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# The Novel Excipient, Dodecyl-2-N, N-dimethylaminopropionate Hydrochloride (DDAIP-HCl) Improves the Flux of Minoxidil in Human Skin

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## Abstract

Transdermal delivery of topically applied compounds is limited by the stratum corneum (SC) protective barrier. Penetration enhancers have been employed to perturb the skin barrier and enhance the transdermal flux of drugs. The novel excipient, dodecyl-2-N, N-dimethylaminopropionate hydrochloride (DDAIP-HCI), was evaluated for penetration activity of minoxidil in human cadaver skin. Minoxidil skin flux through human cadaver skin mounted on Franz cells was investigated with and without the presence of DDAIP-HCI. Enhanced minoxidil permeation levels were observed with DDAIP-HCI at concentrations of 0.05% and 0.5% through human cadaver skin relative to the formulation of the marketed hair loss product Rogaine<sup>®</sup> (5% minoxidil) over a 24-hour period. Increased minoxidil penetration may lead to a better efficacy and clinical response in the treatment of male pattern hair loss or Androgenetic alopecia.

**Keywords:** Minoxidil; Permeation; Excipient; DDAIP-Hcl; Skin flux; Androgenetic alopecia

# Introduction

Minoxidil, the pyrimidine derivative (2,4-diamino-6piperidinopyrimidine-3-oxide), is the only Food and Drug Administration-approved topical treatment with proven efficacy for the treatment of male pattern hair loss (MPHL) or Androgenetic alopecia (AGA). Androgenetic alopecia is hereditary and characterized by the shortening of hair follicles in the frontal and parietal scalp [1]. Despite the use of minoxidil for more than 20 years, little is known about its mechanism on normal human hair growth. Putative modes of action include vasodilatation [2,3], angiogenesis [4], enhanced cell proliferation [5,6] and potassium channel opening [7,8]. In animal studies, topical minoxidil reduces telogen and promotes the extension of anagen, in which the hair follicle regrows and increases in length and thickness [9]. In humans, Han et al. [10], reported that minoxidil stimulates hair growth by prolonging anagen through proliferative and antiapoptotic effects on the dermal papilla cells of human hair follicles. Minoxidil is, however, limited in its usefulness as a potent drug in the use of hair growth treatment due to its poor skin penetration [11].

Drug penetration through the stratum corneum (SC) is a valuable means of drug delivery, as it offers many benefits over other routes, which include oral, intravenous and others. However the SC layer forms an effective barrier for absorption of topically applied substances including drugs, peptides and proteins, thus limiting flux and distribution into the deeper skin layers [12]. Various approaches have been employed to affect the SC layer to increase the permeability of drugs. These include physical enhancers (ultrasound, iontophoresis, electroporation, magnetophoresis, micro needles), vesicles and particulate systems (liposome, niosome, transfersome, microemulsion, solid lipid nanoparticle) [13]. Another approach to increase the permeation of drugs is the use of penetration enhancers, i.e., chemicals that interact with intercellular lipids, keratin and/or improve the dissolving capacity of the SC layer [14,15].

A wide selection of permeation enhancers has been investigated to enhance topical drug delivery, and, as yet, none of them have proven to be ideal. These include surfactants, fatty acids, bile salts, chelating agents, alcohols, fatty alcohols and glycols, fatty acid and esters, sulphoxides, azones and pyrrolidones, essential oils, terpenes and terpenoids [16,17]. Dodecyl-2-N,N-dimethylaminopropionate hydrochloride (DDAIP-HCl) is a novel excipient used in topical formulations to induce permeation through the SC. The proposed mode of action of DDAIP-HCL is to temporarily change the permeation dynamics of the lipid bi-layer, which involves acting on the polar region and loosening the tight intercellular junctions [18].

The aim of this study was to assess whether DDAIP-HCl enhances the permeation of the marketed formulation of minoxidil, Rogaine<sup>\*</sup>. The association between efficacy and cumulative doses of topical minoxidil was investigated by Choi et al. [19], and a significant trend was observed with higher cumulative doses of minoxidil exhibiting a better clinical response in a study containing 24 subjects. Higher doses of minoxidil due to enhanced permeation into the skin layers with DDAIP-HCl could lead to an improved efficacy in the treatment of male pattern hair loss or AGA.

# **Materials and Methods**

All reagents used in this study were analytical reagent grade or better. Rogaine was commercially available (Lot OATUM, Batch 831449000) and contains 5% minoxidil, alcohol, propylene glycol and purified water. The DDAIP-HCl used was obtained from NexMed Lot R1050.

Two minoxodil formulations were developed with the novel excipient, DDAIP-HCl (0.5% and 0.05%) and evaluated for skin penetration against the OTC product, both consisting of a final minoxodil concentration of 5%. Human cadaver skin was obtained from the University of California, San Diego Skin Bank and stored at -80°C. The skin was thawed by immersing in 0.9% sodium chloride for 30 minutes prior to the experiment.

*In vitro* skin flux was conducted by mounting the human cadaver skin sections (US Tissue and Cells-Lot 11-8073233) onto Franz diffusion

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cells (Permegear, Model # VC9). Each experiment was comprised of 6 samples derived from a single cadaver skin donor. The receptor compartment contained a solution of phosphate-buffered isotonic saline (PBS), pH 7.4  $\pm$  0.1 with 0.1% gentamicin sulfate and 0.05% thiourea, maintained at 32.0  $\pm$  0.5°C. The test formulations (100 µL) were applied directly onto the cadaver epidermis (0.64 cm<sup>2</sup>). Samples were collected at specified times from the receptor compartment and the skin was homogenized for determination of minoxidil levels. The skin and receptor samples were analyzed for minoxidil concentrations using liquid chromatography tandem mass spectrometry (LC-MS/MS).

### Analytical method

The quantification of minoxidil was conducted using LC-MS/ MS by High Standard Products, Inc. The Shimadzu 20AD Integrated System was used with a Phenomenex Luna C18 (2) Column ( $50 \times 2$  mm). For the mass spectrometry, the Applied Biosystems API 5000 was used with a TurboIonspray (electrospray) source and samples were prepared using acetonitrile precipitation.

#### Data evaluation

If the result for any sample was less than the limit of quantitation (LLQ), then that sample was treated as a non-data value. Values <LLQ were assigned a value of zero for the purpose of calculating key parameters. A suspected outlier result was confirmed if it was outside the range of the mean  $\pm$  2SD of the replicate values. The amount of permeated drug, Q, was calculated from the following equation:

 $Q = C_t V + \Sigma_{0x} 0.2 (C_{t-x})$ 

Where C<sub>t</sub> is the concentration (µg/mL) of the sample at timepoint t and V is the volume of the cell (5.2 mL) and t-x represent all previous sampling timepoints.  $\Sigma_{0x}$  0.2 (C<sub>t-x</sub>) is the correction for all of the sampling amounts at previous timepoints. The flux is calculated by dividing Q<sub>t</sub> by the area of the Franz Cell, which is 0.64 cm<sup>2</sup> at each timepoint. Skin content was reported as amount minoxidil/g tissue. Statistics were calculated using the ANOVA test method to compare variances.

#### Results

The skin permeation rates of minoxidil over 24 hours, showed that the 5% minoxidil formulation containing 0.05% DDAIP-HCl enhanced minoxidil permeation by 5.7-fold compared to the formulation without DDAIP-HCL, while this permeation was enhanced by 116-fold with the formulation containing 0.5% DDAIP-HCl (Figure 1). Consequently, the concentration of minoxidil present in the skin tissue was increased by 4.2-fold with the 5% minoxidil formulation containing 0.05% DDAIP-HCl, while this concentration increased by 7-fold with the formulation containing 0.5% DDAIP-HCl (Figure 2). Differences in skin concentration between the 5% minoxidil formulation without DDAIP-HCl and the 5% minoxidil formulations containing 0.05% and 0.5% DDAIP-HCL were evaluated using the ANOVA test method and the p values were found to be 0.0002 and <0.0001 respectively, demonstrating strong statistical significance at 95% confidence intervals. A significant difference was found between the 0.05 and 0.5% DDAIP-HCl formulations in the buffer data (p<0.0001), although no significant difference was found between the two formulations in the skin tissue. The optimal DDAIP-HCl concentration to enhance minoxidil permeation would therefore lie within the 0.05-0.5% range and further studies are suggested to investigate this.

# Discussion

Minoxidil, the pyrimidine derivative (2, 4-diamino-6piperidinopyrimidine-3-oxide), is the only Food and Drug Administration-approved topical treatment with proven efficacy for the treatment of male pattern hair loss (MPHL) or Androgenetic alopecia (AGA). The efficacy of the marketed topical 5% minoxidil for men has been confirmed [20-28], but due to the poor solubility of minoxidil in water it has been formulated for topical use in an ethanolbased solution containing ethanol, propylene glycol and water [29]. The formulation displays poor permeation levels into the skin layers,

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possibly due to its propensity to crystallize into an insoluble form [1], as well as displaying other drawbacks such as spreading beyond the intended application site, the increased drying time needed after its application and the potential irritant side effects associated with the propylene glycol [30].

The increased minoxidil levels observed in the cadaver skin using the minoxidil/DDAIP formulation strongly supports the evidence for penetration enhancing properties of DDAIP.

The novel permeation enhancer, DDAIP-HCl (Figure 3), changes the permeation dynamics of the phospholipid bi-layer, acting on the polar region and temporarily loosening the tight intercellular junctions [18,31,32]. DDAIP-HCl up to 2.5% was included in Vitaros<sup>®</sup> formulation which has been approved for the treatment of erectile dysfunction in Canada.

Nonclinical safety studies of DDAIP-HCl were conducted in line with FDA and ICH guidelines [33,34]. Results from these studies support the safety of topical DDAIP-HCl administration at levels of  $\geq 25$  mg/kg/day. DDAIP-HCl is not mutagenic or clastogenic, and its topical administration in mice does not induce local or systemic toxicity at dose levels of 100 or 200 mg/kg/day, respectively. Mice studies have also shown that levels of up to 200 mg/kg/day did not induce tumors in a two-year carcinogenicity assessment.

Clinical safety evaluation of topical DDAIP-HCl revealed no sensitization or phototoxicity potential. During clinical trials in men and women, more than 5,000 patients were exposed to varying concentrations of DDAIP-HCl ranging from 0.05% up to 2.5%. The clinical safety results indicate that topically applied DDAIP-HCl was considered safe and well tolerated.

Two novel minoxidil formulations containing 0.05% and 0.5% DDAIP-HCl were investigated in this study. *In vitro* skin permeation of minoxidil through human cadaver skin was shown to be significantly increased with the minoxidil formulations containing DDAIP-HCl.

Enhanced penetration may lead to an improved efficacy in the treatment of male pattern hair loss or AGA, as well as minimize typical side effects associated with topical minoxidil formulations such as Rogaine<sup>®</sup>, which include application site burn, rash, pruritus, erythema and dryness [35]. Although further formulation optimization is necessary, these results suggest a valuable role for DDAIP-HCl in improving the therapeutic efficiency of minoxidil in the use of hair growth treatment.

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