약제학회지(2005), 제 35 권 제 5 호
J. Kor. Pharm. Sci., Vol. 35, No. 5, 369-373 (2005)

Buccal Delivery of [D-Ala^2, D-Leu^5]Enkephalin Incorporated in Mucoadhesive Poly(acrylic acid) Hydrogels

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(Received September 5, 2005 · Accepted October 18, 2005)

ABSTRACT – The objectives of the current work is to understand the factors impacting the formulation and performance of a Carbopol mucoadhesive buccal delvery system for a model peptide drug, [D-Ala^2, D-Leu^5]enkephalin (DADLE, Mw=569.7) with comparable chemical and enzymatic stability. Specifically, *in vitro* buccal DADLE delivery from the cross-linked poly(acrylic acid) (PAA) hydrogel system was characterized. In addition, the influences of several penetration enhancers on the ex vivo buccal absorption of DADLE were also studied. In this study, the PAA hydrogels generally swell to 100% of their original weight in the phosphate pH 7.4 buffer. The water penetration into the PAA hydrogel occurred based on a zero-order kinetics for the first 60 min and steadily decreased afterwards. From the release study, it can be seen that the initial DADLE release was so rapid and the rate of release of DADLE decreased as the time elapsed. The porcine buccal tissue was found to be permeable to DADLE with a flux value of 0.07 %/cm^2/hr (± 0.01 SD). From the ex vivo diffusion study, it was found that sodium taurodihydrofusidate showed a greater degree of enhancement compared to the phospholipids with an Enhancement Ratio (ER) of 8.7 compared to 2.7 and 1.9 for didecanoylphosphatidylcholine and lysophosphatidylcholine, respectively. The work encompassed within this paper has demonstrated the feasibility of using the PAA hydrogel delivery system with its good mucoadhesive properties for the buccal delivery of peptides.

Key words – Buccal delivery, Mucoadhesive hydrogel, Peptide, Penetration enhancement

Poly(acrylic acid) (PAA) has been demonstrated to be a good mucoadhesive.[1] The interactive groups are carboxyl groups with a pKa of about 4.75. Poly(acrylic acid) shows better mucoadhesive properties at pH's corresponding to the majority of the carboxylic acid groups being protonated. The mucoadhesive properties of poly(acrylic acid) are mainly due to hydrogen binding. Another important aspect for mucoadhesion, when poly(acrylic acid) is crosslinked to form a hydrogel, is the degree of hydration of the polymer chains and their expansion. The degree of expansion depends on the ability of the polymer matrix to swell. Another important aspect is polymer chain mobility, since interpenetration and entanglement of the mucoadhesive polymer with substrates are partly responsible for mucoadhesion. An additional factor to show better mucoadhesion is the hydration of the dry mucoadhesive polymer by the extraction of water from the mucosal surface. When the polymer matrix is hydrated, the matrix swells and mucin strands are drawn inside the polymer network.

Compared to conventional drug compounds, peptide drugs possess unique requirements in terms of formulation and restrictions for delivery.[2] Proteins and peptides have generally large molecular weights. Thus, when formulated into a polymeric delivery system, the polymer must be able to erode or have pores or channels comparable to the size of the molecule. These channels and pores may not be a part of the polymer itself but may be formed as a result of dynamic swelling of chain segment. For this reason, hydrogel form is one of proper choice to deliver peptide and protein drug. Especially, hydrogel for buccal delivery can provide fairly good mucoadhesion, controlled release properties, and a good feel in the mouth.[3]

The objectives of the current work is to understand the factors impacting the formulation and performance of a Carbopol mucoadhesive buccal delvery system for a model peptide drug, [D-Ala^2, D-Leu^5]enkephalin (DADLE, Mw=569.7) with comparable chemical and enzymatic stability.[4] Specifically, *in vitro* buccal DADLE delivery from the cross-linked poly(acrylic acid) hydrogel system was characterized. In addition, the influences of several penetration enhancers on the ex vivo buccal absorption of DADLE were also studied.

Experimental

Materials

DADLE and phosphate-buffered saline pH 7.4 (PBS) tablets were obtained from Sigma-Aldrich Company (MO, USA).

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[*Tyrosyl*-3,5-^{3}H(N)]DADLE ([^{3}H]DADLE, 1 mCi/ml in ethanol) was obtained from American Radiolabeled Chemicals (MO, USA).

For the preparation of the hydrogels, a linear poly(acrylic acid), Carbopol 907 (MW=450,000) was obtained from B.F. Goodrich (NC, USA) and other chemicals were from Sigma Aldrich Company (MO, USA). Liquid scintillation cocktail (HiSafe OptiPhase III) was supplied by Fisher Scientific (Loughborough, UK).

Preparation of Hydrogel

Ten grams of Carbopol was weighed into a 150 m*l* bottle and 10 m*l* of a sucrose solution of 0.1 g/m*l* was added. The mixture was made up to 150 m*l* with water/acetone mixture (25:75, w/w). An aliquot of 15 g the solution was then poured into the casting device. The cast solutions were dried at 45°C for a day. The cured hydrogels were cut into disks of ca. 5 mm diameter and 0.5 mm in thickness.

Swelling of Hydrogel

The swelling ratio of PAA hydrogel was measured after it was swollen to a desired state. The hydrogel disks were immersed in PBS at 37°C. They were taken out of from the PBS solution at predetermined time intervals, wiped with a tissue paper, and then weighed.

Loading of Hydrogel Disks

Loading model peptide drug, DADLE, into a hydrogel was performed in aqueous peptide solution of 5 mg/m*l*. After the hydrogels were swollen in the solution for 24 hr, they were taken out and dried in a vacuum desicator. The loading amount of the peptide was calculated by the concentration of the loading solution and average volume of swollen hydrogels.

***In Vitro* DADLE Release Study**

Three hydrogels were loaded with DADLE in pH 7.4 PBS DADLE solution of 1.25 mg/m*l* for 24 hr. The DADLE-loaded hydrogels were immersed in 30 m*l* of PBS at 37°C. In a special time interval, 0.5 m*l* of the solution released was withdrawn and 0.5 m*l* of fresh PBS solution was added. Samples (0.5 ml) were then removed at set time intervals and replaced with fresh PBS.

HPLC Assay for DADLE

DADLE concentration was measured by isocratic reverse-phase HPLC (RP-HPLC) using a Hypersil C_{18} column (Hichrom Limited, Reading, UK) at ambient temperature, with UV detection at 214 nm. The RP-HPLC system consisted of a ConstaMetric 3000 solvent delivery system (Laboratory Data Control, Stone, UK), SpectraSYSTEM UV1000 detector (Thermoquest, Withenshaw, UK), and Berthold integration software (Berthold Instruments, Herts, UK). The mobile phase was acetonitrile/0.1% trifluoroacetic acid (30/70, v/v).

Ex Vivo Porcine Buccal Transport Study

DADLE (0.5 mg) was dissolved in 0.5 ml of PBS. 1 μl of 1 mCi/ml in ethanol of [^{3}H]DADLE was also added. Three hydrogels form the same batch were immersed in the solution. After 24 hr, the amount of DADLE in the hydrogels was determined by liquid scintillation counting. After dry, the peptide-loaded hydrogel was introduced to the donor compartment of a Franz cell mounted with porcine buccal mucosa. The experiment was initiated by adding PBS to the receiver compartment. Samples were taken at set time intervals and replaced with fresh buffer. Liquid scintillant was added to each sample and the radioactivity determined. To study the effects of various permeation enhancers on buccal absorption of DADLE, lysophosphatidylcholine (LPC), didecanoylphosphatidylcholine (DDPC) and sodium taurodihydrofusidate (STDHF) were added at a concentration of 1.0%(w/w) against the weight of the hydrogel.

Statistical Analysis

The ex vivo buccal transport results were statistically analyzed using one-way ANOVA and P values of 0.05 or less were considered statistically significant.

Results and Discussion

Knowledge of the water uptake profiles and drug release properties of a swelling drug delivery system is fundamental to its evaluation. By studying these, it is possible to determine which factors are important to control drug release. A hydrogel can be defined as a polymeric material which has the ability to swell in the presence of water and retain a significant amount (> 20%) of water within its structure, but does not dissolve in water.[5] The swelling of the hydrogel matrix may have an effect on the diffusion of the drug by increasing the diffusion coefficient and modifying the diffusional path. Therefore, a hydrogel matrix is a solvent driven dosage form.[5]

The degree of swelling of the hydrogel is mainly governed by the degree of cross-linking present in the hydrogel, relaxation of polymer chains, and the availability of hydrating medium. It has been shown that the general uptake into a hydrogel can be represented by the following equation.

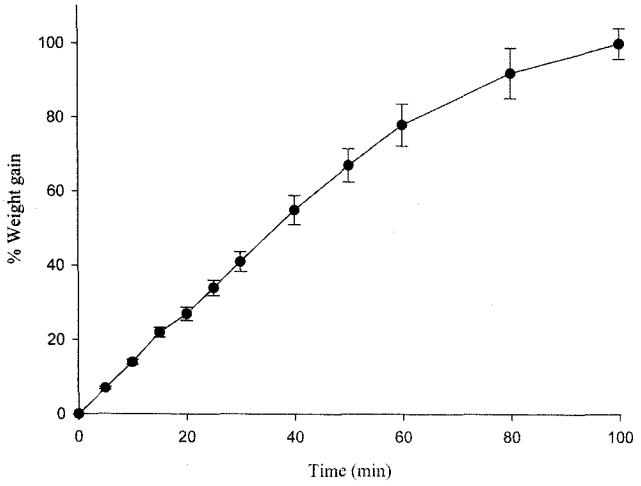

Figure 1–Swelling profile of PAA hydrogels in pH 7.4 phosphate buffer at 37°C. Mean±S.D., n=3.

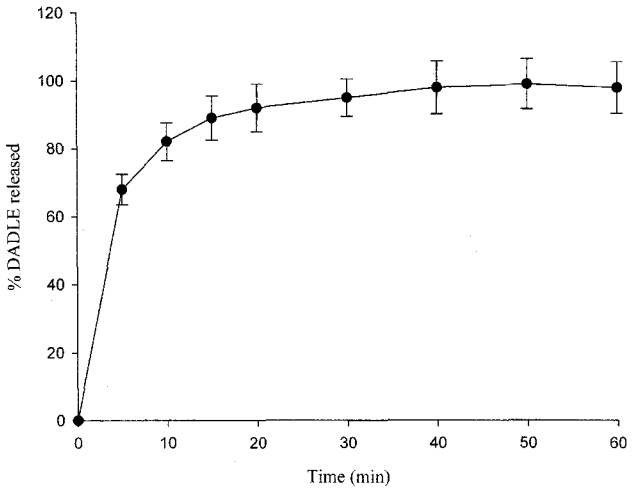

Figure 2–Cumulative % DADLE release from the PAA hydrogel delivery systems in pH 7.4 phosphate buffer at 37°C. Mean±S.D., n=3.

$$M_t/M = kt^n$$

where M_t is the weight of solvent penetrated at time, t and M is the weight at equilibrium, k is the rate of swelling, and n is a physical constant, that indicates the mechanism of swelling. If n = 0.5, diffusion is considered to be Fickian or Case I while for n = 1 diffusion is said to be as Case II or non-Fickian. This method can also be applied to swelling hydrogels but its applicability is limited to the situation that swelling of the system must be less than 25% of its original volume.[6] In this study, the PAA hydrogels generally swell to 100% of their original weight in the phosphate pH 7.4 buffer (Figure 1). Thus, it was unable to describe kinetically using the above equation. It is worthy to note that the water penetration into the PAA hydrogel occurred based on a zero-order kinetics for the first 60 min and steadily decreased afterwards. The high degree of swelling exhibited by the PAA hydrogel may be advantageous in terms of loading the hydrophilic model peptide, DADLE. To fully understand peptide diffusion through a hydrogel, that is assumed to be a passive process, it is important to consider peptide diffusion within a polymeric matrix. Controlling of peptide diffusion within a polymeric matrix can be achieved by changing the degree of cross-linking, controlling the degree of branching and entanglements in the polymer backbone. In addition, the physico-chemical characteristics of the peptide such as molecular size and solubility are another important factors in the penetration through the swollen hydrogel. DADLE consists of 5 amino acids and has the molecular weight of 569.7, along with being regarded as soluble in water. Thus, its diffusion into the PAA hydrogel may have been influenced by the degree of cross-linking. In the present study, to prepare the PAA hydrogel cross-linked by sucrose molecule, the feed mole ratio of PAA to sucrose was only 10:1. Therefore to test the impact of the degree of cross-linking on water uptake and peptide loading amount, further examinations are required.

From the release study, it can be seen that the initial DADLE release was so rapid and the rate of release of DADLE decreased as the time elapsed (Figure 2). This initial burst release phase may be explained by the fact that the peptide was loaded by hydration of the hydrogel. This process involved the removal of excess water from the surface of the hydrogel and may lead to the movement of DADLE to the hydrogel surface which is probably responsible for the initial burst release. The time for 50% release for DADLE was approximately 3 min. There may be a molecular sieving effect within the hydrogel network during the release, which is mainly affected by the molecular size of a penetrant. However, there was no clear evidence that DADLE (Mw=569.7) release was hindered. Thus, it can be supposed that the molecular weight cut-off of the PAA hydrogel system is more than 600, approximately. But caution must be exercised when loading a drug have molecular weight far exceeding approximately 600.

The porcine buccal tissue was found to be permeable to DADLE (Figure 3) with a flux value of 0.07 %/cm^2/hr (± 0.01 SD) (Table I). Using analysis of variance (ANOVA) it was found that for different samples of buccal tissue from the same pig and from different pigs the calculated flux values were not significantly different at the 5% significance level.

From the ex vivo diffusion study, it was found that STDHF showed a greater degree of enhancement compared to the phospholipids with an Enhancement Ratio (ER) of 8.7 com-

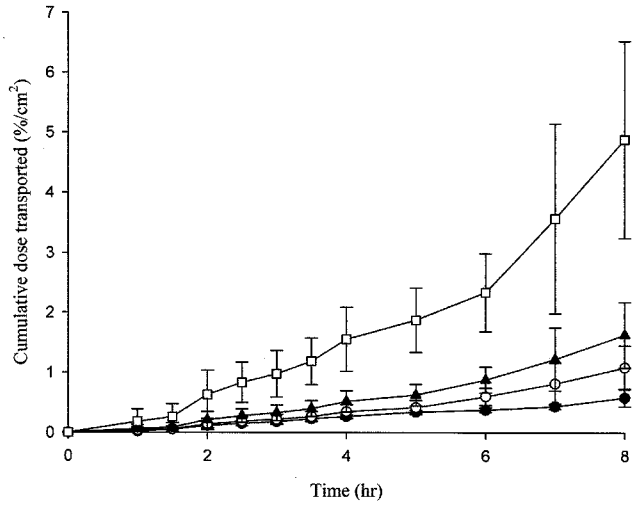

Figure 3–Ex vivo DADLE diffusion (●) across porcine buccal mucosa at 37°C and the effects of STDHF (□), DDPC (▲) and LPC (○). Mean±S.D., n=5.

Table I–*Flux Values of [^{3}H] DADLE Across Porcine Buccal Mucosa at 37°C and the Effects of the Permeation Enhancers Mean±S.D., n=5*

	Flux (%/cm^2/hr)	ER#
DADLE alone	0.07 ± 0.01	-
DADLE + LPC	0.13 ± 0.02*	1.9
DADLE + DDPC	0.19 ± 0.02*	2.7
DADLE + STDHF	0.61 ± 0.07**	8.7

*Significantly different from DADLE alone group at $P < 0.05$.
**Significantly different from DADLE alone group at $P < 0.01$.
#Enhancement ratio = Flux obtained with permeation enhancers/Flux obtained from DADLE alone.

pared to 2.7 and 1.9 for DDPC and LPC, respectively (Table I). A possible explanation for this is that DADLE diffuses via the paracellular route and that STDHF penetration enhancement involves two possible mechanism; extraction of membrane lipids by solubilization within micelles formed above the CMC of 0.15% of STDHF[7] and increasing paracellular spaces by interfering with the connections between cells.[8] The effectiveness of STDHF as a penetration enhancer for hydrophilic macromolecules demonstrated using the in vitro model was substantiated in the literatures. Deurloo et al. showed that inclusion of 1% STDHF in an insulin nasal spray formulation greatly increased insulin absorption in the rabbits and rat models.[9] The degree of enhancement was found to be concentration dependent. Baldwin et al. found that nasal absorption of human growth hormone could be enhanced when STDHF was co-administered and that the degree of enhancement was 3-5 fold greater than that of the cholate derivatives.[10] The efficacy of STDHF has also been shown in human clinical trials, for instance enhanced absorption of octreotide.[11] The above studies demonstrate the usefulness of penetration enhancing technique and these techniques should be used with understanding the mechanism by which drug diffusion is enhanced through biological tissues.

Conclusions

The work encompassed within this paper has demonstrated the feasibility of using the PAA hydrogel delivery system with its good mucoadhesive properties for the buccal delivery of peptides. The study also indicates that a better understanding of the mechanism of action and effectiveness of reversible penetration enhancers on the buccal mucosa is necessary to achieve peptide drug delivery by this route.

Acknowledgments

This Research was supported by the Chung-Ang University Research Grants in 2004.

References

1) J.D. Smart, I.W. Kellaway and H.E.C. Worthington, An in vitro investigation of muco-adhesive materials for use in controlled drug delivery, *J. Pharm. Pharmacol.*, **36**, 295-299 (1984).
2) V.H.L. Lee, Peptide and protein drug delivery: opportunities and challenges, *Pharm. Int. Trends Rev.*, **5**, 208-212 (1986).
3) T. Nagai and Y. Machida, Buccal delivery systems using hydrogels, *Adv. Drug Del. Rev.*, **11**, 179-191 (1993).
4) J. Lee and I.W. Kellaway, Buccal permeation of [D-Ala^2, D-Leu^5]enkephalin from liquid crystalline phases of glyceryl monooleate, *Int. J. Pharm.*, **195**, 35-38 (2000).
5) J.H. Kou, D. Fleisher and G.L. Amidon, Modelling drug release from dynamically swelling poly(hydroxyethylmethacrylate-co-methacrylic acid) hydrogels, *J. Control. Rel.*, **12**, 241-250 (1990).
6) P.L. Ritger and N.A. Peppas, A simple equation for description of solute increase I. Fickian and non-Fickian release from non-swelling devices in the form of slabs, spheres, cylinders or discs, *J. Control. Rel.*, **5**, 23-36 (1987).
7) J.P. Longenecker, A.C. Moses, J.S. Flier, R.D. Silver, M.C. Carey and E.J. Dubovi, Effects of sodium taurodihydrofusidate on nasal absorption of insulin in sheep, *J. Pharm. Sci.*, **76**, 351-355 (1987).
8) B.J. Aungst and N.J. Rogers, Comparison of the effects of various transmucosal absorption promoters on buccal insulin delivery, *Int. J. Pharm.*, **53**, 227-235 (1989).
9) M.J. Deurloo, W.A.J.J. Hermens, S.G. Romeyn, J.C. Verhoef and F.W.H.M. Merkus, Absorption enhancement of intrana-

sally administered insulin by sodium taurodihydrofusidate (STDHF) in rabbits and rats, *Pharm. Res.*, **6**, 853-856 (1989).
10) P.A. Baldwin, C.K. Klingbell, C.J. Grimm and J.P. Longenecker, The effect of sodium tauro-24, 25-dihydrofusidate on the nasal absorption of human growth hormone in three animal models, *Pharm. Res.*, **7**, 547-552 (1990).
11) T. Kissel, J. Drewe, S. Bantle, A. Rummelt and C. Beglinger, Tolerability and absorption enhancement of intranasally administered octreotide by sodium taurodihydrofusidate in healthy subject, *Pharm. Res.*, **9**, 52-57 (1992).