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Research paper

Development of a buccal doxepin platform for pain in oral mucositis derived from head and neck cancer treatment

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ABSTRACT

This study describes the development of semisolid formulations containing doxepin (DOX) for pain relief in oral mucositis, frequently related to chemotherapy and/or radiotherapy treatments in patients with head and neck cancer. Chemical permeation enhancers were evaluated and selected according to the results obtained from rheological studies, drug release, and drug permeation and retention through buccal mucosa. Finally, the selected formulation was compared *in vivo*, with a reference DOX mouthwash, whose clinical efficacy had been previously reported. The obtained findings showed that an orabase® platform loading transcutol® (10%) and menthol (5%) for the buccal vehiculization of DOX exhibited a decreased elastic and viscous behavior improving its application. The main drug release mechanism could be considered as diffusion according to Higuchi model. Obtained DOX permeation rates were considered optimal for an analgesic effect and far below to an antidepressant activity. Similar *in vivo* plasma concentrations were found for the semisolid formulation and the reference mouthwash. However, DOX amounts retained in the mucosa of animals for the semisolid formulation were higher than the reference, which let us hypostatize even stronger potential local therapeutic effect with additional advantages such as, mucoadhesive properties, absence of alcohol, some degree of freshness, as well as, drug palatability improvement.

1. Introduction

Oral mucositis (OM) is a common acute reaction after chemotherapy and/or radiotherapy in head and neck cancer treatment. Although being of ordinary occurrence, current literature reports highly variable incidence (fluctuating from 75% to 99%) [1]. Severity of OM ranges from superficial sore erythema to complete mucosal ulceration in the oral cavity, pharynx and esophagus. Therefore, patient's quality of life is affected since OM is associated with considerable pain and dysphagia, which also complicates the nutritional intake, increases susceptibility to infections and leads to the cancer treatment interruption [2]. All these problems are reflected in the clinical management of cancer, without undertaking any evaluation of associated cost of medical resources [3,4]. Currently, there is no Food and Drug Administration (FDA)-approved intervention for the prevention of OM induced by chemotherapy and/or radiotherapy [5]. Current strategies against OM are directed to limit its extent and to manage its symptomatology, mainly pain relief. Different alternatives are used to alleviate the acute pain in OM and highly severe cases may imply the use of systemic opioids. For mild and less-severe cases, therapeutic alternatives include cryotherapy, use of mouthwashes, coating agents, low laser therapy and topical anesthetic and/or analgesic agents [1,3,4]. Notwithstanding, topical anesthetics and analgesics typically provide less than 30 min of pain relief [6]. In this context, doxepin (DOX), a dibenzoxepin-derivate tricyclic antidepressant (TCA), without being analgesic has shown effective results in the treatment of pain [7,8]. Concretely, some clinical studies have focused on OM pain relief caused by head and neck cancer therapy with DOX oral rinses [9–11]. Its therapeutic effect is believed to be caused by the blockade of sodium channels at local level in cutaneous nociceptors [10].

Considering the promising result of DOX oral rinses and the need of new well supported therapies to manage the OM pain induced by chemotherapy and/or radiotherapy in the treatment of head and neck cancer [12,13], the present study was initiated with the intention to develop a new dosage form for DOX to be

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administered in OM. For this purpose, a semisolid buccal formulation was developed to extend the contact time with the affected tissue, to achieve some degree of coating effect and to provide sustained release of DOX. For this task, mucoadhesive paste Orabase® was selected as the DOX platform for the developed formulations.

Equally, in order to enhance its analgesic effect different penetration enhancers were evaluated. Rheological properties of formulations were assayed, as well as, *in vitro* release and *ex vivo* permeation from which main parameters were calculated. Finally, the semisolid formulation to be tested *in vivo* against the reference mouthwash was selected according to the results obtained from previously mentioned studies.

2. Materials and methods

2.1. Materials

Doxepin hydrochloride, diethylene glycol monoethyl ether (Transcutol®), menthol, myristyl alcohol, sebacic acid, 1-dodecylazacycloheptan-2-one (Azone), sodium lauryl sulfate and N-methylpyrrolidone (NMP) were purchased from Sigma-Aldrich (Madrid, Spain). Orabase® was obtained from Acofarma (Madrid, Spain). Ultrapure water purified using a Station 9000 purification unit was used throughout the work. All other chemical and reagents were of analytical grade.

2.2. Tissue samples

Porcine buccal mucosa was obtained from the Animal Facility at Bellvitge Campus of Barcelona University (Barcelona, Spain). Fresh and frozen buccal mucosa was sourced from freshly sacrificed three- to four-month-old male and female pigs without any instrumental manipulation which could damage them. Immediately after the animals were sacrificed using an overdose of sodium thiopental anesthesia, the buccal mucosa was surgically removed from the cheek region and placed in Hanks balanced salt solution and refrigerated for no longer than 24 h until its use. Unutilized tissues were cryopreserved for further studies [14]. The protocol for the studies was approved by the Animal Experimentation Ethical Committee of the University of Barcelona, Spain (CEEA-UB).

2.3. Liquid formulations

Liquid formulations were used for preliminary studies to select the most promising permeation enhancer and as references. They were prepared by mixing the components in purified water. All formulations contained 5% (w/v) DOX and one of the following enhancers: Azone (A, 5% w/v), menthol (M, 5% w/v), myristyl alcohol (MA, 5% w/v), N-methylpirrolidone (NMP, 5% w/v), sebacic acid (S, 5% w/v), sodium lauryl sulfate (SLS, 5% w/v) or transcutol® (T, 10% w/v).

A plain 2.8% (w/v) DOX aqueous solution was also prepared without the addition of any enhancer for the *in vitro* experiment to discern the type of membrane more appropriate for the release studies. Similarly, a 0.01% (w/v) DOX aqueous solution was prepared for its use in recovery assays.

Finally, a reference DOX mouthwash was prepared by mixing 0.5% DOX, 0.1% alcohol and 0.1% sorbitol with purified water [9–11].

2.4. Optimization

In order to choose those permeation enhancers with the greatest effect in the permeation rate of DOX through porcine mucosa, liquid formulations were submitted to an *ex vivo* permeation study (see Section 2.8), in which 0.1 mL samples of previous liquid formulations were added to the donor compartment. According to the obtained results the three permeation enhancers showing best DOX permeation properties were selected.

2.5. Semisolid formulations

Five DOX semisolid formulations (5%, w/w) were prepared using the bioadhesive platform orabase® instead of water. Three of them contained one of the previously selected penetration enhancer (M, 5%; MA, 5% or T, 10%, w/w). The other two formulations contained a mixture of one of the preselected enhancers (M or MA, 5%, w/w) plus T (10%, w/w). For this task, DOX and the different enhancers or mixtures were weighed and mixed in a mortar with a pestle. Then, pre-weighed orabase® (q.s.) was added to the formulation in increasing amounts by mixing up. The obtained semisolid formulations were further mixed and homogenized by an Ultra-Turrax® T10 basic (IKA, Staufen, Germany) and placed in glass containers. A plain semisolid formulation containing only orabase® was also elaborated as the reference sample for the rheological characterization tests.

2.6. Rheological characterization

The rheological study was conducted in triplicate in a rotational rheometer HAAKE Rheostress 1 (Thermo Fisher Scientific, Karlsruhe, Germany) at 25 ± 0.2 °C, 24 h after preparation. For measurements the device was connected to a thermostatic circulator Thermo Haake Phoenix II + Haake C25P and a computer provided with the Haake Rheowin® Job Manager v. 3.3 software (Thermo Electron Corporation, Karlsruhe, Germany) to execute the tests and Haake Rheowin® Data Manager v. 3.3 software (Thermo Electron Corporation, Karlsruhe, Germany) to perform the analyses of the obtained data, respectively. Two kinds of measurements were made for characterization, rotational measurements and oscillatory tests.

Rotational determinations were addressed with a plate-plate geometry (0.2 mm gap) with a fixed lower plate and a mobile upper plate (Platte PP60 Ti, 60 mm diameter). The shear stress (τ) was measured as a function of the shear rate (γ). Viscosity curves ($\eta = f(\gamma)$) and flow curves ($\tau = f(\gamma)$) at three different shear rates (25, 50 and 100 s^{-1}) and recorded during 60 s after the corresponding three ramp-up periods of 60 s (from 0 to 25 s^{-1}, 25 to 50 s^{-1} and 50 to 100 s^{-1}) were obtained.

On the other hand, rheological oscillatory tests were performed with parallel plate geometry (Haake PP60 Ti, 60 mm diameter, three different gaps separations between plates were tested, 0.2, 0.5 and 1 mm). Firstly, oscillatory stress sweep test was performed at a constant frequency of 1 Hz with an increasing shear stress from 0.01 to 10 Pa in order to determine the linear viscoelastic region (LVR) of the samples. After the determination of LVR, a frequency sweep test was carried out varying the frequency range within 0.1–10 Hz at a constant shear rate within the LVR, in order to determine the related variation of the storage modulus (G'), loss modulus (G''), phase angle (δ) and the complex viscosity (η^*) which were used for sample characterization.

2.7. In vitro release assays

2.7.1. Membrane selection

A pre-study to select the most appropriate artificial membrane was accomplished in triplicate using two different kinds of membranes, nylon (Waters Corporation, Milford, MA, USA) and polysulfone (Pall Corporation, Gelman Sciences, Ann Arbor, MI, USA), both of 45 mm in diameter and 0.45 μm in pore size. Vertical Franz diffusion cells (FDC 400, Crown Glass, Somerville, NY, USA) with an