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Research paper

Development and characterization of novel 1-(1-Naphthyl)piperazine-loaded lipid vesicles for prevention of UV-induced skin inflammation

Ana Catarina Menezes [a,*], Patrícia Mazureki Campos [b], Carla Euletério [a], Sandra Simões [a], Fabíola Silva Garcia Praça [b], Maria Vitória Lopes Badra Bentley [b], Andreia Ascenso [a]

[a] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal
[b] School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

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ABSTRACT

1-(1-Naphthyl)piperazine (1-NPZ) has shown promising effects by inhibiting UV radiation-induced immunosuppression. Ultradeformable vesicles are recent advantageous systems capable of improving the (trans)dermal drug delivery. The aim of this study was to investigate 1-NPZ-loaded transethosomes (NPZ-TE) and 1-NPZ-loaded vesicles containing dimethyl sulfoxide (NPZ-DM) as novel delivery nanosystems, and to uncover their chemopreventive effect against UV-induced acute inflammation. Their physicochemical properties were evaluated as follows: vesicles size and zeta potential by dynamic and electrophoretic light scattering, respectively; vesicle deformability by pressure driven transport; rheological behavior by measuring viscosity and I-NPZ entrapment yield by HPLC. *In vitro* topical delivery studies were performed in order to evaluate the permeation profile of both formulations, whereas *in vivo* studies sought to assess the photoprotective effect of the selected formulation on irradiated hairless mice by measuring myeloperoxidase activity and the secretion of proinflammatory cytokines.

Either NPZ-TE or NPZ-DM exhibited positive results in terms of physicochemical properties. *In vitro* data revealed an improved permeation of 1-NPZ across pig ear skin, especially by NPZ-DM. *In vivo* studies demonstrated that NPZ-DM exposure was capable of preventing UVB-induced inflammation and blocking mediators of inflammation in mouse skin. The successful results here obtained encourage us to continue these studies for the management of inflammatory skin conditions that may lead to the development of skin cancers.

1. Introduction

Skin cancer is the most common malignancy worldwide and as deadly as other forms of cancer [1,2]. The major cause of photocarcinogenesis is the ultraviolet radiation, which can lead to the onset of the most prevalent forms of skin cancer, basal and squamous cell carcinomas, commonly known as nonmelanoma skin cancers [1]. UV radiation is responsible for the induction of irreversible alterations in DNA leading to modifications on several signaling pathways [1]. At skin level, UVB exposure can trigger an acute inflammation process through the recruitment of neutrophil granulocytes and the activation of nuclear factor kappa β (NF-κβ) with subsequent increasing levels of proteins involved in inflammatory and immune responses, including pro-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) [1,3,4]. The abnormal production of these cytokines plays a critical role in multistage cancer development. Furthermore, they induce keratinocyte proliferation and monocyte recruitment to the site of injury, leading to increased secretion of more cytokines. Therefore, modulation of inflammatory processes by downregulating signaling cascades related to tumor promotion may be an interesting approach to prevent photocarcinogenesis [3,5].

1-NPZ is a serotonergic (5-hydroxytryptamine, 5-HT) derivative of quipazine [6]. In the skin, 5-HT is associated with the processes of inflammation and immunomodulation, leading to the production of proinflammatory mediators upon activation of immune cells [1]. 1-NPZ is both a 5-HT2A receptor antagonist and 5-HT1A

Abbreviations: 1-NPZ, 1-(1-Naphthyl)piperazine; NPZ-TE, 1-NPZ-loaded transethosomes; NPZ-DM, 1-NPZ-loaded vesicles containing DMSO; DI, deformability index; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; HPLC, high performance liquid chromatography; IL-1β, interleukin-1beta; MPO, myeloperoxidase; PDI, polydispersity index; 5-HT, serotonin; SPC, soybean phosphatidylcholine; SC, *stratum corneum*; TNF-α, tumor necrosis factor-alpha; UDV, ultradeformable vesicles; UV, ultraviolet radiation.

* Corresponding author.
E-mail address: catarinamenezes@msn.com (A.C. Menezes).

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receptor agonist, comprising a dual mechanism of action in the prevention of immunosuppression and photocarcinogenesis. Hence, 1-NPZ will block the binding of *cis*-urocanic acid to 5-HT2A receptor while eliciting immune cells through 5-HT1A receptor [1]. 5-HT2A receptor is present in melanocytes among other skin cells, whereas 5-HT1A receptor can be found in both keratinocytes and melanocytes [1]. In inflammatory skin diseases, the levels of 5-HT1A receptors may decrease while those of 5-HT2A may increase [1]. Previously, 5-HT1A receptor has shown an anti-inflammatory effect in allergic contact eczema in mice [7]. Furthermore, 5-HT2A antagonists have been shown to reduce the elicitation of delayed-type hypersensitivity reactions in mice [1,7]. The antagonistic functions of both 5-HT1A and 5-HT2A receptors might indicate that they are novel and promising targets for anti-inflammatory therapies in inflammatory skin diseases [7]. Recently, few *in vitro* and *in vivo* experiments have shown promising results with this serotonergic drug [8,9]. In fact, we have already observed such beneficial effects caused by the action of 1-NPZ on tumor development through several *in vitro* studies with human MNT-1 melanoma cell line (data not shown). Notwithstanding, several biomarkers should be studied in order to completely access the mechanism of action of 1-NPZ.

Nanomedicine offers a powerful drug delivery system, allowing a site-specific drug delivery to cancer cells, which significantly increases the treatment efficiency [2]. Lipid nanocarriers display several physicochemical features suitable for delivery of drugs through the skin [10]. The inherent benefits include enhancing drug absorption through the skin, decreasing degradation of the entrapped drug, and provide a sustained drug release [10]. Thus, they are able to increase the intracellular concentration of chemotherapeutic drugs overcoming the heterogeneity of cancer cells and multiple drug resistance [10]. Several lipid nanosystems have been developed over the years for treatment of UV-induced skin diseases including skin cancer, such as liposomes, ultradeformable vesicles (UDV), micelles, micro and nanoemulsions, lipoproteins, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) [2,10]. Conventional liposomes were the first nanocarriers used to enhance the permeation rate of the entrapped active compounds across the skin [11]. However, their use was limited by their confinement in the *stratum corneum* (SC) due to their absence of vesicles flexibility [12]. To overcome such limitation, pioneering vesicular systems have been developed with increased permeation rates of topically administered drugs [11].

Recent strategies in modulating the composition of nanocarriers have resulted in several designs of novel vesicular drug delivery systems, including transfersomes and ethosomes, among others. Transfersomes were the first generation of flexible liposomes introduced by Cevc and Blume in the early 1990s [13]. They contain phospholipids and an edge activator, which is often a single-chain surfactant that destabilizes the lipid bilayers in order to increase vesicles flexibility [14]. Ethosomes were developed by Touitou et al. in 2000 [15], and are formulated with phospholipids, water and ethanol (20–45%) [16]. High ethanol content allows ethosomes to have a much smaller size than liposomes and greater flexibility, disrupting the organization of SC and improving its fluidity [17,18]. *In vitro* and *in vivo* experiments have already demonstrated that both ethosomes and transfersomes are capable of enhancing skin delivery of numerous drugs [18–21]. Given the advantages that transfersomes and ethosomes may provide, liposomal formulations comprising both a surfactant and an alcohol would be desirable as a flexible carrier to deliver drugs into deeper skin layers. Transethosomes (TE) emerged a few years ago and were developed by Song et al. [22]. They consist of phospholipids, water, ethanol and an edge activator or a penetration enhancer [22]. These novel vesicular nanocarriers have shown increased skin permeation and hence superior characteristics compared to conventional liposomes, transfersomes and ethosomes [22,23]. We have also obtained similar results in a previous study [24].

Our research group has recently developed another type of innovative vesicles containing dimethyl sulfoxide (DMSO). DMSO is a powerful aprotic solvent and one of the most popular skin permeation enhancer, being capable of promoting the transdermal delivery of numerous drugs [25]. It increases skin permeability by interacting with the intercellular lipid domains of SC and interfering with the packing geometry [25]. In fact, 1-NPZ has a higher solubility in DMSO rather than in water, which clearly justifies the use of DMSO. In addition, it has also biological activity, being a free radical scavenger and exhibiting anti-inflammatory analgesic effects [26].

The aim of the current study was to formulate and fully characterize NPZ-TE and NPZ-DM for topical administration to uncover the chemopreventive effect against UV-induced acute inflammation.

2. Materials and methods

2.1. Materials

1-NPZ was purchased from Enzo Life Sciences (Farmingdale, NY, USA). Soybean phosphatidylcholine (SPC) was obtained from Lipoid AG (Steinhausen, Switzerland). Sodium cholate, DMSO, dimethylformamide (DMF) and ascorbic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Perchloric acid and ammonium molybdate were purchased from Merck (Darmstadt, Germany) and Riedel-de Haen (Seelze, Germany), respectively. Citric acid monohydrate and trisodium citrate dihydrate were purchased from Panreac Quimica, SA (Barcelona, Spain). Hexadecyltrimethylammonium bromide, ortho-dianisidine dihydrochloride and hydrogen peroxide used in *in vivo* experiments were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a MILLI-Q® System by Millipore (Billerica, MA, USA). All other reagents were of analytical or high performance liquid chromatography (HPLC) grade.

Table 1
Composition of lipid vesicular formulations.

Ingredient	Class	Main action	Composition (% w/v)	
			TE	DM
1-NPZ	Drug	5-HT1AR agonist/5-HT2AR antagonist	0.04	0.04
Soybean phosphatidylcholine (SPC)	Phospholipid	Vesicles forming component	10	20
Sodium cholate (NaCo)	Anionic surfactant	Vesicles flexibility component Skin enhancer	1.3 (3.75:1 SPC:NaCo, Molar ratio)	–
DMSO	Polar aprotic solvent	Solvent Skin enhancer	–	30
Ethanol	Polar protic solvent	Solvent Skin enhancer	30	–
Purified water	Polar protic solvent	Hydrating medium	q.s. 100	–
Citrate buffer 50 mM solution	Buffering agent (pH 5)	Hydrating medium	–	q.s. 100