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Effect of Guanabenz-Loaded Nanoparticles on the Mortality Rate of Mice Infected with Chronic Toxoplasmosis

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A B S T R A C T

Chronic toxoplasmosis is considered as one of the major neglected tropical diseases. Although there are many treatment modalities for acute toxoplasmosis, there is still insufficient knowledge about a safe effective treatment for chronic toxoplasmosis till now. However, guanabenz has shown a promising result for treatment of *Toxoplasma gondii* infection although it could not completely eradicate all brain tissue cysts. This study was designed to test the effect of guanabenz-loaded polyethylene glycol poly lactic-co-glycolic acid (PEG PLGA) nanoparticles on mortality rate of mice infected with chronic toxoplasmosis. The study was conducted in Tanta University, Medical Parasitology laboratory on 160 mice that were divided as follows: Group I (five subgroups each of 20 mice): Ia: non-infected non-treated, Ib: infected untreated, Ic: non-infected mice given nanoparticles alone, Id: infected mice given nanoparticles alone and Ie: infected mice treated by pyrimethamine (4 mg/kg) and sulfadiazine (100 mg/kg). Group II (three subgroups each of 20 mice): IIa: Infected and treated mice with guanabenz alone (5mg/kg/day), IIb: Infected and treated mice with guanabenz-loaded nanoparticles by full dose and IIc: Infected and treated mice with guanabenz-loaded nanoparticles by the half dose (2.5 mg/kg/day). The drugs were given on day 25 post-infection for 19 successive days. On the 20th day the mortality rate was calculated in different subgroups. The data were processed statistically. The Results showed that although guanabenz-loaded nanoparticles were given by intraperitoneal injection which carries much more infection hazards and stress on the mice, it caused less mortality rate in mice than subgroup Ie which was given pyrimethamine and sulfadiazine combination therapy orally, however this was of no statistically significant difference. It could be concluded that guanabenz-loaded PEG-PLGA nanoparticles cause no increase in the mortality rate of mice infected with chronic toxoplasmosis.

Introduction

Toxoplasma gondii is one of the most successful parasites on the earth. Although the cat is the only definitive host, *Toxoplasma gondii* has a wide variety of birds and mammals including man as intermediate hosts. When man becomes infected with *Toxoplasma*, he will go through two main

stages; the acute phase which is usually asymptomatic except for the immunosuppressed patients in which acute toxoplasmosis can cause fatal encephalitis, and the chronic phase in which the parasite becomes dormant as tissue cysts in the deep tissues of the host (Halonenand Weiss, 2013). The gold standard treatment of toxoplasmosis is the pyrimethamine and sulfadiazine combination therapy, however it is directed mainly against acute toxoplasmosis which is characterized by the rapidly replicating tachyzoites with unsatisfactory results regarding chronic toxoplasmosis (Konstantinovic *et al.*, 2019).

Guanabenz, which is an antihypertensive drug was repurposed recently for treatment of toxoplasmosis and showed promising results (Benmerzouga *et al.*, 2015). Nanomedicine is used now to solve many problems as the nanoparticles can be highly concentrated in tissues in addition to their potential ability to penetrate biological barriers as blood brain barrier. Poly lactic-co-glycolic acid (PLGA) is one of the FDA-approved polymers which is used as drug delivery vehicle with enhanced half-life when conjugated with polyethylene glycol (PEG) (Khaledi *et al.*, 2020). So, this preliminary study was designed to estimate the effect of guanabenz-loaded polyethylene glycol Poly lactic-co-glycolic acid (PEG-PLGA) nanoparticles on the mortality rate of mice infected with chronic toxoplasmosis.

Materials and Methods

Parasite

Avirulent ME49 strain of *T. gondii* strain has been kindly provided by Medical Parasitology Department, Faculty of Medicine, Alexandria University, Egypt.

Drug

Guanabenz

Guanabenz was purchased from Sigma Aldrich, USA. It was administered as a suspension by intraperitoneal (IP) injection in a dose of 5mg/kg/day for 19 successive days according to Benmerzouga *et al.*, (2015).

Guanabenz-loaded nanoparticles

Block copolymers of poly [(d,l-lactide-*co*-glycolide)-co-PEG] were purchased from Boehringer- Ingelheim Ltd. (Ingelheim, Germany). Poly vinyl alcohol, phosphate buffered saline (PBS) and dichloromethane were obtained from Sigma Chemical Co. (St. Louis, USA). Guanabenz-loaded nanoparticles were prepared by modified double emulsion, solvent evaporation method according to Haggag *et al.*, (2016). Theywere administered by intraperitoneal (IP) injection in a dose of 5mg/kg/day or 2.5mg/kg/day.

Pyremethamine and sulfadiazine

Pyremethamine and sulfadiazine were purchased from Sigma Aldrich, USA and dissolved in 0.1 ml dimethyl sulfoxide (DMSO) then diluted in distilled water to form a suspension and administered orally daily in a dose of pyrimethamine (4 mg/kg) and sulfadiazine (100 mg/kg) according to Notarangelo *et al.*, (2014) for 19 successive days.

Experimental design

One hundred and sixty laboratory-bred male Swiss albino mice were infected by intraesophageal gavage of 10 cysts/mouse of *Toxoplasma gondii* (ME49) strain according to El-Zawawy *et al.*, (2015). They were divided into two main groups: Group I (five subgroups each of 20 mice): Ia: non-infected non-treated, Ib: infected untreated, Ic: non-infected mice given nanoparticles alone, Id: infected mice given nanoparticles alone and Ie: infected mice treated by pyrimethamine (4 mg/kg) and sulfadiazine (100 mg/kg). Group II (three subgroups each of 20 mice): IIa: Infected and treated with guanabenz alone (5mg/kg/day), IIb: Infected and treated with guanabenz-loaded nanoparticles in a dose of 5mg/kg/day and IIc: Infected and treated with guanabenz-loaded nanoparticles by the half

dose (2.5 mg/kg/day). The treatment started on day 25 post-infection for 19 successive days according to Benmerzouga *et al.,* (2015). On the 20th day the mortality rate was assessed. The data were processed statistically.

All the experimental groups were subjected to estimation of the mortality rate (MR) according to El-Zawawy *et al.,* (2015)

MR = Number of dead mice at the sacrifice time/ Number of mice at the beginning of the experiment × 100.

Statistical analysis

The data were analyzed using SPSS version 20.0. Qualitative data were described using number and percentage. Monte Carlo test was used to compare multiple groups and Fisher's exact test was used and computed for 2x2 tables.

Results and Discussion

Estimation of the mortality rate (MR)

As shown in table 1, the mortality rate of the non-infected non-treated subgroup Ia and also in the infected non-treated subgroup Ib was 10% (two dead mice out of 20). The mortality rate of subgroup Ic (non-infected injected intraperitoneally with nanoparticles alone) was 25% (five dead mice out of 20), while in subgroup Id (infected and injected intraperitoneally with nanoparticles alone for same period), the mortality rate was 30% (six dead mice out of 20). In subgroup Ie (infected and treated orally with a combination of pyrimethamine and sulfadiazine), the mortality rate was 20% (four dead mice out of 20), whereas it was 15% (three dead mice out of 20) in all subgroups treated with guanabenz or guanabenz-loaded nanoparticles (subgroups IIa, IIb, IIc). The difference in MR between different control and infected treated subgroups was not statistically significant (P= 0.741). This study aimed to assess the effect of guanabenz alone or in combination with PEG-PLGA nanoparticles on the mortality rate of mice infected with chronic toxoplasmosis.

The mortality rate (MR) was 10% in both subgroup Ia (healthy control) and subgroup Ib (infected non-treated mice). This result was consistent with that of El-Zawawy *et al.,* (2015) who found a 10% MR in the non-infected non-treated subgroup and the infected non-treated mice. This could be due to the low infective dose (10 cysts for each mouse) beside the a virulent strain used in the study (ME49 strain) as explained by Djurković-Djaković *et al.,* (2002). Nanoparticles alone resulted in mortality rate of 25% and 30% in non-infected mice (subgroup Ic) and infected mice (subgroup Id), respectively with no statistically significant difference compared to other subgroups. This nonsignificant increase in the MR can be explained by the daily stress due to the intraperitoneal injection of mice in these subgroups. The MR of subgroups IIa, IIb and IIc was 15% which is less than that of subgroup Ie (20%), with no statistically significant difference. Although guanabenz-loaded nanoparticles were given by intraperitoneal injection which has more stress on the mice than the oral route, it caused less mortality rate (15%) than subgroup Ie which was treated orally by pyrimethamine and sulfadiazine combination therapy (20%), however this was of no statistically significant difference.

Guanabenz-loaded PEG-PLGA nanoparticles cause no statistically significant difference regarding the mortality rate of mice infected with chronic toxoplasmosis compared to the healthy control group.

Conflict of interest

Authors declare that there was no conflict of interest regarding the publication of this paper.

Table.1 Mortality rate of different control and infected treated subgroups

Mice subgroups / Mortality	Control groups					Infected treated groups		
	Ia N=20	Ib N=20	Ic N=20	Id N=20	Ie N=20	IIa N=20	IIb N=20	IIc N=20
Mortality rate	10%	10%	25%	30%	20%	15%	15%	15%
Number of dead mice	2	2	5	6	4	3	3	3
P 0	0.741							
P 1	------	1.000	0.407	0.235	0.661	1.000	1.000	1.000
P 2	------	------	0.407	0.235	0.661	1.000	1.000	1.000
P 3	-----	------	------	1.000	1.000	0.695	0.695	0.695
P 4	------	------	------	------	0.716	0.451	0.451	0.451
P 5	-------	------	-------	-------	-------	1.000	1.000	1.000
P 6	-------	------	-------	-------	-------	-------	1.000	1.000
P 7	-------	------	-------	-------	-------	-------	-------	1.000

P 0: *P* value for comparing between all subgroup using Monte Carlo test.
P 1: *P* value for comparing between subgroup Ia with each other subgroup (Ib, Ic, Id, Ie, IIa, IIb and IIc).
P 2: *P* value for comparing between subgroup Ib with each other subgroup (Ic, Id, Ie, IIa, IIb and IIc).
P 3: *P* value for comparing between subgroup Ic with each other subgroup (Id, Ie, IIa, IIb and IIc).
P 4: *P* value for comparing between subgroup Id with each other subgroup (Ie, IIa, IIb and IIc).
P 5: *P* value for comparing between subgroup Ie with each other subgroup (IIa, IIb and IIc).
P 6: *P* value for comparing between subgroup IIawith subgroups IIb and IIc.
P 7: *P* value for comparing between subgroup IIb and subgroup IIc.
(*P* 1-7 were performed by using Fisher's exact test)
* significant (*P* value less than 0.05)

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