Assessment of the percutaneous penetration of cisplatin: The effect of monoolein and the drug skin penetration pathway

Leonardo D.D. Simonetti, Guilherme M. Gelfuso, Julie C.R. Barbosa, Renata F.V. Lopez *

Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil

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1. Introduction

Cancer is a health problem, and it is the cause of the death of more than 4 million people per year. Skin cancer is by far the most common of all pathologies related to cancerous disease. Nearly 2 million new cases are diagnosed yearly in the entire world, and this number continues to rise [1]. There are multiple methods of treatment for these tumors, including surgical excision, chemosurgery, cryotherapy, radiation and photodynamic therapy. Besides relatively high recurrence rates, ranging between 5% and 10%, these treatment strategies also cause side effects such as pain, severe inflammation and local irritation, which can remain for several weeks [2,3]. Furthermore, scar formation is frequently observed. Surgical resection is the preferred method in most cases. However, some patients are not good candidates for surgery, due to significant medical problems or because the surgery will result in extensive disfigurement [3,4]. Systemic chemotherapy for thses lesions may eradicate the tumor but with a significant amount of toxic side effects. Therefore, it is important to discover more effective and less destructive therapies, leading to more acceptable aesthetic results, particularly in immunosuppressive patients as well as in patients with multifocal and highly extended lesions [5–9]. Topical chemotherapy for the treatment of cutaneous cancer, such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), could be an alternative to reduce drug systemic toxicity. A smaller residual tumor may permit complete removal with less extensive surgery [4,10–13].

Cisplatin (CIS), cis-diamminedichloroplatinum(II) (Fig. 1), is a widely used antineoplastic drug that is effective against a number of tumors, although its beneficial effects are balanced by significant toxicities [14]. Topical or intra-lesional applications of chemotherapy have been reported to be effective in selected cases of actinic keratosis, BCC and SCC. The major limiting factor to the effectiveness of topical application of antineoplastic drugs appears to be the percutaneous absorption of therapeutic levels of drugs to the sites where invasive tumors are found [15–20] (for a more detailed reading about metals fluxes through the skin, see [21]). In attempting to increase cisplatin delivery into cells, the application of intensive electric pulses (electrochemotherapy) has recently been reported to be effective [4,13,18,19]. A simpler alternative could be the addition of absorption enhancers to the drug formulation.

Monoolein (glycerol monooleate; MO) (Fig. 2) has recently received attention as a percutaneous absorption enhancer especially for polar drugs (see [27] for a general reading about percutaneous absorption enhancers). MO is a metabolite formed during the lipid digestion of oleic triglycerides [22]. It has been suggested that a possible mechanism of action of MO is the increase in the fluidization and extraction of lipids from the SC [23–26]. Lopes et al. [26], for instance, by investigating whether the presence of monoolein in a preparation of propylene glycol could improve cyclosporin A delivery to the skin, found that at 10% this enhancer was able to increase both topical and transdermal deliveries of the drug.
Therefore, the purpose of the present work was to examine the in vitro penetration of CIS through porcine skin in the presence of different concentrations of MO. Pretreatment experiments with MO and experiments through the skin without SC were also carried out in order to understand the behaviour of CIS permeation.

2. Materials and methods

2.1. Materials

CIS was purchased from Eurofarma (Brazil). Myverol 18–99 K, as a source of MO, was kindly granted by Quest International (Cam- pinas, Brazil), and it was used as received. All other chemicals were of analytical grade.

Experiments were performed with full thickness skin from pig ears. Tissue was obtained less than 2 h after the slaughter of the animal (Frigorífico Pontal Ltda., Brazil), and it was stored frozen for a maximum of 7 days before use.

2.2. Analytical chemistry

The amounts of CIS, which had diffused into or through the skin, were determined by a reversed-phase HPLC method [28]. To improve selectivity for total “active Pt(II)” (sum of native drug, mono- and di-aqua species and fraction of complexed Pt(II) still capable of reacting with strong nucleophiles) samples containing CIS were derivatized before analysis as follows: 0.5 mL of CIS sample was reacted with 50 μL of freshly made 10% diethyldithiocarbamate so- dic (DDTC) in 0.1 N NaOH (w/v) for 40 min at 37°C, chilled on ice and extracted with 0.4 mL chloroform. A 20 μL aliquot of the chlo- roformic layer was injected into a high-performance liquid chroma- tography (Shimadzu Instruments LC10-AD) equipped with an automatic sample injector (Shimadzu Instruments SIL10AD) and a 100RP-18 reversed-phase column (Lichrospher 4.0 mm × 125 cm, 5 μm). Elution was performed with a mobile phase consisting of a mixture of methanol water (70:30, v/v), at a flow rate of 1.5 mL/ min. Detection was accomplished at 254 nm (Shimadzu SPD-A). The calibration curve was linear (r = 0.999) for CIS over the concentra- tion range of 0.05–20.00 μg/mL. The intra- and inter-day injection variability was less than 15%.

2.3. In vitro release studies

CIS release rates from different formulations (CIS at 0.05% in propylene glycol (PG), 5% MO in PG, 10% MO in PG and 20% MO in PG) were measured through a 23 μm cellulose membrane (MW 12,000–14,000, Fisher Scientific, USA) using a Franz-type dif- fusion cell with a diffusion area of 0.8 cm² (Laboratory Glass Apparatus, Berkeley, USA). A volume of 0.5 mL of the formulation was placed on the membrane surface in the donor compartment, while the receptor was filled with 6 mL of phosphate buffer solution, pH 7.4. During the experiments, the receptor solution was stirred at 300 rpm and kept at 37°C. The receptor was perfused continuously at 2 mL/h, and samples were collected automatically every hour, up to 12 h. At the end of the experiment, the amount of transported drug was analyzed as described above.

2.4. In vitro permeation studies

Full thickness skin was mounted in a Franz-type diffusion cell (Laboratory Glass Apparatus, Berkeley, USA), with the dermal side facing downwards at the receptor medium: 6 mL of isotonic phos- phate buffer, pH 7.4. The donor compartment was filled with 0.5 mL of solutions containing CIS at 0.05% in (i) propylene glycol (PG), (ii) 5% MO in PG, (iii) 10% MO in PG, and (iv) 20% MO in PG. The experiments were carried out under the same conditions as those used in the release studies, and the amount of CIS in the receptor phase was also determined by HPLC as described above.

2.5. Effect of tape stripping

Stratum corneum (SC) of the full thickness skin was removed by 20 strippings with adhesive tape (3 M-Scotch, Brazil). The removal of the SC was indicated by the glistening of the exposed (epider- mis) surface. The skin without SC was then mounted in a Franz- type diffusion cell with the dermal side facing downwards at the receptor medium. The donor compartment was filled with differ- ent formulations containing CIS at 0.05%, and the experiments were carried out under the same conditions as those used in the re- lease and permeation studies.

2.6. Pretreatment studies

Solutions containing (i) water, (ii) propylene glycol (PG), (iii) 5% MO in PG, (iv) 10% MO in PG, and (v) 20% MO in PG were applied to the skin mounted in the diffusion cell. After 2 h of pretreatment, the skin was carefully washed with distilled water and 0.5 mL of an aqueous solution of CIS at 0.05% was spread on the skin surface. The same system conditions were employed as before, and the samples were analyzed using HPLC.

2.7. Statistic

All results were expressed as the mean ± standard deviation. Statistical comparisons were performed using Mann–Whitney test by GraphPad Prism software. The level of significance was 0.05 (P < 0.05).

3. Results and discussion

Fig. 3 shows the in vitro release profiles of CIS from the different formulations that were investigated. It can be seen that the addition of MO in different concentrations of the propylene glycol solution did not affect CIS release, since the apparent release rates (K) of CIS are very similar among the formulations (Table 1). The influence of MO in the release of drugs has been reported elsewhere. Herai et al. [29] observed that the addition of 5% of MO did not signifi- cantly change the release rate of the chemotherapeutic doxorubi- cin from solutions of this drug in propylene glycol. Although this is the first study to show that higher amounts of MO seemed to decrease the release rate of that drug, contrasting with the results observed for CIS.
Regarding the release kinetics, a linear relationship was observed when the amount of drug released was plotted against the square root of time, showing that the rate-controlling step for CIS release in all formulations is the diffusion process [30].

CIS steady-state flux (J), obtained from the slope of the cumulative amount of drug permeated through intact skin versus time plot, for each formulation, is showed in Fig. 4. It can be observed that the CIS permeation through the impaired skin, especially at 10% (Fig. 4). In contrast, the literature shows that the back diffusion of water increased more than 10-fold when the SC is removed from the skin and the permeation increased from 200- to 400-fold for hydrophilic neutral molecules, as glucose [37] and caffeine [38]. This slight improvement in the CIS permeation in the absence of the SC indicates that the main barrier for this drug permeation through the skin is the low diffusion of CIS from the viable epidermis to the receptor solution. It seems that CIS permeates the SC mainly by an alternative pathway, i.e., the skin appendages, which form shunt pathways through the intact epidermis [39]. In fact, some authors have demonstrated that the transport of drugs across the skin through this route is possible and much common depending on the molecule’s physicochemical properties as well as the delivery system or technique applied to administer the drug [40,41].

In order to sustain the hypothesis of the enhancing effect of MO for CIS skin permeation, pretreatment experiments with MO in PG at the same concentrations already studied (5%, 10%, and 20%) were carried out. Fig. 5 shows the permeation profile of an aqueous long-chain fatty acid with a cis-configuration [25,32–35]. As MO has a structure similar to oleic acid, with a cis-unsaturated double bond in the molecule, it has been proposed that it would act as a penetration enhancer in the same manner as oleic acid [23,24,36]. Steluti et al. [36] noticed an important increase in the penetration of the aminolevulinic acid (ALA), a highly hydrophilic drug, through the hairless mouse skin from formulations containing MO in the same concentrations (5%, 10%, and 20%) used in the present work. Herai et al. [29] studied the penetration of doxorubicin into the SC and through the skin employing the same formulations containing PG and 5%, 10%, and 20% of MO. The authors observed that MO played an effective enhancing role increasing the amount of the drug penetrating into the SC. However, any promotion on transdermal delivery was not verified, since no difference was detected in the receptor solution concerning the drug concentration.

Fig. 4. Flux of cisplatin (CIS) through an intact, impaired and pretreated skin (mean ± SD; n ≥ 3). PG: propylene glycol, MO: monoolein.

**Table 1**

CIS release rates (K) from different formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>K (µg/cm² h)</th>
<th>r²</th>
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<tbody>
<tr>
<td>PG</td>
<td>58.47 (±9.85)</td>
<td>0.999</td>
</tr>
<tr>
<td>5% MO in PG</td>
<td>68.68 (±9.07)</td>
<td>0.996</td>
</tr>
<tr>
<td>10% MO in PG</td>
<td>56.33 (±7.51)</td>
<td>0.994</td>
</tr>
<tr>
<td>20% MO in PG</td>
<td>55.27 (±17.72)</td>
<td>0.998</td>
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* Linear correlation coefficient; PG: propylene glycol; MO: monoolein.

Fig. 5. Permeation profile of cisplatin (CIS) through skin pretreated with different formulations. Each data point represents the mean (±SD) of three determinations. PG: propylene glycol, MO: monoolein.
solution of CIS through skin pretreated with the different MO formulations. It can be seen that neither PG nor MO pretreatment significantly increased the CIS flux from an aqueous solution through a “non-pretreated” skin (skin pretreated with water). The pretreatment with 20% of MO even significantly decreased (∼6-fold) CIS flux through the skin. Therefore, MO does not act as a real penetration enhancer for CIS probably because the SC is not the main barrier for this drug permeation. The absence of lag time for CIS skin penetration is also an indication that the drug is passing through the SC by its shunt pathways. The decrease in CIS penetration when the skin was pretreated with 20% of MO can be explained by a clog of these appendages by the excess of the lipid applied before the administration of the drug. Therefore, the improvement of CIS permeation through the intact skin when MO was added to the formulation can be explained by the permeation of the lipid into the viable epidermis jointly with the drug, altering the solubility properties of this tissue leading to an increase in the drug partition to the receptor solution.

In summary, the results obtained in this study suggest that the main route for CIS permeation is through the skin appendages, which form shunt pathways through the intact epidermis. It can also be suggested that MO has small effects on CIS permeation through the skin, since this enhancer interacts mainly with the lipid domains of SC, altering this barrier, which, nevertheless, seems not to be the main pathway of CIS permeation. On the other hand, due to the benefits that the application of CIS in topical chemotherapy might bring physical techniques, such as iontophoresis [8,43], or delivery systems, such as microemulsions and nanoparticles [44], could be used to improve the permeation of metal coordination compounds by the appendage pathway.

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References


