ISSN 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

Antileishmanial Activity of 2-Phenoxy Nicotinic Acid Hydrazide Sulfonamide against Leishmania (L) Major [MRHO/IR/75/ER] Promastigotes: An In-Vitro Study

Ali Fattahi Bafghi [1], Parisa Kianfar[2]*, Alireza Moradi [3], Arefeh Dehghani [4]

[1]*Associate Professor of Medical Parasitology and Mycology Department, School of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran.*

[2] *Pharmacology Student, Pharmacology Department, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

[3]*Asisstant Professor of Pharmacology Department, School of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

[4] *Instructor of Biostatics and Epidemiology department, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

***Corresponding Author:* Parisa Kianfar**

Abstract

Introduction: cutaneous leishmaniasis is parasitic disease in Iran and a major health problem in the country. The aim of this study was to compare the efficacy-achieved derivatives of 2-phenoxy nicotinic acid hydrazide sulfonamide and glucantime is in the treatment of leishmaniasis. Material and method: In this experimental- laboratory study Leishmania major promastigotes, a standard strain MRHO / IR / 75 / ER were cultured in NNN medium. Then in tubes containing medium, 10^5 Leishmania (equivalently100 ml) and 0.5 ml of dilution of 2-phenoxy nicotinic acid hydrazide sulfonamide and glucantime at a concentration of 2/0, 1, 5, 25 and 125 µg/ml separately added. Cell proliferation assay done by using BrdU (XTT) kit. Result: The results of variance analysis showed that 2-phenoxy nicotinic acid hydrazide sulfonamide derivatives and Glucantime have inhibitory effect on the parasite Leishmania. all concentrations tested 2.0, 1, 5, 25 and 125 µg/ml decreased the number of Leishmania parasites that their effects compared to control group was statistically significant (P = 0.000). Conclusion: 2-phenoxy nicotinic acid hydrazide sulfonamide derivatives able to inhibit the in vitro proliferation of Leishmania major and compared to Glucantime had better inhibitory effect on major promastigotes growth of Iranian rural leishmaniasis.

Keywords: *2-phenoxy nicotinic acid hydrazide sulfonamide, Leishmania major, Glucantime, promastigotes.*

Introduction

Leishmaniasis is one of the most important infectious diseases over the world (WHO, 2552)(1).Leishmaniasis is a disease caused by an intracellular protozoan parasite (genusLeishmania) transmitted by the bite of a female phlebotomine (2).Sand flies while eating human blood transmitted metacyclic promastigotes to humans (3)A few bites leading to clinical disease because most parasites are destroyed by the immune system (4). The disease is clinically varied symptoms that there are three major types of leishmaniasis: visceral (kala-azar), the most important disease; cutaneous, the most common; and mucocutaneous (5).The cutaneous type is prevalent in many Asian countries, including Iran. The parasites that cause diseases acquired from sandfly bites (6).Disease Protests is different based on the type of wound. In rural type cutaneous leshmaniasis that caused by Leishmania major, wound has rapid growth and short courses but in city type that is caused by Leishmania tropica are slow-growing lesions (7). Diagnosis is by microscopic observation of Leishmania in the resulting lesions (8).

Treatment of cutaneous leishmaniasis in resistant cases (more than 4 months), multiple scars (2 to 45 ulcers), larg lesions (1 to 2 cm) and other face lesions to be done. Since treatment varies depending on the type and severity of damages(9).Different methods includings local radiation therapy, burn lesions, cryotherapy, infiltration of topical drugs have been used for the treatment of leishmaniasis in over time (10, 11).Selected treatment is administered pentavalent antimony compounds such as Glucantime or Pntvstam drug (12).

Highly toxic, intravenous administration, long-term use, drug resistance and high price of antimony compounds have caused much research done on alternative medicine (13). Nonsteroidal anti-inflammatory drugs are the most widely used classes of medications in the inhibition of pain and inflammatory reactions, but little is known about its efficacy in treatment of leishmaniasis. They have popularized the notion of inhibiting prostaglandin (PG) biosynthesis as a common anti-inflammatory strategy (14).

Most of the currently available NSAIDs show significant side effects such as gastrointestinal injury, bleeding, and nephrotoxicity in long-term usage that causes some patients to abandon NSAID therapy (14, 15).Thus, a challenging goal for such a study field is the discovery of new safer anti-inflammatory drugs. Synthetic approaches based upon chemical modification of NSAIDs have been taken with the aim of improving the safety profile and, in turn, the therapeutic window of these NSAIDs. Several studies have described the derivatization of the carboxylic function with amide or N-acylarylhydrazone having less acidic amide hydrogen as well as the capacity of stabilizing free radicals (16, 17).

Hydrazone are a class of organic compounds in the Schiff bases family and were used for synthesis of heterocyclic compounds (18). 2-phenoxy nicotinic acid hydrazide sulfonamide derivatives are key intermediates and of hydrazonethat there are antimicrobial compounds are and selectively used to anti-inflammatory assessment. Present study evaluated Leishmanicidal assessment of 2-phenoxy of nicotinic acid hydrazide sulfonamide derivatives on promastigots.

Material and Methods

Standard strain of Leishmania major, MRHO / IR / 75 / ER, provided from Department of Parasitology and Mycology, Faculty of Medical Sciences, Yazd University of medical science and was transferred to pharmacy Laboratory of this university.

Leishmania major strain transmitted from stock medium to Nicole Navy Neal (N.N.N) modified medium to grow sufficiently.

As the liquid phase of 0.2 ml BHI 4 %, 100 mg/ml streptomycin and 100 ml penicillin of for medium were used. The most appropriate stage in medium to preserve of in vivo and in freezing of parasite is logarithmic phase. Thus, the concentration of parasites in the 10^6 promastigotes per ml was prepared.

To the proliferation of parasites, promastigote forms of NNN medium was passaged to RPMI-1640 medium (20 tubes each containing 5 ml) which is supplemented with 10% FCS, L- glutamine, 292 µg/ml and 100 U/ml penicillin and 100 µg / ml streptomycin.
At least one week later of passage and when the parasite promastigote forms were reached to stationary phase, the content of the culture tubes centrifuged and parasites precipitated washed with sterile PBS buffer several times.

Then a little PBS added and number of L. major parasites present counted in Neubauer haemocytometer. Under these culture conditions, the stationary phase of parasite growth was obtained in 6 days as determined.

L. major promastigotes was prepared in in the stationary phase at the rate of 40,000 per 100 ml in RPMI and used were used to cell proliferation assay with BrdU (XTT) mehod.

In was added 100 ml parasite (10^5 promastigotes) in eleven tubes so that the first tube contains µl100 medium RPMI + µl100 of promastigotes, and the pipes of the second to sixth including to 100 µl of

promastigotes concentrations of 0.2, 1, 5, 25 and 125 µg/ml of phenoxy derivatives of nicotinic acid hydrazide sulfonamide and the pipes 7 to 11 µl100 addition the promastigotes concentrations of 0.2, 1, 5, 25 and 125 µg/ml of the meglumine was used as a positive control.

Plates were incubated in condition with temperature ℃ 26, co2 5% and 95% humidity for 72 hours. Then µl10 of BrdU reagent added to all wells, were incubated for 18 to 24 hours after the previous mentioned conditions, and was centrifuged for 10 minutes (1700RPM).

The supernatant removed and the plates were dried at 60 for 30 minutes.200 µl fix Denant added and plate incubated for 30 min at 15-25. Fix Denat discarded and µl / well 100 of the conjugated antibodies as anti- BrdU -POD added and incubated for 90 min at 15-25. Suspension removed and the wells were washed three times with washing solution. 100 µl/well TMB added to the appropriate color is achieved. 25 µl / well H2So4 added as a stop solution and absorption were read at 450nm or 630nm wavelength as reference. Data was analyzed by using SPSS version 17.

Result

Data analysis showed that 2-Phenoxy nicotinic acid hydrazide sulfonamide derivatives had significant effect on decrease of Leishmania proliferation in medium culture (P <0.05). It is found that Glucantime can reduce growth of Leishmania in culture media in dose dependent manner (p=0.000). However, it was observed in the control group, without any treatment, the numbers of parasites were increased.

Different concentrations of Phenoxy derivatives nicotinic acid hydrazide sulfonamide had stronger anti-prolifration effect Compare with glucantime on Leishmania in all concentrations tested 2.0, 1, 5, 25 and 125 µg/ml (p=0.000).

Table1: The Mean proliferation of Leishmania major in the concentration range of 0/2 to 125 µg/ml in the presence of 2-phenoxy nicotinic acid hydrazide sulfonamide and Glucantime

Groups	Con.	Mean con.	SD	P-value	P-value
2-Phenoxy nicotinic acid hydrazide sulfonamide derivatives	0.2	53.600	3	P=0.000	P=0.000
	1	41.600	5		
	5	35.300	2		
	25	23.000	2.5		
	125	4.700	1.75		
Glucantime	0.2	73.300	1.5	P=0.000	
	1	71.300	2		
	5	68.600	2		
	25	59.300	1.5		
	125	46.600	3.5		
control		100.000			

Table 2: Analysis of variance to compare the inhibitory effect of 2-phenoxy nicotinic acid hydrazide sulfonamide with Glucantime afainst Leishmania major

Groups		Sum of Squares	df	Mean Square	F	Sig.
Glucantime	Between Groups	1454.400	4	363.600	70.831	0.000
	Within Groups	51.333	10	5.133		
	Total	1505.733	14			
2-Phenoxy nicotinic acid hydrazide sulfonamide derivatives	Between Groups	4199.269	4	1049.817	104.709	0.000
	Within Groups	100.260	10	10.026		
	Total	4299.529	14			

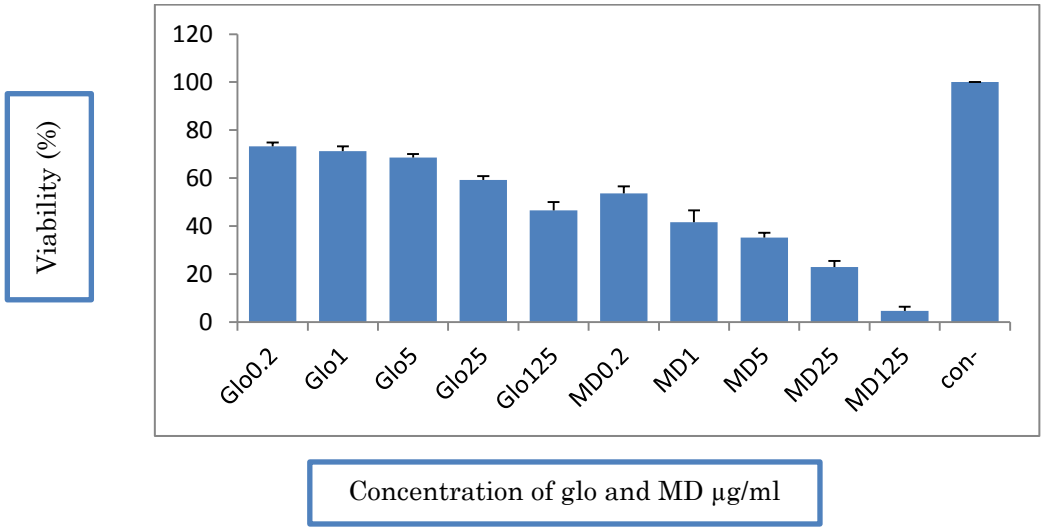

Figure 1: Activity of 2-phenoxy nicotinic acid hydrazide (19) (19) vs glucantime (20) (20) against intracellular amastigotes. The figure describes the effect of 2-phenoxy nicotinic acid hydrazide sulfonamide in wide rwnge (0.2-125) is stronger than glucantime.

Discussion

The results of this study showed 2-phenoxy nicotinic acid hydrazide sulfonamide inhibits proliferation of Leishmania major in a dose-dependent manner. The compare of different concentrations of 2-phenoxy nicotinic acid hydrazide sulfonamide with meglumine as the standard drug for the treatment of Leishmania indicated the inhibitory effect of 2-phenoxy nicotinic sulfonamide acid derivatives is more than the meglumine.

Cutaneous leishmaniasis is endemic in many countries, including Iran; despite of numerous treatments is still lack a simple and effective treatment with fewer side effects (10).

The effects of non-steroidal anti-inflammatory drugs in the treatment of many bacterial infections have been determined which is varying results have been reported.

Jalalian et al studied antileishmanial effect of salicylic acid (6) in susceptible mice Balb/c. Results showed that ASA has limited effects on leishmania by altering the inflammatory immune factors such as CRP and NO on the Balb/c infected with L. major. In addition, the use of ASA reduced visceral parasite replication in target organs and amastigotes inside macrophages; whereas there is not significantly, changing Leishmania wound size, survival rate, hepatomegaly and splenomegaly (21).

Moradi and colleagues, suggested that nicotinic acid 2-phenoxy compounds has a stronger effect compared with mefenamic acid .in addition nicotinic acid has moderate inhibitory effects of cyclo-oxygenase I and weak effect on cyclo-oxygenase II (22).

Gazanion and colleagues in a study in 2011 that examined the effect of combination therapy on Leishmaniasis. They reported that nicotinamide or vitamin B9 had anti leshmania effect. Also synergically, effect of nicotinamide in combination with trivalent antimony compound and amphotericin B were seen (23).

N- Acyl hydrazoneis widely used as an anti-inflammatory, analgesic, and anti-platelet compounds, have been described. This is due to its ability to mimic the BIS-allylic of unsaturated fatty acids and amides.

Some evidences suggest that the hydrazone moiety possesses a pharmacophoric character for the inhibition of cyclooxygenase (19-21). This can be rationalized by the Relative acidity of the amide hydrogen of the NAH group as well as its capacity of stabilizing free radicals.

Narang and colleagues assessed edema induced by Carrageenan in rat model for determining the impact of several hydrazide derivative of nicotinic acid, Naptln-1-acetic acid, Naptoksy acetic acid compared with

diclofenac, as a standard medication. The results showed that nitro-group from nicotinic acid derivatives, had changed in Meta and ortho position had highest anti-inflammatory agents.

Various substituted arylidene-2-phenoxynicotinic acid hydrazide sulfonamide derivatives were synthesized and screened for their potential activities (24).

The results of this study showed that 2-phenoxy nicotinic acid hydrazide sulfonamide derivatives of with displacement in phenolic circuleable to show dose-dependent anti- Leishmania effect. According to anti-inflammatory effects of these derivatives requires further investigation mechanism of anti-inflammatory factors associated with drug effectiveness and change there.

References

1. Savoia D. Recent updates and perspectives on leishmaniasis. The Journal of Infection in Developing Countries. 2015;9(06):588-96.
2. Khyatti M, Trimbitas R-D, Zouheir Y, Benani A, El Messaoudi M-D, Hemminki K. Infectious diseases in North Africa and North African immigrants to Europe. The European Journal of Public Health. 2014;24(suppl 1):47-56.
3. Signorini M, Cassini R, Drigo M, di Regalbono AF, Pietrobelli M, Montarsi F, et al. Ecological niche model of Phlebotomus perniciosus, the main vector of canine leishmaniasis in north-eastern Italy. Geospatial health. 2014;9(1):193-201.
4. Okwor I, Uzonna JE. The immunology of Leishmania/HIV co-infection. Immunologic research. 2013;56(1):163-71.
5. Dawit G, Girma Z, Simenew K. A review on biology, epidemiology and public health significance of leishmaniasis. Journal of Bacteriology & Parasitology. 2013;2013.
6. Salehabadi A, Karamian M, Farzad MH, Namaei MH. Effect of root bark extract of Berberis vulgaris L. on Leishmania major on BALB/c mice. Parasitology research. 2014;113(3):953-7.
7. Hajjaran H, Mohebali M, Mamishi S, Vasigheh F, Oshaghi MA, Naddaf SR, et al. Molecular identification and polymorphism determination of cutaneous and visceral leishmaniasis agents isolated from human and animal hosts in Iran. BioMed research international. 2013;2013.
8. Lombardo G, Pennisi MG, Lupo T, Chicharro C, Solano-Gallego L. Papular dermatitis due to Leishmania infantum infection in seventeen dogs: diagnostic features, extent of the infection and treatment outcome. Parasites & vectors. 2014;7(1):1.
9. Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, Bettaieb J, et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. New England Journal of Medicine. 2013;368(6):524-32.
10. Talari SA, Sadr F. Treatment of cutaneous leishmaniasis: effectiveness, and adverse effects of the drugs. KAUMS Journal (FEYZ). 2005;9(1):85-94.
11. Soto J, Rojas E, Guzman M, Verduguez A, Nena W, Maldonado M, et al. Intralesional antimony for single lesions of bolivian cutaneous leishmaniasis. Clinical infectious diseases. 2013;56(9):1255-60.
12. Jaffary F, Nilforoushzadeh MA, Siadat A, Haftbaradaran E, Ansari N, Ahmadi E. A Comparison between the Effects of Glucantime, Topical Trichloroacetic Acid 50% plus Glucantime, and Fractional Carbon Dioxide Laser plus Glucantime on Cutaneous Leishmaniasis Lesions. Dermatology research and practice. 2016;2016.
13. González U, Pinart M, Reveiz L, Rengifo-Pardo M, Tweed J, Macaya A, et al. Designing and reporting clinical trials on treatments for cutaneous leishmaniasis. Clinical infectious diseases. 2010;51(4):409-19.
14. Almasirad A, Mousavi Z, Tajik M, Assarzadeh MJ, Shafiee A. Synthesis, analgesic and anti-inflammatory activities of new methyl-imidazolyl-1, 3, 4-oxadiazoles and 1, 2, 4-triazoles. DARU Journal of Pharmaceutical Sciences. 2014;22(1):1.
15. Pontiki E, Hadjipavlou-Litina D, Litinas K, Nicolotti O, Carotti A. Design, synthesis and pharmacobiological evaluation of novel acrylic acid derivatives acting as lipoxygenase and cyclooxygenase-1 inhibitors with antioxidant and anti-inflammatory activities. European journal of medicinal chemistry. 2011;46(1):191-200.
16. Lima PC, Lima LM, da Silva KCM, Léda PHO, de Miranda ALP, Fraga CA, et al. Synthesis and analgesic activity of novel N-acylarylhydrazones and isosters, derived from natural safrole. European journal of medicinal chemistry. 2000;35(2):187-203.

17. Duarte CD, Tributino JL, Lacerda DI, Martins MV, Alexandre-Moreira MS, Dutra F, et al. Synthesis, pharmacological evaluation and electrochemical studies of novel 6-nitro-3, 4-methylenedioxyphenyl-N-acylhydrazone derivatives: Discovery of LASSBio-881, a new ligand of cannabinoid receptors. Bioorganic & medicinal chemistry. 2007;15(6):2421-33.

18. Almasirad A, Hosseini R, Jalalizadeh H, Rahimi-Moghaddam Z, Abaeian N, Janafrooz M, et al. Synthesis and analgesic activity of 2-phenoxybenzoic acid and N-phenylanthranilic acid hydrazides. Biological and Pharmaceutical Bulletin. 2006;29(6):1180-5.

19. William H, Markle, MD., and Khal doun, M. cutanous leishmaniasis: Reconition and tretment American Family Phisician 2004;69:1455-60.

20. Glöckner G. Gradient HPLC of copolymers and chromatographic cross-fractionation: Springer Science & Business Media; 2012.

21. Jalalian M. NH, Asmar M.,Esmaeili R.A.,Farahmand M.,Amini M Antileishmanial Immuno Parasitology effects of ASA in Balb/c mice sensitive to Leishmania major by NO and CRP mechanisms. Journal of Biological Sciences Lahijan Branch. 2010;4(2).

22. Moradi A, Navidpour L, Amini M, Sadeghian H, Shadnia H, Firouzi O, et al. Design and Synthesis of 2-Phenoxynicotinic Acid Hydrazides as Anti-inflammatory and Analgesic Agents. Archiv der Pharmazie. 2010;343(9):509-18.

23. Gazanion E, Vergnes B, Seveno M, Garcia D, Oury B, Ait-Oudhia K, et al. In vitro activity of nicotinamide/antileishmanial drug combinations. Parasitology international. 2011;60(1):19-24.

24. Narang R, Sharma S, Narasimhan B. Evaluation of anti-inflammatory activity of acid Hydrazide derivatives. Hygeia. 2012;4(2):21-6.

