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THE ANTI-PARASITE EFFECT OF PODOPHYLLOTOXIN ON GIARDIA LAMBLIA

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ABSTRACT

Giardia lamblia is an intestinal protozoan parasite causing diarrhea in humans and some other mammalians world-wide. Giardia infects about 2% of the adults and 6 to 8% of the children worldwide and is currently responsible for the largest number of water-borne outbreaks of diarrhea. There are a variety of agents used for treatment of Giardiasis caused by G. lamblia, which may have various effects. Podophyllotoxin is a non-alkaloid compound from lignan family that has been used as an anti-viral, anti-fungal and anti-cancer agent. Here we have used PTOX against Giardia lamblia to evaluate its effect as an anti-parasite compound. We have shown that PTOX can inhibit growth of G. lamblia and causes death, suggesting that PTOX can be used against G. lambia.

KEYWORDS: Giardiasis, Giardia lamblia, Podophyllotoxin, Cytotoxicity

Giardia lamblia, causing giardiasis, is a flagellated protozoan parasite that colonizes and reproduces in the small intestine. The parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission (Eissa and Amer, 2012). Giardia infects humans, and is also one of the most common parasites infecting other mammalians such as cat, beavers and sheep. Human infection pathway includes mostly ingestion of untreated sewage, particularly in many developing countries(Lingdan et al., 2012).

Human infection is conventionally treated with metronidazole, nitazoxanide and tinidazole (Khandheria et al.). Although metronidazole is the current first-line therapy, it is considered as a mutagenic agent in bacteria and also carcinogenic in mice, and is recommended not to be used during pregnancy(Rosenkranz and Speck, 1975). It has not been reported that links to cause cancer in humans, therefore it appears safe(Obot et al., 2013). Berberine sulfate is also one of the most common treatments found in Oregon grape root, golden seal, yellow root, and various other plants(Chen et al., 2013). It has been shown that berberine has an antimicrobial and an antipyretic effect causing uterine stimulation. Continuous high dosing of berberine may lead to bradycardia and hypotension in some individuals(Thompson et al., 1993).

Podophyllotoxin (PTOX) is a non-alkaloid toxin in the lignan family that is mostly isolated from genera Podophyllum (M.Wink, 2005). A number of PTOX derivatives and their glucosides have been isolated from these plants. Also, recent researches have demonstrated that plants from Linum species are additional sources of the aforementioned cytotoxic extracts (Farkya et al., 2004). PTOX that was used firstly as an agent for genital warts treatment (Canel et al., 2000) can inhibit growth of epithelial cells infected by human papilloma virus (HPV) in epidermis (Longstaff and von Krogh, 2001). PTOX is the pharmacological precursor of etoposide and teniposide (Cragg and Newman, 2005; Harvey, 1999) which are important anticancer drugs due to a mechanism of action based on the inhibition of cell cycle.

Here, we have used PTOX as an anti-parasite agent against Giardia lamblia. We showed that PTOX can inhibit growth of Giardia lamblia cells and causes a decrease in cell viability.

MATERIALS AND METHODS

Experiments were performed with trophozoites of G. lamblia (Tehran University of Medical Sciences, Iran) which were grown up to log phase in TYI-S-33 medium (pH 7.0) at 37 °C for 72 h, supplemented with 10% fetal bovine serum and 0.1 mg/mL of bovine bile.

Monolayers of rat intestinal epithelial cells (IEC-6 line, Pasteur Institute, Tehran, Iran) were grown in Dulbecco's Modified Eagle Medium (DMEM; Sigma-

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Aldrich, USA) added to 10% fetal bovine serum and 1 U/mL of regular human insulin. IEC-6 cells were trypsinized, added to 24-well and 96-well plates (Nunc Inc., USA) at final concentrations of 1×10^5 cells/mL and 0.8×10^4 cells/mL, respectively, and maintained at 37 °C for 24 h in 5% CO_2 atmosphere.

In vitro Cytotoxicity

In vitro susceptibility assays were performed using a subculture method. Briefly, Giardia lamblia (5×10^4) trophozoites were incubated for 48 h at 37 °C in the presence of different concentrations (0-80 µg/ml) of PTOX in dimethyl sulfoxide (DMSO). Each test included emetine as positive a control (culture medium plus trophozoites and DMSO), and a blank (culture medium). After appropriate incubation, the trophozoites were detached and samples of each tube were subcultured in fresh medium for another 48 and 72 h, without antiprotozoal drugs or extracts. In the time dependent manner, PTOX with the concentration of 10µg/ml was added and the incubation was continued for 24, 48 and 72 h. After removal of the MTT dye solution, cells were treated with 100µl DMSO and the absorbance at 490 nm was quantified using ELISA reader. The cytotoxicity was measured by comparing with the control (treated with 0.1% DMSO). Cytotoxicity is calculated as the concentration of drug inhibiting cell growth by 50% (IC50). All experiments were conducted at least in triplicate.

Statistical analysis

The result of cytotoxicity were analyzed with One way ANOVA followed with T-test was performed using Graphpad Prism 5.0 program and SPSS (SPSS, Chicago, IL, USA). A P-value ≤ 0.05 was considered significant and data were shown as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Despite the recognition of G.lamblia clinical illness for the last 40 years, then early 5,000 people hospitalized with giardiasis annually in the United States, and the large number of infections worldwide, there have been few therapies for this infection.

For treatment of Giardiasis, caused by G. lamblia, numerous agents have been used with different efficacy in both experimental and clinical works(Farthing, 1992) . A large number of chemotherapy agents and drugs including metronidazole, rifampin, bithionol and sodiumfusidate have been developed and showed in vitro cytotoxicity against Giardia lamblia(Rossignol, 2010). There are different classes of agents with different properties. The nitroimidazoles agents including metronidazole, trinidazole, ornidazole and secnidazole have been to treat G. lamblia infections (Leitsch et al., 2012). Metronidazole was used in clinic for treatment of Giardiasis(Argüello-García et al., 2009). In vitro assays have been performed on G.lamblia and showed that they were effective to kill G. lamblia parasite. Tinidazole and metronidazole have shown the greatest anti-parasite activity against G. lamblia(Busatti et al., 2013). There were also several agents used against G. lamblia but they are not effective. Metronidazole has shown some side effects, including headache, vertigo and nausea that could be severe for some patients(Dunn et al., 2010). Also, metronidazole is carcinogenic for rats at high doses. Moreover, it is mutagenic in bacteria but never shown in human(Corrêa et al., 2009).

Lignans are a family of natural products that exist in many plants and are isolated from different species. PTOX as a featured member of this family has different biological properties but is mostly used for synthesis of more applicable derivatives such as etoposide and teniposide with less cytotoxicity(Zhang et al., 2014). Here, we have investigated the effect of PTOX on G. lamblia as an anti-parasite agent.

To evaluate the IC50 of PTOX on Giardia cells, cytotoxicity was performed; increasing drug concentration and the cell viability were determined by MTT assay. Firstly, we have used different concentration of PTOX (0-80 µg/ml) for 24 h against G. lamblia parasites to determine IC50. Using MTT assay, 15 µg/ml doses was calculated as IC50, as shown in Figure 1. In time-dependent manner, 10µg/ml concentration was used and incubated against G. lamblia cells for 24, 48 and 72 h, compared with control cells. As figure 2 shows, increasing incubation time has led to decrease in cell viability of G. lamblia. The viable cells rate was significantly reduced from 55% at 24 h to 30% at 48 h and 20 % at 72 h, suggesting that PTOX functions by time leading to more cell death. According these results, we suggest that PTOX can be used as an anti-parasite agent against G. lambia.

CONCLUSION

Giardiasis still poses critical challenges, including a definitive and efficient chemotherapy. The severe side effects and questionable activity in chronic phase and an increased resistance are the main difficulties in the clinical administration of current treatments. We have shown that PTOX is capable of inducing death of G. lamblia. Accordingly, we suggest that Giardiasis, caused by Giardia lamblia infection, can be treated by PTOX as an anti-parasite agent.

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Conflict of interest: None.

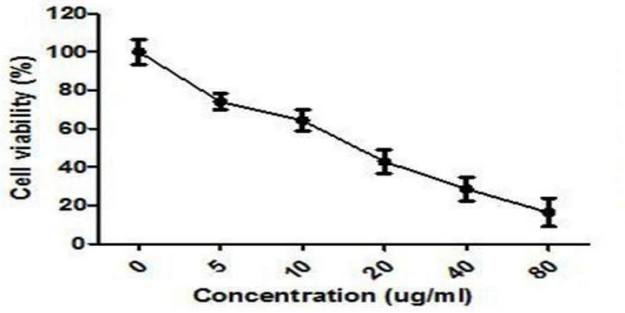

Figure1. Cytotoxicity effect of PTOX on Giardia lamblia. The IC50 was calculated for PTOX after 24 h treatment. Viability values were determined by a MTT assay in triplicate independent experiments (mean ± S.D)

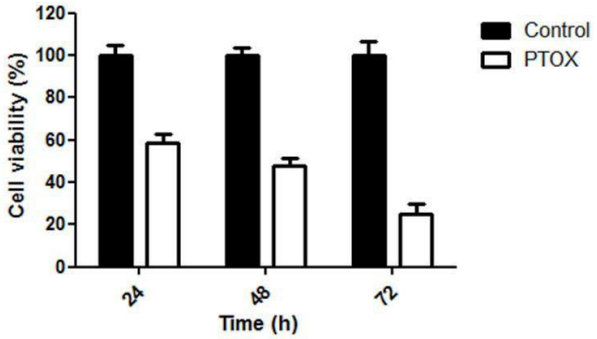

Figure 2. Survival ratios of G. lamblia parasites treated with PTOX. The 80% confluence cell cultures were treated with 10 µg/ml of PTOX for different times. The effect of PTOX in reduction of survival ratio of G. lamblia is time dependent. At 48h after treatment more than 50% of cells die. Results represented means of three independent experiments by MTT assay

REFERENCES

Argüello-García, R., Cruz-Soto, M., Romero-Montoya, L., Ortega-Pierres, G., 2009. In vitro resistance to 5-nitroimidazoles and benzimidazoles in Giardia duodenalis: Variability and variation in gene expression. Infection, Genetics and Evolution 9, 1057-1064.

Busatti, H.G.N.O., Alves, R.J., Santana-Anjos, K.G., Gil, F.F., Cury, M.C., Vannier-Santos, M.A., Gomes, M.A., 2013. Effects of metronidazole analogues on Giardia lamblia: experimental infection and cell organization. Diagnostic Microbiology and Infectious Disease 75, 160-164.

Canel, C., Moraes, R.M., Dayan, F.E., Ferreira, D., 2000. Podophyllotoxin. Phytochemistry 54, 115-120.

Chen, G., Lu, F., Xu, L., Dong, H., Yi, P., Wang, F., Huang, Z., Zou, X., 2013. The anti-diabetic effects and pharmacokinetic profiles of berberine in mice treated with Jiao-Tai-Wan and its compatibility. Phytomedicine 20, 780-786.

Corrêa, G., Vilela, R., Menna-Barreto, R.F.S., Midlej, V., Benchimol, M., 2009. Cell death induction in Giardia lamblia: Effect of beta-lapachone and starvation. Parasitology International 58, 424-437.

Cragg, G.M., Newman, D.J., 2005. Plants as a source of anti-cancer agents. Journal of Ethnopharmacology 100, 72-79.

Dunn, L.A., Burgess, A.G., Krauer, K.G., Eckmann, L., Vanelle, P., Crozet, M.D., Gillin, F.D., Upcroft, P., Upcroft, J.A., 2010. A new-generation 5-nitroimidazole can induce highly metronidazole-resistant Giardia lamblia in vitro. International Journal of Antimicrobial Agents 36, 37-42.

Eissa, M.M., Amer, E.I., 2012. Giardia lamblia: A new target for miltefosine. International Journal for Parasitology 42, 443-452.

Farkya, S., Bisaria, V.S., Srivastava, A.K., 2004. Biotechnological aspects of the production of the anticancer drug podophyllotoxin. Appl Microbiol Biotechnol 65, 504-519.

Farthing, M.J.G., 1992. Giardia comes of age: progress in epidemiology, immunology and chemotherapy. Journal of Antimicrobial Chemotherapy 30, 563-666.

Harvey, A.L., 1999. Medicines from nature: are natural products still relevant to drug discovery? Trends in Pharmacological Sciences 20, 196-198.

Khandheria, M., Snook, E., Thomas, C., Psychotic Episode Secondary to Metronidazole Use. General Hospital Psychiatry.

Leitsch, D., Schlosser, S., Burgess, A., Duchêne, M., 2012. Nitroimidazole drugs vary in their mode of action in the human parasite Giardia lamblia. International Journal for Parasitology: Drugs and Drug Resistance 2, 166-170.

Lingdan, L., Pengtao, G., Wenchao, L., Jianhua, L., Ju, Y., Chengwu, L., He, L., Guocai, Z., Wenzhi, R., Yujiang, C., Xichen, Z., 2012. Differential dissolved protein expression throughout the life cycle of Giardia lamblia. Experimental Parasitology 132, 465-469.

Longstaff, E., von Krogh, G., 2001. Condyloma Eradication: Self-Therapy with 0.15–0.5% Podophyllotoxin versus 20–25% Podophyllin Preparations—An Integrated Safety Assessment. Regulatory Toxicology and Pharmacology 33, 117-137.

M.Wink, A.W.A., R. Franke,B.Wetterauer et al, 2005. Sustainable bioproduction of phytochemicals by plant in vitro cultures: anticancer agents. Plant Gen. Res 3, 90–100.

Obot, I.B., Ebenso, E.E., Kabanda, M.M., 2013. Metronidazole as environmentally safe corrosion inhibitor for mild steel in 0.5 M HCl: Experimental and theoretical investigation. Journal of Environmental Chemical Engineering 1, 431-439.

Rosenkranz, H.S., Speck, W.T., 1975. Mutagenicity of metronidazole: Activation by mammalian liver microsomes. Biochemical and Biophysical Research Communications 66, 520-525.

Rossignol, J.-F., 2010. Cryptosporidium and Giardia: Treatment options and prospects for new drugs. Experimental Parasitology 124, 45-53.

Thompson, R.C.A., Reynoldson, J.A., Mendis, A.H.W., 1993. Giardia and Giardiasis, in: Baker, J.R., Muller, R. (Eds.), Advances in Parasitology. Academic Press, pp. 71-160.

Zhang, Z.-J., Tian, J., Wang, L.-T., Wang, M.-J., Nan, X., Yang, L., Liu, Y.-Q., Morris-Natschke, S.L., Lee, K.-H., 2014. Design, synthesis and cytotoxic activity of novel sulfonylurea derivatives of podophyllotoxin. Bioorganic & Medicinal Chemistry 22, 204-210.