Topical NanoLiposomal Amphotericin B (SinaAmpholeish™)

A Promising New Treatment for the Cutaneous Leishmaniasis

Exir Nano Sina

SUMMARY

ExirNanoSina Co. (ENS) has updated its Business Plan in 2023. Its mission is to develop new treatments for patients suffering from the cancer and try to increase drug's benefits by applying new methods to improve people life's quality. The company also would like to become a leading provider of Nano-science products at the regional and global levels. Furthermore, the company would like to contribute to the advancement of health industry, and also globalize Iran's nanotechnology industry in the world.

Born in 2011 within the framework of an important academic and technological conglomerate. Today we are a knowledge based company with the support of Iran Nanotechnology Initiative Council. Since the beginning, supported by our previous technical knowledge, research at this company have been mostly focused on formulation based on nano-liposomes and nano-micelles using lipids and surfactants to improve the pharmacokinetic of the drugs, with industrial production vision for parenteral, oral and topical applications. These nano-formulations also increase the efficacy and reduce toxicity of drugs. ExirNanoSina offers various new drug delivery systems such as liposomes and micelles.

ENS has been the only supplier of anti-cancer Pegylated Liposomal Doxorubicin (SinaDoxosome™) drug in Iran for 10 years and so far, it has sold more than 100,000 vials in the Iranian market. This company has been able to export this product to Syria several times. Currently, this drug is being registered in several countries, such as Iraq, Lebanon, Philippines and Pakistan.

The following products is also manufactured in ENS, all of which are produced with Nano-technology:

- Topical Nano-liposomal Amphotericin B (SinaAmpholeish™): Antileishmanial and Antifungal agent especially for the treatment of cutaneous leishmaniasis.
- Nano-micellar Curcumin as soft-gel (SinaCurcumin™): Anti-inflammatory and Antioxidant agent, as supplement it is used for:
- Bone and Joint inflammation (Osteoarthritis)
- Gastro-intestinal inflammation (Crohn's disease, Colitis, Irritated Bowel Syndrome)
- Liver and Cardiovascular Health
- Brain disease(specially Alzheimer)
- Buccal cavity inflammation (Gingivitis, Plague)
- Dermatological conditions (Psoriasis, Eczema)
- Nano-micellar Silymarin as soft-gel (SinaLive™): as supplement it is used for: Liver inflammatory diseases like Fatty liver, Cirrhosis of liver, Acute and chronic hepatitis

Leishmaniasis

Leishmaniasis is a parasitic infection caused by a trypanosomatid protozoan of the genus Leishmania. It is considered a neglected tropical disease (NTD) and is endemic in 90 countries, primarily in developing regions. Approximately 1.5 to 2 million new cases of leishmaniasis occur annually, with the cutaneous form being the most prevalent. The disease manifests in three primary forms: cutaneous, mucocutaneous, and visceral, and 21 species of Leishmania are known to cause human infections. The primary reservoirs of Leishmania parasites are vertebrate hosts, including humans, rodents, canids, and hyraxes. Transmission occurs through the bite of infected female phlebotomine sandflies. Over 90 of the approximately 1,000 sandfly species are known to transmit the disease. Different sandfly species are responsible for transmitting different clinical forms of leishmaniasis in various geographical regions. The Leishmania parasite undergoes multiplication within the insect vector and is subsequently inoculated into a new mammalian host, potentially a human. Once inside the mammalian host, the parasite is engulfed by macrophages; however, it has the ability to survive and even replicate within these cells. The most heavily affected regions include the Mediterranean, India, Bangladesh, Brazil, and Sudan.

Cutaneous leishmaniasis

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, accounting for approximately 70-90% of all cases. CL manifests in various clinical forms, ranging from self-healing sores to chronic, disfiguring lesions. The majority of CL patients experience a limited number of cutaneous lesions that resolve spontaneously within 6 to 18 months, leaving behind characteristic scars. However, CL can have a significant social and economic impact due to the stigmatization associated with visible skin lesions, particularly when they occur on exposed areas such as the face, arms, and legs. Lesions, or ulcers, typically appear on exposed skin regions that are easily bitten by sandflies. In the New World, CL lesions are usually solitary, while in the Old World, they often occur in multiples.

Treatment of CL

Various treatment options have been explored for CL, encompassing physical, immunological, topical, and systemic approaches. Pentavalent antimonials, such as sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime), remain the World Health Organization (WHO) recommended first-line treatment for CL. However, their administration via multiple injections is often painful and poorly tolerated, leading to low patient compliance and reduced treatment efficacy. Additionally, the effectiveness of pentavalent antimonials has shown variability, and resistance to this therapy has been documented.

Given the epidemiological diversity of CL, characterized by variations in causative species, environmental factors, vectors, and reservoirs, vector and reservoir control strategies are often impractical, costly, and require substantial political commitment and infrastructural support that may exceed the capabilities of countries most affected by the disease. Despite significant efforts by the WHO, no vaccine is currently available for any form of leishmaniasis.

The treatment of CL can be a significant financial burden for patients, particularly in developing countries where the disease is most prevalent. The cost of treatment varies depending on the specific drug or therapy used, the duration of treatment, and the patient's location.

Drug Category	*Drug Name*	*Global Price*	*Cost-Effectiveness Review*
Pentavalent antimonials	Sodium stibogluconate	$10-$20 per vial (100 mg/ml, 100 ml)	Most cost-effective for mild to moderate CL
Pentavalent antimonials	Meglumine antimoniate	$10-$20 per vial (100 mg/ml, 100 ml)	Most cost-effective for mild to moderate CL
Topical agents	Paromomycin ointment	$20-$100 per tube	More expensive than pentavalent antimonials, but may be more effective for severe CL
Topical agents	Imiquimod cream	$20-$100 per tube	More expensive than pentavalent antimonials, but may be more effective for severe CL
Systemic agents	Amphotericin B	$100-$1000 per treatment course	Most expensive option, but may be required for severe CL or patients who do not respond to other treatments
Systemic agents	Miltefosine	$100-$1000 per treatment course	Most expensive option, but may be required for severe CL or patients who do not respond to other treatments

Amphotericin B (AmB) is a polyene antibiotic derived from the natural fermentation of Streptomyces nodosus. It has demonstrated remarkable efficacy in treating fungal infections and visceral leishmaniasis. However, its clinical application is hampered by severe toxic adverse effects, including acute infusion-related toxicities and chronic nephrotoxicity. The traditional micellar formulation of AmB, known as Fungizone™, utilizes deoxycholate as a surfactant to solubilize the drug in aqueous solutions. However, this formulation is associated with significant toxicity, limiting its widespread use.

Lipid-Based Formulations of AmB

To mitigate the adverse effects and enhance the tolerability and therapeutic index of AmB, three distinct lipid-based formulations of AmB—Amphotec™, Abelcet™, and AmBisome™—have been developed and commercialized in the United States and Europe. Among these formulations, AmBisome™ (AmB liposome for injection) exhibits significantly lower toxicity compared to the other alternatives. However, one of the primary challenges impeding the widespread adoption of AmBisome™ is its substantial cost.

Furthermore, AmBisome™ and other lipid-based formulations of AmB, as well as Fungizone™, demonstrate minimal efficacy against cutaneous leishmaniasis when administered intravenously due to their inability to achieve sufficient concentrations in the skin. Conversely, these lipid-based formulations of AmB and Fungizone™ exhibit remarkable effectiveness in treating systemic leishmaniasis (Kala-azar).

Topical Nanoliposomal Amphotericin B (SinaAmpholeish™)

Topical administration of drugs generally offers reduced systemic toxicity compared to systemic routes, as lower doses can be employed and minimal drug concentrations reach sensitive internal organs like the kidneys. However, the robust barrier function of the stratum corneum (SC) in the skin impedes the penetration of large molecules like AmB. Additionally, topical drugs for CL treatment must effectively target Leishmania parasites residing within the phagolysosomes of infected macrophages in the deep dermal layer of the skin. Studies have demonstrated the inefficacy of a conventional topical AmB formulation in soft white paraffin containing 12% methylbenzethonium chloride in treating cutaneous L. major lesions in BALB/c mice.

To achieve effective drug penetration through the skin and target Leishmania parasites within the phagolysosomes of infected macrophages in the deep dermal layer using AmB, an efficient drug delivery system like liposomes is essential.

Liposomes are colloidal particles typically composed of phospholipids and cholesterol. These lipid molecules form bilayers that entrap water-soluble molecules in their internal aqueous compartment and water-insoluble molecules within their lipid bilayers. When properly formulated and sized, liposomes can deliver drugs to the skin due to the structural similarity of their bilayers to those of natural membranes. This enables them to target macrophages within the dermis. Studies have shown that liposomes can effectively penetrate the SC, reaching the epidermis and deep dermis, and target macrophages within the dermis.

SinaAmpholeish™ is a topical nanoliposomal formulation of AmB with an average particle size of approximately 80 nm. This formulation exhibits remarkable stability, maintaining its efficacy for up to two years when stored at room temperature. It was developed by ExirNanoSina company

with Prof. Mahmoud R. Jaafari as an inventor and is patented as "Topical liposomal compositions for delivering hydrophobic drugs and methods preparing same" (US Patent No. US20150147382A1).

Following topical application of SinaAmpholeish™, some of the flexible, small (80 nm) vesicles penetrate the SC of intact skin, reaching the epidermis and deep dermis. In the dermis, infected macrophages phagocytose these nanoliposomal-AmB vesicles, and the encapsulated drug is released within the macrophage's phagolysosome by acidic lysosomal enzymes. This is where Leishmania parasites reside and multiply. The released AmB then comes into direct contact with Leishmania parasites, effectively eliminating them.

Phase I Clinical Trial

In a Phase I clinical trial, SinaAmpholeish™ was applied twice a day for one week to the left arm of healthy volunteers. Biophysical parameters of the skin, including hydration, transepidermal water loss (TEWL), melanin content, erythema, temperature, sebum production, and pH, were measured before and after application of the product. The right arm of the volunteers served as a baseline control. The results showed no significant differences between the baseline data or the two arms. Therefore, it was concluded that SinaAmpholeish™ does not cause any changes in the biophysical characteristics of the skin in healthy volunteers and is safe for topical application.

This study provides preliminary evidence that SinaAmpholeish™ is safe and well-tolerated in healthy volunteers.

Phase II Clinical Trial

A Phase II clinical trial was conducted to evaluate the efficacy of topical SinaAmpholeish™ in the treatment of cutaneous leishmaniasis (CL). The trial was conducted in accordance with Good Clinical Practice (GCP) guidelines.

Fourteen patients with a total of 84 lesions received national standard treatment of weekly intralesional Meglumine Antimonate (MA) plus biweekly cryotherapy, or daily intramuscular MA (20 mg/kg/day for 14 days) plus topical SinaAmpholeish™ twice daily for 28 days. Twenty-two patients with a total of 46 lesions (7 at most) were treated with topical SinaAmpholeish™ alone twice daily for 28 days. Thirty patients with a total of 68 lesions received national standard treatment of weekly intralesional MA plus biweekly cryotherapy.

Among 22 patients with 46 lesions who received only topical SinaAmpholeish™, 19 completed the study and 18 showed complete cure (95% efficacy). Among 14 patients with 84 lesions who received national standard treatment combined with SinaAmpholeish™, 12 completed the study with complete cure (92%). In the 30 patients with a total of 68 lesions who received national

standard treatment alone, only 33 lesions in 15 patients showed complete cure (48.5%) on day 42 follow-up.
This study provides evidence that SinaAmpholeish™ is an effective treatment for CL.

Phase III Clinical Trial: Evaluating the Efficacy of Topical SinaAmpholeish™ in Combination with Meglumine Antimonate for the Treatment of Anthroponotic Cutaneous Leishmaniasis (ACL) caused by *Leishmania tropica*

The aim of this study was to evaluate the efficacy and safety of SinaAmpholeish™ in addition to national standard treatment (meglumine antimoniate, Glucantime) for the treatment of anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica*. The study was a double blind randomized, comparative, phase 3 efficacy trial to evaluate the efficacy of topical SinaAmpholeish™ in combination with weekly injections of intra-lesional meglumine antimoniate (IL-Glucantime) in comparison with weekly injections of IL-Glucantime alone for the treatment of ACL caused by *L. tropica*. Male (M) and female (F) patient volunteers aged 12-60 years, with ACL caused by *L. tropica* were recruited, ACL diagnosis was based on parasitologically proven cases of CL and species of *Leishmania* was confirmed using PCR. The patients meeting inclusion/exclusion criteria of the trial were randomly divided into two groups according to the randomization list. The two groups were treated with weekly IL-Glucantime. Double blind randomly one group were treated with topical SinaAmpholeish™ twice a day for 28 days and the other group were treated with placebo twice daily for 28 days. Follow up of the enrolled patients was done for 6 months, each patient was visited on days 7, 14, 21, 28, 35, 42, 56, 90 and 180. A total of 130 ACL patients were recruited, 26 patients were withdrawn, 104 (F=57, M=47) patients, age 60-160 years old with 187 lesions have completed the study. At day 90 (visit 10), 42 out of 49 patients who took the topical SinaAmpholeish™ plus IL-Glucantime treatment were cured (effectiveness 85/7%), only 7 patients were not cured (14/3%). Thirty three patients who took the placebo plus IL-Glucantime treatment were cured (effectiveness 60%) and 22 patients were not cured (40%). At the end of the study at day 180 (visit 11), in topical SinaAmpholeish™ plus IL-Glucantime treatment group no patient showed relapse amongst the 42 healed patients (effectiveness 85/7%), but in placebo plus IL-Glucantime treatment group, 4 patients experienced relapse (effectiveness 52/7%). Topical SinaAmpholeish™ significantly ($p<0.01$) increased the efficacy of IL-Glucantime in the treatment of ACL caused by *L. tropica.* The side effects of topical SinaAmpholeish™ were minimal only slight skin irritations was observed that were tolerable for the patients. The results of study showed that topical Lip-AmB 0.4% in combination with IL-Glucantime is safe with a high efficacy rate in the treatment of ACL caused by *L. tropica.*

Phase IV Clinical Trial (Pharmacovigilance)

Open non-randomized, phase IV clinical trial is under way to evaluated safety and efficacy of topical nano-liposomal Amphotericin B alone or in combination with other modalities in treatment of *Leishmania major* or *Leishmania tropica*.

Published Articles

Several studies have investigated the efficacy and safety of SinaAmpholeish in the treatment of CL.

1- **Safety Evaluation of Topical Application of Nano-Liposomal Form of Amphotericin B (SinaAmpholeish) on Healthy Volunteers: Phase I Clinical Trial**

Eskandari SE, Firooz A, Nassiri-Kashani M, et al. Iranian journal of parasitology 13.4 (2019): 517-523.

This study evaluated the safety of SinaAmpholeish, a topical nanoliposomal formulation of amphotericin B (AmB), in healthy volunteers. The study found that SinaAmpholeish was well-tolerated and did not cause any serious adverse events.

2- **Safety Evaluation of Nano-Liposomal Formulation of Amphotericin B (SinaAmpholeish) in Animal Model as a Candidate for Treatment of Cutaneous Leishmaniasis**

Eskandari SE, Firooz A, Nassiri-Kashani M, et al. Journal of Vector Borne Diseases 55.2 (2018): 159-163.

This study evaluated the safety and efficacy of SinaAmpholeish in an animal model of cutaneous leishmaniasis (CL). The study found that SinaAmpholeish was safe and effective in treating CL, with no significant adverse effects.

3- **Combination of Topical Liposomal Amphotericin B and Glucantime in Comparison with Glucantime Alone for the Treatment of Anthroponotic Cutaneous**

Eskandari SE, Khamesipour A, Jaafari MR, et al. Iranian journal of medical sciences 46.4 (2021): 299-304.

This study compared the efficacy and safety of a combination of topical liposomal AmB and Glucantime to Glucantime alone in the treatment of anthroponotic CL. The study found that the

combination therapy was more effective than Glucantime alone, with no significant increase in adverse effects.

4- Development of a Topical Liposomal Formulation of Amphotericin B for the Treatment of Cutaneous Leishmaniasis

Journal of liposome research 11.1 (2016): 29-38.

This study describes the development of SinaAmpholeish, a topical nanoliposomal formulation of AmB. The study found that SinaAmpholeish was stable, effective in vitro against Leishmania parasites, and well-tolerated in animal models.

Conclusion

SinaAmpholeish has demonstrated promising efficacy and safety in preclinical and clinical studies for the treatment of cutaneous leishmaniasis (CL). The drug received manufacturing registration approval from the Ministry of Health of Iran and has been successfully commercialized. SinaAmpholeish has been available in the Iranian market for over six years, providing patients with a novel and effective therapeutic option for CL.

The drug's topical formulation offers localized delivery, reducing systemic exposure and minimizing side effects, while its nano-liposomal encapsulation enhances drug penetration and efficacy. These advantages have contributed to SinaAmpholeish's positive reception among patients and clinicians in Iran.

The successful development, approval, and commercialization of SinaAmpholeish underscore the potential of nanotechnology in the field of medicine. The drug's widespread availability in Iran has brought hope to patients suffering from CL and demonstrates the power of innovation to address unmet medical needs. As SinaAmpholeish continues to gain recognition and adoption, it is poised to make a significant impact on the global fight against CL.

SinaAmpholeish: A Promising New Treatment for Cutaneous Leishmaniasis in the Pan American Health Organization Region

The market for cutaneous leishmaniasis (CL) treatment in the Pan American Health Organization (PAHO) region, including countries like Brazil, Bolivia, Colombia, Peru, has significant potential due to the high prevalence of the disease and the growing demand for effective and safe treatment options.

High Prevalence of Cutaneous Leishmaniasis in PAHO Region

CL is a neglected tropical disease (NTD) endemic in the PAHO region, with an estimated 2 million new cases reported annually. Brazil, Bolivia, Colombia, and Peru are among the countries with the highest burden of CL in the region. For instance, Brazil accounts for over 70% of all CL cases in the Americas.

Inadequate Treatment Options and Growing Demand for Effective Treatments

Current CL treatment options are often associated with limitations, such as long treatment duration, side effects, and poor patient adherence. This has led to a growing demand for more effective and patient-friendly treatment options.

Potential of SinaAmpholeish in PAHO Region

SinaAmpholeish, a topical nano-liposomal amphotericin B, has emerged as a promising treatment option for CL. It offers several advantages over existing treatments, including:

- Topical formulation for localized delivery and reduced systemic exposure
- Nano-liposomal encapsulation for enhanced drug penetration and efficacy
- Proven efficacy and safety in preclinical and clinical studies

Market Potential in PAHO Countries

Considering the high prevalence of CL and the limitations of existing treatments, the market for SinaAmpholeish in PAHO countries holds substantial potential. The drug's efficacy, safety, and patient-friendly formulation make it well-positioned to address the unmet needs of patients and clinicians in the region.

Factors Favoring Market Expansion

Several factors favor the expansion of SinaAmpholeish in PAHO countries:

- Growing awareness of CL and its impact on public health
- Increasing demand for effective and safe CL treatments
- Recognition of SinaAmpholeish's potential as a novel and effective treatment option
- Governmental initiatives to control and eliminate NTDs

Challenges and Opportunities

While the market potential for SinaAmpholeish in PAHO countries is promising, there are also challenges to consider:

- Limited access to healthcare in some areas
- Reimbursement policies that may hinder the adoption of new drugs
- Competition from existing CL treatments

Despite these challenges, the opportunities for SinaAmpholeish in PAHO countries are significant. The drug's efficacy, safety, and patient-friendly formulation make it a strong contender to address the unmet needs of patients and clinicians in the region. With effective

marketing strategies and collaboration with local stakeholders, SinaAmpholeish has the potential to make a significant impact on the fight against CL in the PAHO region.

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