Iontophoresis-Targeted, Follicular Delivery of Minoxidil Sulfate for the Treatment of Alopecia

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ABSTRACT: Although minoxidil (MX) is a drug known to stimulate hair growth, the treatment of androgenic alopecia could be improved by delivery strategies that would favor drug accumulation into the hair follicles. This work investigated *in vitro* the potential of iontophoresis to achieve this objective using MX sulfate (MXS), a more water-soluble derivative of MX. Passive delivery of MXS was first determined from an ethanol–water solution and from a thermosensitive gel. The latter formulation resulted in greater accumulation of MXS in the stratum corneum (skin's outermost layer) and hair follicles and an overall decrease in absorption through the skin. Anodal iontophoresis of MXS from the same gel formulation was then investigated at pH 3.5 and pH 5.5. Compared with passive delivery, iontophoresis increased the amount of drug reaching the follicular infundibula from 120 to 600 ng per follicle. In addition, drug recovery from follicular casts was threefold higher following iontophoresis at pH 5.5 compared with that at pH 3.5. Preliminary *in vivo* experiments in rats confirmed that iontophoretic delivery of MXS facilitated drug accumulation in hair follicles. Overall, therefore, iontophoresis successfully and significantly enhanced follicular delivery of MX suggesting a useful opportunity for the improved treatment of alopecia. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1488–1494, 2013

Keywords: iontophoresis; skin; controlled delivery; targeted drug delivery; drug transport; hair follicles; minoxidil sulfate; alopecia

INTRODUCTION

Androgenic alopecia, or male baldness, is a condition that affects a significant number of both men and women worldwide. The most frequent topical treatment is based on the drug minoxidil [MX; molecular weight (MW) = 209.2 Da] (Fig. 1a),[1,2] a potent antihypertensive agent, which promotes hair growth by various mechanisms including the induction of vasodilation and increasing blood flow in the dermal papillae,[1,3] as well as a direct effect via potassium channels (K_{ATP}) receptors in the follicle,[4] stimulating hair growth via a vellus-to-terminal hair follicle transformation and also via anagen induction and prolongation.[5] MX sulfate (MXS; MW = 289.3 Da) (Fig. 1b), an endogenous metabolite of MX, is a more potent vasodilator,[5] with greater aqueous solubility, and has been investigated as an alternative active moiety in formulations for the topical treatment of baldness.[6]

Current, commercially available formulations of MX are simple ethanol-based solutions of the drug[7] that require at least a twice-daily application to ensure pharmacological effect[8] and provide no specific "targeting" to hair follicles. The efficiency of these products in the treatment of alopecia, therefore, is at best modest.

The contribution of hair follicles to percutaneous drug delivery has been recently reviewed,[9–11] and attention has been drawn to the fact that follicular drug depots may be valuable assets for localized therapy, particularly the treatment of disorders such as acne,[10,11] some types of skin cancer (e.g., Bowen's

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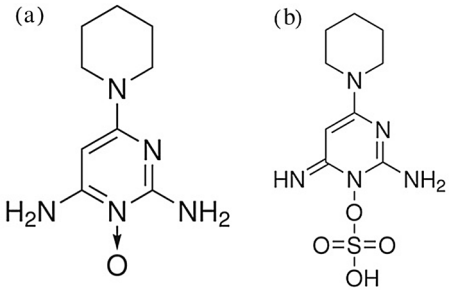

Figure 1. Chemical structure of (a) MX (MW = 209.2 Da; pK_a = 4.3), and (b) MXS (2,4-diamine-6-piperidine-pyrimidine-3-oxide) (MW = 289.3 Da; pK_a = 4.6).

disease),[12] and alopecia.[6,13,14] These perceived benefits, of course, can only be realized through the identification and optimization of drug delivery techniques that promote follicular transport.

Improved follicular delivery has been reported following drug formulation in, for example, liposomes, microspheres, and nanoparticles,[9–11] and specific enhancement of MX delivery incorporated into nanoparticles[14] and cosolvent systems[13] has been observed. In this paper, the use of iontophoresis, a technique that employs a small electric current (not higher than 0.5 mA/cm^2) to increase and control drug delivery through the skin,[15] is explored. The rationale for this approach is that the important contribution of follicular pathways to electrically enhanced topical/transdermal delivery has been clearly demonstrated, and attributed to the lower resistance of these routes across the skin [as compared with the inter- and intracellular pathways through the stratum corneum (SC)] under the influence of a potential gradient.[16–19] The working hypothesis, therefore, is that iontophoresis can "target" MXS to the follicular route and provide an approach to the improved treatment of alopecia.

The specific aim of this research, therefore, was to determine *in vitro* the extent to which iontophoresis is able to facilitate the follicular accumulation of MXS. As a control and benchmark, the passive delivery of MXS was first determined from an ethanol–water solution and from a thermosensitive gel of appropriate viscosity for a typical topical formulation.[20] MXS iontophoresis from the same gel was then undertaken at different pH conditions to assess the added benefit possible from the application of current. A preliminary study in rats was also carried out to investigate whether a short period of iontophoresis could improve the follicular penetration of MXS *in vivo*.

MATERIALS AND METHODS

Materials

Minoxidil sulfate (99%) was kindly provided by Galena Química e Farmacêutica Ltda. (Campinas, Brazil). Ag-wire (99.99%, ø = 1.5 mm), AgCl (99.99%) and Pt-wire, all used to prepare the electrodes, were purchased from Sigma–Aldrich (Steinheim, Germany). Poloxamer®407 (polyethylene–polypropylene–polyethylene triblock copolymer) was purchased from BASF (Lutol F127, Germany); HEPES and NaCl, used in buffer preparation, were obtained from Acros (Fair Lawn, New Jersey). Ethanol was purchased from Fisher Scientific (Leicestershire, UK). The solvents used for extraction and chromatographic analysis were all of HPLC grade: methanol and acetonitrile were purchased from Fisher Scientific (Leicestershire, UK), and acetic acid from Fluka (Steinheim, Germany). The water used in all preparations was of Milli Q grade (Millipore, France).

Skin

Porcine ear skin was used in all *in vitro* experiments. The ears were obtained less than 2 h post-sacrifice of the animal from a local abattoir. The whole skin was removed from the outer region of the ear, separated from its underlying layer, dermatomed to a nominal thickness of 500 μm, and stored frozen at −20°C for a maximum of 1 month before use.

MXS Physicochemical Characterization

Minoxidil sulfate solubility in pH 7.4 HEPES buffer containing 133 mM NaCl (i.e., the receptor solution used in the skin permeation experiments; see *Passive Permeation* section), was determined at room temperature. An excess of MXS (~5 g) was added to 10 mL of buffer and overnight continuously stirred for at least 12 h. The suspension was then filtered and analyzed for drug as described below.

The possible effect of electrical current on MXS stability was evaluated before the iontophoretic studies. A 0.4 mA direct current was passed through 10 mL of a 2% (w/w) MXS aqueous solution containing 89.5 mM of NaCl via Ag and AgCl anode and cathode, respectively, for 6 h. Samples were collected before and after current passage, and the MXS concentration and pH were measured.

The pK_a of MXS was determined via titration of 20 mL of 0.01 M MXS aqueous solutions with (i) 0.1 N NaOH, and (ii) 0.1 N HCl. The pH was measured (Digimed pH meter, model DM-20) after each 0.1 mL addition of the alkali or acid solutions. The pK_a of MXS was deduced from the inflection points of plots of measured pH versus the added volume of acid or base.

Preparation of MXS Formulations

MXS was incorporated at 2% (w/w) (69.1 mM) in (i) an ethanol–water solution (60:40), and (ii) a Poloxamer® gel. In both cases, the aqueous component contained 89.5 mM NaCl. The gel was prepared by cold dispersion of 16% (w/w) polymer in a mixture of