International Journal of Pharmaceutics 429 (2012) 78–83

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

New prospective in treatment of Parkinson's disease: Studies on permeation of ropinirole through buccal mucosa

V. De Caro[a], G. Giandalia[a], M.G. Siragusa[a], F.M. Sutera[a], L.I. Giannola[a,b,*]

[a] Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), Università di Palermo, Italy
[b] Home-based Empowered Living for Parkinson's disease patients (HELP) Consortium supported by EC through the Ambient Assisted Living Joint Programme (AAL-2008-1-022) and MIUR, Italy

ARTICLE INFO

Article history:
Received 7 February 2012
Received in revised form 9 March 2012
Accepted 10 March 2012
Available online 20 March 2012

Keywords:
Buccal delivery
Ropinirole
Parkinson's disease
Absorption enhancement
Porcine buccal mucosa

ABSTRACT

The aptitude of ropinirole to permeate the buccal tissue was tested using porcine mucosa mounted on Franz-type diffusion cells as *ex vivo* model. Drug permeation was also evaluated in presence of various penetration enhancers and in iontophoretic conditions.

Ropinirole, widely used in treatment of motor fluctuations of Parkinson's disease, passes the buccal mucosa. Flux and permeability coefficient values suggested that the membrane does not appear a limiting step to the drug absorption. Nevertheless, an initial lag time is observed but the input rate can be modulated by permeation enhancement using limonene or by application of electric fields. Absorption improvement was accompanied by the important reduction of the lag time; at once the time required to reach the steady state plasma concentration was drastically decreased.

On the basis of these results we could assume that clinical application of ropinirole by buccal delivery is feasible.

1. Introduction

The degeneration of pre-synaptic dopaminergic neurons of the basal ganglia is the main origin of abnormalities in the control of voluntary movement in Parkinson's disease (PD) patients. Currently, L-DOPA is considered as the standard treatment for PD since it initially provides a stable therapeutic response; unfortunately, during long-term treatment, its beneficial effect declines. The main problem with the early use of L-DOPA is its tendency to induce motor complications. This is a particular problem for young onset PD patients who are at a greater risk of developing motor complications and who have to endure this disability over the course of a long and chronic illness. In the management of PD, it is important to minimize the development of motor fluctuations and to postpone them as long as possible. For this purpose, the use of other drugs such as ropinirole (ROP) offers a valid option before initiation of L-DOPA therapy (Ahlskog, 2011; Azeem et al., 2009).

ROP (IUPAC name: 4-[2-(dipropylamino)ethyl]-2-indolinone hydrochloride) is a potent non-ergoline dopamine receptor agonist with high relative specificity and full intrinsic activity at the D_2/D_3 receptors; its affinity for the D_3 receptor is at least 100-fold greater than for the D_2 receptor. ROP is also approved for the treatment of Restless Legs Syndrome (Ahlskog, 2011; Nanaki et al., 2012). ROP stimulates striatal dopamine receptors to produce dopamine. It is being increasingly used as monotherapy in the initial treatment of PD rather than as adjunct to L-DOPA. ROP is also efficacious in the management of more advanced PD in patients experiencing motor complications after long-term L-DOPA use. The usual dose of ROP is 3–9 mg daily to be taken in three divided doses owing to its short half-life (6 h). Multiple daily dosing may lead to decreased medication compliance and unsteady plasma concentrations which can contribute to the occurrence of motor fluctuations (Azeem et al., 2012).

The immediate-release oral formulation of ROP (ROP-IR) is approved for use as monotherapy in the treatment of early-stage PD and as adjunct to L-DOPA therapy in more advanced disease. ROP-IR has already been shown to reduce dyskinesias in long-term studies and is rapidly and almost completely absorbed, with T_{max} generally reached 1–2 h after dosing. Recently, a new oral formulation for 24 h prolonged release (ROP-PR) was introduced in the market. Several benefits of ROP-PR administration were accurately documented, and findings indicate that the formulation may have a relevant impact on the management of PD in early and advanced stages (Onofrj et al., 2009). Nevertheless, ROP-PR allows the achievement of the steady state conditions after 48 h (Thompson and Vearer, 2007; Nashatizadeh et al., 2009).

ROP has a low molecular weight (MW 260), is sufficiently lipophilic (Log P = 3.32), and like L-DOPA suffers from low

* Corresponding author at: Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy. Tel.: +39 091 23891924; fax: +39 091 23891962.
E-mail address: l.italo.giannola@unipa.it (L.I. Giannola).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2012.03.022

bioavailability of about 50% by oral route due to extensive first-pass metabolism (Ahlskog, 2011; Contin and Martinelli, 2010).

The adverse events that occurred in greater than 5% of patient treated with ROP are nausea, dyspepsia, headache, dizziness, dyskinesia and orthostatic hypotension (Nashatizadeh et al., 2009). Moreover, a common side effect of D_3 agonists, encountered in clinical practice, is pathological behavior. This includes excessive gambling, hypersexuality, shopping, hyperphagia or obsessive hobbying, which may develop in up to 30% of people taking higher drug doses (Ahlskog, 2011).

To overcome the limitations of the conventional oral ROP therapy, recently the delivery through the skin has been proposed. Transepithelial delivery might help in reduction of administered doses and in overcoming dose-dependent side effects. Topical formulations would be ideal for the geriatric Parkinson population who may be suffering from dysphagia (difficulty swallowing). Approx. 85% of PD patients are over the age of 65, and over 45% have difficulty in swallowing (Azeem et al., 2009, 2012; Reichmann, 2009).

The buccal mucosa could be a favorable site of drug absorption as an alternative transepithelial route; it is non-keratinized and strongly supplied with blood by a dense capillary vessel network. The tissue covers a relatively large drug absorption area. Drugs can thus reach the systemic circulation directly through the capillary vessels, bypassing the first-pass metabolism in the intestine and liver or avoiding inactivation in the stomach. Moreover, delivery of drugs through the buccal mucosa should be considered like a slow i.v. infusion. These characteristics contribute to higher bioavailability parameters after administration of a smaller dose of the drug than in conventional oral formulations (Campisi et al., 2010).

Really, continuous stimulation of striatal dopaminergic receptors, as opposed to discontinuous or pulsatile stimulation, could delay or prevent the onset of motor complications. This hypothesis has arisen from studies of the normal basal ganglia demonstrating that nigral dopaminergic neurons normally fire continuously and striatal dopamine levels are relatively constant (Stocchi, 2009).

The most important reason of buccal ROP administration is certainly the expectation that a continuous drug delivery through the mucosal tissue could guarantee a continuous dopamine replacement and, as a consequence, a continuous dopamine receptor stimulation should be achieved.

2. Materials and methods

2.1. Materials

ROP, USP grade, was kindly supplied by Teva (USA). Lysine hydrochloride (LysC) and trisodium citrate dehydrate (TNaC) were purchased from Polichimica s.r.l. (Bologna, Italy). Limonene, sodium dehydrocholate (NaDHC), and all components of buffer solutions were purchased from Sigma–Aldrich (Milano, Italy). Menthol was purchased from Carlo Erba (Milano, Italy). Buffer pH 6.8 solution simulating saliva was prepared using NaCl (0.126 g), KC1 (0.964 g) KSCN (0.189 g), KH_2PO_4 (0.655 g), urea (0.200 g), $Na_2SO_4{\cdot}10H_2O$ (0.763 g), NH_4Cl (0.178 g), $CaCl_2{\cdot}2H_2O$ (0.228 g) and $NHCO_3$ (0.631 g) in 1 L of distilled water (Gal et al., 2001). Phosphate buffered saline (PBS) Ca^{2+} and Mg^{2+} free solution, pH 7.4, was prepared by dissolving KH_2PO_4 (0.144 g), anhydrous Na_2HPO_4 (0.795 g) and NaCl (9.0 g) in 1 L of distilled water and used as simulated plasma. Saline isotonic solution (pH 7.0) was prepared by dissolving NaCl (9 g) in 1 L of distilled water. All chemicals and solvents were of analytical grade and were used without further purification.

2.2. Ex vivo permeation of ROP throughout porcine buccal mucosa

Porcine mucosal specimens (kindly supplied by Mattatoio Comunale, Villabate, Palermo) were obtained from tissue removed from the inner cheek (buccal area) of freshly slaughtered domestic pigs. After sampling, all specimens were immediately placed in a refrigerated transport box and transferred to laboratory within 1 h. Some specimens were surgically treated to remove excesses of connective and adipose tissue until slides 500 ± 100 μm were obtained. Other specimens were treated using the heat shock method until slides 250 ± 25 μm thick were obtained. For heat separation of the epithelium, the mucosal tissues were dipped for approximately 1 min in saline solution warmed to 60 °C. Then the connective tissue was carefully peeled off from the mucosa to obtain the heat-separated epithelium. The connective tissue was completely removed and the epithelium remained along with the intact basal lamina. The thickness of the tissues was measured using a digital micrometer. Slicing of the tissue with a dermatome was not performed to avoid preliminary freezing which may alter the barrier properties of the buccal mucosa (Kulkarni et al., 2010). Appropriate sections of mucosa were mounted in vertical Franz type diffusion cells. Tissue disks (0.13 cm^2) were equilibrated for 10 min at 37 °C [Polimix EH 2 bath equipped with a constant-rate adjustable stirrer RECO S5 (Kinematica, Switzerland)] adding simulated plasma in the acceptor compartment. In the donor compartment was then placed a ROP solution (10, 20, 30, 45 or 60 mg of ROP in 1.0 mL of buffer solution simulating saliva). At regular time intervals (30 min), samples (0.5 mL) were withdrawn from the acceptor compartment and the sample volume taken out was replaced by fresh fluid.

In all experiments the drug transferred from the donor to the acceptor compartment was monitored by UV spectrophotometric analysis (see Section 2.5). Each experiment was carried out for 6.5 h. Results are reported as means ± SD of six different experiments in which fractions of the same portion of tissue were used ($P < 0.05$). The integrity of the mucosal tissue was monitored after each permeability experiment, according to the method reported early (De Caro et al., 2008). No significant differences were observed using specimens treated surgically or by heat-separation. Heat treatment did not adversely affect on permeability and integrity characteristics of the buccal mucosa (Diaz Del Consuelo et al., 2005).

At the end of each experiment, the residual ROP into the mucosal tissue was detected by extraction. Each sample of buccal mucosa used in the permeation experiments was washed with simulated plasma (3 mL) and than was dipped for 5 min in warmed (50 °C) methanol (1 mL). The extraction was repeated three times. The extraction mother liquors were collected, quantitatively transferred in a 5 mL flask and brought to volume. The amount of drug extracted was evaluated by UV spectrophotometric analysis using the appropriate calibration curve and blank. The same extraction treatment was performed also on mucosal specimens subjected to experimental phase in absence of ROP and used as control. No absorption was observed in the λ range of ROP.

2.3. Ex vivo permeation of ROP throughout porcine buccal mucosa in presence of chemical enhancers

The permeation behavior of ROP in presence of chemical enhancers was investigated using the same methods described in Section 2.2.

Permeation tests in presence of water-soluble chemical enhancers (NaDHC, LysC, TNaC) were performed placing in the donor compartment 1.0 mL of buffer solution simulating saliva containing 45 mg of ROP and 0.05 mg of enhancer. Permeation tests in presence of water insoluble chemical enhancers (menthol, limonene) were performed placing in the donor compartment 5 μL of a solutions containing 0.042 g of chemical enhancer in 1 mL of