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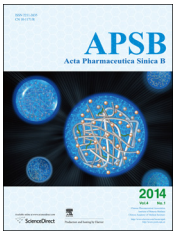

ORIGINAL ARTICLE

The effect of microneedles on the skin permeability and antitumor activity of topical 5-fluorouracil

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Abstract Topical 5-fluorouracil (5-FU) is approved for the treatment of superficial basal cell carcinoma and actinic keratosis. However, 5-FU suffers from poor skin permeation. Microneedles have been successfully applied to improve the skin permeability of small and large molecules, and even nanoparticles, by creating micron-sized pores in the stratum corneum layer of the skin. In this report, the feasibility of using microneedles to increase the skin permeability of 5-FU was tested. Using full thickness mouse skin mounted on Franz diffusion apparatus, it was shown that the flux of 5-FU through the skin was increased by up to 4.5-fold when the skin was pretreated with microneedles (500 μm in length, 50 μm in base diameter). In a mouse model with B16-F10 mouse melanoma cells implanted in the subcutaneous space, the antitumor activity of a commercially available 5-FU topical cream (5%) was significantly enhanced when the cream was applied on a skin area that was pretreated with microneedles, as compared to when the cream was simply applied on a skin area, underneath which the tumor cells were implanted, and without pretreatment of the skin with microneedles. Fluorouracil is not approved for melanoma therapy, but the clinical efficacy of topical 5-FU against tumors such as basal cell carcinoma may be improved by integrating microneedle technology into the therapy.

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1. Introduction

Fluorouracil (5-FU) is an antimetabolite that is used in the treatment of various types of cancers, including breast, head and neck, and colorectal cancer[1,2]. The fluorinated pyrimidine analog is available in topical formulations, which were approved by the United States Food and Drug Administration (US FDA) to treat actinic keratosis (non-cancerous) and superficial basal cell carcinoma (BCC)[3,4]. Approved topical products include 5-FU solutions (*e.g.*, Fluoroplex 1% 5-FU solution, Allergan, Inc., Irvine, CA, USA), creams (*e.g.*, Efudex®, Valeant Pharmaceuticals, Bridgewater, NJ, USA), and a 0.5% microsphere-based cream (Carac®, Valeant Pharmaceuticals). Other reported clinical applications of topical 5-FU include the treatment of nail psoriasis[3,5], cholesteatoma[6], lentigo maligna[7] and some premalignant ophthalmic conditions[8]. Topical treatment with 5-FU, when applicable, is usually more preferred than surgical removal of affected lesions for cosmetic reasons, especially for multiple lesions and/or facial lesions[9]. Unfortunately, the skin permeability of topically applied 5-FU is poor[10–12], likely due to its hydrophilic nature ($\mathrm{Log}P = -0.89$)[13]. The use of topical 5-FU in BCC therapy is only limited to superficial BCC, and it is not recommended for invasive forms of BCC[4,14]. This is based on a study by Mohs et al.[14], who reported that topical application of 5-FU in invasive BCCs can mislead clinicians by showing superficial improvement, while the deeper parts of the cancerous lesions continue to grow unnoticed.

Several approaches to improve the skin permeation of topical 5-FU have been evaluated with different degrees of success. For example, Paolino et al.[11] described the formulation of 5-FU-loaded bola-surfactant-based niosomes to improve the percutaneous permeation and antitumor activity of 5-FU. The proposed niosomes exhibited an 8-fold increase in the percutaneous permeation of 5-FU through human skin, as compared to 5-FU in an aqueous solution. The 5-FU niosomes were significantly more cytotoxic against SKMEL-28 human melanoma cells in culture than 5-FU solution, which was attributed to the improved cellular uptake of 5-FU in the niosomes[11]. Other researchers reported the use of penetration enhancers such as azone, isopropyl myristate and lauryl alcohol[12], or the use of pharmaceutical formulation technologies (*e.g.*, microemulsions[10] and nanogels[13]), to increase the percutaneous permeability of 5-FU. Physical methods to increase the permeability of 5-FU have also been evaluated. For example, Fang et al.[15] studied the effect of a series of physical methods, namely iontophoresis, electroporation, erbium:YAG (erbium: yttrium–aluminum–garnet) laser and their combination, on the permeability of 5-FU. Both iontophoresis and electroporation significantly enhanced the percutaneous permeability of 5-FU, but the controlled removal of the stratum corneum by erbium:YAG laser was most effective[15], confirming that it is the stratum corneum that limits the skin permeability of 5-FU. However, Meidan et al.[16] found that ultrasound unexpectedly lowered the permeability of 5-FU through whole rat skin; an effect that was attributed to the back-diffusion of 5-FU to the ultrasonic coupling gel filled in the donor compartment.

Microneedle technology had been successfully applied to enhance the skin permeability of small molecules, macromolecules and even nanoparticles by creating an array of micro-sized holes in the stratum corneum of skin[13,17–23]. However, it remains unclear whether it is feasible to increase the skin permeability of 5-FU by pretreating skin with microneedles. In the present study, the feasibility of using microneedles to improve the skin permeability of 5-FU was tested *in vitro* using mouse skin mounted on a Franz diffusion apparatus. In addition, the feasibility of using microneedles to improve the *in vivo* antitumor activity of 5-FU was tested by comparing the ability of an FDA-approved topical 5-FU cream in inhibiting the growth of subcutaneously implanted B16-F10 tumors in mice. The 5-FU cream was applied on the mouse skin area where the tumor cells were implanted with or without pretreatment (of the skin area) with a microneedle roller. Topical 5-FU is not approved for melanoma treatment, but 5-FU was reported to be effective against melanoma cells in culture and in animal models[19,20]. The B16-F10 tumor cells are implanted subcutaneously in mice, which allows indirect evaluation of the *in vivo* permeation of 5-FU across mouse skin as well.

2. Materials and methods

2.1. Materials

The Dermaroller® microneedle roller was kindly provided by Cynergy, LLC (Carson City, NV, USA). There are 192 needles (500 μm in length, 50 μm in base diameter) on the roller. The topical 5-FU cream (5%) was from Taro Pharmaceuticals USA, Inc. (Hawthorne, NY, USA). Phosphate buffered saline (PBS, pH 7.4), cell culture medium and antibiotics were from Invitrogen (Life Technologies, Carlsbad, CA, USA). MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and 5-FU were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. In vitro cytotoxicity assay

B16-F10 murine melanoma cells were from the American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco′s modified eagle medium (DMEM) with 10% (*v/v*) fetal bovine serum, 10 U/mL of penicillin and 100 μg/mL of streptomycin. Cells (2000/well) were seeded in 96-well plates and incubated overnight at 37 °C, 5% CO_2. Cells were then incubated in the presence of various concentrations of 5-FU in PBS solution (pH 7.4) for 24 h or 48 h. Cell viability was determined using an MTT assay following the manufacturer′s instruction. The formed formazan crystals were dissolved in 100 μL of dimethyl sulfoxide, and the absorbance of the resultant solution was measured at 570 nm and 630 nm using a BioTek Synergy HT Multi-Mode Microplate Reader (Winooski, VT, USA).

2.3. In vitro permeation of 5-FU in solution through mouse skin

In vitro permeation assay was completed as previously described[19]. Full thickness dorsal skin from C57BL/6 mice was used in the permeation study. Hair was carefully trimmed using an electric clipper 24 h before the collection of the skin. The harvested skins were stored at −20 °C and used within one month. On the day when the permeability study was performed, the skin was also treated with the Veet® hair removal cream (Reckitt Benckiser, Inc., UK) for 5 min and washed three times with water. The skin was then mounted onto the Franz diffusion cells (PermeGear, Inc., Hellertown, PA, USA) with the epidermis side facing upward. The receiver compartment contained 5 mL of PBS (pH 7.4, 10 mmol/L) and was maintained at 37 °C (Haake SC 100 Water Circulator, ThermoScientific, Wellington, NH, USA). The diffusion area of the skin was 0.64 cm^2. The donor compartment was loaded with 400 μg of 5-FU in 400 μL of PBS (pH 7.4,