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Mini-Review Article

Leishmania-ergosterol pathway: a unique drug target

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ABSTRACT

Leishmania is an obligate parasite present intracellularly and survives within the host macrophages by invading the host immune system. It causes *Leishmania*sis, a global health problem. At the moment it is recognized as endemic in 89 different countries throughout five different continents. There are more than 12 million people who are infected in the world and 350 million who are at the risk of infection. The traditional treatments have limitations like high cost, toxicity and painful route of administration. The irregular and inappropriate use of the second line of drugs such as amphotericin B and miltefosine has raised drug resistance in parasites. All these available drugs are toxic to host cells along with the parasite. Therefore, it is an urgent need to develop new anti*Leishmania*l drugs to eradicate *Leishmania*sis completely. The foremost requirement of such a target is to be present only in the parasite and absent in the host. Ergosterol biosynthetic pathway is unique in *Leishmania* but absents in the human being. Ergosterol is essential for growth, proliferation, and virulence of *Leishmania*. In this review, I have thrown light ergosterol biosynthetic pathway of *Leishmania* as the potential target for drug screening.

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INTRODUCTION

*Leishmania*sis has been found to be a major global health problem which affects 1.2 to 2 million people every year. World Health Organization (WHO) has recognized this vector-borne disease as one of the Neglected Tropical Diseases (NTD) (Savioli and Daumerie, 2010; Rub A et al., 2009; 2013). There is no vaccine yet available for the treatment of *Leishmania*sis. The treatment option for *Leishmania*sis relies on chemotherapy but the available chemotherapy is far from satisfactory. The main drugs which are in used are pentavalent antimonials, amphotericin B, and miltefosine. The currently available chemotherapy has the problems like adverse effect, increasing resistance and high cost. In highly endemic areas Pentavalent Antimonial Sbv also called sodium stibogluconate (SSG) is no more effective and resistance has developed in two third of the patient of these areas. Pentamidine was then used to overcome the resistance of SSG, as the second line of drug but a decade after its efficacy reduced from 100% to 70% (12) and shows lower cure rate to Amphotericin B. Thus Amphotericin B (AmB) was then used for treatment of patients having resistance to SSG (Stauch et al, 2013). AmB is highly effective and safe but has several side effects like prolonged hospitalization, dose-limiting renal and nephrotoxicity, the adverse reaction which results in high fever with chills (Stauch et al, 2013). Liposomal AmB is similar in effectiveness as usual AmB, but with negligible adverse effects. High cure rates are possible if the high dose is administered in a short period, but due to its high cost, it is out of reach from most of the patients in endemic countries. An oral drug miltefosine showing good results, have been registered in some

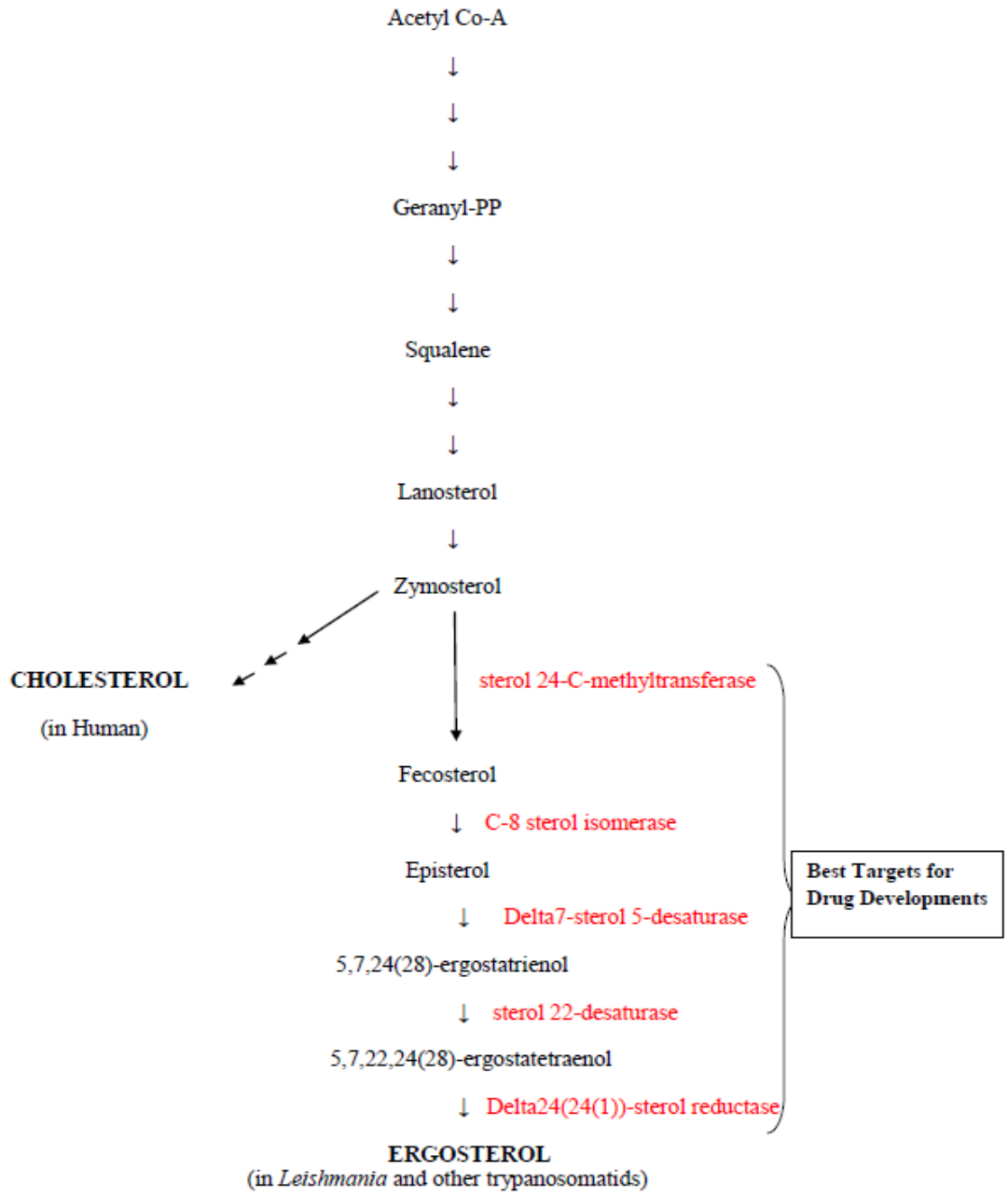

Figure 1. Ergosterol pathway of Leishmania

countries but it has a long half-life of seven days so chances of development of resistance are very high (Arish et al., 2015; Majumdar et al., 2012). Sterol biosynthesis is ubiquitous among the eukaryotes. It also designates the critical step in prokaryotes-to-eukaryotes evolution (Azam et al., 2014). It is well established that there is a special class of sterol (i.e. ergosterol) which is present in the protozoan parasite and important for its growth and proliferation. However, it is completely missing from human cells. In mammals, there is another form of sterol (i.e. cholesterol) which is different from ergosterol having one lesser number of double bond inside B ring. It is also different in the saturation of side chain having no –CH3 group at C24 (Urbina, 1997). In order to combat and cure *Leishmania*sis, novel targets and approaches are being considered. Targeting lipid biosynthetic pathway is one of the strategies made in this direction (Urbina, 1997). It was found that available sterol biosynthesis inhibitors (SBIs) showed the successful result in treatment of fungal diseases (Docampo and Moreno, 2008) but are not powerful enough against parasitological diseases such as *Leishmania*sis and Chagas disease. Treatment of ketoconazole as C14-demethylase inhibitors against amastigotes showed mild antiproliferative activity when compared with epimastigotes stage of Trypanosoma cruzi (Docampo and Moreno, 2008; Urbina et al., 2004). The endogenous sterols are susceptible to the SBIs (such as ketoconazole) due to the smaller pool and higher turnover rate of these sterols in amastigotes (Docampo and Moreno, 2008). These studies suggest that the

combination of SBI with other inhibitors may have promising effect against the sterol biosynthetic pathway of parasites like *Leishmania*. There are many unique enzymes in the ergosterol biosynthetic pathway like S-Adenosyl-L-methionine: △24-sterol methyltransferase, C-8 sterol isomerase, delta-7-sterol 5-desaturase, sterol 22-desaturase and Delta-24(24(1))-sterol reductase (Figure 1).

Epidemiology of *Leishmania*sis

Protozoan parasite *Leishmania* generates a large range of pathologies varying from local cutaneous *Leishmania*sis (CL) to life-threatening visceral *Leishmania*sis (VL) [WHO, 2010]. The occurrence of a distinct clinical manifestation in the infected patient is attributed to the parasite species involved and also the host immune status. The disease is considered to be a serious public health problem mostly in developing countries and rarely in developed countries. Visceral *Leishmania*sis is more common in Somalia, South Sudan, Sudan, India, Brazil, and Ethiopia. It is characterized by spleen enlargement, irregular bouts of fever, weight loss and (Rub et al., 2009; 2013.

Cutaneous *Leishmania*sis is more common in the Middle East, the Mediterranean basin, Central Asia, and America. The most affected countries are Colombia, Algeria, Afganistan, Brazil, Iran, and Syria. It is characterized by skin lesions, ulcers on the body and lifelong scars (Rub et al., 2009; 2013). *Leishmania*sis is also endemic in 18 out of 23 Middle Eastern countries including Saudi Arabia. More than 20,000 cases have been reported in Saudi Arabia in past 10 years (Savioli and Daumerie, 2010). There are many areas of Saudi Arabia like Al-Hassa, Hail, and Madinah where the disease is common. There are three different forms of *Leishmania*sis, visceral, cutaneous and mucocutaneous. Currently available drugs to cure *Leishmania*sis are far from satisfactory outcomes due to their serious side effects and rise in cases of drug resistance [Reithinger et al., 2007; Mishra et al 1992). In view of this, it is pertinent to look into novel biochemical molecules as drug targets to control the detrimental effects of *Leishmania*.

Sterol pathway in trypanosomatids

Sterol biosynthesis is an important metabolic pathway in eukaryotes. It consists of 20 necessary metabolic steps (Figure 1) under three different stages: (i) the formation of isopentenyl pyrophosphate from acetyl CoA or another reserve of carbon like leucine which is reported in trypanosomatids (Urbina, 1997); (ii) formation of squalene the through the reaction of isopentenyl pyrophosphate and dimethylallyl pyrophosphate; and (iii) formation of lanosterol from squalene through ring formation reactions. The major sterols in these organisms consist of △5, 7-compounds related to C28-ergostane or the C29-stigmastane family (Arish et al., 2015; Ishida 2009). *Leishmania* mainly has ergosta-5, 7, 24 (241)-trien-3 β-ol (5-dehydroepisterol) sterol in both of its important stages (promastigotes and amastigotes) of the life cycle. In addition, promastigotes also possess stigmastane type of sterols comprising the 5% of total sterols whereas amastigotes consist of same up to 20 %. Ergosterol in *Leishmania* species is necessary for its development and proliferation, but missing in its human counterparts (Mishra et al 1992). The mammals instead synthesize cholesterol which differs from ergosterol in having only one double bond and in saturation of side chain fatty acid at C24. The cholesterol in the membrane of trypanosomatids is generally incorporated from growth-medium or through the infected person′s body (Urbina, 1997). Formation of ergosterol as well as cholesterol takes several steps in the respective organisms. One of the important step during ergosterol biosynthesis and invariably different from cholesterol synthesis is the transfer of methyl group from S-Adenosyl-L- methionine (SAM) to C-24 of △24 sterols to produce △24 (28)-sterols, which is regulated by a key enzyme sterol 24-c-methyltransferase (Figure 1).

Ergosterol pathway as drug target

Ergosterol is a major sterol in *Leishmania* membrane, so the 24-c- methyltransferase (SMT) is a requisite enzyme during its synthesis. Mammals lack this enzyme and therefore, it may be a valuable target for drug development to cure the *Leishmania*sis. In addition, there are many other enzymes in the ergosterol biosynthetic pathway like C-8 sterol isomerase, delta7-sterol 5-desaturase, sterol 22-desaturase and delta 24(24(1))-sterol reductase which are different from the mammalian sterol biosynthetic pathway [Magaraci et al., 2003; Figure 1]. So far, azasterol and amphotericin B have been reported to exhibit the effective anti-leishmanicidal activity in vitro (Magaraci et al., 2003), which interferes with C-24 alkylation by SMT during the late stage of ergosterol synthesis. However, in vivo significance of these drugs is often associated with unsatisfactory outcome. It is required that all the enzymes of ergosterol biosynthetic pathway be expressed, purified and crystallized. Further structure-based drug screening through bioinformatical tools and biochemical methods are required to develop effective cheap and new drugs against *Leishmania*sis.

CONCLUSION

The available chemotherapeutic drugs against *Leishmania*sis are costly, toxic and less effective. It is very important to search for new drugs which are cheap, effective and have least side effects. All the pathogens of trypanosomatid family have common ergosterol biosynthetic pathway that is why targeting the different enzymes of ergosterol biosynthetic pathway will have broader application in the development of new therapeutics against other infectious diseases also.

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CONFLICTOFINTEREST

No conflict of interest

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