

## Topical Delivery of Lipid Based Amphotericin B Gel in the Treatment of Fungal Infection: A Clinical Efficacy, Safety and Tolerability Study in Patients

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### Abstract

**Objectives:** A novel topical formulation of lipid based amphotericin B (0.1% amphotericin B Gel) was developed to assess the safety, tolerability and efficacy in adult patients with cutaneous and/or mucocutaneous fungal infection.

**Methods:** Amphotericin B gel was formulated using lipids. *In vitro* release assay of amphotericin B was measured using paddle apparatus maintained at  $37.0 \pm 0.5^\circ\text{C}$ . The stability studies of amphotericin B gel were carried out at  $2-8^\circ\text{C}$ ,  $25^\circ\text{C}$  and  $40^\circ\text{C}$ . To test the effect of the drug in clinical setting, 100 patients with recurrent case after failure to standard therapy for cutaneous and/or mucocutaneous fungal infection were treated with amphotericin B Gel. The amphotericin B Gel was applied on the affected area twice daily for 14 days in patients with cutaneous fungal infection and for 7 days in patients of mucocutaneous fungal infection. Response to the amphotericin B Gel treatment was monitored in the patients for up to 14 days and 28 days for mucocutaneous and cutaneous infection respectively.

**Results:** Based on the stability studies, the recommended shelf life of amphotericin B gel is 24 months at  $25^\circ\text{C}$ . *In vitro* studies showed the release of ~90% amphotericin B within two hours and complete release within four hours. A total of 83 patients were assessed for cutaneous fungal infection where 39 patients were cured, 9 patients showed marked improvement, 26 patients showed moderate improvement and 9 patients showed failure after the treatment. For mucocutaneous fungal infection, 100% patients were cured at the end of the treatment. No serious adverse events were reported in patients during the study.

**Conclusion:** Lipid based amphotericin B gel in patients with cutaneous and mucocutaneous fungal infections was found to be safe, tolerable and efficacious.

**Keywords:** Amphotericin B Gel; Topical; Efficacy; Fungal infection

### Introduction

Among the fungal infections, the cutaneous is the most prominent type occurring in human where fungi colonize on dead tissue of the stratum corneum is called dermatophyte. These types of fungi do not produce deep cutaneous or systemic infections. Dermatophyte usually results in an inflammatory host response to the overlying infection and does not lead to actual invasion [1]. Other infections cause gross changes to the surrounding and underlying tissue suggestive of deeper infection.

Several treatment options are available for cutaneous fungal infection [2]. A number of drugs such as miconazole, clotrimazole,

and ketoconazole have been used for the treatment of fungal and yeast skin infections. Many antifungal agents are compounded in different types of excipients/vehicles and have been found to be effective. Most commonly, topical drugs are applied to the surface of the skin in the form of cream, lotion, or spray that can easily penetrate into the skin and prevent them from spreading of infection to the tissues. There are two important factors that drive the use of these drugs. First, patients look for cure in shortest possible time as the infection can appear on any expose part of the skin that could cause irritation and discomfort in the normal routine. Second, patients tend to look for cost effective ways due the availability of several options.

Amphotericin B has long been a gold standard for treatment of patients with invasive fungal infections [3]. Amphotericin B has higher affinity for ergosterol than for cholesterol which results in its binding

predominantly to fungal, leishmania or naegleria cells as they contain ergosterol or resembling compounds. The resulting ergosterol-amphotericin B complex increases membrane permeability of fungal/pathogen cells leading to cell lysis [4]. However, amphotericin B maximal utilization in clinical practice is restricted due to its severe toxicity to the kidney and Red Blood Cells that was found to be overcome by using lipid based delivery system for parenteral use [5]. In addition, topical application is limited due to its low absorption through mucosa or skin. Amphotericin B molecule is highly lipophilic in nature and cannot be dissolved in aqueous medium. Several compounded topical formulations of amphotericin B in the form of cream, lotion, gel and ointment are available but are not approved by regulatory agencies of most countries. Application of these topical formulations of amphotericin B have shown very limited efficacy even at high doses of drug and results in several adverse reactions such as severe blistering, itching, redness, peeling, dryness or irritation of the skin and failed to achieve cure against fungal infections [6].

To circumvent the drawback associated with conventional topical delivery of drugs, lipid-based carrier of therapeutics products were used as delivery system. The small size of lipid nanoparticles ensures a close contact to the stratum corneum and can increase the amount of drug penetrated into the skin [7]. Furthermore, lipid nanoparticles are able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. Advantages of using lipid as carrier systems for skin administration are also related to their physiological nature, which reduces the risk of toxicological problems and local irritancy [8]. Considering the benefits of using lipids as carrier for topical drug delivery, we have developed a lipid based Gel formulation of amphotericin B using natural lipids to reduce adverse reactions, make it compatible to the skin, and minimize allergic reactions.

The present study demonstrates that lipid based Amphotericin B gel is highly effective and safe to treat cutaneous and mucocutaneous fungal infections.

## Materials and Methods

### Material

Amphotericin B was obtained from Alpharma (Denmark). Soy phosphatidylcholine was procured from Lipoid LLC (Newark, NJ) and Sodium cholesteryl Sulfate was obtained from Genzyme Pharmaceuticals (Cambridge, MA). Other inactive ingredients were procured locally.

### Preparation of Lipid based amphotericin B Gel

First, nanosomal amphotericin B, a lipid suspension formulation was prepared by our method described elsewhere [3]. Lipid based amphotericin B gel (0.1%) was prepared as follows: Methyl paraben (18.0 g) and propyl paraben (2.2 g) were dissolved in hot purified water (6.6 L) and allowed its cooling to 25°C. Carbopol Ultrez 10 (100 g) was added slowly and the stirring continued for complete swelling of Carbopol or until the mixture is free from lumps. To this stirring mixture, amphotericin lipid suspension and fragrance were added before the gel was filled into the tubes. Amphotericin B concentration in lipid based amphotericin B gel was determined by a prepacked C18 column (5 µm particle size, 250 × 4.6 mm i.d.) attached with Agilent 1100/1200 Series HPLC system (Agilent technology, Palo Alto, CA) and a UV detector. The clinical batches Amphotericin B Gel (0.1%) as

described above was manufactured under GMP conditions by Intas Pharmaceutical Ltd., India.

The stability studies of lipid based amphotericin B gel (0.1%) were carried out at 2-8°C, 25°C/60% relative humidity (RH) and 40°C/75% RH. The parameters tested include description, pH, assay, related substances, and the contents of lipid excipients.

**In vitro release study:** The *in vitro* release of amphotericin B from amphotericin B gel was measured using Paddle (Enhancer Cell) apparatus maintained at 37.0°C ± 0.5°C. The dissolution medium was prepared by dissolving 1.56 g sodium dihydrogen orthophosphate dihydrate and 10.0 g of sodium lauryl sulfate (SLS) in 950 mL of water. The pH was adjusted to 6.8 with dilute sodium hydroxide and the resulting solution was diluted to 1 L with water. The samples were prepared by weighing accurately 50 mg of amphotericin B gel (0.1%) in each of six separate enhancer cells and covered with Tuffryn membrane filter. Each enhance cell was transferred into a separate 200 mL dissolution bowl filled with dissolution medium and the apparatus was started immediately. At the end of each specified time point, 2 mL of sample medium was withdrawn and replaced with the same volume with fresh dissolution medium equilibrated at 37.0°C ± 0.5°C. Amphotericin B content in the withdrawn samples at each specified time point were analyzed by HPLC.

### Clinical Evaluation

An open label, single arm, multicenter study was conducted to evaluate the efficacy, safety and tolerability of lipid based amphotericin B Gel (0.1%) in the patients with recurrent cutaneous and/or mucocutaneous fungal infection.

Diagnosis of fungal infection was performed during the screening visit (up to 7 days prior to Day 1) by clinical assessment and/or mycological assessment. The clinical diagnosis was conducted by the Investigator as per the judgment based upon the presenting signs and symptoms of the patient. The amphotericin B Gel was applied on the affected area, twice daily for 14 days to patients with cutaneous fungal infection and for 7 days to patients with mucocutaneous fungal infection. Depending on the response of the treatment, Investigator continued the patients' treatment of the cutaneous and mucocutaneous fungal infection for up to 28 days and 14 days respectively. Safety evaluation for the patients was done at all the visits and efficacy evaluation was conducted at the end of the study visit. Efficacy analysis was carried out on primary and secondary efficacy endpoint. All the safety analysis were performed using SAS<sup>®</sup> Version 9.3 (SAS Institute Inc., USA).

The study was conducted in 100 patients of cutaneous and/or mucocutaneous fungal infection where a total of 86 patients were evaluable.

**Inclusion criteria:** Male or female patients aged ≥18 years with willingness to sign and date informed consent form were enrolled in the study. Patients did not use any other topical antifungal therapy during the study. These patients were clinically or mycologically diagnosed for recurrent cutaneous and/or mucocutaneous fungal infection or HIV infected patients with cutaneous and/ or mucocutaneous fungal infection. Female patients were tested for pregnancy and male patients used effective method to avoid pregnancy.

**Exclusion criteria:** Patients with known case of hypersensitivity to amphotericin B. Pregnant or breastfeeding or planning to become

pregnant during the study period. Diagnosed with disseminated Candidiasis or requires systemic antifungal therapy or history of intolerance (e.g., elevation of liver enzymes) to amphotericin B. Patients received any systemic antifungal therapy within 14 days and topical antifungal therapy within 7 days prior to screening. Patients received any other investigational medicinal therapy within 30 days prior to screening. Diagnosed with any concomitant condition that, in the opinion of the investigator, could interfere with the evaluation of efficacy or safety, or would make it unlikely that the subject would complete the study. Women with vaginal *Trichomonas* infection or clue cells of *Gardnerella vaginalis* vaginitis, Chlamydial infection and venereal diseases or with Vulvo vaginal candidiasis, who are in menstruation period or expecting menses during treatment period were also excluded.

**Discontinuation of treatment:** Patients from therapy or assessment were withdrawn in case of adverse events required permanent discontinuation of study drug. Patient suffered from significant inter-current illness or undergoes surgery during the course of the study. Any patient that entered the study in violation of study protocol including pre-study directions regarding alcohol and drug use, requisite fasting or if the patient was uncooperative during the study or any other justifiable reason.

**Mode of administration:** The patients were instructed to clean and dry the affected area prior to the application. The lipid based amphotericin B Gel was applied gently on the affected area. Hands were washed before and after the application. The patients were instructed not to wear tight clothing, use bandages or dressing at the site of application.

**Treatment compliance:** Diary card was given to the patients with proper instructions on filling and bringing back during subsequent visits. The Principal Investigator or his/her designee ensured the compliance to treatment by checking the diary card at each visit for each patient and ensuring that they used at least 75% and not more than 125% of study drug during the study. Based on the quantity of the drug, patient's compliance was evaluated. Drug accountability (Receipt, dispensing and return of drug) was performed by the investigator and/or designee.

### Efficacy and safety measurements

**Efficacy:** Based on the evaluation of clinical efficacy, the primary end point was evaluated for different indications. Assessment of the primary end point was totally based on investigator's judgment. Clinical score was assessed at all visits, weekly once ( $\pm 2$  days) till the end of the study. Patients of cutaneous fungal infection were assessed weekly once ( $\pm 2$  days) from visit 3 (Day 7) until end of the study visit (Day 21 or Day 28) and patients of mucocutaneous fungal infection were assessed weekly once ( $\pm 2$  days) from visit 3 (day 7) until at the end of the study visit (Day 14).

**Safety:** Vital signs and body measurements - Body height, body weight and oral body temperature were obtained at regular intervals during the study. Systolic and diastolic blood pressure (BP), pulse rate and respiratory rate were assessed after the patient had rested in the supine/sitting position for at least 3 minutes. Blood pressure was preferably assessed in the same arm for each time of determination. Physical examination included general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities and basic nervous system evaluation. Significant findings that were present prior to the signing of Informed

Consent Form were included in the relevant medical history/Current medical conditions section of the CRF. Significant findings made after the start of study drug which met the definition of an Adverse Effect were recorded. The Investigator evaluated the clinical significance of each laboratory value outside of the reference range. This decision was based upon the nature and degree of the observed abnormality. The Investigator could choose to repeat any abnormal result ONCE, in order to rule out laboratory error. "NCS" was entered on the original laboratory sheet for those values which were outside the reference range, but were judged as "not clinically significant". In addition all standard clinical laboratory evaluations were also conducted in patients.

**Adverse events (AEs):** Any untoward medical occurrence in a patient administered with amphotericin B Gel was monitored. The Adverse Event was graded as per intensity of the Event, such as, Mild AE, Moderate AE and Severe AE.

### Analysis Sets

The analysis sets were defined as follows:

a. Intention-to-Treat (ITT) set. The intent-to-treat (ITT) population was defined as all patients who received at least one application of study medication.

b. Safety analysis set. The Safety population was defined as all the patients included in ITT population.

The clinical study was carried out in compliance with the protocol and in adherence to good clinical practices, as described in 1) Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects-Tokyo 2004). 2) ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996 (E6, Step 5). 3) ICMR Ethical Guidelines for Biomedical Research on Human Subjects (Year 2000).

### Results and Discussion

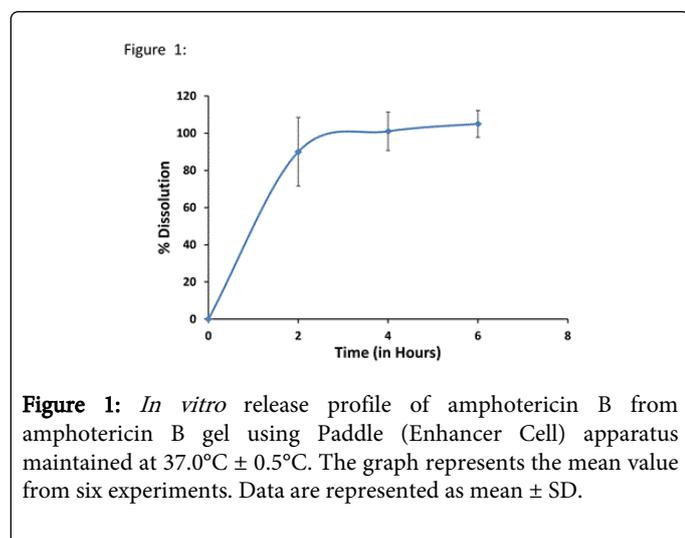
Fungal infections are very common and upon exposure they are also contagious. These infections are estimated to affect more than 20% of the world's population [1]. It occurs frequently in population where common living facilities are used. Even though such infections are not life threatening but chronic infections including those resistant to treatment could cause serious discomfort. These infections are often difficult to treat and persist even after the treatment. If the infection is not 100% cured the genetic predisposition results in a high rate of recurrence [9,10]. Typically topical fungal infections invade outer layers of the skin, the nails and hair. Other infections can affect organ systems in humans but the fungi may only cause dermatological conditions which does not involve tissue invasion. The main groups of fungi causing superficial fungal infections are dermatophytes, yeasts and molds [11].

The responses to superficial fungal infections by using topical agents alone have been found to be relatively poor. For refractory or severe cutaneous fungal infections, uses of systemic antifungal agents are the standard of care. Griseofulvin, terbinafine, and azoles such as fluconazole, itraconazole, and ketoconazole are the most widely used systemic antifungal therapies for cutaneous fungal infections. The use of these agents can result in drug toxicities for which monitoring of hepatic and renal functions are required [1]. Patients generally look for inexpensive and rapid recovery resolution for cutaneous type of infection. Effective treatment depends on various factors including

duration of the treatment, appropriate dosage and frequency of application. Fungicidal agent are a good treatment option as they are of short duration, with high cure rates, minimum relapses and better patient compliance with less adverse effects [12]. Amphotericin B has been demonstrated to be a good fungicidal agent where its fungicidal activity was found to be close to minimum inhibitory concentration resulting in inhibiting morphogenetic transformation of the fungi [13].

Lipid based amphotericin B Gel was developed to provide a better option to the patients with cutaneous and/or mucocutaneous fungal infection. The lipid based amphotericin B Gel is comprised of amphotericin B, soy phosphatidylcholine, sodium cholesteryl sulfate and carbomer homopolymer. The lipid ingredients such as Soy phosphatidylcholine used in the product are known for efficient absorption of the active ingredient across epidermis [14]. Soy phosphatidylcholine is also a naturally occurring lipid and recognized as safe by the United States Food and Drug Administration. The lipid based Amphotericin B gel (0.1%) was found to be stable at accelerated temperature of 40°C up to 6 months and met all the acceptance criteria for the pH, drug, drug related substances, and lipid excipients. Based on the accelerated temperature stability results, the recommended shelf life of amphotericin B gel is 24 months at 25°C.

The *in vitro* dissolution of lipid based amphotericin B Gel was conducted in phosphate buffer pH 6.8 medium containing 1.0% SLS at 37.0°C ± 0.5°C. The results showed the release of about 90% amphotericin B within 2 hours and complete release within 4 hours (Figure 1). This suggests the drug is rapidly available after short time of application that may aid in faster therapeutic effect.



To assess the safety and efficacy of the amphotericin B Gel in clinical setting, an open label study was conducted in 100 patients with cutaneous and/or mucocutaneous fungal infection for 4 weeks. Out of 100 patients, 1 patient discontinued the treatment due to insufficient therapeutic response/treatment failure, 11 patients lost to follow up and 2 patients withdrew their consent. Therefore, a total of 86 patients completed the clinical phase of the study. Amongst them, 83 patients had cutaneous fungal infection and 3 patients suffered from mucocutaneous fungal infection.

There were a total of 12 adverse events (AEs) reported in 7 patients during the conduct of study. The severity of 11 AEs was assessed as

mild while 1 AE related to skin bacterial infection was moderate in nature. The causality assessment of 7 AEs was judged as likely or possibly related to the drug while 5 AEs was judged to be unlikely related to the study drug. The AEs unrelated to drug included skin bacterial, urinary tract infections, haematuria, and pyuria whereas all other AEs were drug related. The most frequently reported AEs for the study medications were burning skin and itching. Twelve post-dose AEs were reported by only 7% of the patients who received at least one dose of the study medication (Table 1). There were no deaths or serious adverse events (SAEs) in the study. The AEs from 0.1% amphotericin B gel is significantly less when compared with other marketed products such as ketoconazole, where 5% of the patients receiving 2% ketoconazole suffered severe irritation, pruritus, and stinging [15].

Adverse Events by system organ class	No. of Events
<b>Investigations</b>	
Hepatic enzyme increased	01
<b>Infections and infestations</b>	
Skin bacterial infection	02
Urinary tract infection	01
<b>Renal and urinary disorders</b>	
Pyuria	01
Haematuria	01
<b>Skin and subcutaneous tissue disorders</b>	
Skin burning sensation	03
Pruritus	03
<b>Total</b>	<b>12</b>

**Table 1:** List of Adverse Events observed after the treatment of patients with Amphotericin B gel.

A summary of the results for Clinical assessment of cutaneous fungal infection is presented in Table 2. If the patients' last visit was prior to the end of the study, then that visit was considered as end of the treatment (EOT). Of the thirteen patients with recurrent fungal infection, 61.5% (8/13) achieved treatment success at the EOT.

Patient's assessment of change from baseline was evaluated at every visit to determine the efficacy. Results of efficacy showed that 89.2% (74/83) of the patients were having favorable outcome at the end of the therapy. Amongst the patients with treatment success, 39/83 of patients with cutaneous fungal infection showing complete cure at the EOT. It was found that 83.1% (69/83) of patients demonstrate improved outcome only after day 7, which would favor patient compliance. Thus, 47.0% of patients were completely cured, 10.8% showed marked improvement and 31.3% patients demonstrated moderate improvement at the EOT (Table 2). There were no patients in un-assessable category. The results from mucocutaneous fungal infection are summarized in Table 3. In Mucocutaneous fungal infection treatment group all patients were found to be cured at the EOT. It was observed that 1 patient showed marked improvement only in 7 days of treatment.

Cutaneous Fungal Infection	Day 7 ( $\pm$ 2 Days)	Day 14 ( $\pm$ 2 Days)	Day 21 ( $\pm$ 2 Days)	End of the Treatment, Day 28 ( $\pm$ 2 Days)
Cure	0	0	1	39
Marked Improvement	9	30	27	9
Moderate Improvement	60	34	28	26
Failure	14	6	7	9
Unassessable	0	0	0	0
Total	83	70	63	83

**Table 2:** Clinical assessment of cutaneous fungal infection after treatment of patients with Amphotericin B gel.

Cutaneous Fungal Infection	Day 7 ( $\pm$ 2 Days)	End of the Treatment, Day 14 ( $\pm$ 2 Days)
Cure	0	3
Marked Improvement	1	0
Moderate Improvement	0	0
Failure	0	0
Unassessable	0	0
<b>Total</b>	<b>1</b>	<b>3</b>

**Table 3:** Clinical assessment of mucutaneous fungal infection after treatment of patients with Amphotericin B gel.

It was reported that treatment with topical 1% Clotrimazole in patients of tinea and cutaneous candidiasis demonstrated 82% (37/45) positive clinical responses [16]. Montero et al. [17] conducted a study to compare efficacy of 1% eberconazole cream and 2% miconazole cream in dermatophytoses patients which demonstrated 76.1% and 75.0% clinical efficacy respectively. The 0.1% amphotericin B gel showed enhanced efficacy [89.2% (74/83)] and may provide better treatment options for patients with cutaneous fungal infections.

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