical micelle concentration, from Calbiochem product information.
[c] Number of animals.
[d] Significantly different ($p < 0.05$) from the control group. Statistical analysis was by analysis of variance and the t-test. If the difference between means is greater than 14.3, the groups are significantly ($p < 0.05$) different.
[e] Adjuvant was not completely soluble at the 5% concentration.

ity only the effect at the 5% concentration was statistically significant.

Various structural analogs of octylglucoside and dodecylmaltoside were evaluated as absorption promoters for buccally administered insulin. Data are summarized in Table 4. In the alkylglucoside series, the CMC decreases with increasing alkyl chain length. Aqueous solubility also apparently decreased with increasing chain length, since decyl- and dodecylglucoside were not completely soluble at the 5% concentrations tested. Octyl- and decylglucoside were the most effective absorption promoters, followed by hexylglucoside; however, nonyl- and dodecylglucoside had no significant effect. The variation of effects among these compounds was unrelated to their CMC. Octylthioglucoside, an octylglucoside analog wherein a sulfur replaces the ether oxygen, was also an effective absorption promoter. Dodecylmaltoside was more effective than decylmaltoside. Decanoyl-*N*-methylglucamide, with an amide linking the alkyl and glycoside portions of the molecule, was also a very effective absorption promoter. Sorbitan monolaurate represents a surfactant wherein an ester links the alkyl and glycoside substituents. It had no permeation enhancing properties. Methyl gluceth 10 is a surfactant comprised of methylglucose and polyoxyethylene substituents. This compound also had no absorption promoting effects for buccally administered insulin, which could be related to its lack of a hydrophobic alkyl group.

4. Discussion

Insulin had low and variable absorption, based on pharmacologic potency, when administered buccally, nasally, or rectally without an absorption promoter. Dodecylmaltoside and octylglucoside are representative of a new category of absorption promoters, alkylglycosides. These compounds enhanced insulin absorption, and their effects were most significant for the buccal route of administration (Table 1). Murakami et al. (1992) reported that various alkylglycosides increased rectal carboxyfluorescein absorption in rats from 7% to as great as 73%, depending on the adjuvant tested and the concentration used. In their study the alkylglycoside concentrations ranged from 1 to 50 mM, each greater than their critical micelle concentration. Dodecylmaltoside had little effect on rectal insulin absorption in our study, but that finding is not necessarily contradictory. Carboxyfluorescein is a low molecular weight model compound. The effects of alkylglycosides could vary depending on the drug being studied. Such site or compound specificity is encouraging in a way, because it suggests that the adjuvants are not merely creating holes in the membrane by extracting a lipid or protein component, an effect that would certainly be unacceptable. Murakami et al. (1992) also observed site specificity of dodecylmaltoside effects. The increase in carboxyfluorescein absorption had the rank order rectum > colon > ileum, and there was no effect of dodecylmaltoside on the jejunal absorption of carboxyfluorescein.

It is well known that the buccal mucosal membrane is structurally different than the nasal and rectal membranes. The buccal membrane is a stratified squamous epithelium, whereas the nasal and rectal membranes are largely columnar epithelia in which the barrier to absorption is a single layer of cells. The buccal mucosa of the rat is keratinized. Buccal absorption is thought to be primarily via intercellular diffusion; the barrier then is the intercellular lipid and glycolipid matrix (Wertz and Squire, 1991). It has also been proposed that the proteinaceous basal lamina may act as a barrier to buccal absorption (De Vries et al., 1991). The buccal membrane is generally less permeable than other mucosal membranes (Rojanasakul et al., 1992), and it is unaffected by permeation enhancers, such as EDTA, that increase membrane permeation by affecting the epithelial tight junctions (Aungst and Rogers, 1988). Permeation enhancement with sodium deoxycholate or sodium lauryl sulfate has been correlated with expansion of the buccal epithelial intercellular space and reduction of lipid or protein peaks on differential scanning calorimetric thermograms of the buccal membrane (Gandhi and Robinson, 1992). Since the alkyl glycosides shown in this study to have absorption promoting activity also have lipid and protein solubilizing

capability, it is possible that they affect buccal absorption by altering the lipid/glycolipid intercellular matrix or the protein of the basal lamina of the buccal membrane.

Most adjuvants that have been shown in other studies of buccal absorption to have absorption enhancing effects, have been either nonionic or ionic surfactants or detergents, such as bile salts. The compounds whose buccal absorption was enhanced by surfactants or detergents include insulin in rats (Aungst et al., 1988) and dogs (Oh and Ritschel, 1990), calcitonin (Nakada et al., 1988), and salicylic acid (Kurosaki et al., 1989). In a study comparing the effects of ether and ester nonionic surfactants with C_{12} hydrophobic groups, esters were ineffective as buccal insulin absorption promoters but ethers were effective enhancers (Aungst and Rogers, 1989). In this study the ester, sorbital monolaurate, was ineffective. However, in the study of Nakada et al. (1988), sucrose palmitate increased the buccal absorption of calcitonin, whereas sucrose laurate had no effect. Structure/effect studies of nasal (Hirai et al., 1981), rectal (Ichikawa et al., 1980) and dermal (Aungst et al., 1986) permeation enhancers have generally shown maximum effects with adjuvants having C_{12} hydrophobic groups. That relationship did not hold true for the results summarized in Table 4, since octylglucoside and decylglucoside were most effective in the alkylglucoside series. There was no consistent relationship between alkyl chain length and insulin absorption promoting effect. The effect was also not related to the adjuvant's CMC.

A possible advantage of using alkylglycosides as absorption promoters for protein or peptide drugs is that they are reportedly non-denaturing. Another possible advantage is the stabilization they could afford protein or peptide drugs. Hovgaard et al. (1992) demonstrated that dodecylmaltoside stabilized insulin against aggregation, enzymatic degradation, and precipitation. Finally, another possible, but yet unproven advantage is reduced irritation potential relative to other enhancers. Membrane irritation severely limits the clinical application of formulations of proteins and peptides containing absorption enhancers. Murakami et al. (1992) reported no histological changes of the rectal mucosa after dodecylmaltoside application at permeation enhancing concentrations. But more thorough studies are required to determine whether the permeation enhancing and membrane damaging effects of alkylglycosides can be separated.

5. Acknowledgement

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