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Assessment of the Activity of Spiramycin Co-administered with Metronidazole and Spiramycin-Loaded Nanoparticles in the Treatment of Acute Murine Toxoplasmosis

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In

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SUMMARY

Toxoplasma gondii (T. gondii) is an obligate intracellular apicomplexan protozoan which represents a global health threat. It has a complex life cycle composed of sexual phase, which is restricted to feline species only and asexual phase that can occur in most warm-blooded animals including man. Humans acquire infection via three main pathways; ingestion of tissue cysts in undercooked meat, ingestion of cats' oocysts in the environment and congenital transmission from an infected mother to her foetus during pregnancy. In most individuals the infection is asymptomatic, whereas severe pathology and lethality are common findings in congenitally infected or immunodeficient individuals.

The wide distribution of *T. gondii* infection makes finding a safe and effective drug a great success. Despite the fact that a variety of approaches have been developed in an effort to find an efficient and well-tolerated therapeutic regimen, the standard therapy is still hampered by severe adverse effects. Current therapeutics do not clear parasite infection and are often not satisfactory to patients. Spiramycin, a macrolide antibiotic, is effective against acute toxoplasmosis, less toxic than other drugs and able to achieve high concentrations in the placenta. In spite of significant tissue penetration, spiramycin demonstrates poor penetration across the blood brain barrier (BBB). In this respect, the search for drug combinations with novel mechanisms of action should be pursued. Thus, it was hypothesized that enhanced spiramycin brain uptake may be attained with the co-administration of a second drug (metronidazole).

In the search for new strategies that overcome the drawbacks of previous treatment regimens, nanomedicine finds its way to provide applicability for old and toxic drugs by improving their biodistribution, modifying bioavailability and decreasing toxicity.

It has been shown that chitosan (CS) exhibit excellent qualities, this is due to its outstanding biological properties. It is a biocompatible, biodegradable, non-toxic, cationic polymer that possesses good mucoadhesivity and the important capacity for increasing the penetration of drugs across barriers. CS has itself many medicinal properties; antimicrobial, antioxidant and immunoadjuvant, which enhance its potential in different biomedical applications. It is envisaged that CS NPs when used either alone or in drug delivery applications may further widen and strengthen their existence in the pharmaceutical area in near future.

In the present study, CS NPs, spiramycin, spiramycin co-administered with metronidazole and spiramycin-loaded CS NPs (400 mg/kg and 100 mg/kg) were evaluated in treatment of mice infected with the virulent RH strain of *T. gondii*, aiming at development of safe and effective drug or drug combination against acute toxoplasmosis.

To fulfil the present work, the NPs were prepared by ionotropic gelation method and characterized using SEM, TEM, Zetasizer and spectrophotometric estimation of loading efficiency. The drug efficacy was then evaluated using 140 male Swiss Albino mice. Mice were divided into control and treated groups. The control group was further subdivided into three subgroups; uninfected control, infected untreated control and infected received CS NPs. The treated group was subdivided into four infected subgroups treated orally with spiramycin, spiramycin-metronidazole, spiramycin-loaded NPs 400 mg/kg and spiramycin-

loaded NPs 100 mg/kg. Infected mice were inoculated intraperitoneally with 2500 tachyzoites of the virulent RH strain.

Drug efficacy was assessed by parasitological, morphological and histopathological studies. The parasitological study was done by clinical observation of mice, mice survival time, mice mortality rate (MR%) on the sacrifice day (8th day post-infection), parasite load (in peritoneal exudates using haemocytometer and in Giemsa stained impression smears of liver, spleen and brain) and parasite percent reduction (%R). The morphological study of the tachyzoites in the peritoneal fluid depended on studying the parasite movement by light microscope and the ultra-structure by SEM. The histopathological study determined the changes in infected tissues (liver, spleen and brain).

The results of characterization of NPs by both SEM and TEM showed nearly spherical NPs with smooth surface and increase in the particle size upon drug loading (average size of CS NPs= 50 nm and average size of spiramycin-loaded NPs= 70 nm). **Zetasizer** was used for measurement of hydrodynamic particle size, size distribution and zeta potential. The mean hydrodynamic particle size of CS NPs and spiramycin-loaded NPs were 177.48 nm (polydispersity index= 0.462) and 385.16 nm (polydispersity index= 0.455) respectively. The respective zeta potential values of CS NPs and spiramycin-loaded NPs were positive. **The drug loading efficiency** estimated by spectrophotometric method was 77.8%. These characteristic properties were considered ideal for systemic drug delivery.

In the drug efficacy assessment results of this study, the **clinical observation** revealed that infected treated mice remained relatively symptom-free with normal behaviour and food intake. Meanwhile, the most significant improvement in mice behaviour was observed among those treated with spiramycin-loaded NPs.

CS NPs, spiramycin, spiramycin-metronidazole, spiramycin-loaded NPs 400 mg/kg and spiramycin-loaded NPs 100 mg/kg were able to induce a statistically significant increase in the mean **survival time** of the infected mice (30.6, 7.4, 10.2, 59.5 and 58.8 days respectively) as compared to the infected untreated control (6.6 days). The maximum survival time of more than 200 days with no mortality on the sacrifice day was observed in mice receiving spiramycin-loaded NPs with no significant difference between the two used doses.

As regards the **parasite load**, a statistically significant reduction was observed in the mean tachyzoites count in the peritoneal exudate, liver, spleen and brain among all treated mice compared to the infected untreated control. Spiramycin-loaded NPs showed the highest significant percent reduction while spiramycin alone revealed the lowest reduction of parasite load as compared to the other used drugs. Both CS NPs and spiramycin-metronidazole showed marked efficiency in decreasing the parasite load with no significant difference between them except in the liver; where CS NPs revealed the least reduction compared to all other treatments.

Light microscopic examination of the peritoneal exudates of all treated mice showed sluggish tachyzoites movement except NPs treated tachyzoites which revealed loss of movement. **SEM performed on the collected tachyzoites** from all treated subgroups showed distortion in the crescent shape, loss of the conoid and various surface irregularities. The tachyzoites were more mutilated and some of them appeared rupturing

in those receiving CS NPs and spiramycin-loaded NPs. All these changes can explain the decrease in parasite burden and the increase in the survival time of treated mice.

Histopathological examination of liver, spleen and brain of all treated mice revealed decrease in the inflammation, congestion, necrosis and tachyzoites within tissue sections. Spiramycin-loaded NPs showed the highest significant reduction in the pathological insult while spiramycin alone revealed the lowest reduction as compared to the other used drugs. Administration of either CS NPs or spiramycin-metronidazole had a moderate reduction in the pathological changes.

The above results proved that spiramycin-loaded NPs have the highest significant promising efficacy in the treatment of acute toxoplasmosis. Besides, the non-toxic nature and anti-parasitic effect of CS make it a potential material either by acting directly or by improving the drug delivery into the tissues, particularly BBB passage.