Topological distribution of a myristoylated peptide throughout the pig buccal mucosa during permeation

Veuillez, Fabienne; Deshusses, Jacques; Falson-Rieg, Françoise; Buri, Pierre

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TOPOLOGICAL DISTRIBUTION OF A MYRISTOYLATED PEPTIDE THROUGHOUT THE PIG BUCCAL MUCOSA DURING PERMEATION

F. Veuillez[1,2], J. Deshusses[3], F. Falson[4] and P. Buri[1,2]

[1]Pharmapeptides, Interuniversity Center Geneva-Lyon, 74166 Archamps, France
[2]School of Pharmacy, University of Geneva, 1211 Geneva 4, Switzerland
[3]School of Pharmacy, University of Lyon I, 69373 Lyon, France
[4]Department of Biochemistry, University of Geneva, 1211 Geneva 4, Switzerland

Introduction

Buccal transmucosal delivery is an alternative administration route which might enable the delivery of higher molecular weight drugs, including peptides, which must currently be delivered via injection. Buccal mucosa has been investigated as a potential site for the controlled delivery of peptides because of its easy accessibility and low enzymatic activity compared to the gastro-intestinal tract [1]. The ex-vivo permeation of an acylated peptide model (myristoyl-tryptophan-leucine or Myr-Trp-Leu) was studied using pig buccal mucosa, a nonkeratinized epithelium, with morphology and permeability comparable to human epithelium [2]. Squier et al. [3] showed that the rate-limiting barrier for the movement of molecules across the buccal (cheek) mucosa was the upper one-third of the epithelium.

In previous permeation experiments [4], we have shown that Myr-Trp-Leu was unable to pass into the receptor medium and accumulated in the tissue. Therefore, experiments were performed in order to define the localization of the acylated peptide as a function of depth in the layers of the buccal epithelium. The depth profile was studied at a number of different time points in order to build a kinetic profile.

Experimental methods

Tissue preparation

Nonkeratinized fresh porcine buccal mucosa was collected from the same region posterior to the angle of the mouth. Slice thickness ranged from 1-1.1 mm. The material was used not later than two hours after the death of the animal.

Permeation experiments

Permeation measurements of Myr-Trp-Leu were performed as described previously[4]. Briefly, the tissue was mounted in a modified Franz diffusion cell (exposed area: 0.78 cm^2). The receptor consisted of 2.2 ml of a 30:70 v/v mixture of ethanol: 0.02 mM phosphate buffer, pH 7.4 (Ph. Helv VII). The device was maintained at 37°C by a circulating water pump and was constantly stirred with a teflon-coated magnetic bar. The tissue was equilibrated for 30 min with the receptor medium, before 0.5 ml of a solution of Myr-Trp-Leu (6mg/ml in ethanol: phosphate buffer pH 7.4, 30/70 v/v) was added to the donor compartment.

The rate of permeation was established by withdrawing 100 µl of the receptor fluid at 8h, 16h, 24h and 48h.

The donor, tissue and receptor compartment were quantified by high performance thin layer chromatography (HPTLC) [4].

Tissue extraction

All specimens were fixed, cryoprotected and sectioned at approximately 60 µm at - 40 °C with a cryostat. Each section was extracted in 2 ml of chloroform for 24h. Solutions were assayed by HPTLC using the Camag Linomat IV [4].

Results and discussion

Upto 70% of the applied Myr-Trp-Leu was found to accumulate in the tissue. The affinity of the peptide for the membrane can be attributed to its high lipophilicity.

In fig. 1 we can see that the peptide progressively distributed through the tissue progressively from the layer in contact with the donor compartment to a maximum at the depth of 360 µm over a period of 24h. It has been reported that the layer of the epithelium functioning as the permeation barrier is restricted to the first micrometers [5]. We observe here that the substance has already passed this layer in 24h. Since the shape of the curve of fig. 1 did not correspond to a simple diffusion process we had to compare the distribution of the acylated peptide in the tissue as a function of time.

As we can see in fig. 2, as expected, the product was found after 8h in the upper epithelial layers. At 18h and above, the slice containing the maximum amount was found to the displaced towards the deeper layers. At 24h, Myr-Trp-Leu was still in the epithelium, but it had almost passed the principal tissue barrier. At 48h, the acylated peptide was found mostly in the conjunctive tissue.

Conclusions

This study shows that the acylated peptide accumulated in the buccal mucosa. As a function of time the variation of its distribution throughout the tissue suggests that a simple diffusion is unlikely and that a dynamic process should be considered. The behavior of the acylated peptide observed in this study presents properties that could be interesting for developing new drug formulations.

References

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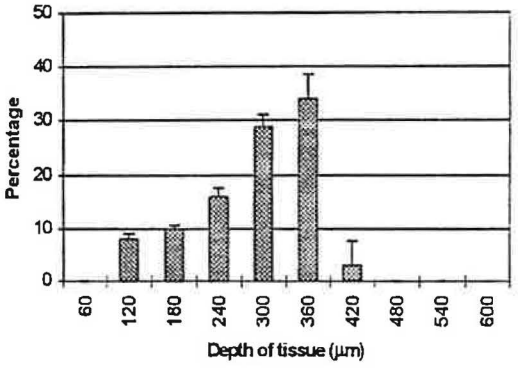

Fig. 1: Percentage of Myr-Trp-Leu in function of depth of the buccal tissue after 24h (n=4).

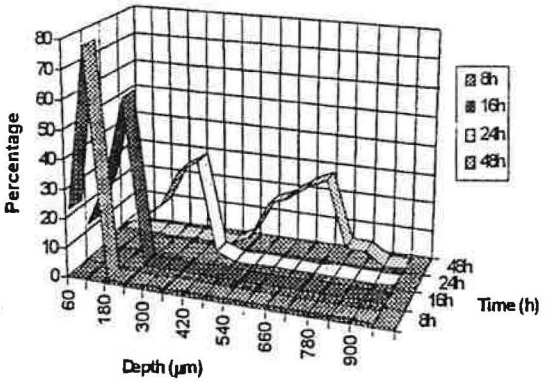

Fig. 2: Percentage of Myr-Trp-Leu in function of time in the buccal tissue (n=4).