Enhanced Bioavailability of Calcitonin Formulated with Alkylglycosides following Nasal and Ocular Administration in Rats

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Purpose. The purpose of this study was to characterize the effects of alkylglycosides on the bioavailability of calcitonin following nasal and ocular administration.

Methods. A salmon calcitonin specific radioimmunoassay kit was used to measure calcitonin levels in anesthetized rats at various times after nasal or ocular administration of calcitonin formulated with saline or with octylmaltoside, a medium chain length alkylglycoside or tetradecylmaltoside, a long chain alkylglycoside. The extent of calcitonin absorption was determined directly from the plasma calcitonin level-time curve and the bioavailability of calcitonin was determined from the area under the plasma calcium level-time curve. The calcium level was determined using a colorimetric method.

Results. When the nasal formulation contained calcitonin plus saline or 0.125% octylmaltoside, little or no calcitonin was absorbed. However, plasma calcitonin levels were increased and plasma calcium levels were decreased when the nasal formulation contained calcitonin plus 0.125% or 0.25% tetradecylmaltoside. Maximal calcitonin levels were observed 7.5–10 min after nasal administration of the formulation. Ocular administration of calcitonin formulated with tetradecylmaltoside also resulted in calcitonin absorption, but less calcitonin absorption was found after ocular administration than after nasal administration.

Conclusion. The experimental data indicate that tetradecylmaltoside, but not octylmaltoside, can be effectively used to enhance the bioavailability of nasally and ocularly administered calcitonin.

KEY WORDS: calcitonin; insulin; blood glucose; alkylglycoside; pharmacokinetics.

INTRODUCTION

The systemic delivery of most peptide- and protein-based pharmaceuticals and their potent synthetic analogues require a parenteral delivery system. However, congenital and acquired metabolic disorders such as diabetes mellitus and osteoporosis require long-term therapy by peptide and protein based pharmaceuticals. Moreover, the rapid disappearance of most peptides and proteins from the body due to first-pass metabolism and degradation by proteolytic enzymes mandates therapeutic regimens that include multiple daily injections to maintain therapeutic efficacy. Research efforts have been directed to explore alternate routes of administration and to develop patient compliant dosage forms for these drugs. The potential routes of administration for systemic delivery of peptide-based pharmaceuticals include the ocular, nasal, pulmonary, buccal, oral, rectal, vaginal, and transdermal routes (1). However, clinically acceptable bioavailability of these drugs generally requires coadministration of an absorption-promoting agent.

Calcitonin, a thyroid polypeptide hormone, is a clinically useful drug in the treatment of a variety of bone diseases characterized by increased bone resorption, such as Paget's disease and postmenopausal osteoporosis. Calcitonin is also used in short-term infusion for the treatment of malignant hypercalcemia. Calcitonin acts by inhibiting osteoclastic bone resorption, thereby reducing plasma calcium levels and increasing bone mineral density in patients with osteoporosis (2). In the United States, salmon and human calcitonin have been available by prescription for delivery by subcutaneous injection. Recently, a nasal formulation of salmon calcitonin, Miacalcin® (Novartis Pharmaceutical Corp., East Hanover, NJ) was released in the US market. Nasal administration of Miacalcin® is indicated for the treatment of postmenopausal osteoporosis. Salmon calcitonin has also been found to be useful in the treatment of primary hyperparathyroidism (3).

Unfortunately, bioavailability of calcitonin from this nasal spray formulation, Miacalcin®, is very limited (approximately 3%) and variable (0.3–30%) when compared with the bioavailability obtained from the intramuscular route (4). Several investigators have shown that permeation-enhancers can increase the bioavailability of nasally administered calcitonin using different surfactants, such as sodium glycocholate, dihydrofusidate, sodium taurocholate, laurylcarnitine chloride, and cyclodextrins, such as dimethyl-ß-cyclodextrin (5–8). Some of these studies have shown that the concentration of calcitonin in blood was increased, with a concomitant decrease in blood calcium levels. In addition, eel-calcitonin formulated with polyacrylic acid gel has been found to produce a prominent hypocalcemic effect in rats (9).

Recently, we have shown that the bioavailability of nasally and ocularly administered peptide drugs such as insulin and glucagon can be improved in anesthetized rodents using long-chain alkylglycosides, such as tetradecylmaltoside, as absorption promoters (10–12). Tetradecylmaltoside, a nonionic detergent with a 14-carbon alkyl chain linked to maltose (Fig. 1), increased insulin absorption even when the concentration of the excipient was very low (0.06–0.25%). In contrast, a shorter chain alkylglycoside, octylmaltoside, which contains an 8-carbon alkyl chain linked to maltose, was not effective at enhancing nasal insulin absorption (10–12). Enhanced insulin absorption was observed when tetradecylmaltoside was added to the formulation and applied either via the nasal route or the ocular route (11,12). Previous studies have shown that insulin applied to the surface of the cornea was not able to cross the corneal surface to a large degree (13). However, insulin removed from the eye by drainage down the nasolacrimal duct after administration as eyedrops was absorbed into the systemic circulation by permeation across the nasal sinus epithelium (14). While the precise molecular mechanism of action remains uncertain, it is believed that the tetradecylmaltoside increased drug absorption through a direct effect on the nasal epithelium, rather than though an interaction with the peptide drug itself (15,16). Tetradecylmaltoside may

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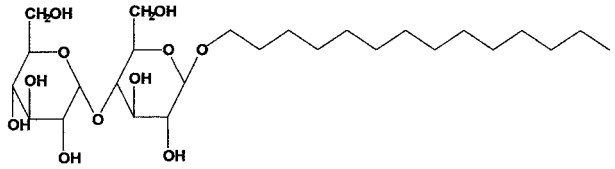
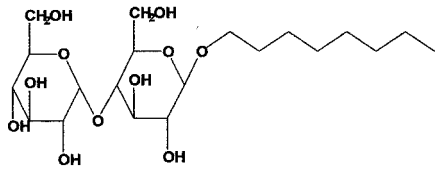


Tetradecyl-β-D-maltoside



Octyl-β-D-maltoside

Fig. 1. Chemical structure of tetradecylmaltoside and octylmaltoside.

intercalate into the lipid bilayer and cause either an increase in the transcellular movement of insulin and other peptide drugs, through partially permeabilized epithelial cells, or an increase in paracellular movement of peptide drugs by loosening tight junctions between cells. If tetradecylmaltoside exerts its effects on insulin permeability in this manner, the absorption of other small protein drugs, such as calcitonin, may also be enhanced.

In this investigation, we report on the results of experiments in which tetradecylmaltoside and octylmaltoside were tested to determine if either of these agents can be used to improve the bioavailability of nasally or ocularly administered salmon calcitonin.

MATERIALS AND METHODS

Materials

Tetradecylmaltoside and octylmaltoside were purchased from Anatrace Corp. (Maumee, OH) and Calbiochem Corp. (La Jolla, CA). Miacalcin® (2200 IU/ml) was a product of Novartis Pharmaceuticals Corp. (East Hanover, NJ).

Preparation of Nasal and Ocular Formulations

Tetradecylmaltoside and octylmaltoside stock solutions were prepared by dissolving the excipients in normal saline at 1% (w/v) and stored for 30 days or less at 4°C. On the day of an experiment, the stock solutions were used to prepare the nasal and ocular formulations. The nasal and ocular formulations were prepared by mixing Miacalcin® (2200 U/ml) with the appropriate concentrations of tetradecylmaltoside or octylmaltoside or saline to achieve a final mixture that contained either 110 U/ml or 1100 U/ml calcitonin.

Absorption Studies in Rats

Nasal, ocular, and intravenous absorption studies were performed in Sprague-Dawley male rats obtained from Charles River Laboratories (Charlotte, NC). Rats were anesthetized by intramuscular injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and anesthesia was maintained with additional xylazine/ketamine as needed throughout the experiment. For nasal administration, rats were placed in the supine position. The nasal formulations, 0.02 ml per rat, were instilled into the left nares, using a pipetter (Pipetman, Gilson, France) with a disposable plastic tip, 20–30 min after the initial dose of anesthetic agents. After 2 min, the rats were turned over and remained in the prone position. For ocular administration rats were placed in the prone position. The ocular formulations (0.02 ml per rat) were administered to the corneal surface of the left eye of anesthetized rats, also using a pipetter. Care was taken to apply one drop at a time to avoid spillage of the formulation. After dosing, rats were left in the prone position during the experiment. For intravenous absorption studies, the right femoral vein of anesthetized rats was ligated and the formulation was infused slowly. Extreme care was taken to avoid any leakage from the vein during this process.

The total amount of calcitonin delivered nasally or ocularly to each rat was either 2.2 or 22 U. The formulations, administered either nasally or ocularly, were allowed to clear from the site of administration by normal physiological processes such as swallowing (nasal administration) or drainage down the nasolacrimal duct (ocular administration). Blood samples were collected from the tips of the tails of anesthetized animals in plastic microfuge tubes containing 5 μl of heparin (1000 U/ml) for determination of plasma calcitonin and calcium levels. Blood samples were immediately placed on ice and within 1 h plasma was separated and stored at –20°C until assayed for calcitonin and calcium content. A salmon calcitonin specific radioimmunoassay kit (DSL Inc., Webster, TX) was used to measure salmon calcitonin levels in the rat at various times after nasal or ocular administration of calcitonin. T_{max} and C_{max} were determined directly from the plasma calcitonin level-time curve and the area under the plasma level-time curve (AUC) was determined by the trapezoidal method using WinNonlin (Pharsight Corp. Mountain View, CA). Calcium was measured in 0.01 ml aliquots of plasma using a colorimetric method (Sigma Chemical, St. Louis, MO).

The animal studies were conducted according to the principles outlined in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

RESULTS AND DISCUSSION

The absorption of calcitonin was negligible when 2.2 U of calcitonin was formulated in saline or in 0.125% octylmaltoside and applied nasally (Fig. 2). The absorption of calcitonin increased when 0.125% tetradecylmaltoside was added to the nasal formulation (Fig. 2). Plasma calcitonin levels were maximal (T_{max}) 10 min after nasal administration of a formulation containing 2.2 U calcitonin plus 0.125% tetradecylmaltoside. The $AUC_{0\text{-}40}$ of the plasma calcitonin-time curve after nasal administration of a formulation containing 2.2 U of calcitonin plus 0.125% tetradecylmaltoside (Table I) was 4-fold greater than that after nasal administration of a formulation containing calcitonin plus saline or octylmaltoside. The $AUC_{0\text{-}40}$ for intranasal administration of 2.2 U of calcitonin formulated with 0.125% tetradecylmaltoside (3,500 pg/ml × min) represented a bioavailability of 53% when compared to the $AUC_{0\text{-}40}$ (6,620 pg/ml × min) for intravenous administration of 2.2 U

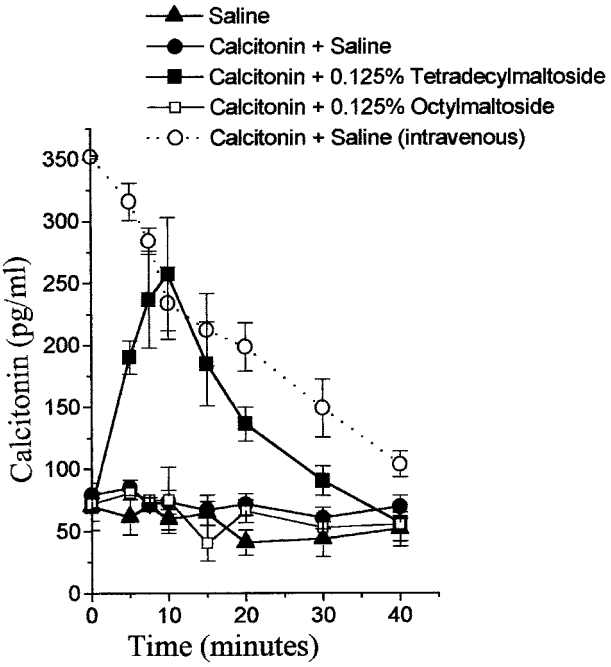


Fig. 2. Changes in plasma calcitonin concentrations after nasal or intravenous administration of 2.2 U calcitonin formulated in saline or in the presence of 0.125% tetradecylmaltoside or octylmaltoside. Data represent mean plasma calcitonin concentrations ± standard error of the mean, n = 3.

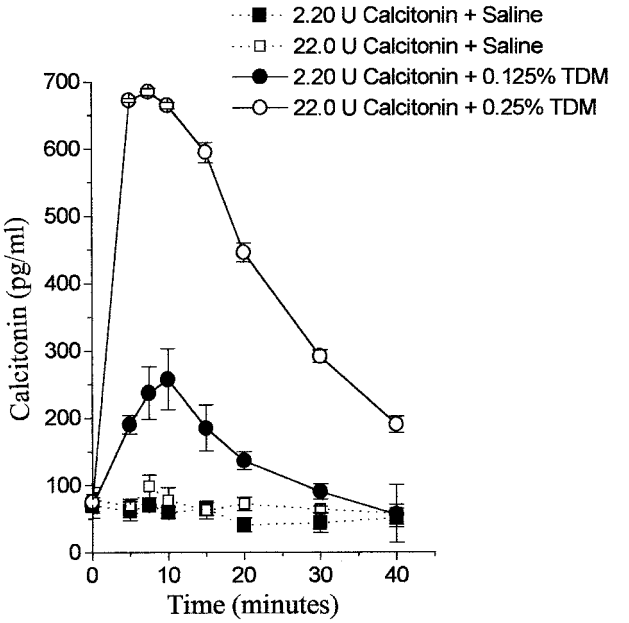


Fig. 3. Changes in plasma calcitonin concentrations are shown after nasal administration of 2.2 or 22 U of calcitonin in saline or in the presence of either 0.125% or 0.25% tetradecylmaltoside. Data represent mean plasma calcitonin concentrations ± standard error of the mean, n = 3.

of calcitonin. When the concentration of tetradecylmaltoside was increased from 0.125% to 0.25%, the $AUC_{0\text{-}40}$ for calcitonin was further increased to a value of 6,250 pg/ml × min (Table I). To determine if more calcitonin could be absorbed from the nasal route, the amount of calcitonin applied to the nose was increased 10-fold from 2.2 to 22 U. In the absence of tetradecylmaltoside, calcitonin absorption remained negligible (Fig. 3). In the presence of 0.25% tetradecylmaltoside, the amount of calcitonin absorbed was increased further by 3.2-fold when compared to the amount of calcitonin absorbed when 2.2 U of calcitonin plus 0.25% tetradecylmaltoside was applied nasally (Table I). By comparison, the $AUC_{0\text{-}40}$ for 22 U of calcitonin in saline with the $AUC_{0\text{-}40}$ for 22 U calcitonin in 0.25% TDM, reflects a 23-fold increase in the relative bioavailability of calcitonin (Table I, Fig. 3).

Table I. AUC of Plasma Calcitonin-Time Curve following Nasal and Ocular Delivery of Calcitonin

	Calcitonin (units)	Tetradecylmaltoside (%)	AUC_{0-40} pg/ml × min (in thousands)
Nosedrops	2.20	0	0.85
	2.20	0.125	3.50
	2.20	0.25	6.25
	22.0	0	0.71
	22.0	0.25	16.37
Eyedrops	2.20	0	0.36
	2.20	0.125	1.07
	22.0	0	0.60
	22.0	0.25	12.58

AUC_{0-40} data were determined by trapezoidal method. Background AUC_{0-40} values were determined in rats that received nosedrops containing only saline. The background value obtained in rats that received saline without calcitonin (2620 pg/ml × min) was subtracted from all values obtained when calcitonin was administered.

An effort was then made to determine if tetradecylmaltoside could be used to enhance the absorption of calcitonin applied topically to the eye. Ocular administration of peptide drugs represents an alternative to nasal administration that some patients consider preferable. Drug absorption after ocular administration actually occurs at the same location as for intranasal administration, in the nasal sinus, following drainage of the ocular formulation down the nasolacrimal duct (13,14). When animals received a formulation containing 2.2 U calcitonin plus 0.125% tetradecylmaltoside, administered ocularly, the amount of calcitonin absorbed was negligible (Fig. 4). When compared to the amount of calcitonin absorbed following nasal administration of the same formulation, ocular administration was ineffective. A more substantial absorption of the peptide was observed after ocular administration of a formulation containing larger amounts of both calcitonin (22 U) and tetradecylmaltoside (0.25%) (Fig. 4, Table I). Again, direct comparison of calcitonin absorption following nasal application and ocular application revealed that the nasal route of administration delivered more of the peptide to the systemic circulation (Fig. 3, Table I).

The calcium concentration in plasma was measured concomitantly in these rodents to determine if immunologically reactive calcitonin recovered in the plasma was also biologically active. Rats receiving nosedrops containing 2.2 U calcitonin formulated with saline did not demonstrate a significant decrease in plasma calcium levels (Fig. 5). In contrast, rats receiving a nasal formulation containing 2.2 U of calcitonin plus 0.25% tetradecylmaltoside displayed a significant decrease in plasma calcium levels (Fig. 5). These data confirm that tetradecylmaltoside increases the biovailability of calcitonin following nasal or ocular instillation.

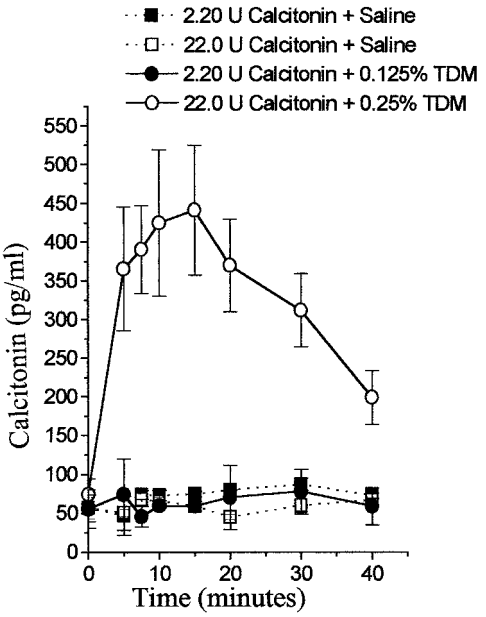


Fig. 4. Changes in plasma calcitonin concentrations are shown after ocular administration of 2.2 or 22 U of calcitonin in saline or in the presence of either 0.125% or 0.25% tetradecylmaltoside. Data represent mean plasma calcitonin concentrations ± standard error of the mean, n = 3.

Overall, the experimental data indicate that tetradecylmaltoside, but not octylmaltoside, can be effectively used to enhance the bioavailability of nasally and ocularly administered calcitonin. It can be argued that the bioavailability of 2.2 U intranasal calcitonin formulated with 0.25% tetradecylmaltoside (6,250 pg/ml × min) can approach the same level observed with intravenous calcitonin administration (6,620 pg/ml × min). The structure/function relationship reported herein for calcitonin absorption is entirely consistent with the earlier studies that measured nasal and ocular insulin delivery. The larger, more lipophilic alkylglycoside was better able to enhance the absorption of the peptide drug than the intermediate length alkylglycoside. The physical and chemical changes that occur during surfactant-induced membrane permeability enhancement have not been rigorously characterized at the molecular level. Several important questions remain to be answered. The hydrophilic/lipophilic balance and critical micelle concentrations of the alkylglycoside family of reagents change as the alkyl chain length increases. The permeability of the nasal sinus epithelium to calcitonin, as well as to insulin, increases when a very low concentration of TDM is added to the nasal formulation. The finding that TDM increases the bioavailability of two distinct peptides, calcitonin and insulin, reinforces the hypothesis that the effect of the surfactant agent is to alter the permeability of the nasal epithelium as opposed to selectively modifying insulin or calcitonin uptake. There is likely to be an upper limit of enhanced membrane permeability beyond which irreversible toxicity occurs. Additional studies in this laboratory have shown that hexadecylmaltoside, which contains a sixteen carbon alkyl chain, is no more potent than tetradecylmaltoside in enhancing nasal delivery of insulin (unpublished data). Because hexadecylmaltoside is more lipid-soluble than TDM and has a lower CMC value, the permeability enhancement cannot solely be explained by the surfactant's hydrophobic properties as represented by the HLB or CMC (17).

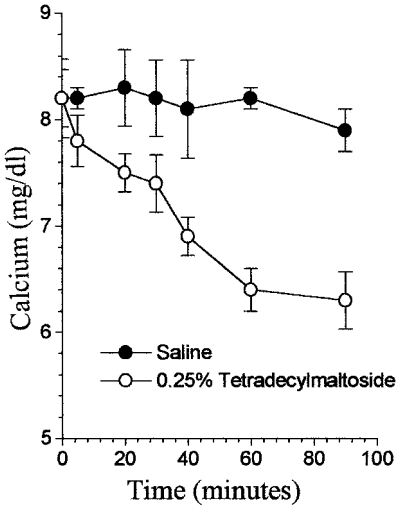


Fig. 5. Changes in plasma calcium levels after nasal administration of 2.2 U calcitonin in the presence of saline or 0.25% tetradecylmaltoside. Data represent mean calcium concentrations ± standard error of the mean, n = 3.

The toxicity of surfactant agents is of great concern in the development of formulations to be used for the nasal and ocular delivery of peptide drugs. At present, little is known about the toxicity of alkylglycosides. Based on the fact that very low concentrations of long-chain alkylglycosides (0.05–0.25%) can effectively increase the absorption of peptide drugs such as insulin and calcitonin, it can be argued that local toxicity will likely be less problematic than observed with other, less potent absorption enhancing agents. Finally, caution must be used when extrapolating from the results of these animal studies in terms of identifying reagents that may, in the future, prove useful in improving the bioavailability of calcitonin and other peptide drugs administered via the nasal or ocular route to humans.

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