Leishmanicidal Activity of Nystatin (Mycostatin): A Potent Polyene Compound

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Abstract

The susceptibility of promastigote of Leishmania major to Nystatin in vitro was examined. L. major (MHOM/PK/88IDESTO) promastigote were cultured in medium 199 supplemented with 10% heat inactivated foetal bovine serum and 2% urine. The growth of the promastigote was monitored in the absence and presence of the experimental compound (Nystatin) for upto 5 days post- inoculation. The EC5O value (the concentration of drug necessary to inhibit the growth rate of cells to 50% of the control value) obtained for Nystatin against the promastigote of L. major was less than 9.76 iu ml1. Certain polyene compounds like Amphotericin-B and Nystatin (mycostatin) are familiar for their fungicidal activity. Amphotericin-B is used since long as antileishmanial drug as well, Results obtained suggest that Nystatin has a very good anti leishmanial activity in vitro. The mode of action proposed for this drug is same as for Amphotericin-B as both of these polyene compounds interact with the various sterols present on the surface of the parasite, thus unusual gaps and pores are formed on the surface that results in the leakage of the ions. This leakage finally leads to the destruction of the parasite (JPMA 47:246,1997).

Introduction

The parasitic protozoan Leishmania is the causative agent of a group of parasitic disease i.e., Leishmaniases[1,2]. Leishmaniases is generally classified in three major types: the cutaneous leishmaniasis generally caused by L. major/tropica species of the parasite; the mucocutaneous leishmaniasis which is caused by L. brazeliensis species of the parasite and visceral leishmaniasis or more commonly known as “kala azar” which is caused by L. donovani species[1,2]. Cutaneous, mucocutaneous and macrophages of spleen, liver and bone marrow are infected by the parasite in above mentioned three types of leishmaniases respectively[1,2]. In Pakistan, only two manifestations of disease are prevalent i.e., cutaneous leishmaniasis caused by L. major (MHOM/PK188/DESTO) or L, tropica (MHOM / PKJ91 / ABDULLA) and visceral leishrna.niasis caused by L. infantum (MHOM/PK/ 91/RAB-KARAMATVL). Cutaneous form is much more prevalent in Pakistan mainly in Balochistan and NWFP, in Punjab, the disease is restricted to Multan while in Sindh, cases reported so farare from Karachi. Whereas, visceral fonn is restricted to the far northern areas like Gilgit and Baltistan1. The Leishmania spp. are transmitted primarily by female sandflies of genus Phiebotomus in endemic areas[3], although transmission via blood transfusion has also been.documented. Leishmania spp. have two distinct forms, the intracellular aflagellated amastigote form which resides in macrophages in the mammalian host and the extracellular flagellated promastigote which is found in the gut of insect vector (sandfly) and in the in-vitro culture[4]. The promastigote enter the mammalian host from the sandfly’s mouthparts during a blood meal.
The pentavalent antimonial drugs introduced in the early days of the chemotherapy of leishmaniases

are still the drugs of choice[5,6]. However, the recent clinical report show an alarming trend. A considerable pmportion of cases 15-25% are becoming unresponsive to antimonial therapy[7]. In such cases, pentamidine, biguanidine and amphotericin-B serve as second line of defence[8]. All of these drugs however, are highly toxic and needs monitored conditions that can only be provided in an advanced hospital situation Due to these limitations, reseaithers are looking for new antileishmanial drugs that are effective, cheap and above all exhibit least toxic side effects. Nystatin or more commonly known mycostatinis a polyene compound which is obtained from Streptomyces nouresi. This compound is closely related to famous leishmanicidal polyene drug Amphotencin-B. Nystatin is used widely as antifungal drug in conditions like monaliasis and candidiasis for a long time[9,10] but its antileishmanial activity was not investigated. We have tested this polyene compound on the promastigote of leishmania in-vitro. This study reports the effectiveness of Nystatin against L. major promastigote.

Materials and Methods

Amastigote major (MHOM/PK/88/DESTO)] were isolated from open ulcerated lesion and maintained in modified Tobie's medium between 21-23°C in dark where they transfonned in promastigote form. Promastigote were then subcultured and cultivated in bulk in medium 199 (Sigma) supplemented with 10% that inactivated foetal bovine serum (HIFBS) and 2% sterile human urine between 21-22°C ma cooled incubatorfor72 hours. The parasites were counted under 40X magnification on a modified neubaur chamber and were sedimented down at 3000 gfor 15 minutes. The sedimented parasites were diluted to a concentration of 2x106 parasites per ml with fresh medium and were transferred aseptically into disposable culture tubes (coming) of a capacity around 5 ml.
Nystatin suspension was obtained from Lederle/Cyanamid Pakistan. Each ml of Nystatin suspension contained 100,000 iu (international units) of the active compound. The nystatin suspension was aseptically introduced into the culture tube and was serially diluted so that different tubes contained 1250, 625, 312.5, 156.15, 78, 39,19.5,9.76 iu of the compound. The tubes were placed in an incubator between 2 1-22°C for upto 3-5 day's. The parasites were counted on modified neubaur chamber[11].

Results

The results obtained are graphically shown in the figure 1.

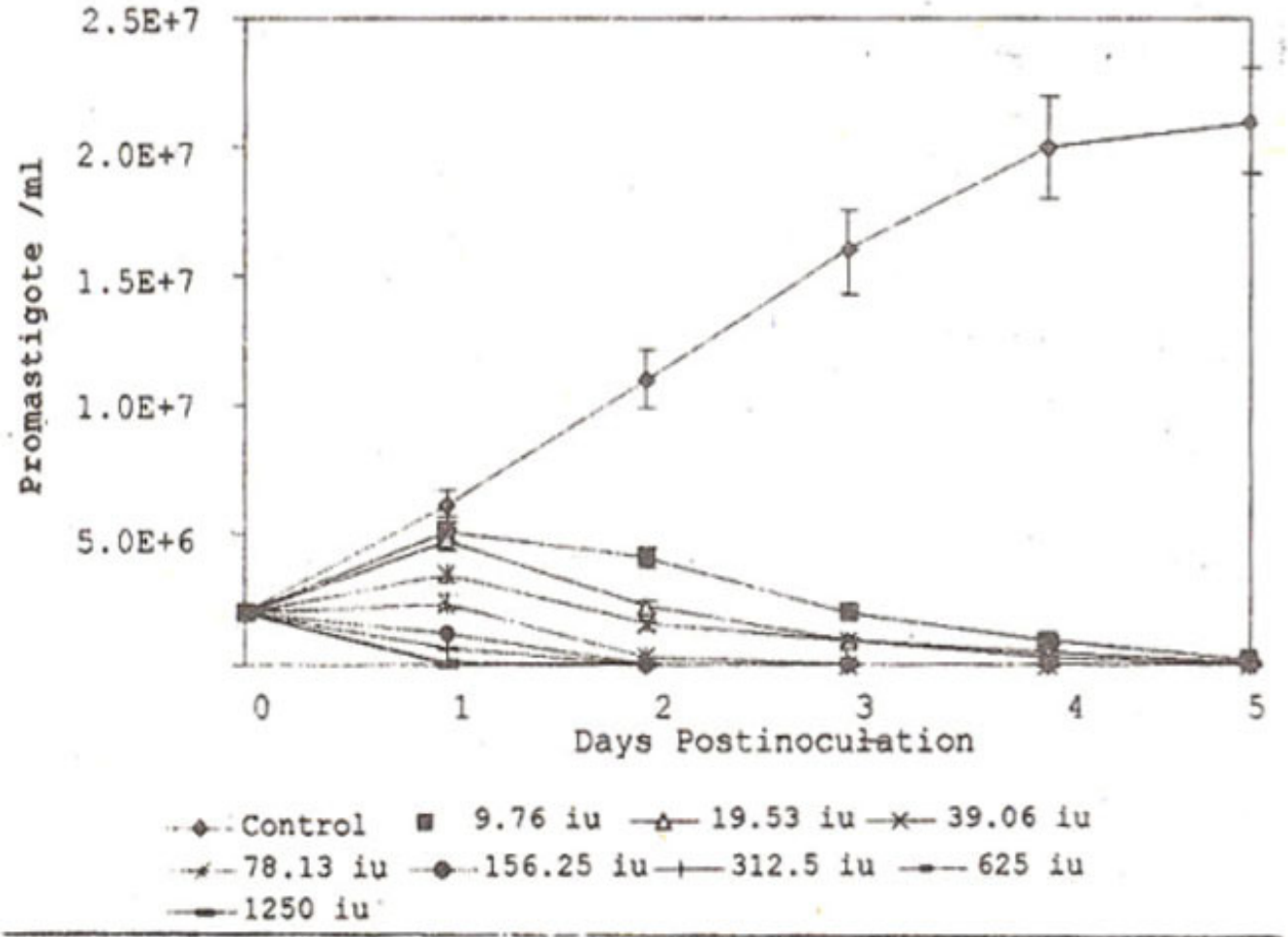

Figure 1. Growth of L. major promastigote in the absence (control) and presence of various concentrations of Nystatin.

The tests were made in triplicate and the mean was considered as the final result. The EC50 value was foundtobe less than 9.75 iu ml-1 while less than 19.5 iu of the drug suspension was enough to completely inhibit the growth of promastigote within 3 days post- inoculation in vitro.

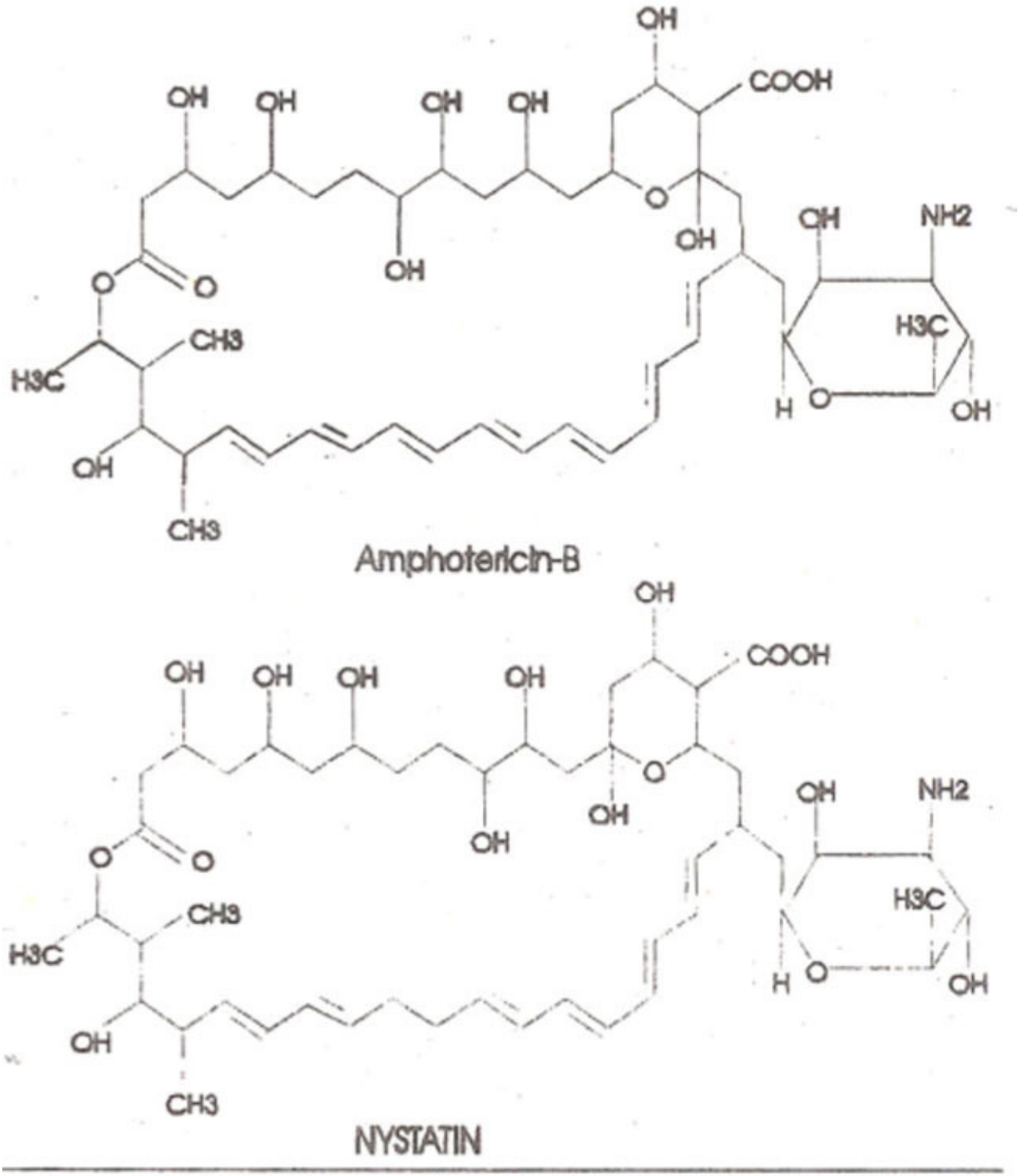

Figure 2. Structure of Amphotericin-B and Nystatin.

In next 24-48 hrs of incubation, the results were even more pronounced and approximately all parasites were found dead at aconcentrationof 19.5 iu mF1.

Discussion

The cytoplasm of all living things is bounded by the cytoplasmic membrane, which serves as selective permeability barrier, carries out active transportfunctions and thus controls the internal composition of the cell. If the functional integrity of the cell membrane is disrupted, macromolecule and ions escape from the cell and cell damages or death ensues. As microbial cell surface is different than the animal cell surface, itis possible to disrupt itby certain agents. Consequently, selective chemotherapy is possible[13]. Nystatin or more commonly known as Mycostatin is a polyene drug10. This is produced by the growth of Streptomyces nouresi. Nystatin-A is closely related to the Amphotericin-B, another polyene antifungal drug. Like Amphotericin-B, the Nystatin is a macmcyclic lactone with a ketal ring, an all-trans-polyene system and a mycoseaniine (3- amino-3-deoxyrhamnose) moiety (Figure 2). Nystatm is yellow incolor, havinganodour suggestive of cereals. It is hydroscopic and light, heat and air sensitive. While in aqueous media, has a pH between 6.5-8.0. It is very slightly soluble inwater, slightly/sparingly soluble in alcohols and n-butyl alcohols and completely insoluble in organic solvents like chloroform and benzene. Nystatin is completely ineffective againstbacteria[9,10], however, it is quite active againstvarious type of fungus andyeast[10]. The mode of action proposed for this drug is similar to that of Amphotericin-B[8].

Amphotericin-B and Nystatin specifically interacts with ergosterol in fungal membranes[12], Leishmania sterols are comparable to fungal sterols[12,13] and efficacy of this drug against Leishmania may similarly be due to interaction with ergosterol in Leishmania membrane. Leishniania synthesizes sterols. The major sterol synthesized by the parasite is 5-dehydroepisterol, episterol, ergosterol and some other sterols[12,13]. . Like Amphotericin-B, the Nystatin strongly and selectively binds to the sterols present on the surface of the parasite particularly the ergosterol and results inthefonnation of unusual pores/gaps on the surface of the parasite that results in the leakage of the ions. This leakage eventually leads to the destruction of the cell. At the same time, it is also suggested that while on the surface of the parasite, the Nystatin and Amphotencin-B also inhibit the further synthesis of the sterols[12-14]. The Amphotericin-B is the second line of the drug used against the parasites when initial treatment of the antimonial drugs fail, however, the toxic effects[14] of this drug like fever, chills, nausea, vomiting, renal failure, hypokalemia and anaemia are quite prominent as well. On the other hand, the Nystatin is of course not as active as the Amphotericin-B but is far less toxic and much more suitable for the oral administration As antileishmanial drug, Nystatinis at its very initial stages, however, the strong leishmanicidal activity of the compound on promastigote in vitro was observed. Whether such inhibition occurs in amastigotes and accounts for antileishmanial effect of this compound invivo remains to be detennined.

Acknowledgement

This work received financial support from UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

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