MEDDOCS
International

Animal science

Leishmaniasis

Corresponding Author: Azar Shokri

Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran.

Tel: 0098-05832297182;

Email: azar_sh1969@yahoo.com

Published Online: Jul 08, 2021

eBook: Animal science

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abstract

Leishmaniasis is a common parasitic disease in tropical and subtropical regions of the world that is caused by about 21 species and subtypes of Leishmania parasites [1]. Leishmania species are present on all continents except Australia and Antarctica. The disease is found in 98 countries except for South Asia. The disease is transmitted through the bite of sandflies and infects humans and other mammals. It is estimated that 12 million people are infected with the parasite and the annual incidence is 0.2 to 0.4 million visceral cases and 0.7 to 1.2 million skin and 350 million people are at risk [2,3]. Various forms of the disease including cutaneous, mucocutaneous, and visceral form which can be fatal if left untreated or improperly treated [4].

Introduction and classification of leishmania parasite

The Leishmania parasite belongs to the Trypanosomatidae family and belongs to the Protista family. The classification of parasites (position of parasites in the animal hierarchy) is as follows:

Table 1: Classification of Leishmania parasite.

Kingdom	Protista	Haekel, 1866
Subkingdom	Protozoa	Goldfuss, 1817
Phylum:	Sarco astigophora	Honigberg and Balamuth, 1963
Subphylum:	Mastigophora	Diesing, 1866
Class:	Zoo mastigophora	Calkins, 1909
Order:	Kinetoplastida	Honigberg1963, Emend Vickerman 1976
Suborder:	Trypanosomatina	Kent, 1880
Family:	Trypanosmatidae	Doflein, 1901, Emend Grobben, 1905
Genus:	Leishmania	Ross, 1903

Different forms of leishmaniasis

Leishmaniasis occurs in humans in three main forms, including the skin, mucous membranes, and viscera. Different forms of the disease are classically caused by a wide variety of Leishmania species. Depending on the specific characteristics of the genus and species and the immune status of the host, clinical forms may take many forms. For example, "species that cause cutaneous leishmaniasis cause visceral form, or species that cause visceral leishmaniasis may cause skin damage. Leishmania infantum reports of skin forms in Mediterranean countries such as Italy, Tunisia, Turkey, etc. There have also been reports of *Leishmania tropica* developing visceral form [5]. Some species of Leishmania in endemic regions of the world are presented simultaneously so that clinical findings alone cannot be relied on for species-specific diagnosis [6].

Treatment methods for various types of leishmaniasis

Before explaining the treatment of leishmaniasis, we summarize the potential drug targets that are important in designing anti-leishmania drugs.

Design of anti-leishmaniasis drugs

The first step in achieving new anti-leishmania compounds is to identify the drug targets. For this purpose, it is necessary to know the biological pathways in the parasite. Fortunately, the Trypanosomatidae family is phylogenetically isolated from higher cells, so their cell configuration is distinctly different from that of mammalian cells, making it easy to find unique drug targets in them. It is important to consider the targets, to consider the biochemical properties of the protein molecules, and specific inhibitors can be designed if the target is a protein enzyme so that inhibition of the relevant enzyme kills the parasite.

Citation: Shokri A, (2021). Leishmaniasis. Animal Science, MedDocs Publishers. Vol. 1, Chapter 4, pp. 39-51.

Sterol biosynthesis pathway

Sterols are important constituents of cell membranes and are crucial for maintaining cell structure. Unlike mammalian cells, where cholesterol is the major component of cell membranes, Trypanosomatide produces ergosterol and other 24 methyl sterols that are essential for their growth and development. These sterols are not present in mammalian cells. An important enzyme in this pathway is Squalene Synthetase (SQS) (EC 2.5.1.21), which performs the first step in sterol synthesis by pairing two molecules of franyl and squalene synthesis. Zaragonic acid and quinoclidines inhibit this enzyme. Terbinafine and allylamine inhibit squalene epoxy. Squalene is converted to 2 and 3 oxidoqualene by the enzyme squalene epoxide. The enzyme converts squalene chains into the tetracyclic skeleton of sterols. Terbinafine has been shown to inhibit the growth of amastigotes and promastigotes of the Leishmania parasite, which is caused by the altered mitochondrial structure. This effect has a synergistic effect in combination with Ketoconazole, which is an inhibitor of ergosterol biosynthesis. An important and well-known drug target in the pathway of ergosterol biosynthesis is delta 24 and 25 Sterol Methyl Transferase (SMT), which is present only in the Trypanosomatide family and is not present in mammals, so it can be a suitable drug target in leishmaniasis. Azoestrols inhibit the enzyme and exert their inhibitory effect by changing the mitochondrial membrane and its swelling and loss of matrix contents. The combination of azoestrol with other azoles causes a synergistic effect and should be considered in combination therapy.

Enzyme lanosterol 14-alpha dimethylase

Azole compounds that inhibit sterol biosynthesis by inhibiting the enzyme lanosterol 14-alpha-demethylase have been shown to potentially inhibit the growth of leishmaniasis in the body. The enzyme lanosterol 14-alpha-demethylase or CYP51 is a key enzyme in the sterol biosynthesis of plasma membranes of the eukaryotic cells. This enzyme removes the methyl carbon group of 14 lanosterol molecules, which is an important step in the conversion of lanosterol to sterol for the plasma membrane. Accordingly, compounds that can inhibit this enzyme and this stage of the sterol production pathway can potentially prevent membrane formation and ultimately parasite growth. At present, the nucleotide sequences of the genomes of 13 species of the genus Leishmania are being identified, and the genome sequences of several species, including L. braziliensis, L. infantum and L. major, have been completed. Studies of these sequences have shown that this parasite has the necessary genes for sterol biosynthesis, including the lanosterol 14-alpha dimethylase enzyme gene [7]. However, for the design and synthesis of lanosterol-14-alpha-demethylase inhibitor molecules, the structural form and three-dimensional structure of this enzyme are absolutely essential. On the other hand, to date, the three-dimensional structures known for the enzyme lanosterol 14-alpha-demethylase is belonging to fungi. Fortunately, in 2011 the crystallographic structure of the enzyme lanosterol 14-alpha-demethylase was identified by *L. parantum*, but a comparison of the initial sequence of this enzyme in *L. infantum* and *L.major* reveals important differences in the structure of this enzyme. Conclusion: The susceptibility of the species is different due to the difference in the activity of C14α-lanosterol demethylase due to the enzymatic structure. Similarly, flavanones exhibit a wide range of activities against enzymes [8]. Glycolytic pathway: The metabolism of the Trypanosomatidae family relies heavily on the carbon sources present in the host. Glycolytic enzymes are located in proximal-like organelles. Due to the phylogenetic distance of the *Leishmania* parasite and mammals, this structure is quite unique. This property can be considered in the design of inhibitors of these enzymes as anti-leishmaniasis compounds. Adenosine is a weak inhibitor of the enzyme, but its analogue affects its anti-enzymatic effects by being substituted at the 2-position of ribose or at the 6-nitrogen position of adenosine. One of the adenosine analogues, N6- (1-naphthalenemethyl) -20- (3-methoxybenzamido) inhibits the growth and proliferation of *Leishmania mexicana* at a concentration of 0.28 μM [9]. This study shows that the energy uptake pathway through glycolytic enzyme inhibitors can be blocked. Because this pathway is the only way to receive energy, this method can lead to the destruction of the parasite. Purine Receipt Path: Because the Trypanosomatidae family is unable to make the purine nucleotides they need; they receive these substances from their mammalian host. Purine bases are transported through the parasite membrane by transporter nucleotides. These transporters are involved in transmitting purines through parasite membranes, and two types, including LdNT1 in promastigotes and amastigotes and LdNT2 in amastigotes, are specific to *Leishmania* parasites [10]. LdNT1 is responsible for the transport of adenosine and pyrimidine nucleosides, and LdNT2 is responsible for the transport of pyrine nucleosides. The function of these transporters is essential for parasite survival within human macrophages. Because there are different pathways for purines to be obtained, it is very difficult for the inhibitor to target them. However, they are pharmacologically important because they absorb toxic nucleoside analogs that inhibit cell growth [10].

Glycosyl Phosphatidyl Inositol (GPI) pathway

This compound is a component of the *Leishmania* parasite that acts as an anchor for surface glycoproteins. *Leishmania* promastigotes are coated with glycocalyx, which contains GPI-linked glycoproteins and GPI-linked lipophosphoglycans, and a group of free GPIs called high-density Glycinoinositol Phospholipids (GIPLs). The role of these compounds is to protect the parasite from the complement pathway and external hydrolase. Lipophosphoglycan is essential for the infectivity of *Leishmania major* promastigotes in both mammalian and insect cells [11]. The first step in GPI synthesis is the formation of GlcNAc-PI by the transfer of N-acetylglucosamine from UDP-GlcNAc to UPN-acetylglucosamine and binding to the phosphatidyl inositol PI. The reaction is catalyzed by the protein transferase complex: (GPI-GnT) GPI-N-Acetylglucosaminyl transferase then GlcNAc-PI is obtained by the desaccation of GlcN-PI by the enzyme decylase. This pathway is essential for GPI biosynthesis, and in trypanosomes a zinc metalloenzyme is involved in the reaction [11,12]. As in *Trypanosoma brusei* and *Leishmania major*, a subtle specificity has been observed in the substrate, which is of a specific mammalian type [13]. The difference between GPI of parasites and mammals in the time of acylation and deacylation of inositol is between them. The next step in GPI biosynthesis in parasites is mannosylation of GlcN-PI and continues with inosyl acylation, while in yeasts and humans inosyl acylation precedes mannosylation. The nucleus of the three mannosyl GPIs is amplified in three steps and involves three different Mannosyl Transferase (MT) enzymes. Type III of this enzyme (MT III) has a specific substrate and the structure of this substrate is different in humans and trypanosomatidae parasites and a specific inhibitor of the species can be synthesized for inhibitory purposes [14]. Thiolactomycin is an inhibitor of fatty acid synthesis in the parasite *Leishmania* and leads to parasite death. Myristate-

specific deformation has also been reported for parasitic GPIs [15]. Protein kinases: Cyclin Dependent Kinases (CDKs) play an important role in cell division. It has been observed that abnormal regulation of this enzyme occurs in cancer cells and serves as a drug target. In the *Leishmania* parasite, the Cdc2-Related Kinase (CRK) family has received a great deal of attention as a drug target. These are homologues of CDKs and are essential for the continued life cycle of the parasite. Two types of these CDKs have been identified in *Leishmania*, Mexico, called LmexCRK1 and LmexCRK3, which are essential for the parasitic stage of the parasite. Attempts to create null mutants from CRK3 have led to changes in the parasite ploidy [13]. Inhibition of CRK3 in *Leishmania donovani* inhibits parasite growth and proliferation within peritoneal macrophages. These compounds also cause abnormal DNA and abnormal cell morphology, which has been proven by flow cytometry [16]. MAP kinases Mitogen-Activated Protein (MAP) kinases are mediators of message transmission and play an important role as regulators in the proliferation and differentiation of eukaryotic cells. Ten of these kinases have been identified in *Leishmania*, Mexico, where LmxMKK, LmxMPK, LmxMPK9 have been well studied [17], of which only LmxMPK can be a drug target [24].

Proteinases The four most important types of this enzyme are cystine, serine, aspartate, and metalloproteins. In parasitic protozoa, Cystine Proteinase (CPs) is the most well-known, which is homologous to mammalian cathepsin. For several reasons, cysteine proteinases have attracted much attention as drug targets: Significance of parasite-host interaction 2. Approved virulence factor and 3. Separate mammalian type [15]. Folate biosynthesis. The folate route is of interest to researchers as a drug target and has been used in anti-cancer and anti-malarial therapies. Folate is an important cofactor used in a wide range of metabolic pathways such as DNA and RNA synthesis and amino acid metabolism. Because the enzymes involved in their synthesis are vital for growth, they are considered as medicinal targets There have been many, especially Thymidine Synthetase (TS) and Dihydrofolate Reductase (DHFR), which convert dihydrofolate to tetrahydrofolate (an important cofactor for thymine synthesis). These two enzymes are present in trypanosomatidae. In studies of *Leishmania major*, some inhibitors inhibited these two enzymes and prevented the growth of the parasite. These studies have shown that these two are effective inhibitors and inhibit both enzymes simultaneously [18].

Glyoxalase system: This system is necessary for the removal of toxins and mutagenic and methylglyoxal intermediates (glycolysis by-products). This system has two important enzymes: glyoxalase I (lactoyl glutathione lyase) and Glyoxalase II (hydroxyacyl glutathione hydroxylase), which use glutathione as a cofactor. Trypanosomatida uses its own Glyoxalase system and relies on it, which is unique to these parasites. glyoxalase I is known in Leishmania donovani [19] and Leishmania major [20]. The enzyme has its own substrate and is dependent on trypanothione as a substrate while in the mammalian host it relies on glutathione. Glyoxalase II has been identified in Leishmania donovania [21]. Due to its unique properties as a possible drug target, this system has attracted more attention and research.

Trypanothione route

In the metabolism of *Leishmania* and *Trypanosoma* parasites, spermidine (trypanothione bis- glutathionyl) is a key molecule in the oxidative stress pathway, a parasite-specific enzyme that is critical to its survival, so this pathway is a promising drug target [20].

Topoisomerases

DNA topoisomerases are unique enzymes that play a key role in some essential steps such as DNA replication, translation, synthesis, and regeneration. They are divided into two important groups, type I and type II, which separate single-stranded and double-stranded DNA. These enzymes have been used for medicinal purposes in bacteria and parasites. Topoisomerase I in the parasite *Leishmania donovani* and *Trypanosoma cruzi* is known and not dependent on ATP [22]. This enzyme is present in *Leishmania donovani* in kintoplasts and nuclei (23). Topoisomerase I inhibitors in *Leishmania* parasitic drugs are sodium leishmaniasis, acetaminophen and acetaminophen. A plant alkaloid called Camptothecin, which inhibits topoisomerase I in eukaryotes, also has an inhibitory effect on the parasites *T. brucei, T. cruzi and L. donovani*.

Topoisomerase II in *T. brucei, T. cruzi* and *L. donovani* parasites has been identified [25,26]. Topoisomerase II in *Leishmania* and *Trypanosoma* products is strongly inhibited by 9-anilinoacridine, which is anti-cancer, and all acridines [27]. The three isoflavanoids 8-prenylmucronulatol, lyasperin H and smiranicin have anti-leishmaniasis activity that kill the parasite by preventing kDNA linearization and inhibition of topoisomerase II [28]. Structural analysis of these enzymes can lead to an understanding of their catalytic mechanisms and ultimately the design of new anti-Leishmania drugs.

Hypusine pathway

Hypusine (Ne- (4-amino-2-hydroxybutyl) lysine) is an unusual amino acid derived from polyamine spermidine present in all eukaryotes. Its synthesis occurs during a post-translational process exclusively in a cellular protein called eukaryotic initiation factor 5A (eIF5A) in two enzymatic steps. Hypocyanation of eIF5A is essential for cell function and survival. Recently, "hyposin biosynthesis has been identified in *Leishmania donovani* [29]. This pathway can be considered as a drug target.

Treatment of cutaneous leishmaniasis: Cutaneous leishmaniasis caused by *Leishmania major* sometimes resolves spontaneously and even preferably is not "treated" to provide long-term immunity. *Leishmania tropica* infections usually require treatment. Among the traditional treatments that can be mentioned are the use of the kernel of the plant Floss and Enzrut, burnt cotton bolls, cherry and date kernel ash, burnt hair, dandruff ink (nitric acid) and hell stone in Iran. The World Health Organization does not recommend the treatment of wet forms unless the lesions are more than 2, the size of the lesion is more than 5 cm, or in the face and near vital organs of the body. Of course, this advice is not practical in most cases because the patient or his parents want to treat the disease [30]. In general, three methods have been suggested for the treatment of cutaneous leishmaniasis: physical methods, topical drugs, systemic drugs Physical methods: Among the physical methods used to treat leishmaniasis are: Lesion curettage: In this method, an attempt is made to shave the entire lesion to its bed and completely "separate" it, but today, due to the scar, this method is almost "left out". Radiation therapy: In some areas, including Palestine, granz rays have been used to treat cutaneous leishmaniasis, but given the risks of tumorigenesis, this radiation is not very suitable for benign lesions and is mostly used for large and resistant lesions [31].

Thermotherapy: Thermotherapy is a simple and cost-effective method that can increase the effectiveness of antimony

compounds and improve treatment outcomes. The potential mechanism in this method is to increase the activity of macrophages and lymphocytes at a temperature of 37-40 ^{o}C to kill parasites and direct destruction of parasites by heat. Leishmania parasites are said to lose their ability to reproduce at temperatures above 39^{o}C; There are also other reports that support the proper effectiveness of this treatment. There are many different treatments for thermotherapy in studies, including the topical application of a pad soaked in hot water with a temperature of 39-41 degrees on the lesions, the use of infrared waves for 6-He mentioned 5 weeks to stimulate the immune response, create hyperthermia with ultrasound waves and use direct current. But arguably the biggest breakthrough in thermotherapy came with the introduction of local current field radiofrequency devices. These devices were approved in 2558 by the US Food and Drug Administration (FDA) for the treatment of leishmaniasis. Disadvantages of thermotherapy include the need for local anesthesia, the high cost of devices, and side effects such as grade 2 superficial burns, painless edema and blisters for a week, and secondary bacterial infections [32-34].

Cryotherapy: Cryotherapy is not a new treatment for cutaneous leishmaniasis. In a study in 1892, cryotherapy using CO_2 in 85 patients improved 100% without any scarring. It was later found that liquid nitrogen had similar effects. For cryotherapy, cotton swabs should be prepared according to the size of the lesions before treatment, and the therapist must wear gloves and glass to protect himself; then pour enough liquid nitrogen into a disposable glass and hold a cotton swab for a few seconds until completely soaked. Then, swap quickly on the lesion and press for 15 seconds until the lesion turns white. This should be done on the entire surface of the lesion up to a millimeter of healthy skin around it to whiten the entire lesion. This method is superior to cryospray. In the cryospray method, with the help of a spray device, liquid nitrogen is sprayed on the lesion from a distance of 1 to 2 cm, so that, like the previous method, the entire lesion is whitened with a margin of 2 mm from the surrounding healthy skin for 10 seconds. This method is more effective on primary papular lesions and has little effect on inflammatory and wound lesions, and in addition, the presence of specialized staff is essential in this case [35].

laser therapy: The first report of laser treatment of cutaneous leishmaniasis was published in Russia in 1970. Although the use of destructive lasers, especially CO_2 and non-destructive lasers such as erbium glass, in the treatment of leishmaniasis scars has been relatively successful, the CO_2 laser has been used many times for topical treatment of leishmaniasis and rarely causes complications in patients.

CO_2 laser kills tissues and thermophilia (thermolysis) kills macrophages and parasites. Various reports have been published on the effectiveness of this method. For the treatment of dry cutaneous leishmaniasis, CO2 laser has shown higher efficiency and faster healing rate in just one treatment session compared to the combined method of cryotherapy with intralesional injection of glucantime [36].

Photodynamic therapy: Elimination of amastigote and promastigote forms of Leishmania parasite with the help of light is a new technique for the treatment of cutaneous leishmaniasis. Use of photosensitizers such as aluminum phthalocyanine chloride Aluminum phthalocyanine chloride (can make photodynamic therapy more effective. It seems that the high adsorption of the negatively charged parasite membrane and the high amount of single oxygen along with the immunomodulatory effects can be the main factors in increasing the effectiveness of photodynamic therapy in the presence of photosensitizing drugs [36].

Topical chemical treatments for cutaneous leishmaniasis: The most important topical treatments used in this disease are:

Interferon-gamma: Interferon-gamma injections have been used to treat leprosy, some cancers, AIDS, and Granulomatous Chronic Disease (CGD). In laboratory studies, the use of this compound has been shown to increase the lethal capacity of the leishmaniasis parasite in human monocytes *in vitro*. Complications of interferon include injection site pain, which is mostly mild, and headache has been reported [36].

Metronidazole: Intralesional metronidazole was the first line of treatment for trichomoniasis and was later used for ambiosis and giardiasis.

Intralesional injection of 5-valent antimony: This method of treatment has been proposed by the World Health Organization. In this procedure, 1-3 ml of glucantime is injected around the wound 2-3 times a week, depending on the extent of the lesion. The drug should be injected into the dermis so that the area around the lesion is completely white. The drug should be applied in different directions to the wound margin with a fine needle number 27 or 85 at an angle (at a 45-degree angle) so that the tip of the needle is up and The center of the lesion is intact, at the border of the healthy west and at the beginning of induration at the periphery of the lesion. It is injected into the upper-middle area of the lesion dermis. “The effectiveness of this treatment depends on the therapist’s technical skill, and one of the main causes of treatment failure is incomplete infiltration of the lesion during injection. Therefore, the Dermojet device, which is an efficient and safe method, can be used to inject these drugs into the lesion. Symptoms improve after 3-4 injections, but treatment may be continued for 7-10 weeks, which firstly “shortens the course of the disease and secondly” reduces the leishmaniasis in the lesion. This method can be used to treat non-injured nodular lesions. It is difficult to inject medicine with this method in children, as well as injections in the nose and eyelids. The first complication of topical treatment with pentavalent derivatives of antimony is that direct injection of these drugs into the lesion and its infiltration leads to severe pain and can even lead to vasovagal shock. Infusion pain and itching, itching and inflammation, other complications of injection treatment. They are inside the lesion. In 15% of cases, local tenderness is seen at the injection site and significant edema and erythema develop at the site. In this case it is recommended to stop the injection [37].

Topical paromomycin ointment: Paromomycin, or aminocidin, is a broad-spectrum aminoglycoside derivative (AML) that has no antiparasitic effects on other aminoglycosides. Paramomycin inhibits protein synthesis by binding to 16S rRNA in Kenya in 1990 against visceral type and in 1996 in the treatment of cutaneous type. The required dose of the drug for topical treatment varies between two to three times a day for 10 to 30 days. Although topical paromomycin may be effective in the treatment of ulcerative lesions caused by *L. Major*, its topical forms have shown different therapeutic results in terms of improvement in cutaneous leishmaniasis. There was a position. The use of an ointment containing 15% paramomycin sulfate in 12% methyl benzotonium chloride dissolved in white Vaseline based on the treatment of skin lesions caused by *Leishmania infantum, Leishmania mexicanana, Leishmania enriettii* (*L.*

Enriettii) and to a lesser extent is effective against Leishmania. However, it is recommended to use topical parmomycin as an acceptable alternative treatment in cases where glucantime cannot be used. In another study, paramomycin ointment and metronidazole cream were used [37]. Recently, 15% paramomycin ointment and 0.5% gentamicin ointment have been shown to be effective in treating the skin form caused by *Leishmania major* [38]. Researchers also believe that topical application of the liposomal form of paromomycin, which has been tested experimentally *in vivo* It can promise effective treatment for cutaneous leishmaniasis [36]. Side effects and side effects of paromomycin treatment have been reported to be mild and tolerable in all studies, and have been most common in most studies of erythema and redness. Adverse effects of this drug in studies include: inflammation, itching, burning, edema, discharge and local pain, which has a very low prevalence and is mild and tolerable. Miconazole ointment: It is a group of imidazole compounds that has recently been used in the treatment of cutaneous leishmaniasis, 2% cream of this drug has been used twice a day for two weeks in the treatment of cutaneous leishmaniasis, which has had good results [39]. Chlorpromazine ointment: This drug has been used in the treatment of diffuse leishmaniasis. The only possible complication of this method is photosensitivity, which can also be prevented by closing the lesion [40].

Quinacrine Solution (Atebrin): Infiltrates around the wound as a 5-10% solution. This is done every 3 to 5 days with a total of three injections [41].

Imiquimod: It is one of the regulatory factors of the immune system. It is a potent inducer of cytokines, INF_ɣ and TNF. It also kills the leishmaniasis parasite by activating macrophages and releasing nitric oxide. 5% ointment under the brand name Aldaras®, which is also used in the treatment of genital warts, has been used daily for 20 days on skin leishmaniasis lesions, but due to its high cost, it cannot be used in poor countries [37]. A randomized controlled clinical trial conducted for New World leishmaniasis found that in all patients treated with Imiquimod alone, the disease recurred after discontinuation of the disease, but in all patients combined with Imiquimod topical 5 Percentage and systemic glucantime were treated, the disease was completely cured. However, reports on the performance of this drug do not seem to be sufficient to reach a comprehensive conclusion and further studies are needed. Injection of intramuscular injection of leukemia [42] Berberine acid sulphate alkaloid: Concentration of 1.8000 This alkaloid kills Leishmania parasite [43].

Systematic treatments in cutaneous leishmaniasis

Antimony salts (antimonials): pentavalent compounds of antimony, glucantime (meglumine antimonate), pentostam (sodium acetylgluconate), methylgluclemine antimonate, fuadine and tartaramatic, etc. are among the best drugs for us so far. Have been identified and used. Two important drugs in this category include:

Glucantime: It is one of the 5 valence compounds of antimony and is the most common drug used today in the treatment of cutaneous and even visceral leishmaniasis. The pentavalent antimony salt (Sb) v in these drugs has a more lethal effect on amastigotes compared to the sugar part of these drugs, but the mechanism of this effect and its biochemical activity are not fully understood [44]. Pentavalent antimony compounds. They should be reduced to a trivalent form to have a toxic effect against the parasite. Glucantime is in the form of 5 ml ampoules containing 1.5 g of the active substance (methylglucamine antimony). 4 to 3 mg/kg body weight) daily or every other day for 10 to 15 doses, in which case the treatment program can be repeated after a break of two to four weeks. In the form of leishmaniasis tuberculosis, a mixture Glucantime and corticosteroids (5 to 15 mg prednisolone acetate) are used as topical injections. The total frequency of these drugs is 10 to 15 injections, which are given once or twice a week. Prednisolone acetate may cause atrophy. When taking Glucantime, care should be taken that the drug is fresh and that its original color is z The trace of amber is not lost or discolored [37,45,46]. In the human body, antimony compounds are reduced to a trivalent form that inactivates enzymes with the sulfhydryl group present in the parasite. *In vitro* studies have shown that exposure to *L. tropica* promastigotes by trivalent antimony reduces glucose uptake into the Krebs cycle, leading to the accumulation of glycolysis by-products and some end products that may prevent the activity of enzymes in the krebs cycle, therefore, it seems that antimony salts prevent the first stages of glycolysis and interrupt the activity of some enzymes in the Krebs cycle, which ultimately reduces energy production by the parasite (Also, the use of pentostam on mouse macrophages infected with *Leishmania tropica* parasite has been shown to have an effect on the mitochondrial membrane of the cell and prevent the proper functioning of the macrophage cell membrane by affecting macrophages infected with the parasite [47].

Side effects reported so far include allergies, localized muscle aches, and changes in the electrocardiogram. This drug should be avoided in people with myocarditis, nephritis and hepatitis [46,48]. Of course, at present, this drug is widely used in our country. According to some reports, after taking this drug, the recovery was up to 94% in patients [49]. Generally, people who suffer from chronic forms of ulcers that do not respond to treatment, as well as patients with visceral leishmaniasis, respond less to this drug and it is better to use it in combination with other drugs to get better results. Pentostam: It is one of the compounds that is more common in American countries. The drug is in the form of a white powder containing 36% of pentavalent antimony, which is completely soluble in water and stable in the dark, but decomposes due to light and loses its effect. The drug is in solution, inside the ampoule, and Each ampoule contains 190 mg, which is less toxic than other pentavalent substances recently used to treat leishmaniasis [50]. The mechanism of action of pentostam on *Leishmania tropica* is not exactly known, and in visceral form it appears to have a direct toxic effect on macrophages that carry the parasite [47]. Recently, when liposome beads have been used to deliver drugs to different parts of the body, liposome attachment to antimycotic drugs has been used in the treatment of leishmaniasis. Is alon [37]. Dosage and method of injection: The maximum dose is 600 mg daily for 10 days as an intramuscular injection. The manufacturer also recommends a dose of 17-28 mg per kilogram of body weight daily for 10 days and, if necessary, repeat it after a rest period of 15 days [47]. Although the drug can be administered intramuscularly, it may be locally painful and the intravenous route is preferred when the volume to be injected is large. Intravenous thrombosis has been reported during intravenous injection of pentostam, which may be reduced by more precise intramuscular injection. There have been reports of bursitis following intravenous injection of pentostam at 17 mg/kg [50]. If the skin lesion due to leishmaniasis is small, it can be used as a topical injection. Some believe that the use

of pentostam in higher doses and for a longer time or with or without allopurinol or cold therapy may be effective in treating lupus type leishmaniasis. It is given in successive doses: 20 days for cutaneous leishmaniasis, 28 days for visceral and mucosal leishmaniasis, and in cases of recurrence the same dose should be repeated twice as long as the previous treatment [50]. Side effects of the drug: Although at first there are few side effects, but the likelihood of side effects increases with the accumulation of doses. The most common effects are gastrointestinal symptoms, fever, rash, hemolytic anemia. Liver, kidney and heart damage are rare [51].

Glucantime treatment failure: Although there are no epidemiological studies on the susceptibility of strains of the parasite, failure to treat leishmaniasis, especially *L. infantum*, is very common. For example, in a report from Spain, more than 50% of patients had the first failed treatment. The causes of treatment failure are: 3- Host safety response 2- Differences in the activity of the compound or drug used 2- There is a difference in the susceptibility of the parasite strains [52]. Tartarmatic: In 1995, the Brazilian scientist Gaspar Vienna determined the potential value of the drug against leishmaniasis, and despite its toxicity, tartarmetic was considered the standard drug for the treatment of all forms of leishmaniasis. Tartaramethic has also been widely used in the treatment of Leishmania infantum infections and *Leishmania tropica* wounds [53,54]. Stibofen: The pronounced toxicity of tartaramethics led to research into low-risk derivatives, and acetaminophen, a trivalent antimony, was widely used until the advent of pentavalent compounds, and its greater effectiveness in the treatment of cutaneous leishmaniasis than visceral leishmaniasis was determined. However, its side effects are major forms and are currently rarely used despite pentavalent antimonial [54,55]. Neostasis: It is a derivative of estibanilic acid and is relatively non-toxic and has been used in the treatment of leukemia and visceral leishmaniasis caused by Leishmania infantum [56]. Fouadin: It is one of the trivalent compounds of antimony, which is less used today due to its high toxicity. This drug is used in the form of a 5 ml ampoule containing 8.5 mg of active ingredient in 1.1 ml. This compound is also used in the treatment of trypanosomiasis. It has been reported that regular use of this drug in the amount of 8-10 injections in the treatment of leishmaniasis has not had satisfactory results in more than 40% of cases [53].

Miltefosine or (hexadecylphosphocholine): It is an anti-cancer drug that was introduced in 1980 and was discarded very quickly due to its high toxicity to blood cells. It was tested in 1992 as an anti-leishmaniasis drug and had excellent results. Then it was prescribed in the amount of 50-100 mg/body weight/day for 28 days for the treatment of cutaneous leishmaniasis. Despite side effects such as gastrointestinal problems, increased transaminases and creatinine, spelling problems, and eventually miscarriage, the drug was well tolerated by the patient. This drug was tested as a 6% topical ointment in Iran and showed 90% improvement in cutaneous leishmaniasis caused by *Leishmania major* [37]. Aromatic diamides These drugs are derivatives of benzoic acid and their use shows different results in success in the treatment of leishmaniasis. Pentamidine isothionate: Pentamidine is an aromatic diamidine prepared in the form of isothionate and methane sulfonate salts and is used as a second-line drug in the treatment of leishmaniasis. Also, diffuse ethiopic skin leishmaniasis responds better to pentamidine [53]. It is given as an injection because it is not well absorbed from the gastrointestinal tract. It rapidly leaves the bloodstream and binds with high affinity to fetuses, especially the liver, spleen, and kidneys. The mechanism of action of pentamidine and its antiparasitic function are not well understood. *In vitro* studies show The drug may interfere with the production of DNA, RNA, phospholipids, and proteins, causing its effect on kinetoplast DNA to be broken down, two important compounds of which are pentamidine isothionate and pentamidine dimethane sulfate. For sodium acetylgluconate in the treatment of several forms of leishmaniasis [37]. Acetylpamidine isothionate: The use of this drug as the last resort treatment for antimony-resistant harassment products. Other drugs: In the treatment of cutaneous leishmaniasis, drugs such as anti-malarial drugs, antibiotics, some imidazole compounds and purinol compounds are used when necessary. Antimalarial drugs: Chloroquine: 600 mg daily for 2 days and then 300 mg daily for 2-3 weeks [49]. Quinacrine (Atabrine): 91% improvement was shown by consuming 10% quinacrine solution in a group of 206 people. Kamular (cyclooganyl pamoate): In a study of 30 patients treated for cutaneous leishmaniasis, the cure rate was up to 77%. The dose of the drug, depending on the age of the patient, is between 1.2-2 ml as a single injection. The following antibiotics have been used to treat leishmaniasis of the skin and have had promising results.

Monamycin: This drug is second only to antimony in effective cases. It is structurally similar to paramomycin and has been particularly effective in wet skin leishmaniasis. It is administered at a dose of 500 units per kilogram of body weight three times a day for three weeks and intramuscularly. The results were satisfactory. Side effects of this drug appear as autotoxicity and nephrotoxicity [53]. Tetracycline: orally at a dose of one gram per day for three weeks, which helps control secondary infections and heal the lesion. Rifampicin: A class of antibiotics that affect the parasitic kinetoplast RNA of the parasite and cause the parasite to die. Although this drug is better known as an anti-tuberculosis drug, it is an effective and low-complication drug for the treatment of cutaneous leishmaniasis on fresh Leishmania major wounds and old Leishmania tropica wounds. The therapeutic effect of rifampicin depends on the type of leishmaniasis parasite. Side effects include renal failure, hepatitis, and impaired blood coagulation [57]. Amphotericin B: It is a type of macronutrient, the first of which is the effective antifungal drug B amphotericin, which is still widely used. This drug has the effect of inhibiting the growth and development of fungi in normal concentrations. By binding to cell membrane sterols, the drug alters cell membrane permeability and leaks some intracellular contents. It has common side effects such as headache, chills, fever, hypotension, slow breathing, lethargy, pain, weight loss, nausea and anorexia, and sometimes allergies to the drug that can interfere with its use. Show. Recently, the use of this drug against leishmaniasis has shown acceptable effects and is used as the second drug of choice in the treatment of antimony-resistant leishmaniasis. The mechanism of its antiparasitic effect is related to the action of voltage-dependent channel formation in The liposomal form of this drug is almost 100% effective in treating skin lesions. Amphotericin B is injected slowly intravenously over 6 hours every day or every other day. The initial dose is increased from 0.25 to 0.5 and then to 1 mg per kg of body weight daily and continues until the total dose is approximately 30 mg / kg body weight. Patients should be closely monitored in the hospital as side effects of the drug may be severe. Less common side effects of the drug include thrombophlebitis, vomiting and severe nephrotoxicity in addition to the common side effects and show that unfortunately the drug is very toxic and Arrives

Dapsone (Diaminophenyl Sulfonone) (DDS) is a drug from the group of sulfonates used in the treatment of leprosy. It is used to treat cutaneous leishmaniasis at a dose of 21 mg/kg body weight for about three weeks [58].

Emtin: Intra lesion injection has been used in the treatment of cutaneous leishmaniasis, but today this drug is not used in the treatment of leishmaniasis due to its high toxicity and the presence of other drugs [58]. Allopurinol: The mechanism of action of this drug by acting on parasite enzymes inhibits the activity of adenyl succinate synthetase and adenine phosphoribosyl transferase and ultimately causes the death of the parasite. Because allopurinol is an extremely weak substrate for mammalian hypoxanthine, guanine phosphoribosyl transferase, it has selective anti-leishmaniasis effects *in vitro* and is specifically non-toxic to the mammalian host. After being used as the purine substrate for the parasite phosphoribosyl transferase, it is converted to allopurinol ribotide, which accumulates in the parasite and is amino, converted to triphosphate, and eventually inserted into RNA. This abnormal RNA apparently does not contribute to the growth of the parasite. Allopurinol also exacerbates the anti-leishmanic effects of sodium stibucluconate [37,59]. Berberine Chloride: There is little information on the use of this plant alkaloid derivative. However, several successes have been reported after injection of the drug in and around Leishmania tropica lesions [60]. Azoles: and their mechanism of action: Azoles act by inhibiting the enzyme lanosterol 14-α-dimethylase dependent on cytochrome 450-P (P-450DM). These enzymes catalyze various biological reactions. In this way, these enzymes bind to oxygen and in stages, by breaking its double bond, introduce an oxygen atom into the inactive C-H bond. Some enzymes of the 450-P subgroup (especially aromatase and lanosterol 14-α-demethylase) can catalyze a series of reactions that result in the breakdown of the C-C bond by hydroxylation and oxygenation. P450-DM catalyzes the first step in the conversion of lanosterol to cholesterol (mammals) or ergosterol (fungi) and parasites by removing the 14-α-methyl lanosterol group and converting it to unsaturated sterol 15-14. Cholesterol and ergosterol are essential components of the cell wall of mammals and fungi, respectively. Figure 1 shows this process and the steps of ergosterol biosynthesis.

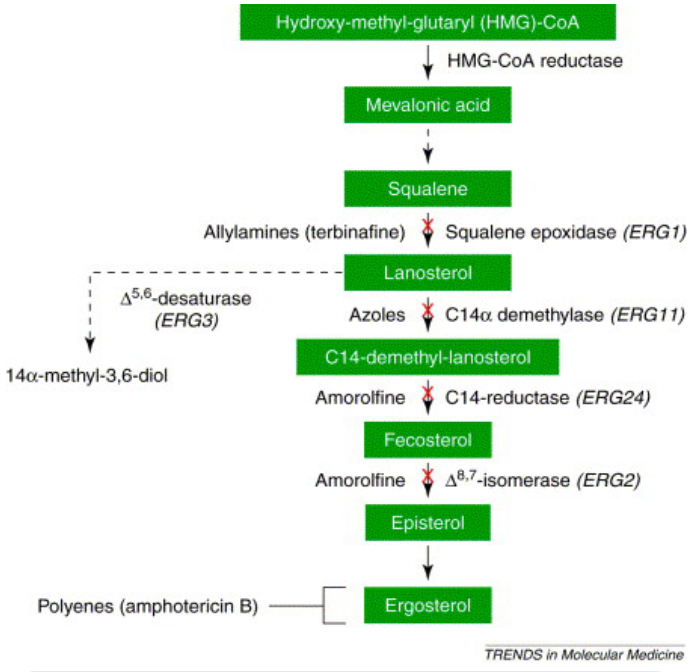

Figure 1: Ergosterol biosynthesis steps.

Demylation reactions at C-14 of sterol nucleus has three stages, the most important of which is the inhibition of demethylation at C-14 as a result of the binding of heterocyclic nitrogen of the azoles to the protohem iron atom, which prevents oxygen activity. Non-heterocyclic moieties of anti-parasitic and antifungal compounds bind to cytochrome 450-P lipophilic sites. The different inhibition of cytochrome 450-P enzyme between fungi and parasites of pathogens and humans is the main reason for the clinical use of antifungal azoles and also due to the differentiation of the inhibitory effect of azoles on fungal and plant enzymes, they can be used as antifungals [61-63].

Types of azoles Azoles are divided into two groups based on their structure: imidazole and triazole derivatives. 1- Imidazoles: Imidazoles, or azoles, are relatively new drugs that have been around for almost four decades and are now widely used. They are mainly used to treat fungal infections and their use in the treatment of leishmaniasis is limited. The first azole drugs included imidazoles, of which chlorobenzyl imidazole was the first azole to be used as a 5% topical cream and was effective against many gram-positive fungi and bacteria. Also in 1969, with the introduction of three combinations of clotrimazole, miconazole and econazole, the importance of antifungal imidazoles became apparent, and finally, with the introduction of ketoconazole (the only oral drug in the group of imidazoles), it developed significantly [64]. Drugs such as Oxyconazole, Thioconazole, Sulconazole, Butoconazole, etc. are also among the imidazoles. The effectiveness of these drugs on Leishmania promastigotes has been proven in laboratory studies. Some azoles have been used systematically in the treatment of leprosy in the Old World and have had successful results. However, the topical use of these compounds in the treatment of leishmaniasis is not yet widespread and few studies have been done in this regard. Ketoconazole: This drug and other non-toxic imidazole compounds that inhibit synthase ergosterol are effective against *Leishmania tropica* amastigotes in human macrophages *in vitro*, but have severe hepatotoxicity effects. Ketoconazole is an imidazole [8]. Due to the appropriate antifungal response, some azoles such as Miconazole, Ketoconazole, Econazole and Clotrimazole appear to be effective on a wide range of infectious agents. As mentioned earlier, the antifungal analogue imidazole Clotrimazole has an inhibitory effect on the growth of Leishmania parasite. This compound has better effects when combined with metal ions [64]. *In vitro* studies on *Leishmania major* parasite in the form of pro-mastigot and amastigote have been very effective. Other analogues, namely miconazole and econazole, along with a number of synthetic azoles on *Leishmania donovani* parasite have been studied *in vitro* and their good effect has been observed in intracellular amastigotes, which caused 100-94 % of the parasite eradication. For example, two compounds in this category of synthetic azole in hamsters reduce parasitism by 52-60 % and indicate their potentially beneficial effect on the parasite [65]. Levamisole: This drug improves with two mechanisms. In the first mechanism, it destroys it by acting on intracellular metabolism, and in the second mechanism, it strengthens the immune system by acting on T lymphocytes [66-69]. Metronidazole: This drug is used to treat protozoa such as Giardia and Entamoeba histolytica. In the treatment of cutaneous leishmaniasis, and especially in the treatment of cutaneous leishmaniasis with Mexican leishmaniasis, a 78% improvement has been reported. It seems that taking this drug is effective in accelerating healing in the late period of the wound [37].

2- Tri azoles in the structure of azoles, due to the replace-

ment of imidazole ring with triazole, major changes were made and also the presence of tri azole ring led to improved solubility, increased polarity and reduced binding to plasma proteins. Replacing the imidazole ring with a triazole due to the lower nucleophilic properties of the triazole ring, this increases the drug's resistance to metabolism, and ultimately the triazole ring increases the specificity and affinity of these drugs for the enzymatic systems of parasites and fungi and increases its potency [8,65]. The first triazole to be used topically to treat vaginal candidiasis and dermatomycosis was turconazole. Fluconazole, Voriconazole, Itraconazole and Terconazole are among the triazole drugs that have a special place in treatment [65]. The effectiveness of the combined use of azoles and anti-leishmaniasis drugs and their effect on the pharmacokinetics and pharmacodynamics of these drugs have been investigated, which in some cases has a significant increase in half-life and plasma concentration compared to antimonials. Azoles also affect some parasitic protozoa such as Toxoplasma, Giardia, Naegleria, Trypanosoma and inhibit the growth of the parasite [70,71]. The anti-leishmaniasis effect of this group of compounds in very low concentrations confirms their suitable effectiveness in this field and introduces them as low-harmful compounds. Azole antifungals selectively inhibit cytochrome 450-P (P-450DM) -dependent lanosterol 14-α-demethylase enzyme and bind N3 or N4 nitrogen atoms (in the N3 atom region on the imidazole ring and in the N4 atom region on the triazole ring, respectively). They also act on the enzyme CYP51 with ring iron [65].

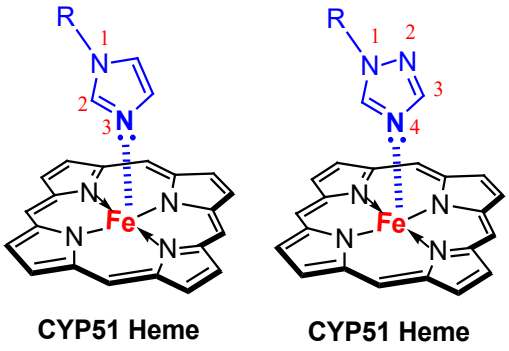

Figure 2: The process of binding nitrogen atoms of azoles.

In the past few years, in order to achieve new azole compounds, several studies have been conducted in the Faculty of Pharmacy of Mazandaran University of Medical Sciences by Dr. Emami and his colleagues. An interesting group of these compounds that have been designed and synthesized are derivatives of azole flavanone. The pharmacophore required for its antiparasitic and antifungal effect is the skeleton of phenethyl azole, which has these compounds on both sides, while their structure is rigid. The study of antifungal effects of these compounds has proven their effectiveness in comparison with fluconazole on different fungi [72,73]. The general formula of these compounds with imidazole substitution is as follows, and the difference between these categories of derivatives is related to the substitution of different functional groups (X, R).

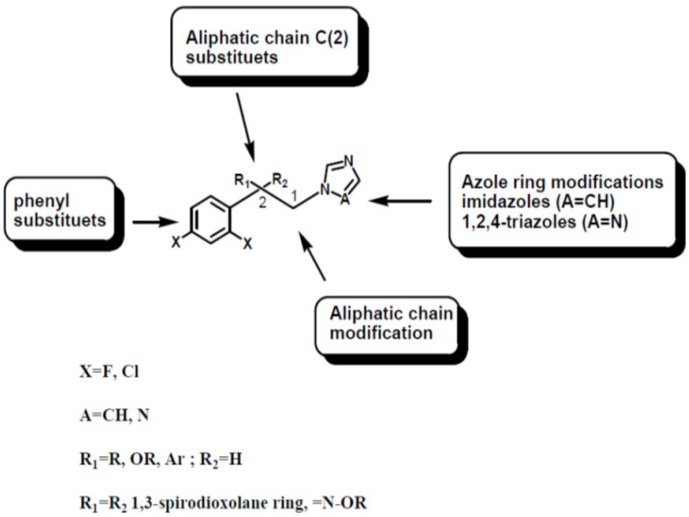

Figure 3: SAR studies of antifungal azoles.

Pharmacophoric structure of azoles the structure of N-phenethyl azole is the main pharmacophore for azole antifungal compounds whose phenyl ring is attached to the nitrogen of the azole ring by an ethyl chain. Having a weak imidazole or 1,2,4-triazole ring is one of the most important structural requirements for antifungal azoles that bind to the rest of the molecule structure through nitrogen [73]. After the discovery of the first imidazole compounds, many studies were conducted on the Structure-Activity (SAR) relationship of antifungal azoles, which led to the achievement of compounds with more effective biological or pharmacological properties. Figure 10-1 shows an overview of the structural changes of azoles in which the pharmacophore consists of the following sections: a. (4) 3-N azole ring B. A phenyl ring centroid attached to the azole ring nitrogen by a carbon bridge. In the 2-C ethyl chain, there are often substituents such as the ether group (miconazole and econazole) or the 1,3-spiro dioxalane ring (ketoconazole and itraconazole) or the oxime group (oxiconazole) or the methyl triazolyl and hydroxyol groups (hydroxy) has.

Compounds with higher potencies often have fluorine or chlorine substitution at 2,4 positions in the phenyl ring attached to the 2-C ethyl chain, and this non-polar structure can produce high lipophilicity in the compound [73].

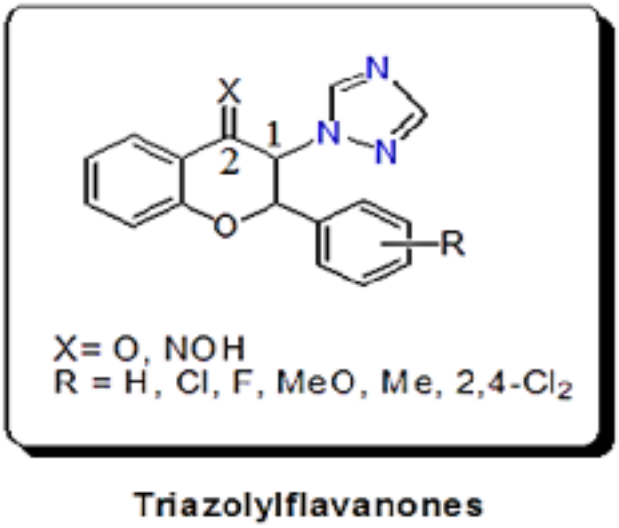

Figure 4: Structure of more azolyl flavanone compounds.

Other treatments for leishmaniasis Transfer factors: The results show that if factor transfer is obtained from people who have recently had "leishmaniasis and recovered", it has a better therapeutic effect. They are used in blood banks that are injected into the skin and around the lesion after dissolving the extract in one milliliter of normal saline. Because it is expensive, it is used in special cases such as immunosuppressed patients [74]. Immunotherapy: This method has received a lot of attention due to factors such as the high cost of 3 and 5 valent antimony drugs, high side effects and drug resistance [37]. In immunotherapy with the use of immune system boosters, the period of Leishmania infection can be altered by non-specific activation of macrophages. In this method, two general programs are followed: one is the stimulation of specific and non-specific immune responses using antigens mixed with b. (As a helper and modulator) and the use of cytokines derived from Th1 lymphocyte cells, especially interferon-gamma, TNF-α and IL-12 [37]. Creating resistance to disease: Protecting healthy people from infection has not yet become practical. Older methods include leishmaniasis, in which a new culture is inoculated from the leishmaniasis parasite at a fixed stage in a covered area of the body. At the site of inoculation, the scar remains, so there is no longer a risk of spreading a part of the face or nose or ears. This program should be considered a successful program in spite of the side effects that in some cases cause large and long wounds. Permanent immunity against re-infection after recovery, prevention by leishmaniasis and various other reasons encourage researchers to find an effective vaccine has fought against this disease. Currently, there are two categories of vaccines designed against leishmaniasis: the first group consists of the whole body of the Leishmania parasite or part of it, most of which are vaccines, and the second group has no direct relationship with the Leishmania parasite. Of this group, only sand fly saliva has been evaluated as an immunogen in the animal model. It should be noted that despite many efforts in the field of leishmaniasis vaccine in Iran and the world, but so far the ideal vaccine for this disease has not entered the market, but according to the available evidence and information, it is not impossible to prepare such a vaccine. And we will see such progress in the near future [75,76].

Method of sampling lesions suspected of leishmaniasis and smear preparation and parasitological examination in humans: Inflamed and swollen sides of the skin lesion are the most important part with the highest density of amastigotes. The important point is that the more samples are taken from the tissue, the more likely the parasite is in the sample (Figure 1). Because skin lesions may be secondary to bacterial or fungal infections, it is necessary to thoroughly clean the area of the lesion from which the sample is intended to be taken and, if necessary, change the cotton swab several times. Sampling and staining is done as follows: 1. Wear gloves every time sampling 2- Harvesting cobras on the lesion and any pus on it 3- Selecting a suitable place for sampling, including the outer edge of the swollen and inflamed part of the lesion, and avoiding sampling from open and injured areas of the lesion. 4. Using ethanol 70 to sterilize and wash the lesion, wait until the alcohol dries before sampling. The use of substances such as mercurocorum at the site of the lesion should be avoided as it may cause the death of amastigotes or their deformation. If iodinated compounds are used to disinfect the lesion, the lesion should be cleaned with an alcohol swab before sampling. 6. The site of the lesion to be sampled should be held firmly with two thumbs and forefinger. 7- Using a sterile vaccine and a sterile scalpel of the tip of the load, make a gap of 1 mm in the area taken with the fingers. 8- With the sharp edge of a scalpel or vaccinosteel, a few scratches should be given from the depth of the split site to the surface and center of the lesion to remove the appropriate amount of tissue and blood. 9- Remove the scalpel and prepare at least 3 smears from the materials on it and engrave the patient's specifications with a diamond pen on the lambs (if culture is needed, transfer the sample to the culture medium next to the flame).

How to paint with Giemsa: Giemsa paint is sold as a concentrated commercial solution. This product can be completely different, so each sample should be tested before use. Normally, if the staining of white and red blood cells is sufficient, it can be assumed that the dye is suitable for the parasite. The painting method is as follows: 1- Allow the samples to dry without using a flame and in room air. 2- Then pour on 70% methanol on slide for 30 - 60 seconds. 3- Dry the slide in air. 4- Depending on the type of Giemsa, dilute it in a ratio of 1: 30 to 1 to 50 with water with a pH set of 7.2. 5- Place the slide on the paint bridge and pour the prepared Giemsa solution on it for 30 to 50 minutes or immerse it in it for the same time. 6-Slides are immersed in water with a set pH of 7.2 for a short time and is quickly removed and dried in the presence of air. 7- Using ocular lens 10 and object lens 10, 40 and then 100 and immersion oil and without using a lamellar is studied under a microscope. Positive diagnosis includes seeing *Leishmania* parasite clearly. In each slide, until the Leishman body is observed, at least 30 suitable fields are examined and seen. If the sample is negative, the second or third slide is examined. If you see a lot of red blood cells and you do not see macrophage cells and you do not see Leishman's body, this sample is not suitable for evaluation and the sampling place is pressed with cotton and alcohol to clean the blood and the new sample is free of blood from this or another area. Sampling must be prepared again [77]. Methods of culturing parasites *in vitro Leishmania* parasites need special substances and minerals to grow and multiply naturally in mammals. In order for *Leishmania* to grow and multiply in the laboratory, it is necessary to provide the required materials by parasite-specific culture media. In this chapter, we examine the parasite culture media.

Types of leishmania culture media

Leishmania culture media environments include two-phase and single-phase environments, which we examine the components and how to build them.

NNN Novy-Mac Neal-Nicolle medium (the most well-known two-phase culture medium for the parasite, abbreviated to 3N medium) consists of a solid phase containing blood and a liquid phase which can be RPMI-1640 liquid medium, physiological serum and to prepare the solid phase, mix 1.4 g of agar with 0.6 g of NaCl and 90 ml of distilled water in an Erlenmeyer flask and put it on heat and stir regularly until the melting agar is uniform and clear, then close the Erlenmeyer flask and autoclave for 15 minutes at 121^{o}C and 15 Ib / in2 pressure. After autoclaving, set the Ben Marie at 50^{o}C and Place the Erlenmeyer agar to cool to 50^{o}C, then aseptically prepare the rabbit defibrillation blood, add 15% of the final concentration to the agar and stir until blood Spread evenly in the medium, add 100 g of Iu/ml penicillin and 100 g/ml of streptomycin to the medium and stir, then add the blood-containing medium to a volume of 1.3 ml in the end tubes. Pour the icing and place on a ramp to harden the agar, then place them upright in the refrigerator. The prepared medium can be used for up to 4 weeks and is ready for patient sample transfer. To check the non-contamination of the culture

medium, incubate a number of tubes for 24 hours at 37°C (Figure 2.1).

Figure 5: Two-phase environment 3N.

Biopsy specimens of lesions, peripheral blood, bone marrow, or specimens prepared from the margins of the lesions and even aspirated material from the skin lesion bed can be cultured in this suitable medium. The samples enter the food agar to a depth of 2 mm from the lowest slope. After transferring the samples, the medium is kept in an incubator at a temperature of 22 to 25°C. The parasites accumulate and grow in the liquid phase at the bottom of the sloping part of the environment. The tubes are examined once a week for a month and are considered negative if no parasites are observed under phase contrast microscopy. But if the parasite is rare, it needs more time to grow. In case of contamination with bacteria or fungi, the parasite is not able to grow in the environment.

How to prepare rabbit defibrillation blood?

Blood is collected directly from the rabbit heart by observing sterile conditions and disinfecting the blood collection site. Transfer the prepared blood into a sterile Erlenmeyer flask containing 20-30 glass beads. Shake the Erlenmeyer continuously to rotate the fibrin fibers from the blood to obtain defibrillated blood. Anticoagulants such as heparin can also be used, but due to the inhibitory effect of these substances, they are less used [77].

References

1. Wheeler RJ, Gull K. The cell cycle of Leishmania: morphogenetic events and their implications for parasite biology. Molecular Microbiology. 2011; 79: 647-662.
2. Croft SL, Fairlamb AH. Drug Resistance in Leishmaniasis. Clinic Microbiol Rev. 2006: 111-126.
3. Alvar JD, Herrero M, Desjeux P, Cano J, Den Boer M. Leishmaniasis Worldwide and Global Estimates of Its Incidence. PLoS ONE. 2012; 7: e35671.
4. Agarwal KC, Shakya N, Gupta S. Design and synthesis of novel substituted quinazoline derivatives as antileishmanial agents. Bioorganic & Medicinal Chemistry Letters. 2009; 19: 5474-5477.
5. Alborzi A, Shahmirzadeh A. Leishmania tropica–isolated patient with visceral leishmaniasis in souththern Iran. Am J Trop Med Hyg. 2006;74: 306-307.
6. Gerlind A. PCR Diagnosis of leishmaniasis in the west bank.development of a field applicable procedure for epidemiological studies edoc.huberli.de. 2003.
7. Coelho AC, Trinconi CT, Costa CH, Uliana SR. In vitro and in vivo miltefosine susceptibility of a Leishmania amazonensis isolate from a patient with diffuse cutaneous leishmaniasis. PLoS Negl Trop Dis. 2014; 8: e2999.
8. Shokri A, Emami S, Fakhar M, Hosseini Teshnizi S, Keighobadi M. In vitro antileishmanial activity of novel azoles (3-imidazolylflavanones) against promastigote and amastigote stages of Leishmania major. Acta Trop. 2017; 167: 73-78.
9. Rodrigues JC, Attias M, Rodriguez C, Urbina JA, de Souza W. Ultrastructural and biochemical alterations induced by 22, 26-azasterol, a Δ24 (25)-sterol methyltransferase inhibitor, on promastigote and amastigote forms of Leishmania amazonensis. Antimicrobial agents and chemotherapy. 2002; 46: 487-499.
10. Aronov AM, Suresh S, Buckner FS, Van Voorhis WC, Verlinde CL, Opperdoes FR, et al. Structure-based design of submicromolar, biologically active inhibitors of trypanosomatid glyceraldehyde-3-phosphate dehydrogenase. Proceedings of the National Academy of Sciences. 1999; 96: 4273-4278.
11. Vasudevan G, Carter NS, Drew ME, Beverley SM, Sanchez MA, Seyfang A, et al. Cloning of Leishmania nucleoside transporter genes by rescue of a transport-deficient mutant. Proceedings of the National Academy of Sciences. 1998; 95: 9873-9878.
12. Späth GF, Epstein L, Leader B, Singer SM, Avila HA, Turco SJ, et al. Lipophosphoglycan is a virulence factor distinct from related glycoconjugates in the protozoan parasite Leishmania major. Proceedings of the National Academy of Sciences. 2000; 97: 9258-9263.
13. Urbaniak MD, Crossman A, Chang T, Smith TK, van Aalten DM, Ferguson MA. The N-acetyl-D-glucosaminylphosphatidylinositol De-N-acetylase of glycosylphosphatidylinositol biosynthesis is a zinc metalloenzyme. Journal of Biological Chemistry. 2005; 280: 22831-22838.
14. Smith TK, Sharma DK, Crossman A, Dix A, Brimacombe JS, Ferguson MA. Parasite and mammalian GPI biosynthetic pathways can be distinguished using synthetic substrate analogues. The EMBO Journal. 1997; 16: 6667-6675
15. Pink R, Hudson A, Mouriès M-A, Bendig M. Opportunities and challenges in antiparasitic drug discovery. Nature Reviews Drug Discovery. 2005; 4: 727-740.
16. Hassan P, Fergusson D, Grant KM, Mottram JC. The CRK3 protein kinase is essential for cell cycle progression of Leishmania mexicana. Molecular and biochemical parasitology. 2001; 113: 189-198.
17. Grant KM, Dunion MH, Yardley V, Skaltsounis A-L, Marko D, Eisenbrand G, et al. Inhibitors of Leishmania mexicana CRK3 cyclin-dependent kinase: chemical library screen and antileishmanial activity. Antimicrobial agents and chemotherapy. 2004; 48: 3033-3042.
18. Wiese M. A Mitogen-Activated Protein (MAP) kinase homologue of Leishmania mexicana is essential for parasite survival in the infected host. The EMBO Journal. 1998; 17: 2619-2628.
19. Hardy L, Matthews W, Nare B, Beverley S. Biochemical and genetic tests for inhibitors of Leishmania pteridine pathways. Experimental parasitology. 1997; 87: 158-170.
20. Padmanabhan PK, Mukherjee A, Singh S, Chattopadhyaya S, Gowri VS, Myler PJ, et al. Glyoxalase I from Leishmania donovani: a potential target for anti-parasite drug. Biochemical and biophysical research communications. 2005; 337: 1237-1248.
21. Vickers TJ, Greig N, Fairlamb AH. A trypanothione-dependent glyoxalase I with a prokaryotic ancestry in Leishmania major. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101: 13186-13191.
22. Werbovetz KA. Target-based drug discovery for malaria, leishmaniasis, and trypanosomiasis. Current medicinal chemistry. 2000; 7: 835-860.

23. Das A, Dasgupta A, Sengupta T, Majumder HK. Topoisomerases of kinetoplastid parasites as potential chemotherapeutic targets. Trends in Parasitology. 2004; 20: 381-387.

24. Bengs F, Scholz A, Kuhn D, Wiese M. LmxMPK9, a mitogen-activated protein kinase homologue affects flagellar length in Leishmania mexicana. Molecular microbiology. 2005; 55: 1606-1615.

25. Brata Das B, Sen N, Ganguly A, Majumder HK. Reconstitution and functional characterization of the unusual bi-subunit type I DNA topoisomerase from Leishmania donovani. FEBS letters. 2004; 565: 81-88.

26. Das A, Dasgupta A, Sharma S, Ghosh M, Sengupta T, Bandopadhyay S, et al. Characterisation of the gene encoding type II DNA topoisomerase from Leishmania donovani: a key molecular target in antileishmanial therapy. Nucleic aci.

27. Strauss PR, Wang JC. The TOP2 gene of Trypanosoma brucei: a single-copy gene that shares extensive homology with other TOP2 genes encoding eukaryotic DNA topoisomerase II. Molecular and biochemical parasitology. 1990; 38: 141-150.

28. Figgitt D, Denny W, Chavalitshewinkoon P, Wilairat P, Ralph R. In vitro study of anticancer acridines as potential antitrypanosomal and antimalarial agents. Antimicrobial agents and chemotherapy. 1992; 36: 1644-1647.

29. Salem MM, Werbovetz KA. Antiprotozoal Compounds from Psorothamnus p olydenius. Journal of natural products. 2005; 68: 108-111.

30. Saebi E. Parasitic diseases in Iran. Fourth edition. Tehran: Awige publisher. 2005, 472.

31. Chawla B, Jhingran A, Singh S, Tyagi N, Park MH, Srinivasan N, et al. Identification and characterization of a novel deoxyhypusine synthase in Leishmania donovani. Journal of biological chemistry. 2010; 285: 453-463.

32. Eskandari SE, Bahar M, Safai Naraghi Z, Javadi A, Khamesipour A, Miramin Mohamadi A. Efficacy of Microwave and Infrared Radiation in the Treatment of the Skin Lesions Caused by Leishmania major in an Animal Model. Iranian J Publ Health. 2012; 41: 80-83.

33. Neva FA, Corsey R, Bogaert H, Martinez D. Observations on local heat treatment for cutaneous leishmaniasis. Am J Trop Med Hyg. 1984; 33: 800-804.

34. Valencia BM, Witzig RS, Boggild AK, Llanos-Cuentas A. Novel Low-Cost Thermotherapy for Cutaneous Leishmaniasis in Peru. PLOS Neg Trop Dis. 2013; 7: e2196.

35. Navin TR, Arana FE, Demerida M ,Castillo AL, Pozuleos JL. Placebo - controled clinical trial of meglumine antimoniate(glucantim) vs localozed controled heat in the treatment cutaneous leishmaniasis in Guatmala. Am J Trop Med Hyg. 1990; 42: 89-153.

36. Amlashi SV, Taghavi F, Taheri AR. Local therapies in cutaneous leishmaniasis: A review study. J Birjand Uni Med Sci. 2020; 27: 1-21.

37. Zerehsaz A, Hanjani F. A double blind randomized clinical trial of topical herbal extract (Z-HE) vs. systemic meglumineantimoniate for the treatment of cutaneous leishmaniasis in Iran. Int J Dermatol. 1993; 38: 610-612.

38. Masmoudi A, Marrekchi S, Amouri M, Turki H. Old World cutaneous leishmaniasis: diagnosis and treatment. J Dermatol Case Rep. 2013; 2: 31-41.

39. Nagle AS, Khare S, Kumar AB, Supek F, Buchynskyy A, Mathison CJ, et al. Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis. Chemical reviews. 2014; 114: 11305-11347.

40. Larbi EB, al-Khawajah A, al-Gindan Y, Jain S, Abahusain A, al-Zayer A. A randomized, double-blind, clinical trial of topical clotrimazole versus miconazole for treatment of cutaneous leishmaniasis in the eastern province of Saudi Arabia. Am J Trop Med Hyg. 1995; 52: 166-168.

41. El-On J, Rubinstein N, Kernbaum S, Schnur L. In vitro and in vivo anti-leishmanial activity of chlorpromazine alone and combined with N-meglumine antimonate. Annals of tropical medicine and parasitology. 1986; 80: 509-517.

42. Kurban AK, Farah FS, Chaglassian HT. The treatment of cutaneous leishmaniasis. International Journal of Dermatology. 1967; 6: 168-171.

43. Cohen HA, Livshin R. Treatment of leishmaniasis nodosa (oriental sore) with intralesionally injected emetine hydrochloride. Journal of the American Academy of Dermatology. 1987; 17: 595-599.

44. Modaber F. First generation leishmaniasis vaccine clinical development: moving but what next? . Curr Opin Anti_infect Invest Drug. 2000; 2: 35-39.

45. Cook G. Leishmaniasis: some recent developments in chemotherapy. Journal of Antimicrobial Chemotherapy. 1993; 31: 327-330.

46. Blum J, Schwartz E, Beck B, Hatz C. Treatment of cutaneous leishmaniasis among travellers. J Antimicrob Chemo. 2004; 53: 158-166.

47. Mahmoodi M, Nosratabadi SJ, Fekri AR, Haghparast A, Sharifi I. Evaluation of resistance in treatment of cutaneous leishmaniasis with antimony meglumine (glucantime): Comparison of susceptibility of the parasite isolated from the patient to the drug in culture medium with the patient's clinical response to the drug. J Semnan Uni Med Sci. 2003; 4: 143-150.

48. Langreth SG, RiordanGP, Lee LS. Fine structural alternation in Leishmania tropica with in human macrophage exposed to antileishmanial drugs in vitro. J Parasitol. 1983; 30: 555-561.

49. Zamen momeni A, Nadim AH, Javadian EO, Mohebali M. Leishmania parasite and leishmanioses. University Publication Center. 2009.

50. de Menezes JPB, Guedes CES, Petersen ALdOA, Fraga DBM, Veras PST. Advances in development of new treatment for leishmaniasis. BioMed research international. 2015; 2015.

51. Faris RM, Jarallah JS, Khoja TA, al-Yamani MJ. Intralesional treatment of cutaneous leishmaniasis with sodium Stibogluconate antimony. Int J Dermatol. 1993.

52. Adib A. Translation of basic and clinical pharmacology. 1996.

53. Roberts WI RP. Antileishmanial activity of sodium stibogluconate fractions. Antimicrob Agents Chemo. 1993; 37: 1842-1846.

54. Carrio J , Callego M.et al. In vitro susceptibility of Leishmania infantum to meglumine antimonite in isolates from repeated leishmaniasis episodes in HIV-Co infected patients. J antimicrob chemo. 2001: 120-121.

55. Sun H, Yan SC, Cheng WS. Interaction of antimony tartrate with the tripeptide glutathione. European Journal of Biochemistry. 2000; 267: 5450-5457.

56. Berman JD. In vitro susceptibility of antimony-resistant Leishmania to alternative drugs. Journal of Infectious Diseases. 1982; 145: 279.

57. Özsoylu Ş. Treatment of visceral leishmaniasis. Turkish J Pedia. 2003; 45: 280.

58. Rezai H, Behbehani A, Gettner S, Ardehali S. Effect of levamisole on the course of experimental leishmaniasis in guinea-pigs and mice: haematological and immunological findings. Annals of tropical medicine and parasitology. 1988; 82: 243-249.

59. Dogra J. A double-blind study on the efficacy of oral dapsone in cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1991; 85: 212-213.

60. Martinez S, Marr JJ. Allopurinol in the treatment of American cutaneous leishmaniasis. New England Journal of Medicine. 1992; 326: 741-744.

61. Kalinin SV, Gruverman A. Scanning probe microscopy: electrical and electromechanical phenomena at the nanoscale: Springer Science & Business Media. 2007.

62. Xi P, Xie H, Liu Y, Ding Y. Optical nanoscopy with stimulated emission depletion: CRC Press. 2014.

63. Dufrêne YF, Pelling AE. Force nanoscopy of cell mechanics and cell adhesion. Nanoscale. 2013; 5: 4094-4104.

64. Galluzzo C, Eperon G, Mauris A, Chappuis F. [Old World cutaneous leishmaniasis]. Revue medicale suisse. 2013; 9: 990-995.

65. Keighobadia M, Emami S, Fakhar M, Shokri A, Mirzaei H, Hosseini Teshnizi S. Repurposing azole antifungals into antileishmanials: Novel 3- triazolylflavanones with promising in vitro antileishmanial activity against Leishmania major. Parasitol Inte. 2019; 69: 103-109.

66. Vennerstrom JL, Lovelace J, Waits V, Hanson W, Klayman D. Berberine derivatives as antileishmanial drugs. Antimicrobial agents and chemotherapy. 1990; 34: 918-921.

67. Patra M, Joshi T, Pierroz V, Ingram K, Kaiser M, Ferrari S, et al. DMSO-Mediated Ligand Dissociation: Renaissance for Biological Activity of N-Heterocyclic-[Ru (η6-arene) Cl2] Drug Candidates. Chemistry–A European Journal. 2013; 19: 14768-14772.

68. Parreek S.S. Combination therapy of sodium stibogloconate and rifampin in cutaneous leishmaniasis. Int J Dermatol. 1984; 23: 970-971.

69. Butler P. Levamisole therapy of chronic Leishmania tropica. The Journal of tropical medicine and hygiene. 1978; 81: 221-224.

70. Oxberry M, Thompson R, Reynoldson J. Evaluation of the effects of albendazole and metronidazole on the ultrastructure of Giardia duodenalis, Trichomonas vaginalis and Spironucleus muris using transmission electron microscopy. International journal for parasitology. 1994; 24: 695-703.

71. Patterson S, Wyllie S. Nitro drugs for the treatment of trypanosomatid diseases: past, present, and future prospects. Trends in parasitology. 2014; 30: 289-298.

72. Nazarian Z, Emami S, Heydari S, Ardestani SK, Nakhjiri M, Poorrajab F, et al. Novel antileishmanial chalconoids: Synthesis and biological activity of 1-or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones. European journal of medicinal chemistry. 2010; 45: 1424-1429.

73. Emami S, Behdad M, Foroumadi A, Falahati M, Lotfali E, Sharifynia S. Design of Conformationally Constrained Azole Antifungals: Efficient Synthesis and Antifungal Activity of trans-3-Imidazolylflavanones. Chemical biology & drug design. 2009; 73: 388-395.

74. Takasu K, Terauchi H, Inoue H, Takahashi M, Sekita S, Ihara M. Antileishmanial activities of rhodacyanine dyes. Heterocycles. 2004; 64: 215-221.

75. . Delgado O, Romano E, Belfort E, Pifano F, Scorza J, Rojas Z. Dialyzable leukocyte extract therapy in immunodepressed patients with cutaneous leishmaniasis. Clinical immunology and immunopathology. 1981; 19: 351-359.

76. Modaber F. Development of vaccine against leishmaniasis. Scand Infect Dis. 1990; 76: 42-48.

77. Shirzadi MR. Cutaneous Leishmaniasis Care Guide in Iran. Ministry of Health, Treatment and Medical Education, Deputy Minister of Health, Center for Disease Management Infectious, the management of human-animal communicable diseases. 2012.