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Review

Cutaneous/Mucocutaneous Leishmaniasis Treatment for Wound Healing: Classical versus New Treatment Approaches

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Abstract: Cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (ML) show clinical spectra that can range from a localized lesion (with a spontaneous healing process) to cases that progress to a generalized systemic disease with a risk of death. The treatment of leishmaniasis is complex since most of the available drugs show high toxicity. The development of an effective topical drug formulation for CL and ML treatment offers advantages as it will improve patient's compliance to the therapy given the possibility for self-administration, as well as overcoming the first pass metabolism and the high costs of currently available alternatives. The most common dosage forms include solid formulations, such as membranes and semi-solid formulations (e.g., ointments, creams, gels, and pastes). Topical treatment has been used as a new route of administration for conventional drugs against leishmaniasis and its combinations, as well as to exploit new substances. In this review, we discuss the advantages and limitations of using topical drug delivery for the treatment of these two forms of leishmaniasis and the relevance of combining this approach with other pharmaceutical dosage forms. Emphasis will also be given to the use of nanomaterials for site-specific delivery.

Keywords: cutaneous leishmaniasis (CL); mucocutaneous leishmaniasis (ML); topical formulations; drug delivery; nanomaterials

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1. Introduction

Leishmaniasis, a chronic parasitic disease is caused by the flagellate protozoa belonging to the genus *Leishmania*. It is classified as a neglected disease by the World Health Organization (WHO) and is globally distributed mainly in developing countries reaching at least 2 million new cases and causing around 30,000 deaths per year [1]. Last few years have seen an increase in the number of cases mostly because of urban development, deforestation, climate change, and the migration of people to endemic areas. According to the Tropical Diseases Research Program, leishmaniasis is classified as one of the six most important endemic diseases in the world, due to its complexity in the clinical spectrum and epidemiological diversity [1].

More than 20 *Leishmania* species have been described to cause infection in humans, transmitted by the invertebrate vector of the *Psychodidae* family [2]. The parasite can adapt

to different habitats and climate and presents two forms (promastigote and amastigote) during its life cycle. The promastigotes are flagellated, mobile, and elongated in shape, and develop in the digestive tract of the invertebrate vector. The amastigotes, on the other hand, are non-flagellated, measuring about 2–5 μm in diameter and develop inside the phagocytic cells. There are no morphological differences between parasite species, but geographical, biological, and clinical criteria, are used to identify them [3].

The clinical manifestations of leishmaniasis vary according to the species of *Leishmania* and its virulence, as well as the clinical condition of the host, including their nutritional status and immune response [4]. Clinical spectrum can range from a localized lesion, with spontaneous cure, to cases that progress to a generalized systemic disease with a risk of death. Based on the clinical symptoms leishmaniasis can be classified as visceral leishmaniasis (VL) or kala-azar, cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (ML), with respect to the infection of macrophages throughout the reticuloendothelial system, localized in the dermis, or spread in the naso-oropharyngeal mucosa, respectively. For all three forms, infection can range from asymptomatic to severe. CL and ML can cause substantial morbidity, whereas VL can be life-threatening [5].

The transmission of the disease occurs when the female sandflies undertake blood meal in the mammals and inoculate promastigotes in the host. The promastigotes are encompassed by macrophages and are transformed into amastigotes within a period of 24 to 72 h. The amastigotes, inside the macrophages, multiply intensely until promoting the rupture of phagocytic cells. The released amastigotes infect other macrophages, completing the cycle [6]. The opposite is also possible, that is an insect bites an infected host and ingests the macrophages parasitized by amastigotes. Then, the amastigotes present in the intestine of the invertebrate are transformed into promastigotes, which are infectious to the vertebrate host [7].

2. Cutaneous and Mucocutaneous Leishmaniasis

Worldwide cases of CL and ML are estimated to be over 1 million per year. CL is the most common form of leishmaniasis and is caused mainly by *Leishmania (Leishmania) amazonensis*, *Leishmania (Viannia) guyanensis,* and *Leishmania (Viannia) braziliensis* [8]. The skin lesions usually appear within several weeks or months after the exposure especially on the exposed parts of the body, such as the face, arms, and legs. They evolve from papules to nodular plaques that may result in ulcerative lesions, showing a raised border and central depression, that can be covered by scab or crust. Some lesions persist as nodules. The lesions are usually painless but can become painful, when infected with bacteria or when present near a joint. The healing process typically results in atrophic scarring [9]. Mucosal leishmaniasis is a metastatic sequela of the cutaneous form, due to the dissemination of the parasites from the skin to the naso-oropharyngeal mucosa, caused by species in the Viannia subgenus (especially *L.* [V.] *braziliensis* but also *L.* [V.] *panamensis* and sometimes *L.* [V.] *guyanensis*. The risk factors for mucosal dissemination are poorly understood and reported to vary among geographic regions. ML becomes evident within several years of the original cutaneous lesions, especially those not treated at all or treated poorly. Nasal and oral mucosa are the most frequently affected body lesions. The lesions in the oral cavity can spread to the oropharynx and larynx and may affect cartilage and vocal cords. ML lesions are ulcerated, and treatment is essential to control infection. ML is a potentially life-threatening and highly disfiguring condition, due to the late-stage destruction of the oral-nasopharyngeal mucosa and cartilage.

Also, the development of experimental models for the study of leishmaniasis has contributed to understanding the pathogenesis. In recent years, several studies have explored patterns of resistance and susceptibility of different strains of mice infected with different species of *Leishmania* sp. The best-studied model for leishmaniosis tegumentary is the infection of mice with *L. major*. This model employs a large number of parasites, which are inoculated subcutaneously in the paw of the animals. Using this model, susceptibility and resistance to leishmaniasis were created, due to a Th1-type cellular immune response

in which lymphocytes produce high levels of IFN-α and low levels of IL-4, there is the development of a small lesion at the site of inoculum, non-ulcerated that heals spontaneously. Otherwise, experimental infections in mice with various species of *Leishmania* sp. can mimic several forms of human cutaneous leishmaniasis. Depending on the parasite species and mouse strain, a considerable disease spectrum can be produced. Some strains of mice such as BALB/c, are highly susceptible to *L. major* infection and fail to develop a Th1 response against the parasite. In contrast, other mouse strains such as C3H and C57BL/6, infected with *L. major*, develop spontaneously healing lesions associated with strong cellular immunity. Infections in mice with other species of *Leishmania* sp. can lead to different models of resistance and susceptibility. For example, strains of mice resistant to *L. major* infection (such as CBA, C3H or C57BL/6) are susceptible to infection with *L. amazonensis* or *L. mexicana*, suggesting that parasite-specific factors play an important role in the course of the disease [10].

Although *L. braziliensis* induces a disease that is serious in public health in South America, there are few experimental works that characterize the immune response to this parasite, probably because mice are unlikely. Moura et al., 2005 developed a model of inoculation of *L. braziliensis* in the ear dermis of BALB/c mice leading to the development of an ulcerated lesion, with raised edges and necrotic fundus that heals spontaneously, regional lymphadenopathy and persistence of the parasite in lymphoid tissues and development of a Th1 response. In this way, this model is used in Brazil as a reference [11].

3. Classical Treatments

The treatment of leishmaniasis is complex [4]. Several drugs are described in the literature. The primary treatment can be traced back to 1920, based on trivalent antimony salts (Sb III), but their toxicity, narrow therapeutic window, and resistance to parasites resulted in their discontinuous use in several countries [12].

Pentavalent antimonials were developed with improved treatment potential and less toxicity. Antimonials are well-tolerated, but some side effects, such as pain at the injection site, gastrointestinal dysfunction, muscle pain, stiffening of joints, arrhythmias, and pancreatitis have been reported [13]. In Brazil, N-methyl glucamine antimonate (Glucantime®) is the drug distributed by the Ministry of Health. The prescribed dose of Glucantime® varies between 10–20 mg/kg/day for children and adults for 20 to 30 days uninterrupted via intravenous or intramuscular administration routes. However, N-methyl-glucamine antimoniate has been associated with some mutagenic effects [14]. Activation of antimonial drug through reduction from Sb(V) to Sb(III), followed by inhibition of trypanothione reductase and oxidative stress, is one or the reasons why *Leishmania* parasites are susceptible to organic antimonial drugs [15,16]. From the latest version of the guideline for the treatment of Leishmaniasis in America (2nd Edition), pentavalent antimonials are yet recommended for the treatment of mucosal or mucocutaneous leishmaniasis, either with or without oral pentoxifylline [17].

Amphotericin B and pentamidine isethionate are also used especially during treatment failure with pentavalent antimonial. Amphotericin B is not suggested in patients with heart disease, liver disease, and nephropathy. Its recommended dose is 1–4 mg/kg/day, administered intravenously daily until maximal dosage or tolerance. More recently, amphotericin B was loaded in unilamellar vesicles, consisting of hydrogenated soy phosphatidylcholine, cholesterol, disteroyl phosphatidyl glycerol, denominated commercially, Ambisome®. This nanotechnology-based formulation was found useful to reduce undesirable effects, such as fever, chills, stiffness, drowsiness, slight elevation of liver function tests, renal dysfunction, and cardiopulmonary toxicity. This drug is effective in VL and ML [18]. Pentamidine isethionate is also well-tolerated and effective in the treatment of ML caused by *L. braziliensis*. Pentamidine isethionate is suggested in cases of poor response to N-methyl glucamine antimonate and intolerance to amphotericin B [19].

Miltefosine is the first oral drug used for both CL and ML. Its mechanism of action is not clear, however, Lux et al. (2000) [20] demonstrated that this drug inhibits the enzyme 1-

acyl-2-lysoglycero-3- phosphocholine acyltransferase in promastigote stage. Some adverse effects such as nephrotoxicity, hepatotoxicity, and teratogenicity have been observed [21].

The current therapeutic options for the treatment of leishmaniasis are linked to high toxicity, low efficacy, difficulty in administration, and parasitic drug resistance [4]. The pharmacotherapy has been mostly unchanged for decades, with few drug options, in spite of species diversity and numerous forms of disease manifestation [22]. Over the last decades, new drugs for leishmaniosis treatment have been developed focusing on the improvement of efficiency, while keeping low cost and reduced collateral effects. An example is isopentyl caffeate which was described as promising against the mechanisms that parasites undertake for their survival [23–26]. More recently, Novais et al. (2021) [27] discussed the use of host-directed therapies either to enhance protective immune responses or to ameliorate excessive cutaneous inflammation, i.e., host-specific delivery is thus proposed to be tailored according to the type of leishmaniosis.

The loading of conventional drugs into innovative formulations (e.g., topical solid dosage forms, films and membranes, nanomaterials) has been proposed to improve the clinical outcomes, reduce the toxicological risk and costs, with the ultimate aim to improve the patients' life quality (Figure 1). Table 1 summarizes relevant examples of drugs commonly used for the classical treatment of CL and ML.

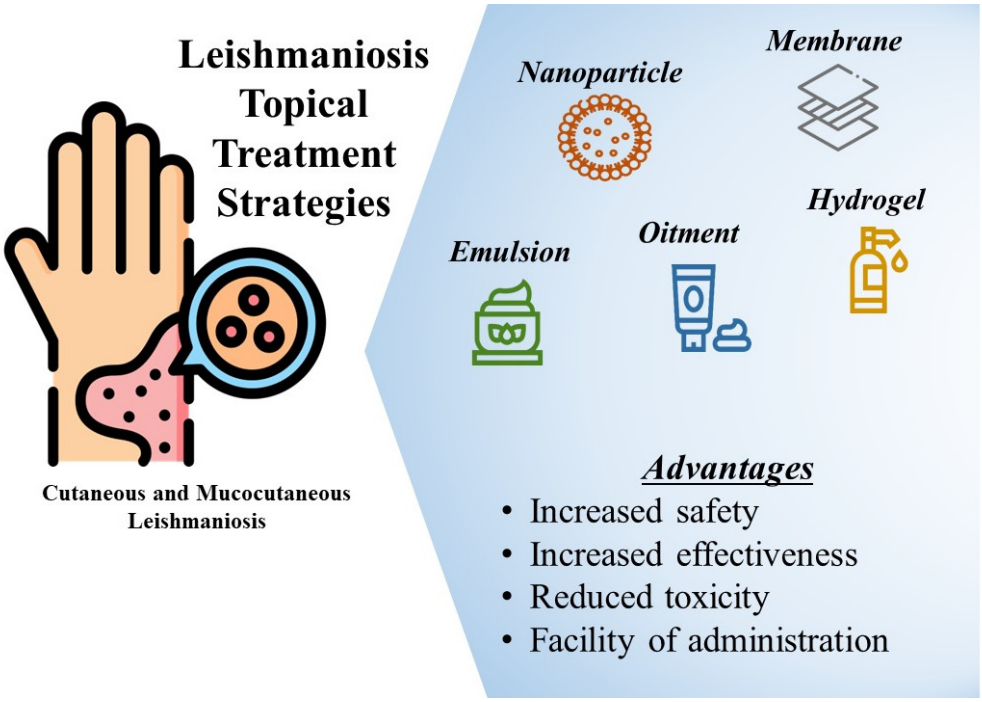

Figure 1. Examples of topical strategies for the treatment of cutaneous and mucocutaneous Leishmaniosis and their advantages.

Table 1. Drugs used for the classical treatment of cutaneous (CL) and mucocutaneous leishmaniasis (ML).

Drug	Administration Route	Dose	Mechanism of Action	Side Effects	References
N-methylglucamine antimoniate	Intravenous, or intramuscular	CL: 15 mg/kg/day (20 days); ML: 20 mg/kg/day;	Two mechanisms: (i) it binds with ribonucleosides forming a complex, preventing topoisomerases from carrying out their function in the process of DNA replication and transcription; (ii) it increases pro-inflammatory cytokines in the host, enhancing the phagocytic action of neutrophils and monocytes.	Myalgia, liver changes, abdominal pain and cardiac disorders.	[28]
Amphotericin B deoxycholate	Intravenous	CL and ML: 1 mg/kg/day;	It binds with the ergosterol of the pathogens' plasma membrane. It will cause the dysfunction of the cells through forming of ion pore channels. The pore formation will cause inhibition of glycolysis and rapid efflux of K+ and Mg+ ions inside cells leading to an increase in acidity of these cells and cells death	Fever and chills at the moment of the infusion. Anemia, neutropenia, thrombocytopenia, and changes in liver enzymes.	[29–31]
Amphotericin B	Intravenous	CL and ML: 1–4 mg/kg/day (daily);	It is the same as Amphotericin B deoxycholate, the difference happens with the addition of lipid formulations that help to decrease side effects and to reach only target tissues with maximum concentration and selectivity, serum concentration of the drug should be kept low.	Loss of potassium and magnesium, anaphylaxis, fevers. Anemia and nephrotoxicity	[29,31]
Pentamidine isethionate	Intravenous or intramuscular	CL and ML: 4 mg/kg/day;	It interferes production of polyamine, RNA polymerase activity, causing the inhibition of protein and RNA synthesis. It has ability to enter the pathogen's cell and bind the RNA transfer is carried out and thus block the synthesis of proteins, nucleic acids, phospholipids and folate.	Hypoglycemia, hypotension, arrhythmias, prolonged QT interval, fatigue, night sweats, anorexia, nausea, vomiting, syncope, rash, nephrotoxicity, hepatotoxicity.	[32]
Miltefosine	Oral	CL and ML: 2.5 mg/kg/day.	It activates cytotoxic macrophages, the ability to interfere with cell signaling pathways, carry out modifications in the lipid membrane, as well as programmed cell death (apoptosis). When administering the drug, it will have the ability to interfere with the pathogen's cell membrane, influence the lipid composition, permeability, and fluidity of the membrane, as well as the metabolism of phospholipids, causing apoptosis to be stimulated.	Anorexia, nausea, vomiting and diarrhea, skin allergy, high concentrations of liver transaminases and, in rarer cases, renal failure.	[33]
Imiquimod	Topical	5%	The off-label use of topical imiquimod has been evaluated as an agent in the treatment of several infectious diseases. In cutaneous leishmaniasis, it acts on the stimulation process, causing TCD4 lymphocytes to secrete interferon-y, activating the release of macrophages that will follow the infection site to phagocytose the amastigote forms of leishmania.	Some local side effects may occur in high dose situations, such as itching, erythema, burning, local irritation.	[34,35]
Paromomycin	Topical and parenteral		It inhibits the synthesis of proteins present in the protozoan structure. It binds to the 30S ribosomal unit causing an accumulation of abnormal ribosomal complexes leading to the death of the protozoan.	Nephrotoxicity, ototoxicity and liver dysfunction	[21,36,37]

Table 1. *Cont.*

Drug	Administration Route	Dose	Mechanism of Action	Side Effects	References
Azithromycin	Oral	500 mg/day (20 days)	It is an antibacterial that works by preventing protein production and interfering with bacterial growth. Its antiparasitic action is possibly associated with its immunomodulatory activity preventing the production of cytokines and pro-inflammatory mediators.	nausea, vomiting and diarrhea	[38]
Azoles	Oral	Ketoconazole: 200 to 400 mg/twice a day (for three months) Fluconazole: 5 to 8 mg/kg (for 4–12 weeks) Itraconazole: ML: 4 mg/kg/day (for 6 weeks)	Its mechanism of action is based on blocking the synthesis of ergosterol, an essential molecule for bioregulation and cell membrane integrity.	Itching, nausea, vomiting, allergic and anorexia	[21]
Sodium Stibogluconate	Intravenous [or Intramuscular	20 mg/kg/day (for 20 days)	Its mechanism of action is based on the inhibition of glycolysis and oxidation of fatty acids in protozoan cells.	Nausea, vomiting, abdominal pain, fatigue, muscle pain, arrhythmias, liver disorders.	[39]
Zinc sulfate	Oral	10 mg/kg/day	The effect of zinc sulfate shows relatively positive but variable results in the treatment of leishmaniasis. The evaluation of the effect of zinc sulphate in the treatment of leishmaniasis is still not well understood, the effect may be related to the action of zinc sulphate on protozoan enzymes interfering with DNA synthesis.	-	[40]

4. Topical Treatment Strategies

The skin is a barrier to external environmental conditions, protecting the body against ultraviolet radiation, chemical agents, microorganisms, and allergens. In addition to regulating the loss of moisture and some nutrients, it maintains body homeostasis, controls body temperature and blood pressure, and allows the administration of pharmaceuticals for a systemic effect.

The development of an effective topical dosage forms for the treatment of CL and ML would represent a significant advance in the therapy of this neglected disease [4]. The drug must be released from the formulation and remain retained in the wound. The action of a drug into wound healing depends on a range of factors, including the drug's physicochemical properties, the characteristics of the pharmaceutical dosage form, and the conditions of the skin [41].

While challenging to treat topical leishmaniasis, several advantages can be pointed out for the topical treatment, such as the possibility for self-administration, avoidance of the first-pass metabolism which helps to increase the drug's bioavailability, in particular, those with a short half-life and a narrow therapeutic window, less plasma fluctuation, improved effectiveness with a lower dosage, reduced costs, all promoting patient's [42,43]. Moreover, the possibility to target the drug into the site of action (e.g., with nanomaterials) reduces the toxicological risks. The most common dosage forms include films/membranes and semi-solid formulations (e.g., ointments, oil-in-water emulsions and hydrogels). Topical treatment has been used as a new route of administration for conventional drugs against leishmaniasis and its combinations, and also for the delivery of new compounds with anti-leishmaniosis activity [41]. Table 2 summarizes CL treatments according to the type of formulation for skin route of administration.

5. Semi-Solid Formulations

Ointments are stable, semi-solid pharmaceutical dosage forms, soft in consistency, intended for external use, consisting of one or more drugs and monophasic excipients with lipophilic or hydrophilic characteristics. Ointments must be plastic and thermo-reversible, so that with the increase of the temperature upon topical application, they become less viscous, allowing the drug to be released and reach the skin. According to the degree of penetration and the excipient used, they are classified as epidermal (if the drug acts superficially on the skin and the excipients used are petroleum and mineral oil), endodermal (the drug penetrates deeper into the skin reaching the dermis, and the excipient is a vegetable oil) and hypodermic (it absorbed and can trigger a systemic effect; the excipient is lanolin).

Bilbao-Ramos et al. (2020) [44] described the first-time in vivo study reporting the use of ursolic acid to treat leishmania. Ursolic acid was loaded in a semi-solid formulation (e.g., cream or ointment) and compared to the commercial Orabase® (Fagron) in which the ursolic acid was dispersed with glycerin:propylene glycol. The semi-solid formulation using 0.2% of ursolic acid promoted a reduction of ~50% of lesion size compared control group, after 28 consecutive days. The treatment was however not efficient in completely reducing the *L. amazonensis* infection, attribute to the occlusive effect of the ointment which promoted ursolic acid permeability across the skin with a prolonged the drug delivery.

In another study, Copas-López et al. (2016) [45] infect foot of Syrian hamster model using *L. tropica* strain. The effect of (−)-α-bisabolol by topical, oral and intralesional administration was evaluated. The ointment was composed of cetyl alcohol, lanolin, white petroleum jelly and (−)-α-bisabolol (1%, 2.5%, 5%) was applied on the footpad lesion to prevent microbial infection and reduce the risk of scars. (−)-α-bisabolol is a natural origin with anti-inflammatory, anti-microbial and healing properties.

Table 2. Examples of formulations proposed for cutaneous leishmaniosis, type of drug, production method and main results.

Type of Formulation	Drug	Production Method	Results	References
Polymeric nanoparticles	Meglumine antimoniate	Nanoemulsification	The formulation was able to control a leishmaniasis infection as the same level than the reference injected Glucantime®.	[46]
Film-Forming (spray formulation)	Nitroimidazole DNDI-0690	Dispersion in water/ethanol medium of polymer and plasticizer	The formulation reduced the parasites in the skin, but did not influence the lesion size compared with the control.	[47]
Emulsions	(PEI25-CAN-γ-Fe2O3 NPs) Nano-Leish-IL	Emulsion	The elimination of infection by L. major in vivo assays was observed.	[48]
Emulsion	Amphotericin B	Emulsion	Cure rates of 39.4% were observed, showing that topical Amphotericin B was not effective for the treatment of CL.	[49]
Nanostructured lipid carriers (NLCs) incorporated into a hydrogel	Amphotericin B	NLC was produced by the emulsification method, polymer and plasticized was added and homogenized.	The formulation was around five times slower in the IC50 values in vivo assays.	[50]
Nanotransfersomes incorporated in chitosan gel	Rifampicin	Film hydration method follow, chitosan added and homogenized	Nanotransfersomes were more effective compared to drug pristine. Furthermore, the nanotransfersomes incorporate in chitosan gel reduced the wound healing significantly.	[51]
Membranes	Diethyl dithiocarbamate (DETC)	Bacterial cellulose membranes were obtained from cultivation of *Gluconacetobacter hansenii*	Reduction in significance of parasite load and of infection of *L. braziliensis* in macrophages	[52]
Membranes	amphotericin B (AmB)	A polyvinyl alcohol, (PVA) hydrogel produced by casting	The leishmanicidal, antifungal, and cytotoxic activity of the system loaded with AmB were signaled an efficient pharmacological activity and adequate biocompatibility of PVA-AmB hydrogels with great potential in the topical treatment of CL.	[53]
Self-nanoemulsifying drug delivery systems	buparvaquone (BPQ)	Dispersion of drug, oil, surfactant in solvent	Reduction of parasitism and indicated healing in animals	[54]
Liposomes	Amphotericin B (AmB)	Film hydration method	Liposomal formulation was considerably higher than that observed for pristine AmB	[55]
Liposomes	stibogluconate and ketoconazole	Film hydration method	In vitro and in vivo anti- indicated a 10.67-fold lower IC_{50} value	[56]

Table 2. *Cont.*

Type of Formulation	Drug	Production Method	Results	References
Liposomes	Azithromycin and glucantine	dehydration–rehydration vesicle; (DRV) method dehydration–rehydration vesicle; (DRV) method Dehydratation-rehydratation vesicle method	In vivo assays showed a cure rate of 77% for azithromycin and 76% for glucantime.	[57]
Liposomes	Amphotericin B	-	Liposomal formulation was stable and showed capacity to penetrate into the skin. It was also efficient against *L. major* in vitro and in vivo.	[58]
Hydrogels	Miltefosine	Dispersion of drug and polymer in water.	The topical 0.5% miltefosine gel formulation was efficacious and non-toxic when administered topically in vivo assays.	[59]
Hydrogels	Meglumine antimoniate	Polymer homogenization in aqueous medium	It showed high retention on the skin and reduction of IC50 compared the control.	[60]
Hydrogels	Amphotericin B	Homogenization of polymer with constant mechanical stirring in aqueous medium	No cytotoxic effects were observed in macrophages. No in vitro and in vivo assays were done yet.	[61]
Ointment	Ursolic acid	melting	Reducing the *L. amazonensis* infection, attribute to the occlusive effect of the ointment, which promoted ursolic acid permeability across the skin with a prolonged the drug delivery	[44]
Ointment	(−)-α-bisabolol	Melting	In vivo assays prevented microbial infection and inflammation, leishmanial and healing properties.	[45]

After infection, the animals were treated for 7 days. The most promising result was obtained with the topical treatment using 2.5% (−)-α-bisabolol ointment, which reduced ~83% the lesion size and 80% parasite load. This outcome was attributed to the anti-inflammatory activity of drug, together with the enhanced drug bioavailability upon topical administration in the wound.

Regarding the use of ointments for leishmaniasis treatment Soto et al. (2019) [62] reported a relevant result with topical application of pentamidine in leishmaniasis caused by *L. brazilensis*. A 15% pentamidine ointment was formulated in a hydrophilic vehicle give adsorption properties. The hydrophilic formulation of pentamidine had a cure rate of 77.5% compared to the positive control (intralesional injection of pentamidine administered on days 1, 3 and 5 at a dose of 120 μg/mm^2 at the lesion site) which had a rate of 70%. These results suggest that the ointment acts positively, increasing the drug bioavailability. In a Phase III trial described by Sosa et al. (2019) [63], an efficacy of 79% and 78% was observed for the group treated with 15% pentamidine and 0.5% gentamicin ointment and 15% pentamidine ointment, respectively, when applied once a day for 20 days [35].

The first medicine containing paromomycin to treat CL was an ointment based on 15% of paromomycin and 12% of methyl benzethonium showing a satisfactory result in vivo, eliminating the parasites and healing the lesion in 100% of the animals [64]. This formulation is available commercially in Israel, as Leoshcutan®. More recently, Veraldi et al. (2020) [65] developed paromomycin (15%) ointment content glycerin, vaseline, sepigel 305,

sodium bicarbonate, and sodium hydrate. The clinical assays tested ointment occlusive dressing with two applications/day for 3 weeks. In some patients, the treatment was ineffective, suggesting drug resistance; thus not demonstrating that drug delivery could influence the release profile, suggesting a low dose concentration.

Emulsions are a mixture between two immiscible liquids, one of which is in the form of fine globules within the other liquid, forming a stable mixture. Emulsions show reduced viscosity being easier to spread, and the drug can be dissolved or suspended in the aqueous or oily phases, and this versatility is one of their main advantages. Lopez et al. (2018) [49] studied the effect of an oil-in-water (o/w) emulsion containing 3% Amphotericin B cream evaluated in patients with CL caused by *L. panamensis*, *L. braziliensis* and *L. guyanensis* in Colombia. Cure rates of 39.4% were observed, showing that topical Amphotericin B was not effective for the treatment of CL. Kawakami et al. (2021) [66] evaluated the efficacy of emulsion with *Pterodon emarginatus* Vogel oleoresin for the treatment of tegumentary leishmaniasis and observed that the topical use of a nanoemulsion combination with *Pterodon emarginatus* Vogel oleoresin and intraperitoneal meglumine antimoniate reduced the size of the lesion. mice by 41%. Despite these results, more studies need to be carried out based on new formulations with the aim of improving results in clinical practice.

Another pharmaceutical form, hydrogels are networks of three-dimensional polymers, with a hydrophilic structure, capable of absorbing and releasing water in response to environmental conditions, that is, they have the ability to swell in an aqueous medium without dissolving it [67]. The amount of water retained in the hydrogel network mesh is defined by the structure of the polymer network and other factors such as temperature, pH and ionic strength of the water solution in contact with the polymer. Due to this water-absorbing property, polymeric hydrogels began to be used for several applications: aid in wound healing, substrate for cell growth and substrate for controlled release of chemical substances. Such hydrogels have many advantageous characteristics regarding their use in medical applications, such as: non-toxicity; ability to swell in water and biological fluids, which resembles living tissue; elastomeric consistency, which reduces friction between the tissues and the hydrogel; high permeability; ease of obtaining in different forms and possibility of incorporation and controlled release of drugs of different polarities [68].

A study was also found, evaluating the effect of miltefosine in the 0.5% Carbopol gel pharmaceutical form [59]. The authors observed that this formulation reduced lesion size by 84% to 100% without parasite detection in BALB/c mice infected with LC species, L. (Viannia) *braziliensis* and L. (Viannia) *panamensis*. Although effective, there was some variation in results which may be due to different causative species, treatment duration and formulation components.

6. Films and Membranes

The recovery and healing process of damaged skin tissue involves several molecules such as growth factors, chemokines, cytokines, different cell lines, and other tissues [69,70]. The stages involved in the healing process can be divided into four, which overlap with time and space, namely, inflammation, migration, proliferation, maturation and additionally, hemostasis, which should be considered a fifth step if the lesion reaches capillaries or larger blood vessels and causes bleeding [70,71].

American CL (ACL) is a disease known to trigger limited ulcerative skin lesions and disfiguring lesions in the mucous membranes of the nose, mouth, and pharynx in the mucocutaneous form [72]. The diverse patterns of the disease may vary with the phlebotomine sand flies, the human population involved, and the level of exposure or/and the diversity of hosts [73]. Topical, dermal, transdermal treatment of ACL lesions represents an up-and-coming alternative to reduce systemic toxicity associated with the use of available pharmaceutical forms administered intravenously, aiming at removing the parasite load, skin regeneration, protection against infections secondary without the need for hospitalization, that is, the entire procedure performed on an outpatient basis safely

and efficiently [53,74]. In addition, the treatment cost is reduced and provides increased patient compliance to treatment by the ease of drug administration.

Nowadays, the so-called "smart" wound dressings have also been extensively studied to treat skin lesions, including ACL treatment. Wound dressings can provide an additionally moist environment, remove excess exudate with the possibility of mucoadhesion, avoid maceration, protect the damage against secondary fungal and bacterial infections and maintain an adequate gas exchange (i.e., exchange of O_2 and H_2O) are great features for innovative CL treatment [75,76]. In addition, these dressings can also have an adjusted drug release profile, high porosity, and malleability [53].

Several studies have demonstrated the potential of these systems as support matrices for drug delivery systems as well. Among the materials used in the production of dressings, examples include the use of clays [71], natural [77–80] or synthetic polymers [78,81,82] or a combination of both that can form thin films and gels, classified as hydrocolloid dressings, alginate dressings [83,84] and chitosan-based dressings [76,85].

Alexandrino-Junior et al. (2019) [53] developed a poly(vinyl alcohol, PVA) hydrogel loaded with amphotericin B (AmB) to be used as a dressing system for the treatment of CL. The microstructure of the hydrogel was characterized by evaluating the state of AmB aggregation to the system, surface morphology, degree of swelling, drug release kinetics, and water and microbial permeability. The leishmanicidal, antifungal, and cytotoxic activity of the system loaded with AmB were signaled an efficient pharmacological activity and adequate biocompatibility of PVA-AmB hydrogels with great potential in the topical treatment of CL. Seeking the exact purpose of developing a new dressing to treat ulcers caused by *L. braziliensis*, Celes et al. (2016) [52] evaluated bacterial cellulose and diethyldithiocarbamate membranes with parasite reduction after three weeks of treatment.

Pereira et al. (2020) [86] used anti-leishmanial drugs in their studies, provided by the Brazilian Ministry of Health (Glucantime, Amphotericin B, Pentamidine) to develop alternatives to the treatment of CL, administered through flexible Band-Aid-type dressings based on a porous non-woven fabric (TNT) covered with Polyvinyl Acetate (PVA) and glycerol. The authors demonstrated good results from a topical treatment containing Glucantime and Pentamidine reduction of the promastigote forms of *L. amazonensis*.

Another innovative treatment based on wound dressings is nanofibers, designed with larger pores; however, with an adequate and necessary barrier against opportunistic infections [87]. Additionally, the surfaces and microstructures of electrotrophied nanofibers enable a significant absorption of fibroblasts in the epidermis, boosting the regeneration of damaged tissue, accelerating the formation of extracellular matrix [88]. Alishahi et al. (2020) [89] developed a topical drug delivery system that can release glucantime at the site of Leishmania skin wounds. Therefore, the electrospinning method was used to prepare core-shell nanofibers composed of polyethylene oxide, gelatin, poly (vinyl alcohol), and chitosan. The proposed system can eliminate more than 78% of the Leishmania (L. major) promastigotes forms and is cyto-compatible with assays with fibroblast cells.

7. Nanomaterials

For the topical treatment of leishmaniasis, nanotechnology has been exploited as an optimization tool, and the reported results using liposomes and polymeric nanoparticles are promising as drug delivery strategies. The reduced size favors internalization into the infected cell macrophages; moreover, the positive surface electrical charge (i.e., positive zeta potential values) promote complexation with nucleic acids through electrostatic interactions. Furthermore, it promotes the condensation of it structure, decreasing its size, and also neutralizes the negative charge, favoring the entry of nucleic acids into the cell through the cell membrane [90].

Various other nanocarrier strategies have been used for leishmaniasis treatment demonstrating their own advantages and disadvantages. As is known, the main cell targets in leishmaniasis are macrophages. Thence, the most employed nanoparticles in the disease are polymeric nanoparticles and liposomes because of the easy and fast way of

internalization by the infected cells. Liposomes are nanocarriers that have unique properties to load and deliver hydrophobic and/or hydrophilic molecules by surface activation. Moreover, because they are positively charged, liposomes are promptly internalized by the macrophages. Due to these properties, they are the most commonly nanocarriers employed in leishmaniasis (Saleem et al. (2019) [90]).

Monteiro et al. (2019) [91] described the preparation and evaluated in vitro a modified nanostructured lipid carrier using chitosan and dextran for the co-administration of buparvaquone and polymyxin B against leishmaniasis. The carrier developed proved to be a promising formulation to overcome the disadvantages of the current treatment of leishmaniasis by co-delivering two distinct drugs.

Redesigning the use of sodium stibogluconate, Dar et al. (2018) [92] integrated it into a nanodeformable liposomal drug delivery system for topical application among BALB/c mice infected with *L. tropica*. The authors observed effectiveness in this treatment from the significant reduction in the size of the lesion (four-fold increase in activity) and also by the reduction in the IC50 value, which was 1.65 mg/mL for ointment with a simple drug delivery system, but in this new formulation it was 1.3 mg/mL. Kavian et al. (2019) [93] showed that a 4% liposomal miltefosine formulation had no significant effects on cure rate. Further investigation is needed for its use in the treatment of CL.

Another study also showed a similar result from the use of a nanodeformable liposomal drug delivery system combining sodium stibogluconate with ketoconazole, evaluated in BALB/c mice infected with *L. mexicana*. This combination exhibited synergistic interaction and flow cytometry revealed greater clearance of *L. mexicana* in infected macrophages. In vitro and in vivo anti-leishmania experiments indicated a 10.67-fold lower IC50 value and a 35.33-fold lower parasite load compared to the plain sodium stibogluconate solution [92]. Using a deformable lipid vesicle, Carvalheiro et al. (2021) [55] observed that the formulation of Amphotericin B with this lipid vesicle was able to dose-dependently reduce the viability of the promastigote, as well as the number of intracellular amastigotes in THP-macrophages 1. The value of the selectivity index observed for Amphotericin B included in the deformable lipid drug delivery system was considerably higher than that observed for free Amphotericin B.

Peralta et al. (2021) [94] prepared dispersions containing miltefosine and liposomes of different compositions to optimize transepidermal penetration and in vitro and in vivo experiments evaluated the efficacy and toxicity, the drug release rate and the stability of particle size over time. Treatments were administered topically to BALB/c mice infected with *Leishmania amazonensis*. Dispersions containing 0.5% miltefosine eliminated 99% of the parasites and healed the lesions with complete re-epithelialization, no visible scarring and hair growth.

Dare et al. (2020) [95] with the aim of optimizing and evaluating amphotericin B and miltefosine co-loaded in second-generation ultra-deformable liposomes for the topical treatment of CL, observed a synergistic interaction between amphotericin B, miltefosine and their anti-leishmanial activity liposomes against *Leishmania mexicana* amastigotes. The results indicated IC50 values about 8.62 to 6.12 times lower of this co-load compared to free-form drug solutions. Regarding the in vivo results, it was observed that there was a significant reduction in the parasite load in an experimental BALB/c model with LC.

Another recent study aiming to develop topical nano-liposomal Amphotericin B (AmB) for the treatment of CL suggested the topical use of this formulation at 0.4% Amphotericin could be a very useful tool [58]. The authors developed and characterized liposomes containing 0.1, 0.2 and 0.4% Amphotericin B for size, trapping efficiency, long-term stability and skin penetration properties using Franz diffusion cells. Since the 0.4% formulation had an ED50 (necessary to kill 50% of *L. major* amastigotes) of 0.0856 (μg/mL), it caused an 80% reduction in the fluorescence intensity of macrophages infected with GFP +. They applied all formulations twice daily for 4 weeks to the skin of BALB/c mice to treat lesions caused by L. major and observed superiority of the 0.4% nano-liposomal Amphotericin B formulation compared to Lip-AmB 0.2 and 0.1%, so that the parasite was completely

eliminated from the site of infection of the skin and spleen at weeks 8 and 12 post-infection in treated mice. Thus, the use of liposomal delivery systems has been shown to be a promising approach for the cutaneous delivery of substances, representing a new safe and low-cost therapeutic option in the treatment of leishmaniasis.

Miltefosine, meglumine antimoniate and imiquimod were loaded in cationic nanovesicles (cNVs) for combined therapy, producing cNVs of mean size of approximately 85 nm [96]. A synergic activity of drugs was described to be efficient to reduce local skin irritation, reduce cytotoxicity and reduce local parasites.

8. Conclusions

CL and ML pathologies are complex, therefore, when proposing a possible treatment for the disease, it is necessary to jointly evaluate both the elimination of the parasite and the intense inflammatory process. Topical drug administration or in combination with other therapies may be an important strategy for the treatment of leishmaniasis. In addition, it should be noted that the topical treatment does not require the patient to be hospitalized, it is easy to apply and is of low-cost. It is emphasized that a simple modification of the pharmaceutical dosage form can improve the treatment of leishmaniasis and promote the quality of life of patients.

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References

1. World Health Organization. Available online: https://www.who.int/health-topics/leishmaniasis#tab=tab_1 (accessed on 3 August 2022).
2. Kaye, P.M.; Cruz, I.; Picado, A.; Van Bocxlaer, K.; Croft, S.L. Leishmaniasis immunopathology-impact on design and use of vaccines, diagnostics and drugs. *Semin. Immunopathol.* **2020**, *42*, 247–264. [CrossRef]
3. Thakur, S.; Joshi, J.; Kaur, S. Leishmaniasis diagnosis: An update on the use of parasitological, immunological and molecular methods. *J. Parasit. Dis.* **2020**, *44*, 253–272. [CrossRef]
4. Souto, E.B.; Dias-Ferreira, J.; Craveiro, S.A.; Severino, P.; Sanchez-Lopez, E.; Garcia, M.L.; Silva, A.M.; Souto, S.B.; Mahant, S. Therapeutic Interventions for Countering Leishmaniasis and Chagas's Disease: From Traditional Sources to Nanotechnological Systems. *Pathogens* **2019**, *8*, 119. [CrossRef] [PubMed]
5. Torres-Guerrero, E.; Quintanilla-Cedillo, M.R.; Ruiz-Esmenjaud, J.; Arenas, R. Leishmaniasis: A review. *F1000Research* **2017**, *6*, 750. [CrossRef]
6. Santana, W.; Oliveira, S.C.; Ramos, M.H.; Santos, A.L.S.; Dolabella, S.S.; Souto, E.B.; Severino, P.; Jain, S. Exploring Innovative Leishmaniasis Treatment: Drug Targets from Pre-Clinical to Clinical Findings. *Chem. Biodivers.* **2021**, *18*, e2100336. [CrossRef]
7. Burza, S.; Croft, S.L.; Boelaert, M. Leishmaniasis. *Lancet* **2018**, *392*, 951–970. [CrossRef]
8. Mokni, M. Cutaneous leishmaniasis. *Ann. Derm. Venereol.* **2019**, *146*, 232–246. [CrossRef] [PubMed]
9. Handler, M.Z.; Patel, P.A.; Kapila, R.; Al-Qubati, Y.; Schwartz, R.A. Cutaneous and mucocutaneous leishmaniasis: Differential diagnosis, diagnosis, histopathology, and management. *J. Am. Acad. Derm.* **2015**, *73*, 911–926. [CrossRef] [PubMed]
10. Barral, A.; Petersen, E.A.; Sacks, D.L.; Neva, F.A. Late metastatic Leishmaniasis in the mouse. A model for mucocutaneous disease. *Am. J. Trop. Med. Hyg.* **1983**, *32*, 277–285. [CrossRef]

11. de Moura, T.R.; Novais, F.O.; Oliveira, F.; Clarêncio, J.; Noronha, A.; Barral, A.; Brodskyn, C.; de Oliveira, C.I. Toward a novel experimental model of infection to study American cutaneous leishmaniasis caused by *Leishmania braziliensis*. *Infect. Immun.* **2005**, *73*, 5827–5834. [CrossRef]
12. Casalle, N.; de Barros Pinto Grifoni, L.; Bosco Mendes, A.C.; Delort, S.; Massucato, E.M.S. Mucocutaneous Leishmaniasis with Rare Manifestation in the Nasal Mucosa and Cartilage Bone Septal. *Case Rep. Infect. Dis.* **2020**, *2020*, 8876020. [CrossRef]
13. Ponte-Sucre, A.; Gamarro, F.; Dujardin, J.C.; Barrett, M.P.; López-Vélez, R.; García-Hernández, R.; Pountain, A.W.; Mwenechanya, R.; Papadopoulou, B. Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0006052. [CrossRef] [PubMed]
14. Capela, R.; Moreira, R.; Lopes, F. An Overview of Drug Resistance in Protozoal Diseases. *Int. J. Mol. Sci.* **2019**, *20*, 5748. [CrossRef] [PubMed]
15. Denton, H.; McGregor, J.C.; Coombs, G.H. Reduction of anti-leishmanial pentavalent antimonial drugs by a parasite-specific thiol-dependent reductase, TDR1. *Biochem. J.* **2004**, *381*, 405–412. [CrossRef] [PubMed]
16. Wyllie, S.; Cunningham, M.L.; Fairlamb, A.H. Dual Action of Antimonial Drugs on Thiol Redox Metabolism in the Human Pathogen *Leishmania donovani*. *J. Biol. Chem.* **2004**, *279*, 39925–39932. [CrossRef] [PubMed]
17. Pan American Health Organization. *Guideline for the Treatment of Leishmaniasis in the Americas*, 2nd ed.; PAHO: Washington, DC, USA, 2022. [CrossRef]
18. Adler-Moore, J.P.; Gangneux, J.P.; Pappas, P.G. Comparison between liposomal formulations of amphotericin B. *Med. Mycol.* **2016**, *54*, 223–231. [CrossRef]
19. Piccica, M.; Lagi, F.; Bartoloni, A.; Zammarchi, L. Efficacy and safety of Pentamidine isethionate for tegumentary and visceral human leishmaniasis: A systematic review. *J. Travel Med.* **2021**, *28*, taab065. [CrossRef]
20. Lux, H.; Heise, N.; Klenner, T.; Hart, D.; Opperdoes, F.R. Ether–lipid (alkyl-phospholipid) metabolism and the mechanism of action of ether–lipid analogues in Leishmania. *Mol. Biochem. Parasitol.* **2000**, *111*, 1–14. [CrossRef]
21. Sundar, S.; Chakravarty, J. An update on pharmacotherapy for leishmaniasis. *Expert Opin. Pharm.* **2015**, *16*, 237–252. [CrossRef]
22. Santos, S.S.; de Araújo, R.V.; Giarolla, J.; Seoud, O.E.; Ferreira, E.I. Searching for drugs for Chagas disease, leishmaniasis and schistosomiasis: A review. *Int. J. Antimicrob. Agents* **2020**, *55*, 105906. [CrossRef]
23. Oliveira, S.S.; Marques, C.S.; de Sousa, D.P.; Andrade, L.N.; Fricks, A.T.; Jain, S.; Branquinha, M.H.; Souto, E.B.; Santos, A.L.; Severino, P. Analysis of the mechanisms of action of isopentenyl caffeate against Leishmania. *Biochimie* **2021**, *189*, 158–167. [CrossRef] [PubMed]
24. Marques, C.S.F.; Barreto, N.S.; Oliveira, S.S.C.; Santos, A.L.S.; Branquinha, M.H.; Sousa, D.P.; Castro, M.; Andrade, L.N.; Pereira, M.M.; Silva, C.F.D.; et al. β-cyclodextrin/isopentyl caffeate inclusion complex: Synthesis, characterization and antileishmanial activity. *Molecules* **2020**, *25*, 4181. [CrossRef] [PubMed]
25. Oliveira, S.S.; Ferreira, C.S.; Branquinha, M.H.; Santos, A.L.; Chaud, M.V.; Jain, S.; Cardoso, J.C.; Kovačević, A.B.; Souto, E.B.; Severino, P. Overcoming multi-resistant leishmania treatment by nanoencapsulation of potent antimicrobials. *J. Chem. Technol. Biotechnol.* **2021**, *96*, 2123–2140. [CrossRef]
26. Oliveira, S.S.C.; Marques, C.S.F.; Sousa, D.P.; Andrade, L.N.; Fricks, A.T.; Jain, S.; Branquinha, M.H.; Souto, E.B.; Santos, A.L.S.; Severino, P. Corrigendum to "Analysis of the mechanisms of action of isopentyl caffeate against Leishmania". Biochimie 189 (2021) 158–167. *Biochimie* **2022**, *201*, 213–214. [CrossRef] [PubMed]
27. Novais, F.O.; Amorim, C.F.; Scott, P. Host-Directed Therapies for Cutaneous Leishmaniasis. *Front. Immunol.* **2021**, *12*, 660183. [CrossRef]
28. Carvalho, S.H.; Frézard, F.; Pereira, N.P.; Moura, A.S.; Ramos, L.; Carvalho, G.B.; Rocha, M.O.C. American tegumentary leishmaniasis in Brazil: A critical review of the current therapeutic approach with systemic meglumine antimoniate and short-term possibilities for an alternative treatment. *Trop. Med. Int. Health* **2019**, *24*, 380–391. [CrossRef] [PubMed]
29. Shirzadi, M.R. Lipsosomal amphotericin B: A review of its properties, function, and use for treatment of cutaneous leishmaniasis. *Res. Rep. Trop. Med.* **2019**, *10*, 11–18. [CrossRef] [PubMed]
30. Al-Khikani, F.; Al-Janabi, A. Topical amphotericin B formulas: Promising new application. *Int. J. Med. Sci. Curr. Res.* **2019**, *2*, 187–196.
31. Falci, D.R.; Pasqualotto, A.C. Anfotericina B: Uma revisão sobre suas diferentes formulações, efeitos adversos e toxicidade. *Clin. Biomed. Res.* **2015**, *35*, 65–82. [CrossRef]
32. Bray, P.G.; Barrett, M.P.; Ward, S.A.; de Koning, H.P. Pentamidine uptake and resistance in pathogenic protozoa: Past, present and future. *Trends Parasitol.* **2003**, *19*, 232–239. [CrossRef]
33. Ribeiro, J.B.P.; Miranda-Vilela, A.L.; Amorim, A.A.S.; Garcia, R.D.; Moreira, J.R.; Gomes, C.M.; Takano, G.H.S.; de Oliveira, G.M.F.; Lima, A.V.; da Silva, I.C.R.; et al. Study of the efficacy of N-methyl glucamine antimoniate (SbV) associated with photodynamic therapy using liposomal chloroaluminium phthalocyanine in the treatment of cutaneous leishmaniasis caused by *Leishmania* (L.) *amazonensis* in C57BL6 mice. *Photodiagnosis Photodyn. Ther.* **2019**, *26*, 261–269. [CrossRef] [PubMed]
34. Hanna, E.; Abadi, R.; Abbas, O. Imiquimod in dermatology: An overview. *Int. J. Derm.* **2016**, *55*, 831–844. [CrossRef] [PubMed]
35. Fuentes-Nava, G.; Tirado-Sánchez, A.; Fernández-Figueroa, E.A.; Sánchez-Montes, S.; Becker, I.; Bonifaz, A. Efficacy of imiquimod 5% cream as first-line management in cutaneous leishmaniasis caused by *Leishmania mexicana*. *Rev. Soc. Bras. Med. Trop.* **2021**, *54*, e0305-2020. [CrossRef] [PubMed]

36. Kip, A.E.; Schellens, J.H.M.; Beijnen, J.H.; Dorlo, T.P.C. Clinical Pharmacokinetics of Systemically Administered Antileishmanial Drugs. *Clin. Pharm.* **2018**, *57*, 151–176. [CrossRef] [PubMed]
37. Santos, A.M.; Noronha, E.F.; Ferreira, L.A.; Carranza-Tamayo, C.O.; Cupolillo, E.; Romero, G.A. [Effect of a hydrophilic formulation of topical paromomycin on cutaneous leishmaniasis among patients with contraindications for treatment with pentavalent antimonials]. *Rev. Soc. Bras. Med. Trop.* **2008**, *41*, 444–448. [CrossRef] [PubMed]
38. Amer, E.I.; Eissa, M.M.; Mossallam, S.F. Oral azithromycin versus its combination with miltefosine for the treatment of experimental Old World cutaneous leishmaniasis. *J. Parasit. Dis.* **2016**, *40*, 475–484. [CrossRef]
39. Solomon, M.; Pavlotzky, F.; Barzilai, A.; Schwartz, E. Liposomal amphotericin B in comparison to sodium stibogluconate for Leishmania braziliensis cutaneous leishmaniasis in travelers. *J. Am. Acad. Derm.* **2013**, *68*, 284–289. [CrossRef] [PubMed]
40. Fattahi Bafghi, A.; Noorbala, M.; Noorbala, M.T.; Aghabagheri, M. Anti Leishmanial Effect of Zinc Sulphate on the Viability of Leishmania tropica and L. major Promastigotes. *Jundishapur J. Microbiol.* **2014**, *7*, e11192. [CrossRef]
41. Azim, M.; Khan, S.A.; Ullah, S.; Ullah, S.; Anjum, S.I. Therapeutic advances in the topical treatment of cutaneous leishmaniasis: A review. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009099. [CrossRef]
42. Santos, R.S.; Loureiro, K.C.; Rezende, P.S.; Andrade, L.N.; de Melo Barbosa, R.; Santini, A.; Santos, A.C.; Ferreira da Silva, C.; Souto, E.B.; de Sousa, D.P.; et al. Innovative nanocompounds for cutaneous administration of classical antifungal drugs: A systematic review. *J. Dermatol. Treat* **2019**, *30*, 617–626. [CrossRef] [PubMed]
43. Severino, P.; Fangueiro, J.F.; Ferreira, S.V.; Basso, R.; Chaud, M.V.; Santana, M.H.; Rosmaninho, A.; Souto, E.B. Nanoemulsions and nanoparticles for non-melanoma skin cancer: Effects of lipid materials. *Clin. Transl. Oncol.* **2013**, *15*, 417–424. [CrossRef] [PubMed]
44. Bilbao-Ramos, P.; Serrano, D.R.; Ruiz Saldaña, H.K.; Torrado, J.J.; Bolás-Fernández, F.; Dea-Ayuela, M.A. Evaluating the Potential of Ursolic Acid as Bioproduct for Cutaneous and Visceral Leishmaniasis. *Molecules* **2020**, *25*, 1394. [CrossRef]
45. Santos, A.M.d.; Noronha, E.F.; Ferreira, L.A.M.; Carranza-Tamayo, C.O.; Cupolillo, E.; Romero, G.A.S. Efeito de uma formulação hidrofílica de paromomicina tópica na leishmaniose cutânea em pacientes com contra-indicações de tratamento com antimonial pentavalente. *Rev. Soc. Bras. Med. Trop.* **2008**, *41*, 444–448. [CrossRef]
46. Aragão Horoiwa, T.; Cortez, M.; Sauter, I.P.; Migotto, A.; Bandeira, C.L.; Cerize, N.N.P.; de Oliveira, A.M. Sugar-based colloidal nanocarriers for topical meglumine antimoniate application to cutaneous leishmaniasis treatment: Ex vivo cutaneous retention and in vivo evaluation. *Eur. J. Pharm. Sci.* **2020**, *147*, 105295. [CrossRef]
47. Van Bocxlaer, K.; McArthur, K.-N.; Harris, A.; Alavijeh, M.; Braillard, S.; Mowbray, C.E.; Croft, S.L. Film-Forming Systems for the Delivery of DNDI-0690 to Treat Cutaneous Leishmaniasis. *Pharmaceutics* **2021**, *13*, 516. [CrossRef] [PubMed]
48. Kannan, S.; Harel, Y.; Levy, E.; Dolitzky, A.; Sagiv, A.E.; Aryal, S.; Suleman, L.; Lellouche, J.-P.; Michaeli, S. Nano-Leish-IL: A novel iron oxide-based nanocomposite drug platform for effective treatment of cutaneous leishmaniasis. *J. Control. Release* **2021**, *335*, 203–215. [CrossRef] [PubMed]
49. López, L.; Vélez, I.; Asela, C.; Cruz, C.; Alves, F.; Robledo, S.; Arana, B. A phase II study to evaluate the safety and efficacy of topical 3% amphotericin B cream (Anfoleish) for the treatment of uncomplicated cutaneous leishmaniasis in Colombia. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006653. [CrossRef] [PubMed]
50. Riaz, A.; Hendricks, S.; Elbrink, K.; Guy, C.; Maes, L.; Ahmed, N.; Kiekens, F.; Khan, G.M. Preparation and Characterization of Nanostructured Lipid Carriers for Improved Topical Drug Delivery: Evaluation in Cutaneous Leishmaniasis and Vaginal Candidiasis Animal Models. *AAPS Pharm. Sci. Tech.* **2020**, *21*, 185. [CrossRef]
51. Rabia, S.; Khaleeq, N.; Batool, S.; Dar, M.J.; Kim, D.W.; Din, F.U.; Khan, G.M. Rifampicin-loaded nanotransferosomal gel for treatment of cutaneous leishmaniasis: Passive targeting via topical route. *Nanomedicine* **2020**, *15*, 183–203. [CrossRef] [PubMed]
52. Celes, F.S.; Trovatti, E.; Khouri, R.; Van Weyenbergh, J.; Ribeiro, S.J.L.; Borges, V.M.; Barud, H.S.; de Oliveira, C.I. DETC-based bacterial cellulose bio-curatives for topical treatment of cutaneous leishmaniasis. *Sci. Rep.* **2016**, *6*, 38330. [CrossRef] [PubMed]
53. Alexandrino-Junior, F.; Silva, K.G.d.H.e.; Freire, M.C.L.C.; Lione, V.d.O.F.; Cardoso, E.A.; Marcelino, H.R.; Genre, J.; Oliveira, A.G.d.; Egito, E.S.T.d. A Functional Wound Dressing as a Potential Treatment for Cutaneous Leishmaniasis. *Pharmaceutics* **2019**, *11*, 200. [CrossRef] [PubMed]
54. Lalatsa, A.; Statts, L.; Adriana de Jesus, J.; Adewusi, O.; Auxiliadora Dea-Ayuela, M.; Bolas-Fernandez, F.; Dalastra Laurenti, M.; Felipe Domingues Passero, L.; Serrano, D.R. Topical buparvaquone nano-enabled hydrogels for cutaneous leishmaniasis. *Int. J. Pharm.* **2020**, *588*, 119734. [CrossRef] [PubMed]
55. Carvalheiro, M.; Vieira, J.; Faria-Silva, C.; Marto, J.; Simões, S. Amphotericin B-loaded deformable lipid vesicles for topical treatment of cutaneous leishmaniasis skin lesions. *Drug Deliv. Transl. Res.* **2021**, *11*, 717–728. [CrossRef] [PubMed]
56. Dar, M.J.; Khalid, S.; Varikuti, S.; Satoskar, A.R.; Khan, G.M. Nano-elastic liposomes as multidrug carrier of sodium stibogluconate and ketoconazole: A potential new approach for the topical treatment of cutaneous Leishmaniasis. *Eur. J. Pharm. Sci.* **2020**, *145*, 105256. [CrossRef]
57. Rajabi, O.; Layegh, P.; Hashemzadeh, S.; Khoddami, M. Topical liposomal azithromycin in the treatment of acute cutaneous leishmaniasis. *Dermatol. Ther.* **2016**, *29*, 358–363. [CrossRef]
58. Jaafari, M.R.; Hatamipour, M.; Alavizadeh, S.H.; Abbasi, A.; Saberi, Z.; Rafati, S.; Taslimi, Y.; Mohammadi, A.M.; Khamesipour, A. Development of a topical liposomal formulation of Amphotericin B for the treatment of cutaneous leishmaniasis. *Int. J. Parasitol. Drugs Drug Resist.* **2019**, *11*, 156–165. [CrossRef] [PubMed]

59. Neira, L.F.; Mantilla, J.C.; Escobar, P. Anti-leishmanial activity of a topical miltefosine gel in experimental models of New World cutaneous leishmaniasis. *J. Antimicrob. Chemother.* **2019**, *74*, 1634–1641. [CrossRef] [PubMed]
60. Berenguer, D.; Sosa, L.; Alcover, M.; Sessa, M.; Halbaut, L.; Guillén, C.; Fisa, R.; Calpena-Campmany, A.C.; Riera, C. Development and Characterization of a Semi-Solid Dosage Form of Meglumine Antimoniate for Topical Treatment of Cutaneous Leishmaniasis. *Pharmaceutics* **2019**, *11*, 613. [CrossRef] [PubMed]
61. Berenguer, D.; Alcover, M.M.; Sessa, M.; Halbaut, L.; Guillén, C.; Boix-Montañés, A.; Fisa, R.; Calpena-Campmany, A.C.; Riera, C.; Sosa, L. Topical Amphotericin B Semisolid Dosage Form for Cutaneous Leishmaniasis: Physicochemical Characterization, Ex Vivo Skin Permeation and Biological Activity. *Pharmaceutics* **2020**, *12*, 149. [CrossRef]
62. Soto, J.; Soto, P.; Ajata, A.; Luque, C.; Tintaya, C.; Paz, D.; Rivero, D.; Berman, J. Topical 15% Paromomycin-Aquaphilic for Bolivian *Leishmania braziliensis* Cutaneous Leishmaniasis: A Randomized, Placebo-controlled Trial. *Clin. Infect. Dis.* **2019**, *68*, 844–849. [CrossRef] [PubMed]
63. Sosa, N.; Pascale, J.M.; Jiménez, A.I.; Norwood, J.A.; Kreishman-Detrick, M.; Weina, P.J.; Lawrence, K.; McCarthy, W.F.; Adams, R.C.; Scott, C.; et al. Topical paromomycin for New World cutaneous leishmaniasis. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007253. [CrossRef] [PubMed]
64. El-On, J.; Jacobs, G.P.; Witztum, E.; Greenblatt, C.L. Development of topical treatment for cutaneous leishmaniasis caused by Leishmania major in experimental animals. *Antimicrob. Agents Chemother.* **1984**, *26*, 745–751. [CrossRef]
65. Veraldi, S.; Benzecry Mancin, V.; Faraci, A.G.; Nazzaro, G. Unsuccessful treatment of cutaneous leishmaniasis by Leishmania infantum with topical paromomycin. *Int. J. Dermatol.* **2020**, *59*, e251–e252. [CrossRef] [PubMed]
66. Kawakami, M.Y.M.; Zamora, L.O.; Araújo, R.S.; Fernandes, C.P.; Ricotta, T.Q.N.; de Oliveira, L.G.; Queiroz-Junior, C.M.; Fernandes, A.P.; da Conceição, E.C.; Ferreira, L.A.M.; et al. Efficacy of nanoemulsion with Pterodon emarginatus Vogel oleoresin for topical treatment of cutaneous leishmaniasis. *Biomed. Pharm.* **2021**, *134*, 111109. [CrossRef] [PubMed]
67. Zielińska, A.; Eder, P.; Rannier, L.; Cardoso, C.J.; Severino, P.; Silva, M.A.; Souto, B.E. Hydrogels for Modified-release Drug Delivery Systems. *Curr. Pharm. Des.* **2022**, *28*, 609–618. [CrossRef]
68. Francesko, A.; Petkova, P.; Tzanov, T. Hydrogel Dressings for Advanced Wound Management. *Curr. Med. Chem.* **2018**, *25*, 5782–5797. [CrossRef] [PubMed]
69. Debone, H.S.; Lopes, P.S.; Severino, P.; Yoshida, C.M.P.; Souto, E.B.; da Silva, C.F. Chitosan/Copaiba oleoresin films for would dressing application. *Int. J. Pharm.* **2019**, *555*, 146–152. [CrossRef]
70. Santos, T.S.; Santos, I.; Pereira-Filho, R.N.; Gomes, S.V.F.; Lima-Verde, I.B.; Marques, M.N.; Cardoso, J.C.; Severino, P.; Souto, E.B.; Albuquerque-Júnior, R.L.C. Histological Evidence of Wound Healing Improvement in Rats Treated with Oral Administration of Hydroalcoholic Extract of Vitis labrusca. *Curr. Issues Mol. Biol.* **2021**, *43*, 335–352. [CrossRef]
71. García-Villén, F.; Souza, I.M.S.; de Melo Barbosa, R.; Borrego-Sánchez, A.; Sánchez-Espejo, R.; Ojeda-Riascos, S.; Iborra, C.V. Natural Inorganic Ingredients in Wound Healing. *Curr. Pharm. Des.* **2020**, *26*, 621–641. [CrossRef] [PubMed]
72. Murback, N.D.N.; Hans Filho, G.; Nascimento, R.A.F.d.; Nakazato, K.R.d.O.; Dorval, M.E.M.C. Leishmaniose tegumentar americana: Estudo clínico, epidemiológico e laboratorial realizado no Hospital Universitário de Campo Grande, Mato Grosso do Sul, Brasil. *An. Bras. Dermatol.* **2011**, *86*, 55–63. [CrossRef] [PubMed]
73. de Barros, N.B.; Aragão Macedo, S.R.; Ferreira, A.S.; Tagliari, M.P.; Kayano, A.M.; Nicolete, L.D.F.; Soares, A.M.; Nicolete, R. ASP49-phospholipase A(2)-loaded liposomes as experimental therapy in cutaneous leishmaniasis model. *Int. Immunopharmacol.* **2018**, *55*, 128–132. [CrossRef] [PubMed]
74. Carneiro, G.; Aguiar, M.G.; Fernandes, A.P.; Ferreira, L.A. Drug delivery systems for the topical treatment of cutaneous leishmaniasis. *Expert Opin. Drug Deliv.* **2012**, *9*, 1083–1097. [CrossRef] [PubMed]
75. Sood, A.; Granick, M.S.; Tomaselli, N.L. Wound dressings and comparative effectiveness data. *Adv. Wound Care* **2014**, *3*, 511–529. [CrossRef] [PubMed]
76. Hissae Yassue-Cordeiro, P.; Zandonai, C.H.; Pereira Genesi, B.; Santos Lopes, P.; Sanchez-Lopez, E.; Garcia, M.L.; Camargo Fernandes-Machado, N.R.; Severino, P.; Souto, E.B.; Ferreira da Silva, C. Development of Chitosan/Silver Sulfadiazine/Zeolite Composite Films for Wound Dressing. *Pharmaceutics* **2019**, *11*, 535. [CrossRef] [PubMed]
77. Pereira, R.; Carvalho, A.; Vaz, D.C.; Gil, M.H.; Mendes, A.; Bártolo, P. Development of novel alginate based hydrogel films for wound healing applications. *Int. J. Biol. Macromol.* **2013**, *52*, 221–230. [CrossRef]
78. Meng, X.; Tian, F.; Yang, J.; He, C.N.; Xing, N.; Li, F. Chitosan and alginate polyelectrolyte complex membranes and their properties for wound dressing application. *J. Mater. Sci. Mater. Med.* **2010**, *21*, 1751–1759. [CrossRef]
79. do Nascimento, M.F.; Cardoso, J.C.; Santos, T.S.; Tavares, L.A.; Pashirova, T.N.; Severino, P.; Souto, E.B.; Albuquerque-Junior, R.L.C. Development and Characterization of Biointeractive Gelatin Wound Dressing Based on Extract of *Punica granatum* Linn. *Pharmaceutics* **2020**, *12*, 1204. [CrossRef]
80. Souto, E.B.; Yoshida, C.M.P.; Leonardi, G.R.; Cano, A.; Sanchez-Lopez, E.; Zielinska, A.; Viseras, C.; Severino, P.; Silva, C.F.d.; Barbosa, R.d.M. Lipid-Polymeric Films: Composition, Production and Applications in Wound Healing and Skin Repair. *Pharmaceutics* **2021**, *13*, 1199. [CrossRef]
81. Kim, J.O.; Park, J.K.; Kim, J.H.; Jin, S.G.; Yong, C.S.; Li, D.X.; Choi, J.Y.; Woo, J.S.; Yoo, B.K.; Lyoo, W.S.; et al. Development of polyvinyl alcohol-sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *Int. J. Pharm.* **2008**, *359*, 79–86. [CrossRef]

82. Elsner, J.J.; Zilberman, M. Novel antibiotic-eluting wound dressings: An in vitro study and engineering aspects in the dressing's design. *J. Tissue Viability* **2010**, *19*, 54–66. [CrossRef]
83. Diniz, F.R.; Maia, R.; Rannier, L.; Andrade, L.N.; Vinicius Chaud, M.; da Silva, C.F.; Corrêa, C.B.; de Albuquerque Junior, R.L.C.; Pereira da Costa, L.; Shin, S.R.; et al. Silver Nanoparticles-Composing Alginate/Gelatine Hydrogel Improves Wound Healing In Vivo. *Nanomaterials* **2020**, *10*, 390. [CrossRef] [PubMed]
84. Oliveira, D.M.L.; Rezende, P.S.; Barbosa, T.C.; Andrade, L.N.; Bani, C.; Tavares, D.S.; da Silva, C.F.; Chaud, M.V.; Padilha, F.; Cano, A.; et al. Double membrane based on lidocaine-coated polymyxin-alginate nanoparticles for wound healing: In vitro characterization and in vivo tissue repair. *Int. J. Pharm.* **2020**, *591*, 120001. [CrossRef]
85. Yoshida, C.M.P.; Pacheco, M.S.; de Moraes, M.A.; Lopes, P.S.; Severino, P.; Souto, E.B.; da Silva, C.F. Effect of Chitosan and Aloe Vera Extract Concentrations on the Physicochemical Properties of Chitosan Biofilms. *Polymers* **2021**, *13*, 1187. [CrossRef] [PubMed]
86. Pereira, V.; de Barros, N.B.; Macedo, S.R.A.; Dos Santos Ferreira, A.; Kanis, L.A.; Nicolete, R. Drug-containing hydrophobic dressings as a topical experimental therapy for cutaneous leishmaniasis. *J. Parasit. Dis.* **2020**, *44*, 79–87. [CrossRef]
87. Goyal, R.; Macri, L.K.; Kaplan, H.M.; Kohn, J. Nanoparticles and nanofibers for topical drug delivery. *J. Control. Release* **2016**, *240*, 77–92. [CrossRef] [PubMed]
88. Tchemtchoua, V.T.; Atanasova, G.; Aqil, A.; Filée, P.; Garbacki, N.; Vanhooteghem, O.; Deroanne, C.; Noël, A.; Jérome, C.; Nusgens, B.; et al. Development of a Chitosan Nanofibrillar Scaffold for Skin Repair and Regeneration. *Biomacromolecules* **2011**, *12*, 3194–3204. [CrossRef]
89. Alishahi, M.; Khorram, M.; Asgari, Q.; Davani, F.; Goudarzi, F.; Emami, A.; Arastehfar, A.; Zomorodian, K. Glucantime-loaded electrospun core-shell nanofibers composed of poly(ethylene oxide)/gelatin-poly(vinyl alcohol)/chitosan as dressing for cutaneous leishmaniasis. *Int. J. Biol. Macromol.* **2020**, *163*, 288–297. [CrossRef] [PubMed]
90. Saleem, K.; Khursheed, Z.; Hano, C.; Anjum, I.; Anjum, S. Applications of Nanomaterials in Leishmaniasis: A Focus on Recent Advances and Challenges. *Nanomaterials* **2019**, *9*, 1749. [CrossRef] [PubMed]
91. Monteiro, L.M.; Löbenberg, R.; Fotaki, N.; de Araújo, G.L.B.; Cotrim, P.C.; Bou-Chacra, N. Co-delivery of buparvaquone and polymyxin B in a nanostructured lipid carrier for leishmaniasis treatment. *J. Glob. Antimicrob. Resist.* **2019**, *18*, 279–283. [CrossRef] [PubMed]
92. Dar, M.J.; Din, F.U.; Khan, G.M. Sodium stibogluconate loaded nano-deformable liposomes for topical treatment of leishmaniasis: Macrophage as a target cell. *Drug Deliv.* **2018**, *25*, 1595–1606. [CrossRef] [PubMed]
93. Kavian, Z.; Alavizadeh, S.H.; Golmohamadzadeh, S.; Badiee, A.; Khamesipour, A.; Jaafari, M.R. Development of topical liposomes containing miltefosine for the treatment of Leishmania major infection in susceptible BALB/c mice. *Acta Trop.* **2019**, *196*, 142–149. [CrossRef] [PubMed]
94. Peralta, M.F.; Usseglio, N.A.; Bracamonte, M.E.; Guzmán, M.L.; Olivera, M.E.; Marco, J.D.; Barroso, P.A.; Carrer, D.C. Efficacy of topical Miltefosine formulations in an experimental model of cutaneous leishmaniasis. *Drug Deliv. Transl. Res.* **2022**, *12*, 180–196. [CrossRef] [PubMed]
95. Dar, M.J.; Khalid, S.; McElroy, C.A.; Satoskar, A.R.; Khan, G.M. Topical treatment of cutaneous leishmaniasis with novel amphotericin B-miltefosine co-incorporated second generation ultra-deformable liposomes. *Int. J. Pharm.* **2020**, *573*, 118900. [CrossRef]
96. Khalid, S.; Salman, S.; Iqbal, K.; Rehman, F.u.; Ullah, I.; Satoskar, A.R.; Khan, G.M.; Dar, M.J. Surfactant free synthesis of cationic nano-vesicles: A safe triple drug loaded vehicle for the topical treatment of cutaneous leishmaniasis. *Nanomed. Nanotechnol. Biol. Med.* **2022**, *40*, 102490. [CrossRef] [PubMed]