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Formulation and evaluation of selected transmucosal dosage forms containing a double fixed-dose of acyclovir and ketoconazole

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ABSTRACT

Viral and fungal dermatological manifestations are often the first and only sign of HIV/AIDS. They are cosmetically disfiguring and threaten the quality of life, but can be treated by acyclovir and ketoconazole, correspondingly. This study attempted the formulation of stable, double fixed-dose acyclovir and ketoconazole novel transmucosal dosage forms, which are able to provide an efficient flux for both compounds. A cream, gel and lip balm formulation containing both drugs were formulated that can be applied to mucosal tissue. Compatibility experiments between acyclovir and ketoconazole were conducted. A six month stability study was performed on the formulations which included visual appearance; mass variation; assay of the drugs; pH; viscosity; zeta potential; and particle size distribution. Flow-through diffusion tests, utilizing vaginal porcine mucosal specimens, were conducted to determine *in vitro* permeation. No physicochemical interactions exist between the actives. Stability testing revealed that the formulations could be considered stable and may easily be stored at 25 °C/60% RH. The following rank order could be established for the average cumulative acyclovir amount that permeated the mucosa: Acitop® ≥ gel > cream > lip balm; and for the average cumulative ketoconazole amount: lip balm > > > Ketazol® > gel > cream. Both drugs depicted release rates that obeyed the Higuchi model, affirming release from a matrix system.

Stable transmucosal dosage forms containing a double fixed-dose of acyclovir and ketoconazole; and targeting mucosal tissue could thus be prepared. These formulations were able to provide an efficient flux for both compounds.

1. Introduction

Since the first reported cases of acquired immunodeficiency syndrome (AIDS) more than 35 years ago, we are still unable to fully grasp the complexity of this disease. Infection caused by the human immunodeficiency virus (HIV) may lead to numerous clinical manifestations on the skin (Ramdial, 2010; Cedeno-Laurent et al., 2011; Altman et al., 2015). Dermatological manifestations are often the first and only sign of HIV/AIDS; and in developing countries skin lesions can be used as a marker in HIV/AIDS progression (Vusadevan et al., 2012; Oninla, 2014; Schwartz, 2016) as they are almost inevitable and occur in approximately 90% of the patients (Dlova and Mosam, 2004; Cedeno-Laurent et al., 2011; Halder et al., 2012; Oninla, 2014; Schwartz, 2016).

The dermatological lesions seen in HIV/AIDS patients are caused by decreased mucocutaneous immunity (Uthayakumar et al., 1997; Han et al., 2013). Severity of dermatological conditions increases as the cluster of differentiation 4 (CD4) lymphocyte cell count decreases. The CD4 cell count is important in HIV/AIDS as it serves as an indication to assess progression of the disease as well as the time to initiate treatment (Rigopoulos et al., 2004; Sharma et al., 2004; Akinbami et al., 2012). In 2013 the WHO guidelines changed HIV therapy initiation to 500 cells/μl. However, pathological skin conditions often manifest long before such a low CD4 cell count (Olubajo et al., 2014), including fungal, viral and bacterial infections. Though most of these cutaneous manifestations are not life threatening, they are cosmetically disfiguring, cause emotional distress and threaten the quality of life of HIV infected patients as the skin is the most visible organ (Ramdial, 2000; Ramdial, 2010; Moskowitz et al., 2013).

The most commonly found dermatological manifestations that present in HIV/AIDS patients are fungal infections, for example candidiasis, dermatophytosis, cryptococcosis, histoplasmosis and sporotrichosis. The defective immune system of HIV/AIDS patients provides an ideal environment for fungi to invade skin tissue and cause damage (Durden and Elewski, 1997; Dlova and Mosam, 2004; Han et al., 2013; Altman et al., 2015). As a result opportunistic viral infections also increase among these patients, for example the *herpes simplex* virus (HSV)

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has a high prevalence in low immunity patients. However, the *molluscum contagiosum* virus and human papillomavirus infections also become progressively more prevalent in these patients (Dlova and Mosam, 2005; Schwartz, 2016). In third-world countries the prevalence of opportunistic fungal infections has moreover significantly increased; and these infections are one of the foremost causes of morbidity and mortality in HIV-AIDS patients (Duffalo, 2006; Kaur et al., 2016).

Azole antifungal agents dominate antifungal drug development and use (Bennett, 2011; Kim, 2010). Ketoconazole, a broad spectrum synthetic imidazole derivative (Skiba et al., 2000; Bethesda, 2010) displays antifungal activity against *Candida* species; *Blastomyces dermatitidis*; *Coccidioides immitis*; *Cryptococcus neoformans*; *Histoplasma capsulatum*; *Paracoccidioides brasiliensis*; and to a lesser extent against *Sporothrix schenckii* (Bennett, 2011). It is also the treatment of choice for non-life-threatening mycoses (Maertens, 2004; Kim, 2010). The HSV (oral and genital *herpes*) syndrome, on the other hand, is used as a Centers for Disease Control (CDC)-defined index in establishing an AIDS diagnosis (Gide et al., 2013; CDC, 2015; Schwartz, 2016). Acyclovir has become the standard therapy for HSV and *varicella zoster* virus infections; and it is the treatment of choice in *herpes simplex encephalitis*, however, acyclovir demonstrates minimal activity against the cytomegalovirus (King, 1988; Acosta and Flexner, 2011; Porter and Kaplan, 2011; van der Plas and Hardie, 2011; Gide et al., 2013; Krishnaiah et al., 2014; Gandhi et al., 2014; Safrin, 2015).

The concurrent use of two or more active pharmaceutical ingredients (APIs) can expressively modify the therapeutic and side-effect profile of the individual APIs (Bickers, 1994). Fixed-dose combinations are capable of offering various benefits. First, they eradicate questions regarding application time of more than one separate topical product in addition to stability and chemical compatibility concerns of these formulations. Secondly, enhanced efficacy may be achieved if the APIs possess synergistic and complementary pharmacological properties. The use of fixed-dose combinations may furthermore be more convenient and simplify the treatment regimen; thereby possibly refining treatment adherence and consequences (Thiboutot et al., 2007; Gollnick et al., 2009; CS, 2013). In 1986 Pottage et al. (1986) investigated the *in vitro* activity of ketoconazole in combination with acyclovir on the replication of HSV-1 and 2 utilizing a yield reduction assay. They found a synergistic antiviral effect against these viruses when ketoconazole and acyclovir were used in combination. Jacobs et al. (2016) attempted topical delivery of acyclovir and ketoconazole by means of the Pheroid™ delivery system when incorporated into semi-solid formulations. Although the Pheroid™ technology enhanced dermal and transdermal delivery of these APIs using human skin as model, these formulations were considered instable. An essential consideration for topical pharmaceutical and cosmetic products is the stability of the product. The physical stability of these products is characterized by the absence of coalescence or creaming, and the maintenance of appearance, odor, color and other physical properties. The objective of stability testing is to ensure that a given product has an adequate shelf-life in its container, before it can be marketed and sold (Sheraz, 2009; Sheraz et al., 2011; Aulton, 2013; Williams, 2013).

Oral and transdermal administration of compounds can be challenging due to difficulty in keeping the medicament at the desired location for effective absorption, distribution and metabolism; and permeation through these routes may also be problematic. Due to excellent permeability and rich blood supply, interest in using the mucosal lining of cavities for local or systemic compound delivery has glimmered. The cavities include nasal, rectal, vaginal, ocular and oral cavities (Hussain and Ahsan, 2004; Madhav et al., 2009; Patel et al., 2011; Shakya et al., 2011; Katz et al., 2015). Mucosa is described as non-keratinized epithelium, *i.e.* the stratum corneum, of which the principle barrier of the skin is removed; and the mucosa consists of a less structured lipid barrier which creates a lower resistance to molecular diffusion compared to normal skin, rendering mucosa five times more permeable to water than normal skin tissue (Kobayashi and Tagami, 2004). Some parts of the vulva for example, even show thinner epithelial tissues which results in faster diffusion of a component due to a shorter path length (Elsner, 2011; Farage and Scheffler, 2011).

Transmucosal drug delivery on the lips and vaginal mucosa may provide attractive advantages and pharmaceutical research and formulators have shown increased interest (Madhav and Yadav, 2014; Katz et al., 2015). Drug delivery by means of the vaginal area has been applied for numerous years and for various reasons. Currently ample activity is focused on development of topical products that deliver drugs in the vaginal environment in order to inhibit infection by several pathogens, including HSV. The vaginal wall or rugae is well suited for the absorption of drugs as it contains many folds that increase surface area and also comprises a vast network of blood vessels. This area additionally consists of some noteworthy features with regards to vaginal secretion, pH (45), enzyme activity (however, the human genital tract has very limited enzyme activity) and micro-flora. Formulation of a relevant dosage form; as well as release, distribution, retention and absorption of drugs at the site of application may, however, be affected by these features (Vermani and Garg, 2000; Hussain and Ahsan, 2004; Ranade and Hollinger, 2004; Lopez et al., 2005; Elsner, 2011; Sahoo et al., 2013; Katz et al., 2015). Labial mucosa (*i.e.* lips), on the other hand, form part of the oral mucosa and comprise epidermis, subcutaneous tissue, orbicularis oris muscle fibers, and mucosa. The vermilion border consists of non-keratinized squamous epithelium covering many capillaries, which provide the vermilion with its characteristic red color. Here the stratum corneum is significantly thinner or absent compared to all other sites of the human body (Blistex, 2009; Madhav and Yadav, 2011; Madhav and Ojha, 2012; Madhav and Yadav, 2014). Both these routes offer definite advantages, for example, gut and hepatic first-pass metabolism are circumvented, gastrointestinal and hepatic side effects are reduced, and local drug targeting may be achieved (Elsner, 2011; Madhav and Ojha, 2012; Madhav and Yadav, 2014; Katz et al., 2015).

This study attempted the formulation of selected novel topical dosage forms containing both acyclovir (5% w/w) and ketoconazole (2% w/w) targeting mucosal tissue; and which are stable products able to provide an efficient flux for both compounds. Not only has it been stated that these compounds have a synergistic antiviral activity (Pottage et al., 1986; Bickers, 1994), but they are furthermore able to produce a single, stable product that is capable of permeating mucosal tissue. These formulated products will have the potential to treat various uncomplicated topical lesions as a result of several fungal and viral infections, as discussed; and patient compliance may be enhanced (Barry, 2007; Bethesda, 2010; Sweetman, 2011; Langer and Maibach, 2012; Williams, 2013; Sheppard and Lampiris, 2015). No commercial product containing both acyclovir and ketoconazole is available yet. We have therefore attempted to formulate selected fixed-dose combination products containing these APIs and furthermore compared them to Acitop® and Ketazol®, which are two commercial products available on the South African market, containing 5% w/w acyclovir and 2% w/w ketoconazole, respectively.

2. Materials and methods

2.1. Chemicals and reagents

Acyclovir (225.2 Da) and ketoconazole (MW = 531.4 Da) were obtained from DB Fine chemicals (Pty) Ltd. (Rivonia, Johannesburg, South Africa). Acitop® (5% w/w acyclovir), Lovire® (5% w/w acyclovir) and Ketazol® (2% w/w ketaconazole) were purchased from a local pharmacy. Potassium dihydrogen orthophosphate (Merck, Wadeville, South Africa) and sodium hydroxide (Merck, Wadeville, South Africa) were utilized in the preparation of phosphate. Octane-1-sulfonic acid sodium salt (Merck, Darmstad, Germany) and methanol AR (Merck, Darmstad, Germany) were used in the mobile phase during high-performance liquid chromatography (HPLC) analysis.