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Novel perspectives in the tuberculosis treatment: Administration of isoniazid through the skin

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ABSTRACT

Despite its high efficacy in anti-tuberculosis therapy, the oral administration of isoniazid (INH) may lead to poor patient compliance due to hepatotoxicity events. In this context, the transdermal administration of INH was evaluated, for the first time, since this route avoids hepatic first pass effect. INH was applied to porcine skin in Franz diffusion chambers alone and with 5% menthol, limonene or Transcutol®. Infrared and DSC analyses were selected for mechanistic studies. The transdermal absorption of INH was sufficient to ensure a systemic therapeutic effect. Menthol was not able to improve the absorption of INH, but it increased the drug accumulation in skin compared to the control (1.4-fold). Transcutol® reduced permeation flux of INH (2.2-fold) and also increased the amount of drug retained in skin (1.7-fold). Limonene was the most effective excipient since it increased permeation flux of INH (1.5-fold) and lag time was greatly shortened (2.8-fold). DSC and FTIR analyses of limonene-treated skin suggest higher degree of disorder in lipid bilayers. Transdermal delivery of INH was positively correlated with log *P* of chemical enhancers. INH can be efficiently delivered by skin route and specific excipients may be selected depending on intended use.

1. Introduction

Although new drugs are being developed to face the challenge of emerging multidrug-resistant strains of *Mycobacterium tuberculosis*, isoniazid (INH) remains a widely used and effective first-line agent. Acute tuberculosis is mostly treated with a multidrug therapy approach, including rifampicin or pyrazinamide, but INH monotherapy is typically used in the treatment of latent tuberculosis (Boelsterli and Lee, 2014). It has an efficacy of more than 90% in latent tuberculosis if treatment is completed properly (Acton, 2012).

Oral therapies have been effective in the treatment of tuberculosis; however, undesirable side effects leading to treatment interruption have been reported (Gülbay et al., 2006). INH-induced liver injury ranges from a mild to severe form (Cai et al., 2012). The risk of severe hepatotoxicity caused by isoniazid occurs in approximately 1–2% of patients and 20% of patients are associated with elevated plasma levels of hepatic enzyme activity (Sarich et al., 1999). Histopathological analyses revealed the occurrence of hepatocellular focal or confluent necrosis, often with periportal inflammatory components and hydropic degeneration of hepatocytes (Pessayre et al., 1977). This fact limits the concomitant administration of other drugs, which are metabolized by *via* hepatic, such as those used in antiretroviral therapy.

In this context, various interventions have been taken in order to reduce drug-induced hepatotoxicity and improve patient compliance. Hepatoprotective effects of different plant preparations and other well-known antioxidants have been evaluated in *in vitro* and *in vivo* assays (Hussain et al., 2012; Prabakan et al., 2000; Yue et al., 2009), but further clinical studies should be carried out to confirm these benefits. Another well-designed way to solve this problem would be the selection of an administration route able to avoid the hepatic first-pass metabolism and to ensure that therapeutic concentrations may be achieved. Taking this into consideration, particulate drug-delivery systems have been proposed aiming a pulmonary administration. A major breakthrough expected out of these formulations is their capacity to enter into and act directly on *Mycobacterium* by a variety of mechanisms (Kaur and Singh, 2014). The particle size needs to be

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tightly controlled so that a suitable lung deposition and permeability in *Mycobacterium* can be observed. Despite the advantage of a significant surface area, the pulmonary route presents the limitation of nonreproducible placement of the drug at the site of absorption in the alveoli (Cereijido, 1992) and the duration of action is often short-lived (drug can be quickly removed from the lung through various clearance mechanisms) (Byron, 1986). Alternatively, transdermal systems for drug delivery may be considered, given that they are non-invasive and self-administered, which improve patient compliance, avoid hepatic first-pass metabolism, and may also provide drug release for long periods of time (Prausnitz and Langer, 2008). Moreover, cases of cutaneous tuberculosis have been recently reported (dos Santos et al., 2014), which makes this route even more interesting for the INH administration. In this context, the purpose of this study was to investigate, for the first time, the potential of the skin for the systemic delivery of INH and the impact of different well-known chemical enhancers (limonene, menthol and Transcutol®) on its absorption using Franz diffusion cells, a model previously established in our laboratory (Caon et al., 2010, 2014). Mechanistic studies were carried out using Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC). Based on these findings, the use of these excipients may be targeted to topical or systemic formulations.

2. Materials and methods

2.1. Materials

Isoniazid, L-menthol, limonene and Transcutol® were purchased from Sigma–Aldrich (St. Louis, MO, USA). Krebs bicarbonate Ringer (KBR) and PBS buffers were prepared according to standardized protocols. Acetonitrile was of HPLC grade and all other chemicals and reagents were of analytical grade and used as received.

2.2. Methods

2.2.1. Ex vivo *skin permeation studies*

Once pig skin is anatomically, physiologically and biochemically similar to human skin, this animal model was selected for the permeation studies (Simon and Maibach, 2000). Furthermore, the follicular structure of pig skin also resembles that of humans (Jung and Maibach, 2015), which is particularly relevant in the context of polar molecules as isoniazid. The full thickness of pig ear skin (1.00 ± 0.05 mm) was obtained from a local abattoir immediately after slaughter and was transported in ice-cold KBR buffer (pH 7.4). Unlike INH, the permeability coefficient of highly lipophilic compounds may be overestimated when full thickness skin is used (EPA/US, 1992). Given that the investigated compound has polar nature, in these situations, the dermal layer acts significant barrier properties, and thus the full thickness skin was considered for the experiments. The skin was initially cleaned with tap water and the hair and subcutaneous fat tissue were removed. The skin was placed in a two-chamber glass Franz diffusion cell with the stratum corneum toward the donor compartment. An available diffusion area of 1.77 cm^2 was considered. Before the permeation experiments, the donor and receptor chambers were filled with 2 and 10 mL of PBS buffer (pH 7.4), respectively. No solubilizing agents were added in the receptor chamber because INH presents high aqueous solubility. The system was kept at 37 °C by circulating heated water through an external water jacket, and the solution in the receptor chamber was continuously stirred at 800 rpm using Teflon® coated magnetic stirrers. After a 30 min-equilibration period to maintain the tissue hydration level, the PBS buffer solution was removed from donor chamber and the impact of different well-known absorption enhancers (menthol, limonene and Transcutol®) (Dragicevic and Maibach, 2015) on the INH transport was evaluated by comparing with those of the controls (500 μg/mL INH in PBS or in 40/60 ethanol/PBS). Once some of these enhancers present a more lipophilic nature, a previous solubilization in ethanol was performed (5% enhancer in a 40/60 ethanol/PBS buffer solution containing INH at 500 μg/mL). The selection of penetration enhancers was based on not only its efficacy in enhancing skin permeation but also on its dermal toxicity. Terpenes are generally considered less toxic and with low irritation potential compared to surfactants and other synthetic skin penetration enhancers. Furthermore, menthol and limonene are included in the list of Generally Recognized As Safe (GRAS) agents issued by US FDA (Sapra et al., 2008). Transcutol® is listed in the FDA Inactive Ingredient Database for transdermal applications given that it has been considered a nonirritant compound to skin even after prolonged and repeated contact (Smith and Maibach, 2005; Sullivan et al., 2014). At fixed time intervals (every 1 h, for 6 h), samples (400 μL) were withdrawn from the receptor chamber, replaced by the same volume of fresh medium and quantified by a previously validated HPLC method (see Section 2.2.2). At the end of the permeation experiment, samples of the skin were placed in separate pre-weighed tubes to determine the amount of INH in each tissue sample collected. INH was extracted from the skin with 3 mL of acetonitrile, centrifuged ($22{,}000 \times g$; 10 min) and sonicated (15 min). The extraction method was validated by spiking the skin with a known amount of INH (concentrations ranging from 1 to 100 μg/mL and recovery values from 98 to 101%). Critical mass balance was also determined by considering the total amount of drug in each of the compartments of the experiment (receptor compartment, that remaining in the donor compartment and that found in the skin layers). The percentages of recovery found were 101.2, 98.4, 100.9, 97.1 and 95.8 for treatments only with drug, INH coapplied with ethanol, ethanol/menthol 5%, ethanol/Transcutol® 5% and ethanol/limonene 5%, respectively. Regarding the permeation parameters, the steady-state permeation flux (J_s) was determined from the linear slope of the cumulative amount of INH permeated over time. The lag time represents the time required to achieve the steady-state flux, and the permeation coefficient (P) was obtained from the relation between the flux and the initial concentration of INH added to the donor compartment ($P = J_s/C_d$). J_s,P and lag time from different treatments were compared using a one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test. The target flux to ensure a therapeutic effect was calculated using the following Eq. (1) (Gannu et al., 2010)

$$\text{Target flux} = \frac{C_{ss} \times CL_t \times BW}{A}$$

where C_{ss} = the INH concentration at therapeutic level (3–5 μg/mL) (Fahimi et al., 2011); CL_t = total body clearance (5.025 mL/min/kg = 0.084 mL/h/kg; mean value regarding the limits for both rapid and slow acetylators) (Kergueris et al., 1986); BW = standard human body weight of 60 kg; and A = surface area of the diffusion cell (*i.e.*, 1.77 cm^2). The calculated target flux value for INH was 8.52–14.19 μg/cm^2 h (therapeutic window). This result is a rough calculation to estimate the amount of IZH in the plasma, but the effect of particular physiological factors and other aspects related to the formulation should also be evaluated by more sophisticated pharmacokinetic models, including *in vivo* experiments. In view of the fact that an increased dermal perfusion rate contributes to increase percutaneous permeation of drugs and the *in vitro* model is static (Crutcher and Maibach, 1969), in *in vivo* conditions, permeation rates are expected to be even larger.