The Effects of pH and Ionic Strength on Topical Delivery of a Negatively Charged Porphyrin ($TPPS_4$)

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ABSTRACT: *Meso*-tetra-[4-sulfonatophenyl]-porphyrin ($TPPS_4$) is a charged porphyrin derivate used in photodynamic therapy (PDT) by parenteral administration. This study means to investigate potential enhancement for its topical delivery by determining the $TPPS_4$ dependence on the environmental characteristics and applying iontophoresis. In order to accomplish this task, cathodal and anodal iontophoresis as well as passive delivery of the drug were studied *in vitro* and *in vivo* in function of its concentration, pH and ionic strength. A reduction in drug concentration as well as the NaCl elimination from donor formulation at pH 2.0 increased $TPPS_4$ passive permeation through the skin *in vitro*. Iontophoresis improved $TPPS_4$ delivery across the skin when applied in solutions containing NaCl at pH 2.0, regardless electrode polarity. However, at pH 7.4, the amount of $TPPS_4$ permeated by iontophoresis was not different from that one permeated after passive experiments from a solution containing NaCl. Despite the fact that iontophoresis did not improve $TPPS_4$ transdermal delivery at this specific condition, *in vivo* experiments showed that 10 min of iontophoresis quickly and homogeneously delivered $TPPS_4$ to deeper skin layers when compared to passive administration, which is an important condition for topical treatment of skin tumors with PDT.
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Keywords: iontophoresis; topical administration; skin penetration; photodynamic therapy; $TPPS_4$

INTRODUCTION

Photodynamic therapy (PDT) is a new form of local cytotoxic treatment that may be effective for a wide variety of conditions: corneal neovascularization, sterilization of freshly frozen plasma, actinic keratosis, hair removal, psoriasis, Kaposi's sarcoma and several types of cancer which includes: lung, T-cell lymphoma, breast, esophageal, bladder, gastric, cervical, head and neck, brain, intestine and skin cancer among others.[1] Fundamentally, a photosensitizing agent (PS), which preferentially accumulates in target tissues, interacts with visible light and molecular oxygen. Then, two types of photodynamic reactions can occur: one involves the generation of free radicals (type I photochemical reactions), and the other involves the production of singlet molecular oxygen O_2 (type II), which is the main photoproduct responsible for cell inactivation. The type II reaction has an important effect on the cell death, although the photodynamic mechanism of the PS on neoplastic tissues is not fully understood.[2] PDT efficiency is affected by various factors, including PS photo physical properties, wavelength of the activation light, depth of the

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light penetration in the biological tissue and drug distribution in the specific tissue.[3]

The most widely applied PSs for PDT in human medicine are porphyrin derivatives. They are composed by tetrapyrrolic macro cycles, with distinct effects in biological systems.[4] *Meso*-tetra-[4-sulfonatophenyl]-porphyrin ($TPPS_4$; Fig. 1) was first used by Winkelman, in 1962.[5] It is a stable drug, water soluble at room temperature, which provides a greater yield of singlet oxygen.[6] However, depending on its synthesis and purification $TPPS_4$ can be neurotoxic.[6,7] Therefore, its topical administration, especially for the treatment of skin diseases, can avoid this problem. Nevertheless, $TPPS_4$ molecule can be protonated in acid media, which changes drastically its spectral and energetic properties[8] and can also affect its skin penetration. In order to administer this porphyrin topically with higher efficacy, it should be dispersed in an adequate formulation or drug delivery system and a complete understanding of its behavior in the formulation is required.

Iontophoresis is a method which transports hydrophilic and charged molecules into and through tissues, by application of a small direct current (no more than 0.5 $mA\,cm^{-2}$).[9] It has been widely used for topical anesthesia[10] and for topical and transdermal delivery of a great variety of drugs, including aminolevulinic acid (ALA), which is a porphyrin precursor widely used in PDT.[11–14] The two main mechanisms involved in iontophoretic delivery of drugs are electromigration and electroosmosis. Both positively or negatively charged molecules are typically delivered by electromigration, as the charged drug stands in the same charged compartment, while neutral molecules can be transported via electroosmosis.[15]

In the present study, the potential of iontophoresis as a method to enhance the $TPPS_4$ delivery for topical PDT was analyzed *in vitro* and *in vivo* in function of drug concentration, pH of the formulation and ionic strength in the donor solution by comparing the passive delivery with cathodal and anodal iontophoresis.

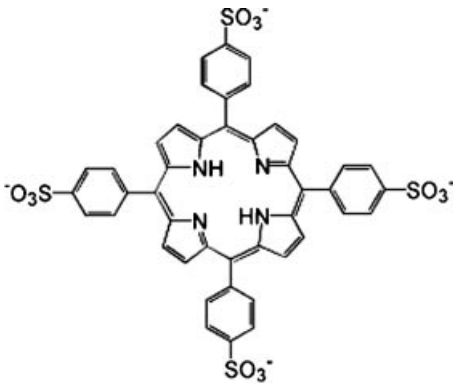

Figure 1. Chemical structure of $TPPS_4$.

MATERIALS AND METHODS

Materials

The $TPPS_4$ 2HCl ($TPPS_4$ acid dihydrochloride) was obtained from Frontier (Logan, USA), Ag-wire (99.99%, $\phi = 1.5$ mm), AgCl (99.99%) and Pt-wire were all purchased from Sigma-Aldrich (Steinheim, Germany), HEPES from J.T. Baker (Phillipsburg, USA), NaCl from Synth (Diadema, Brazil), Tissue Tek® (O.C.T. Compound) from Sakura (Torrance, CA) and *p*-phenyllenediamine from Sigma-Aldrich. All other reagents were BDH or HPLC reagents. The water used in all preparations was of Milli-Q grade (Millipore, France).

The membrane used for the *in vitro* experiments was fullthickness skin from porcine's ear. It was obtained less than 2 h after the slaughter of the animal (Frigorífico Pontal Ltda., Pontal, Brazil). The whole skin was removed from the outer region of the ear, it was separated from the underlying layer and stored frozen for a maximum of 7 days before use.

Analytical Chemistry

A method for $TPPS_4$ quantification was developed using an UV/Vis Spectrophotometer (Femto—800XI) operated at 412 nm. A linear calibration graph ($y = 0.274x + 0.001$; $r = 0.999$) was obtained over the working concentration range 0.1–1.0 μg/mL. Intra- and interdays precision and accuracy of the method showed a variation coefficient and a relative error not greater than 1.3% and 4.2%, respectively. The method was validated and showed precision and accuracy. It was also sensitive and selective during all the analyses.

In Vitro Iontophoretic Experiments

The skin was mounted between the upper and lower parts of vertical, flow-through iontophoretic cells (LG-1088-IC—Laboratory Glass Apparatus, Inc., Berkeley, CA). The area of skin exposed in