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Research paper

In vitro and *in vivo* evaluation of the delivery of topical formulations containing glycoalkaloids of *Solanum lycocarpum* fruits

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ABSTRACT

The glycoalkaloids solasonine (SN) and solamargine (SM) have been studied for their antiparasitic, antifungal, and anticancer properties, especially *in vitro* and *in vivo* against non-melanoma skin cancer. Thus, the alkaloidic extract of *Solanum lycocarpum*, which contains approximately 45% each of SN and SM, was used to define the best experimental conditions for *in vitro* and *in vivo* assays. The *in vitro* assays were performed with the Franz cell diffusion porcine skin model to evaluate the effects of different pHs and the presence of monoolein, ethoxydiglycol or ethanol penetration enhancers on the skin penetration and retention of SN and SM after 3, 6, 9 and 12 h of exposure. The *in vivo* assay was performed on hairless mice with the formulation selected in the *in vitro* assays. The results showed that pH 6.5 was optimal for SM penetration. The formulation containing 5% alkaloidic extract, 5% propylene glycol, 5% monoolein and a hydroxyethyl cellulose gel base (Natrosol®) (pH 6.5) was optimal for the delivery of SN and SM into the skin, and this formulation is potentially useful for the topical therapy of several skin disorders.

1. Introduction

Solanum lycocarpum A. St.-Hil. (Solanaceae) is a native species of Brazilian Cerrado belonging to the genus *Solanum* that is known for the biosynthesis of glycoalkaloids. The glycoalkaloids solasonine (SN) and solamargine (SM) (Fig. 1) are found in the fruits of more than a hundred species of *Solanum* [1], but *S. lycocarpum* stands out for its production of these compounds. SN and SM can be obtained by selectively extracting them from the fruits of this species [2]. These compounds are structurally similar because they contain the same steroidal moiety, solasodine, differing only in their sugar chain moieties: solatriose for SN and chacotriose for SM [3].

SN and SM have been studied as antiparasitics [4,5], antidiabetics [3], anti-virals against herpes [6], antifungals [7–9], immunomodulators [10], and anticancer agents in several cell lines [11–15] and *in vivo* against human skin cancer [16,17].

Studies have shown that SN and SM are selective for cancerous cells due to the sugar chain moiety, mainly rhamnose, of which SN and SM contain one and two units, respectively [18,19]. These compounds have been studied to treat non-melanoma skin cancer by employing a mixture of glycoalkaloids extracted from *Solanum* species [18]. However, the efficacy of these compounds not only depends on the quantity used but also on the composition of the topical formulation. For instance, cetomacrogol-based cream with 10% glycoalkaloids and 10% dimethylsulfoxide (DMSO) effected an 83% skin carcinoma cure rate [16], while another formulation with 0.005% glycoalkaloids and keratolytic agents was 100% curative [20]. In contrast, a 66% cure rate was achieved with 0.005% glycoalkaloids and keratolytic agents in emulsifying wax and white soft paraffin base [17]. It is important to note that these studies were performed with an alkaloidic mixture from another *Solanum* species, which is composed of 33% SN, 33% SM and 34% di- and mono-glycosides of solasodine [18], while the alkaloidic extract from *S. lycocarpum* studied in this work contained approximately 45% SN and 45% SM [21].

It is desirable that topical formulations adequately allow for skin penetration and retention with minimal systemic absorption [22]. In this context, penetration enhancers have been widely used in formulations to improve the skin penetration of several compounds [23]. Moreover, it is known that glycoalkaloids have a basic character, which plays an important role in their skin permeability, depending on their degree of ionization, solubility in the applied

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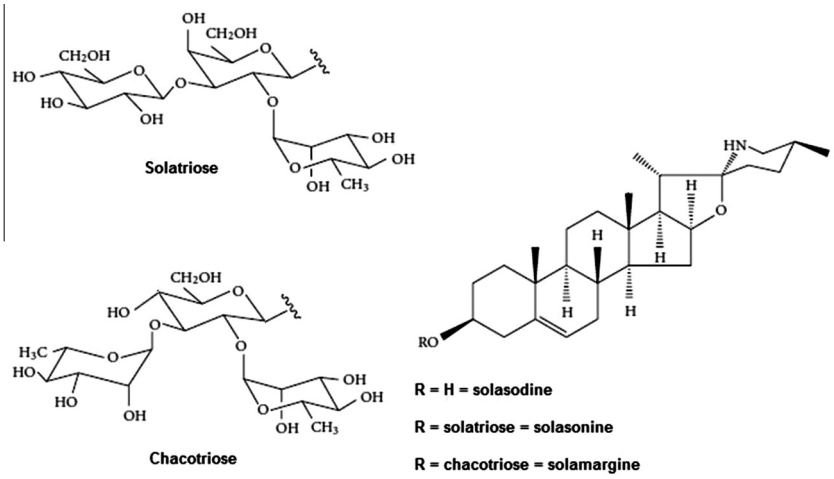

Fig. 1. Structures of solasodine and their glycosides: solasonine (SN) and solamargine (SM).

phase and partitioning into the skin [24]. Taking into consideration that penetration and the consequent effects of glycoalkaloids in the skin could be greatly influenced by the formulation pH [25], it is mandatory to understand the physicochemical properties of these compounds and the skin penetration of SN and SM in the development and optimization of new skin anticancer therapies.

Therefore, considering the remarkable activity of glycoalkaloids on cancer cells and the lack of data on their topical delivery, as well as their high content in the alkaloidic extract of *S. lycocarpum* fruits, we have performed studies on the development of a topical formulation with the ability to deliver these compounds into deeper skin layers so that they can be used in the treatment of non-melanoma skin cancer.

2. Material and methods

2.1. Chemicals and reagents

High performance liquid chromatography (HPLC)-grade acetonitrile (MeCN) and methanol (MeOH) were obtained from Mallinckrodt Co. (Xalostoc, Mexico). Anhydrous disodium hydrogen phosphate was acquired from Carlo Erba Reagents (Brazil). Deionized water was purified by Milli-Q-plus filter systems (Millipore, USA). The analytical grade cetylpyridinium chloride, ethanol (EtOH), propylene glycol, hydrochloric acid, sodium hydroxide, sodium phosphate, methylparaben (Nipagin®) and propylparaben (Nipasol®) were purchased from Synth (Brazil). Hydroxyethyl cellulose gel (Natrosol®) was purchased from Galena (Netherlands). Ethoxydiglycol (Transcutol CG®) was obtained from Gatefosse (France). Monoolein 18-99K (Myverol™) was obtained from Quest (Netherlands). The standard compounds, SN and SM, with 96% purity, were kindly provided by Dr. James D. McChesney from Cypress Creek Pharma.

2.2. Glycoalkaloid extraction

The alkaloidic extract incorporated into the topical formulation, containing 45.09% ± 1.14 SN and 44.37% ± 0.60 SM, was obtained using a selective acid–base extraction method and was quantified by analytical HPLC with ultraviolet (UV) detection, as described previously [2].

2.3. Analytical HPLC conditions

The analysis with SN and SM standards was performed using a validated reverse-phase HPLC-UV method with gradient elution, as described in our previous work [21]. Briefly, a Zorbax SB-C18 analytical reverse phase column was used as stationary phase, and the binary gradient consisted of sodium phosphate buffer (pH 7.2, 0.01 M) (pump A) and MeCN (pump B). Thus, the calibration curves were constructed for both glycoalkaloids, and linearity was achieved for concentrations in the range of 0.77–990.00 μg/mL for SN and 0.78–1000.00 μg/mL for SM, with correlation coefficients $\geqslant$ 0.999. The retention times of SN and SM were 10.35 and 12.35 min, respectively. The intra-assay and inter-assay variations were less than 9.18% for SN and 8.74% for SM. The method recoveries were evaluated for samples of stratum corneum (SC) and epidermis plus dermis (EP + D), determined at three concentrations, and the values were higher than 88.94% for SN and 94.75% for SM. The limits of detection and quantification were 0.29 μg/mL and 0.86 μg/mL for SN, and 0.57 μg/mL and 1.74 for SM, respectively [21].

2.4. Evaluation of physicochemical properties of SN and SM at different pHs

To evaluate the influence of pH on the solubility and partition coefficient of these compounds, a buffer was prepared by mixing different volumes of sodium citrate (0.1 M) and sodium phosphate (0.2 M) solutions, obtaining pHs of 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0 and 8.0, according to Macilvaine [26]. To determine the saturation concentration (Cs), excess of alkaloidic extract were mixed in these solutions, filtered, and the amounts of SN and SM were quantified by HPLC, as described in Section 2.3.

The partition coefficient between octanol and water ($K_{octanol/w}$) was determined using a shake flash method ($n = 3$) [27]. Both buffer solutions at different pHs were saturated with octanol, and octanol was saturated with buffer solutions. The buffer phases were then separated, and an excess of extract was added. After achieving equilibrium, the dispersions were filtered, and the alkaloids were quantified before partition ($A1$). To each remaining buffer solution sample (1.5 mL), 1.5 mL of buffer-saturated octanol was added. This system was shaken for 24 h at room temperature. After this period, an aliquot of the aqueous phase was removed for analysis and quantification ($A2$).

The $K_{octanol/w}$ values were determined for glycoalkaloids at each pH and were obtained by the following equation:

$$K_{octanol/w} = (\text{Conc. } A1 - \text{Conc. } A2)/\text{Conc. } A2 \quad (1)$$

where Conc. is the concentration of each glycoalkaloid [27].