ABSTRACT SUMMARY

The purpose of this study was to evaluate the feasibility of iontophoresis (ITP) on transbuccal peptide drug delivery in vivo. sCT was chosen as a model drug and N-acetyl-L-cysteine (NAC) as well as sodium deoxyglycocholate (SDGC) were used as Chemical enhancer(CE), and 0.5 mA/cm² of fixed anodal electric current was employed as a physical enhancer. ITP and ITP with SDGC combination showed not only fast onset of action but sustained hypocalcemic effect compared to nasal and intravenous groups. FT-IR and H&E staining supported that iontophoresis was thought to be a safe and potential strategy to enhance and sustain transbuccal peptide delivery in vivo.

INTRODUCTION

Delivering therapeutically active biopharmaceuticals via oral route has been challenged for decades because of the low bioavailability due to enzymatic degradation or acidic condition of gastrointestinal tract. Therefore they are unsuitable for oral delivery, and are mostly delivered via parenteral route so far. Although parenteral route has many benefits, injection has been claimed with some serious problems. As peptides have a short half-life in human body, repeated injections are indispensable to get expected therapeutic effects. Another problem is side effects such as phlebitis or tissue necrosis by repeated injection. Thus potential alternative delivery methods via nasal, vaginal, rectal, oral, transdermal and buccal routes have been considered in order to find solution for the raised problems by injection.

Buccal tissue can avoid the first-pass hepatic metabolism, gastrointestinal degradation and has a fast onset of action. It also may provide easy administration with little irritation, thereby improves patient compliances. The absorption of drug via buccal route is not interfered by the potential variations in the gastric-emptying rate or the presence of foods. In addition, buccal mucosa has rapid recovery property compared to other mucosal sites and has been taken into account for sustained drug delivery method especially for peptide drugs.

Despite the non-keratinization of buccal mucosa, however various permeation enhancing strategies are often required because the mucosal lining protects the body from external environments and the intercellular lipid within buccal epithelial tissue acts as a physical barrier. Iontophoresis applies a low electric current and it is advantageous in trans-membrane delivery of charged, hydrophilic and macromolecular drugs. In these regards, buccal tissue may be an appropriate alternative delivery route for sCT because it has been considered for sustained release of drugs.

EXPERIMENTAL METHODS

Male New Zealand white rabbits of 2.5~3.0 kg were. Each rabbit was anaesthetized with mixed solution of equal amounts of Zoletil 50® and Xylazine HCl. 15% of Poloxamer 407 was used and the mucoadhesive property of the hydrogel was obtained by adding 2 % (w/v) chitosan (control hydrogel). The test hydrogels were prepared by the addition of enhancers into control formulation. Each hydrogel formulation contained 200 IU of sCT. Two chemical enhancer(CE) groups were used; i) mixture of 1% sodium deoxyglycocholate (SDGC) with 10% ethanol and ii) mixture of 5 % N-acetyl-L-cysteine (NAC) with 10% ethanol. The rabbit was anaesthetized with Zoletil 50® and Xylazine HCl (Rompun®), and then sCT formulations were placed onto the rabbits’ buccal mucosa.

We investigated the single effect of electrical assistance and combination effect with CE for buccal permeation of sCT. An Ag/AgCl electrode was used. 0.5 mA/cm² of fixed electric current was constantly applied by a DC power supply and controlled using a current controller. 200 IU of sCT was injected via ear vein and nasal mucosa of rabbit. The blood samples were collected at the desired time points and calcium levels of serum samples were measured using Ca²⁺ kit.

The buccal samples were mixed with KBr powder and FT-IR spectra of rabbit's buccal samples were obtained in the frequency range of 4,000-800 cm⁻¹.
RESULTS AND DISCUSSION

Iontophoresis enhanced significantly the hypocalcemic effect of sCT compared with no enhancer group and especially the combination of iontophoresis and SDGC dramatically enhanced hypocalcemic effect (Figure 1).

Figure 1. Effect of electrical assistance on the transbuccal delivery of sCT (n = 3). ●; negative control (no sCT formulation), ○; sCT with no enhancer, ▼; ITP with no enhancer, △; ITP with CE (5% NAC with 10% ethanol), and ■; ITP with CE (1% SDGC with 10% ethanol).

Intranasal or transbuccal delivery of sCT with no enhancing method decreased the blood calcium level compared to negative control but the effect was statistically insignificant. Figure 2. The comparison of various administration routes (n = 3). ●; intravenous administration of sCT with no enhancer, ○; intranasal administration of sCT with no enhancer, ▼; transbuccal delivery of negative control (no sCT formulation), △; transbuccal delivery of sCT with no enhancer, and ■; transbuccal delivery of sCT by ITP with CE (1% SDGC with 10% ethanol).

Parenteral administration of sCT exhibited fast and the strongest hypocalcemic effect and the values were statistically significant (p<0.01) among the no enhancer groups. But the hypocalcemic effect by intranasal and parenteral delivery diminished after 4 h. On the other hand, iontophoretic transbuccal delivery of sCT applied 1% SDGC with 10% ethanol increased dramatically the hypocalcemic effect (p<0.01) and it was sustained for 8 h which supports that the iontophoresis could sustain the delivery of sCT, which reflects buccal delivery suitable for sustained peptide drug delivery.

Figure 3 represents the results of FT-IR studies. The use of iontophoresis or CE caused the broadening and reduction of C-H stretching absorbance peaks which resulted from the increase of lipid bilayer fluidity and the epithelial lipid extraction, respectively. Such effects in C-H stretching peaks were dramatically enhanced by combination of iontophoresis with SDGC, and finally this formulation exhibited the strongest hypocalcemic effect because the transbuccal drug flux will be closely related to the lipid fluidity.

Figure 3. FT-IR spectra of the excised rabbit’s buccal mucosa. Symmetric and asymmetric C-H bond stretching absorbances were observed after the application of enhancing methods.

CONCLUSION

Iontophoretic buccal delivery showed sustained hypocalcemic effect compared to i.v. injection and nasal delivery. Although the FT-IR study has shown the changes in the barrier function of the buccal epithelial lipids, the combination of SDGC and iontophoresis was thought as a safe and optimal strategy to enhance the transbuccal sCT delivery.

REFERENCES