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Research paper

Simultaneous controlled iontophoretic delivery of pramipexole and rasagiline *in vitro* and *in vivo*: Transdermal polypharmacy to treat Parkinson's disease

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ABSTRACT

Effective treatment of Parkinson's disease (PD) involves administration of therapeutic agents with complementary mechanisms of action in order to replenish, sustain or substitute endogenous dopamine. The objective of this study was to investigate anodal co-iontophoresis of pramipexole (PRAM; dopamine agonist) and rasagiline (RAS; MAO-B inhibitor) *in vitro* and *in vivo*. Passive permeation of PRAM and RAS (20 mM each) across porcine skin after 6 h was 15.7 ± 1.9 and 16.0 ± 2.9 μg/cm^2, respectively. Co-iontophoresis at 0.15, 0.3 and 0.5 mA/cm^2 resulted in statistically significant increases in delivery of PRAM and RAS; at 0.5 mA/cm^2, cumulative permeation of PRAM and RAS was 613.5 ± 114.6 and 441.1 ± 169.2 μg/cm^2, respectively – corresponding to 38- and 27-fold increases over passive diffusion. Electromigration was the dominant mechanism for both molecules (> 80%) and there was no effect on convective solvent flow. Statistically equivalent delivery was observed with human skin. The co-iontophoretic system showed high delivery efficiency with 29% and 35% of the applied amounts of PRAM and RAS being delivered. Preliminary pharmacokinetics studies in rats confirmed that the input rate *in vivo* was such that therapeutic amounts of the two drugs could be co-administered to humans by transdermal iontophoresis using reasonably sized patches and moderate current densities.

1. Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease; it is characterized by symptoms of tremor, rigidity and bradykinesia [1,2]. Levodopa is considered to be the most effective agent for the treatment of PD although clinical and pathologic studies have shown that it cannot slow disease progression [3]. However, the development of motor complications (e.g., on-off effect, dyskinesia) can affect its long-term use. Therefore, dopamine agonists including ergot and non-ergot agents are frequently prescribed in early stages of disease and levodopa is initiated when dopamine agonist monotherapy is no longer able to provide satisfactory clinical outcomes [4].

Pramipexole (PRAM) is a non-ergot dopamine agonist acting at the D_2 and D_3 receptors that was approved for the treatment of PD in 1997 and is the most widely prescribed dopamine agonist, both as monotherapy and as an adjunctive therapy with L-DOPA [5,6]. Another class of drugs effective in PD are the inhibitors of monoamine oxidase B (MAO-B), an enzyme that regulates metabolism of catecholamine neurotransmitters (e.g., dopamine) [7,8]. Rasagiline (RAS) is a selective irreversible dose-dependent MAO-B inhibitor that also exhibits neuroprotective effects [9]. PRAM and RAS have complementary mechanisms of action and several studies have shown clinical improvements in symptoms and quality of life for patients when they are used together [10,11].

The mean intake of tablets for advanced PD patients is estimated to be 9.9 tablets per day corresponding to ~3600 tablets per year [12]. Drowsiness and cognitive impairment along with a rigorous dosing regimen impose a strain on the patient's life and mean an extra

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workload for caregivers. It is therefore not surprising that patients' principal request for improvement in treatment relates to the reduction in daily tablet intake and this opinion is shared by physicians. Furthermore, patients can have swallowing difficulties and the high tablet burden can lead to non-compliance and contribute to reduced therapeutic efficacy. This was one of the drivers behind the development of products such as Stalevo®, which is a tablet containing L-DOPA and inhibitors of dopamine decarboxylase (carbidopa) and catechol-O-methyltransferase (entacapone) – enzymes responsible for its biotransformation – thus, creating a "polypharmacy" approach in a single tablet.

Transdermal drug delivery systems offer a promising alternative to oral administration for chronically ill patients especially in circumventing difficulties associated with swallowing, and in addition to increasing compliance (and efficacy), they can significantly improve quality of life and bring down treatment costs by reducing care-givers' work-load. Transdermal administration of drugs also prevents problems associated with hepatic first-pass metabolism, poor absorption from the gastrointestinal tract and variable bioavailability as observed with RAS. Constant plasma concentrations may also be achieved as a result of controlled zero order drug input even when the drug has a short elimination half-life thereby reducing peak-trough variations in blood levels. This is of particular relevance for PD therapy since motor fluctuations seen in PD patients are attributed to pulsatile stimulation of dopamine receptors when drugs are given orally [13]. Transdermal administration would provide a continuous "physiologic" stimulation of dopamine receptors and thereby help to reduce these complications [14,15].

Transdermal patches have been used to deliver selegiline (MAO-B inhibitor) and rotigotine (dopamine agonist) [16,17]. With these systems, plasma concentrations of the drug begin to rise after 4–6 h application and steady states are reached in 12–24 h. This would mean a long lag phase before achieving a pharmacodynamic effect and a greater risk of irritation due to longer contact time of the patch with the skin. The physicochemical properties of PRAM and RAS are shown in Table 1; considering their polar and ionic nature, it would be difficult to achieve the required flux for delivering the therapeutic amounts of either drug by passive transdermal delivery [18].

In contrast, transdermal iontophoresis can exploit these physicochemical properties – i.e. good aqueous solubility and ionized state – to deliver these drugs efficiently through the skin [19]. Iontophoresis involves application of an electric potential across the membrane that will significantly increase the transport rates of ions as compared to passive diffusion. Control over drug input kinetics is achieved by modulation of current density, which can be used to increase delivery of therapeutics in advanced disease state without changing the drug loading or patch size. It can be used to provide highly individualized treatment regimens and hence improve efficacy and patient compliance. We have previously demonstrated the feasibility of iontophoresing PRAM and RAS individually [20,21]. The goal of this work was to investigate their co-iontophoretic delivery in order to optimize their transdermal administration for the treatment of PD and at the same time demonstrate that iontophoresis could be used as a means to provide controlled transdermal polypharmacotherapy (cf. the L-DOPA-carbidopa-entacapone combination tablet) [22]. The specific aims of the study were (i) to investigate the effect of experimental parameters on anodal co-iontophoresis of RAS and PRAM in porcine skin *in vitro* and to deduce the relative contributions of electromigration (EM) and electroosmosis (EO), (ii) to study co-iontophoretic delivery of RAS and PRAM in rats *in vivo* and hence (iii) to evaluate the feasibility of simultaneously delivering therapeutic amounts of the two anti-Parkinson's drugs *in vivo*.

Table 1
Comparing physicochemical and pharmacological properties of PRAM and RAS.

	PRAM	RAS
M_W (Da)	211.33 (302.26)[a]	171.24 (267.34)[b]
Aqueous solubility (g/L)	> 20[a]	3.4[b]
pKa	5.0, 9.6	7.2
log P	2.34	1.67
log $D_{pH7.0}$	0.024	1.40
Half-life (h)	8–12 h	3
Oral bioavailability	90%	35%
Dosage form	Oral: 0.75–4.5 mg	Oral: 0.5–1 mg
Mechanism of action	Dopamine agonist	MAO-B inhibitor

[a] Dihydrochloride monohydrate salt.
[b] Mesylate salt.

2. Materials and methods

2.1. Materials

Pramipexole dihydrochloride monohydrate and rasagiline mesylate were purchased from Nectar Industrial Co. Ltd (Shenzhen, China) and Jinan Jinao CDC Ltd. (Jinan City, China), respectively. Acetaminophen (ACE), sodium chloride, 2-(*N*-morpholino)-ethanesulfonic acid (MES), sodium metabisulfite, citric acid, sodium hydroxide and sodium citrate were all purchased from Sigma-Aldrich (Buchs, Switzerland). Silver wire and silver chloride used for the fabrication of electrodes were also sourced from Sigma-Aldrich. Potassium dihydrogenphosphate, sodium salt of heptanesulfonic acid and diethyl ether were purchased from Acros Organics (Geel, Belgium). Methanol and PVC tubing (ID 3.17 mm; OD 4.97 mm) were purchased from VWR International (Nyon, Switzerland). All solutions were prepared using deionized water (resistivity > 18 MΩ cm). All other chemicals were at least of analytical grade.

2.2. Skin source

Porcine ears were obtained from a local abattoir (CARRE; Rolle, Switzerland), the skin was excised (thickness 750 µm) with an air dermatome (Zimmer; Etupes, France), wrapped in Parafilm™ and stored at −20 °C for a maximum period of 1 month. Human skin samples were collected immediately after surgery from the Department of Plastic, Aesthetic and Reconstructive Surgery, Geneva University Hospital (Geneva, Switzerland), fatty tissue was removed and the skin was wrapped in Parafilm™ before storage at −20 °C for a maximum period of 7 days. The study was approved by the Central Committee for Ethics in Research (CER: 08–150 (NAC08-051); Geneva University Hospital).

2.3. In vitro studies

Skin was clamped in two compartment diffusion cells (area = 2 cm^2). After equilibration with phosphate buffered saline (PBS, pH 7.4), 1 ml of buffered formulation containing PRAM and RAS (20 mM each in 25 mM MES pH 5.3 with 26 mM sodium metabisulfite) was placed in the donor compartment. To decrease competition between cationic charge carriers, the anodal and formulation compartments were connected by means of a salt bridge. Acetaminophen (ACE; 15 mM) was included in the formulations to report on electroosmosis (EO) and the effect of cation transport on skin permselectivity [23,24]. The receptor compartment (which also contained the cathode) was filled with PBS. Samples (0.6 ml) were collected from the receiver compartment hourly and replaced with the same volume of fresh buffer. A constant current density (0.15, 0.3 and 0.5 mA/cm^2) was applied for 6 h via Ag/AgCl electrodes connected to a power supply (APH 1000 M; Kepco, Flushing, NY).

In a separate study, formulations containing different concentrations of PRAM and RAS were iontophoresed at 0.5 mA/cm^2 for 6 h to investigate the effect of formulation composition on their co-transport. Five different formulations with the following concentrations were tested: Formulation A (10 mM each), B (20 mM each), C (40 mM each),