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Communication

Buccal permeation of [D-Ala^2, D-Leu^5]enkephalin from liquid crystalline phases of glyceryl monooleate☆

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Abstract

The ex vivo buccal permeability of a [D-Ala^2, D-Leu^5]enkephalin (DADLE) and glyceryl monooleate (GMO) was examined from the cubic and lamellar liquid crystalline phases of GMO and aqueous phosphate-buffered saline (pH 7.4, PBS) solution across excised porcine buccal mucosa mounted in a Franz cell. GMO was released in vitro from the liquid crystalline phases indicating the erosion of the liquid crystal matrices. GMO released from the liquid crystalline matrices permeated the porcine buccal mucosa with fluxes of 0.10 ± 0.03 and $0.07 \pm 0.00\%/cm^2$ per h for the cubic and lamellar phases, respectively. The flux of DADLE (1.21 ± 0.32 and $1.15 \pm 0.11\%/cm^2$ per h for the cubic and lamellar phases, respectively) from the liquid crystalline phases was significantly enhanced by the GMO compared with PBS solution ($0.43 \pm 0.08\%/cm^2$ per h) during the initial permeation phase ($t < 3$ h). Our results suggest that the cubic and lamellar liquid crystalline phases can be considered as promising buccal drug carriers for peptide drugs as well as acting as permeation enhancers.

Keywords: Buccal permeation; Peptide and protein; Liquid crystalline phase; Glyceryl monooleate; Permeability enhancement of peptide

1. Introduction

Glyceryl monooleate (monoolein-GMO) is a polar and sparingly water-soluble lipid (Sadhale and Shah, 1998) that can form lyotropic liquid crystalline phases such as cubic (Q), lamellar (L_α), hexagonal (H_{II}) and reversed micellar (L_2) phases. The glyceryl monooleate-water system has pharmaceutically attractive characteristics such as amphiphilicity, temperature-induced phase transition, gel-like texture and low toxicity, which have led to its use as a drug carrier in various dosage forms such as a semisolid matrix, a subcutaneous or intramuscular depot and intravenous injection. Furthermore, the cubic and lamellar liquid crystalline phases have bioadhesive properties although the mechanism of bioadhesion has

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not yet been identified (Engström et al., 1995; Nielsen et al., 1998). In addition, the cubic phase showed protective action against enzyme related degradation of peptide drugs (Ericsson et al., 1991) and also has improved the chemical stability of compounds containing amide bonds such as cefazolin and cefuroxime (Sadhale and Shah, 1998). The cubic and lamellar phases are therefore promising mucoadhesive buccal drug carriers for peptide and protein drugs. In this study, we evaluated simultaneously the ex vivo buccal permeability of a [D-Ala^2, D-Leu^5]enkephalin (DADLE, $M_w = 569.7$) and GMO from the cubic and lamellar liquid crystalline phases through porcine buccal mucosa.

2. Materials and methods

2.1. Materials

A commercially available grade of distilled GMO was purchased from Danisco Ingredients (Copenhagen, Denmark) and used as received. DADLE and phosphate-buffered saline pH 7.4 (PBS) tablets were obtained from Sigma-Aldrich Company (Poole, UK). [*Tyrosyl*-3,5-^{3}H(N)]-DADLE ([^{3}H]DADLE, 1 mCi/ml in ethanol) and monooleoyl-*rac*-glycerol [oleic 1-^{14}C] ([^{14}C]GMO, 50 μCi/ml in toluene:ethanol 1:1) were obtained from Du Pont (Hertfordshire, UK) and American Radiolabeled Chemicals (St. Louis, MO), respectively.

2.2. Preparation of liquid crystalline phases

The aqueous phase containing DADLE, [^{3}H]DADLE (6 μCi/g), [^{14}C]GMO (3 μCi/g) and water and molten GMO were used at 50°C to form the liquid crystalline phases for an ex vivo buccal permeation study of a system that contained 35% (cubic) and 16% (lamellar) water by weight, respectively. Samples were kept in an incubator for 3 days and then allowed to equilibrate at room temperature in the dark for 5 days. [^{14}C]GMO (0.5 μCi/g) -containing cubic and lamellar phases were also prepared for an in vitro [^{14}C]GMO release study.

2.3. In vitro release of [^{14}C]GMO from the cubic and lamellar phases

A polypropylene cylindrical mould (5.0 mm internal diameter, 3.0 mm thickness) was filled with cubic and lamellar phases containing [^{14}C]GMO and maintained at 4°C for 0.5 h. Samples were removed from the mould and placed on top of a sieve and supporter. This platform was immersed in 10 ml PBS in a glass bottle with a cap and the medium was constantly agitated with a magnetic stirrer at 37°C. The amount of radioactivity released into the medium was measured periodically by liquid scintillation counting.

2.4. Ex vivo buccal permeation study

2.4.1. DADLE permeation from aqueous PBS solution

The porcine buccal tissue was equilibrated in a Franz cell by placing 0.5 ml of PBS in the donor compartment and 2.2–2.4 ml of PBS in the receiver compartment for 0.5 h. The experiment was initiated at 37°C by replacing the PBS in the donor compartment with 0.5 ml of the test solution containing [^{3}H]DADLE (4 μCi/ml) and DADLE (0.5 mg/ml) in PBS. Then 0.2 ml of samples were withdrawn from the receiver compartment over 8 h and replaced with the same volume of pre-warmed fresh PBS. The radioactivity of [^{3}H]DADLE permeated was measured by liquid scintillation counting.

2.4.2. DADLE permeation from cubic and lamellar phases

To produce thin films of liquid crystalline phases in direct contact with the buccal mucosa, the following procedure was employed. The excised porcine buccal mucosa was hydrated and placed on a piece of Parafilm (9 cm^2). A washer (1.6 mm thick, 5.1 mm i.d., 30.1 mm o.d.) was placed on the buccal tissue and both weighed. The cubic and lamellar phases were applied to the surface of the buccal mucosa and re-weighed to determine the mass of liquid crystalline phases applied. The assembled tissue, washers and test phases were mounted into the Franz cell maintained at 37°C and the receiver compartment was

filled with PBS to initiate the experiment. The radioactivity of [^{3}H]DADLE and [^{14}C]GMO permeated into the PBS was assayed simultaneously by liquid scintillation counting.

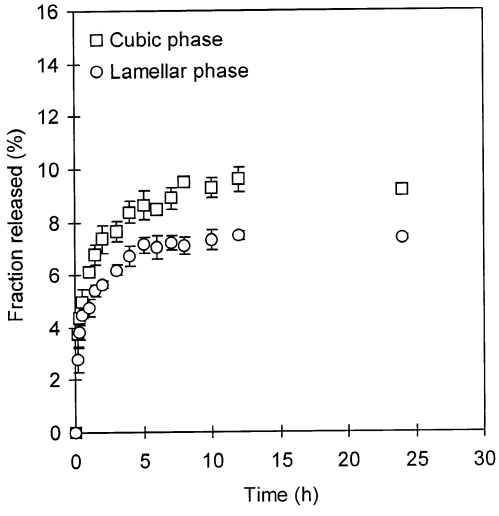

Fig. 1. Release of [^{14}C]GMO to the PBS medium from the cubic and lamellar phases. Each point represents mean ± S.D. of three determinations.

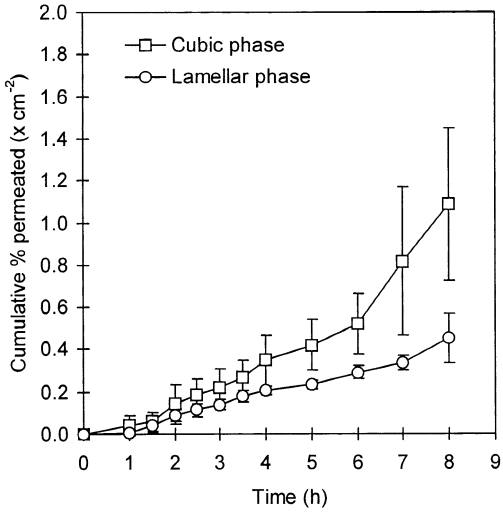

Fig. 2. Permeation of [^{14}C]GMO to the receiver fluid (PBS) across porcine buccal mucosa (0.21 cm^2) from the cubic and lamellar phases in the Franz cell. Each point represents mean ± S.D. of five determinations.

3. Results and discussion

The initial in vitro [^{14}C]GMO release rate from the cubic phase was greater than that from the lamellar phase (Fig. 1). The maximum release of [^{14}C]GMO from the cubic and lamellar phases was ~9 and 7% of initial dose of matrix, respectively. The cubic and lamellar phases were found to be eroded without the action of an enzyme such as lipase, or bile salts and liberated into the aqueous medium.

[^{14}C]GMO released from the cubic and lamellar phases permeated the porcine buccal mucosa with fluxes of 0.10 ± 0.03 and $0.07 \pm 0.00\%/cm^2$ per h for the cubic and lamellar phases, respectively calculated from data collected in the first 4 h (Fig. 2). [^{14}C]GMO flux across the porcine buccal mucosa was significantly greater ($P < 0.05$) when applied as the cubic phase than as the lamellar phase which is probably due to the faster in vitro release of [^{14}C]GMO from the cubic phase compared to the lamellar phase (Fig. 1).

The permeation of [^{3}H]DADLE from liquid crystalline phases demonstrated significantly higher fluxes (1.21 ± 0.32 and $1.15 \pm 0.11\%/cm^2$ per h for the cubic and lamellar phases, respec-

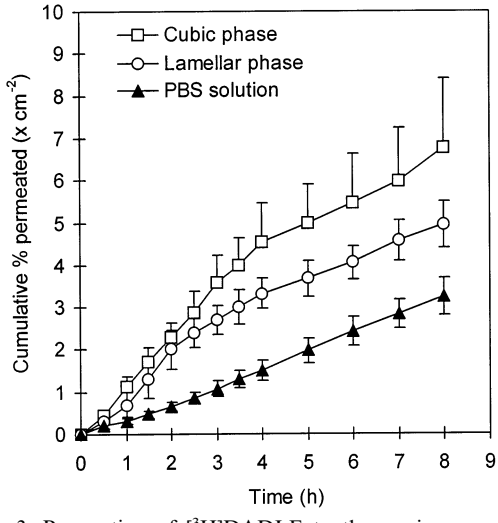

Fig. 3. Permeation of [^{3}H]DADLE to the receiver compartment across porcine buccal mucosa (0.21 cm^2 for liquid crystalline phases, 0.52 cm^2 for PBS solution formulation) from the liquid crystalline phases and aqueous PBS solution in the Franz cell. Each point represents mean ± S.D. of five determinations.

tively) ($P < 0.01$) compared with PBS solution ($0.43 \pm 0.08\%/cm^2$ per h) during the initial permeation phase ($t < 3$ h) (Fig. 3). This would suggest that rapidly released GMO from both the cubic and lamellar phases is acting as a permeation enhancer for the DADLE. The greater in vitro release rate of GMO from the cubic phase resulted in higher permeation of DADLE compared with the lamellar phase. There is no evidence of breakdown of the liquid crystalline phases giving rise to increased DADLE release. Indeed, both cubic and lamellar phases exhibited minimal loss of GMO following 4 h exposure to PBS medium (Fig. 1). At the end of the experiment a greater amount of DADLE had permeated from the cubic phase compared with the lamellar phase, a result similar to that previously reported for in vitro drug release (Lee and Kellaway, 1999). The correlation between GMO release and DADLE permeation indicates that GMO can enhance the buccal permeation rate of DADLE by a co-transport mechanism of lipid and peptide with a concomitant increase in the percentage of DADLE permeated. This result is in agreement with some attempts in introducing GMO as a percutaneous absorption enhancer (Hastewell et al., 1994; Roy and de Groot, 1994) and improving bioavailability (Nishihata et al., 1986; Balandraud-Pieri et al., 1997).

In conclusion, the cubic and lamellar liquid crystalline phases can be considered as promising drug carriers for the buccal delivery of peptide drugs as well as acting as permeation enhancers.

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