EFFECTIVENESS OF MYRRH AND SPIRAMYCIN AS INHIBITORS FOR TOXOPLASMA GONDII TACHYZOITES IN VIVO

BY

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ABSTRACT

The antitoxoplasmonic activity of Spiramycin and Myrrh were evaluated in murine models of intraperitoneal infection using the RH strain of Toxoplasma gondii. In mice infected with $2 \times 10^5$ tachyzoites / ml, treatment with those drugs at 100 and 200 mg/kg/day had a good effect. Administration of tested drugs intraperitoneally reduced the mean number of tachyzoites present in the peritoneal fluid of experimented mice 4 days after infection, with the treatment of 100 and 200 mg/kg/day of Myrrh the percentage of growth inhibitions were 96.6%, 100 % respectively, as that of the reference drug, Spiramycin, (% growth inhibitions were 96.6%, 75.9% correspondingly) compared by the control infected untreated mice.

Key words: Antitoxoplasmonic Activity, Spiramycin, Myrrh, Toxoplasma Gondii, Growth Inhibition.

INTRODUCTION

Toxoplasma gondii is an intracellular apicomplexan protozoan parasite related to the causative agents of malaria (Plasmodium spp) and coccidiosis (Eimeria spp). It is found world-wide, at high prevalence and in an exceptionally broad host range, making it one of the most ‘successful’ parasite on earth (Cook et al., 2000 ; John et al., 2002 ; Montoya and Liesenfeld, 2004).

The life cycle of Toxoplasma includes two subcycles. The asexual cycle takes place in virtually any warm-blooded animal, ranging from chickens to sea otters and humans. This portion of the life cycle consists of just two stages, the rapidly dividing tachyzoites and the more slowly dividing bradyzoites, which can encyst in the brain, heart and other tissues. Transmission occurs by ingestion of bradyzoite-infected tissue through carnivoreism or scavenging. The sexual cycle includes full gametogenesis and mating within the intestinal epithelium and culminates in the generation of oocysts shedding in the cat’s feces. These oocysts are highly infectious and extremely stable in the environment (John et al., 2002).

Toxoplasma gondii is able to persist in
its host by conversion from the proliferative tachyzoite stage into quiescent encysted bradyzoites, controlled in this stage by the host's immune response (Dubey, 1977; Dubey and Beattie, 1988). Although infection in immunocompetent humans is usually asymptomatic, toxoplasmosis may cause severe complications in immunocompromised individuals (Israelnski and Remington, 1993). Infection is generally benign with few symptoms, occasionally causing eye disease (Roberts and Mcleod, 1999). However, an acute infection can cause devastating disease in the fetus (Remington et al., 1995), AIDS patients (Luft and Remington, 1992), recipients of organ transplants (Machado et al., 1992) and patients receiving cancer therapy (Dagher and Lucas, 1996). At the veterinary level, toxoplasmosis is also one of the main causes of infectious reproductive wastage in many countries, causing fetal resorption, abortion, stillbirth, and neonatal mortality in sheep, pigs, and goats (Dubey and Beattie, 1988).

An ideal drug for treatment would show effective penetration and concentration in the placenta, transplacental passage, parasiticidal properties against the different parasitic stages, penetration into cysts, distribution in the main sites of fetal infection and a total lack of fetal toxicity and teratogenic effects. No available drug fulfills all these criteria. A sulfonamide alone, such as sulfadiazine, lacks parasiticidal activity when it is not used in combination with a dihydrofolate reductase (DHFR) inhibitor such as pyrimethamine (Derouin et al., 2002; Montoya and Liesenfeld 2004). The current use of pyrimethamine for treatment of T. gondii infection is associated with suppression of bone marrow and can result in neutropenia even when accompanied with leucovorin supplements. Furthermore, this treatment is not used to treat congenital toxoplasmosis in the first trimester of gestation when folate depletion can have additional detrimental consequences for early fetal development. Moreover, the combination of pyrimethamine with sulfadiazine can give rise to further concern due to allergy, kidney stones, or hepatic or renal complications (Mui et al., 2005).

Atovaquone, a new DHFR inhibitors such as epiroprim and antibiotics such as fluoroquinolones are effective in vitro and in vivo against T. gondii. However, they cannot be used in pregnant women because their potential harmful effects on the embryo or fetus have not been properly examined (Derouin et al., 2002).

An alternative drug used in this setting, particularly in Europe, is spiramycin. It is a potent bacteriostatic, established macrolide that has long ago been shown to be effective in murine toxoplasma infection (Garin and Eyles, 1958). The antitoxoplasmic activity of Spiramycin was evaluated...
in murine models of infection using a type-1 (RH) or type-2 (Me 49) strain of Toxoplasma gondii. In mice infected with 2x102 tachyzoites of the RH strain, treatment with spiramycin at 100 and 200 mg/kg/day had only a limited effect, despite some dependent prolongation of survival, it was unable to protect mice against death (Grujić et al., 2005).

Mui et al. (2005) reported that Triazine was a potent inhibitor for gondii tachyzoites in vitro. Also, in vivo, the administration of Triazine intraperitoneally reduced the mean number of RH strain tachyzoites present in peritoneal fluid substantially 4 days after intraperitoneal infection of mice. The medicinal plant Juniperus procera was tested by using fruit, leaves and stems extracts as a growth inhibitor for Toxoplasma tachyzoites in dose of 400 mg/kg/day, (Al-Zanbagi, 2008a).

The oleo-gum resin of Myrrh (Commiphora molmol) has been used in Middle Eastern medicine for treatment of infected wounds and bronchial complaints for thousands of years (Bown, 1995). The extract of Myrrh produced significant inhibition of carrageenan induced inflammation and cotton pellet granuloma (Tariq et al., 1986). The extract also showed significant antipyretic activity in mice (Massoud and Labib, 2000). The mosquitocidal extracts of Myrrh plant, oil and oleo-resin, were proved to demonstrate larvicidal activity against Culex pipiens larvae (Massoud et al., 2001).

Two reports recorded that Myrrh is effective in the treatment of human schistosomiasis and human fascioliasis (Abo-Madyan et al., 2004a; Abo-Madyan et al., 2004b). The actual effect of Myrrh for the treatment of schistosomiasis and heterophyidiasis was also studied (Hassan et al., 2003; Fathy et al., 2005). Myrrh extract was successfully used in the treatment of human dicrocoeliasis dendriticum patients (Al-Mathal and Fouad, 2004). Also, it was proved to be very effective in sheep fascioliasis and sheep monieziaisis expansa (Haridy et al., 2003; Haridy et al., 2004). As well it has a good effect against Trichomonas vaginalis (Al-Zanbagi, 2007). By using Myrrh as antitoxoplasmic drug, Al-Zanbagi, (2008b) recorded that it reduced the number of Toxoplasma tachyzoites after initiation of the infection in the experimental mice by 2 x 10^2 tachyzoites / ml.

Myrrh has molluscicidal effect on Bulinus truncatus and Biomphalaria alexandrina snails at low concentrations of 10 and 20 ppm respectively after 24 hours exposure (Massoud and Habib, 2003), on Biomphalaria arabica snails at 40 ppm after 48 hours exposure (Al-Mathal and Fouad, 2006) and on Bithynia connollyi at 80 ppm after 72 hours exposure (Shoukry, 2006).

The present study was conducted to ex-
amine the antitoxoplasmic activity of spiramycin and Myrrh in murine models of intraperitoneal infection using the RH strain of Toxoplasma gondii.

**MATERIAL AND METHODS**

**Mice model:**
Throughout this study, CBA/J inbred female mice strain was used, they weighed 18 - 20 gram. Mice were obtained from Experimental Animal Unit of Medical Research Center, Faculty of Medicine, Ain Shams University, Cairo, Egypt. They were offered drinking water and regular mouse feed ad libitum (standard conventional rodent chow).

**Parasite under study:**
Tachyzoites of the RH Toxoplasma gondii strain were used which being maintained through serial intraperitoneal passages in female mice. For experimental infection, tachyzoites were obtained from mouse peritoneal fluid 72 hours post-infection as described by Djurkovi - Djakovi et al. (2005). After counting in a haemocytometer, suspensions were adjusted to $2 \times 10^2$ tachyzoites / ml with saline and 0.4 ml aliquots were inoculated intraperitoneally into mice according to Grujić et al. (2005).

**Myrrh extract:**
Myrrh was purchased from the local market, suitable weight was crushed well and dissolved in specific water for injection (Egypt otsuka pharm. Co.) to have a concentration of 1:1 and 2:1, then administered to female mice at doses of 100 and 200 mg/kg body mass per day.

**Spiramycin:**
This reference drug (Rovamycin®500, Rhone-poulenc Rorer Aebe) was also administered at the same doses with the same concentrations after weighing, crushing and dissolving in the specific water appropriate for injection.

**Experiment Design:**
According to the method of Mui et al. (2005), groups of female mice (almost three mice for each concentration) were injected intraperitoneally with $2 \times 10^2$ tachyzoites of the Toxoplasma RH strain. These mice were assigned to treatment with 100 or 200 mg of the tested drug/kg/day. Treatment was initiated 15 minutes post infection and sustained for the next three days. Control mice left untreated but received an equivalent amount of the injection water.

**Quantization of inhibitory drugs:**
Since murine infection with RH strain parasites is habitually lethal, growth inhibition of tachyzoites in the peritoneal fluids of treated mice was the parameter of treatment effect, which showed the effectiveness of Myrrh extract for the tested parasite. On the fifth day of experiment,
peritoneal fluids of all female mice were examined for Toxoplasma gondii tachyzoites by using a hemocytometer to record the number of parasites present in each mouse peritoneal fluid. The percentage of growth inhibition was calculated with respect to the growth control as (1 - growth rate for extract / growth rate control) x 100, as recorded by Muelas-Serrano et al. (2000).

**Statistics analysis:**
The effect of the different extracts used in this study on Toxoplasma gondii tachyozoites growth was analyzed by using one way of variance (ANOVA). To perform pair wise comparisons following a significant ANOVA test, least significant difference (L.S. D.) was used as Post Hoc Test at the level of statistical significance of 0.05.

**RESULTS**
All female mice under experiment were killed and dissected at the end of 4 days, the test duration. Treatment with spiramycin and myrrh gave a considerable growth inhibition for the tachyzoites of T. gondii. Treatment with spiramycin at 100 or 200 mg/kg/day as of day 1 post infection for three days later, did not eliminate T. gondii tachyzoites in the peritoneal fluids of all examined animals (Table 1, Figures 1 & 2). The growth inhibition of tachyzoites in mice treated with spiramycin at 100 or 200 mg/kg (mean ± S.D., 66.7 ± 115.5 and 466.7 ± 416.3 respectively) was limited, being as 96.6 and 75.9% at concentrations of 1:1 and 2:1 respectively. The estimated probabilities of tachyzoites growth inhibition differ significantly among all groups of mice treated by 100 or 200 mg/kg of spiramycin (p = 0.29). Also, between untreated (controls) mice and spiramycin treated groups, there is a significant difference at 0.5 levels.

Myrrh extracts at doses of 100 or 200 mg/kg showed higher activities than that recorded by using spiramycin at the same doses, giving the percentage of growth inhibition as 96.6 and 100% correspondingly. This effect of Myrrh as growth inhibitor in the treated mice was highly limited at the two doses used (mean ± S.D. 66.7 ± 115.5 and 0.00 ± 0.00 respectively). The growth inhibition did not differ significantly among all mice groups treated by myrrh at the different doses used (p = 0.68), in spite of a significant differences at the level 0.5 between the treated mice by the two doses of Myrrh and that of untreated mice (p = 0.001).

When the specific water for injection was injected intraperitoneally into experimental mice, it reduced the number of Toxoplasma gondii tachyzoites in comparison with that of untreated control mice. The percentage of growth inhibition was 72.4 and 86.2% for the concentrations of 1:1 and 2:1 correspondingly.
DISCUSSION

The present study showed the activity of myrrh and spiramycin in murine infection with RH strain of Toxoplasma gondii. In infection induced with $2 \times 10^2$ tachyzoites/ml spiramycin in doses of 100 and 200 mg/kg exerted only a limited effect (96.6 and 75.9% respectively) and it was possibly unable to protect mice against death. This result appears to be better than that recorded by Grujić et al. (2005). However, Al-Zanbagi, (2008a) showed that spiramycin injected intraperitoneally into mice at dose of 400 mg/kg/day for seven days, gave a growth inhibition as 98.5% indicating a result better than that of the present study this may be due to the spiramycin dose or to the duration of experiment. Aqueous extract of spiramycin proved to be effective as a growth inhibitor (91 and 96% in doses of 100 and 200 in that order) for Toxoplasma gondii when used in female mice infected by 1× 104 tachyzoites despite of the difference in the number of tachyzoites used for initial infection and the strain of experimental mice which were MFI (Al-Zanbagi, 2008b).

Myrrh treatment significantly enhanced protection and decreased the tachyzoites number in experimental animals (96.6 and 100%), which is comparable to that recorded by Mui et al. (2005), who observed that Triazine was highly effective against T. gondii tachyzoites in a mouse model which was infected intraperitoneally with 10,000 tachyzoites. Al-Zanbagi, (2008b) observed similar results by using Myrrh extract that inhibited the growth of Toxoplasma tachyzoites when it dosed at 100 and 200 mg/kg and gave the percentage of inhibition as 97 and 91% in mice infected intraperitoneally with 1× 104 tachyzoites. Also, it is superior than that recorded by Al-Zanbagi, (2008a) who found that the percentage of growth inhibition of the fruit, leaves and stems of Juniperus procera was 53.5, 50 and 48% at concentration of 4:1 respectively.

Regarding the results recorded here, the aqueous extract of Myrrh used as antitoxoplasmosis drug proved a considerable effect, and it should be tested by using distilled water instead of the specific water for injection used in this study. To be convinced for myrrh effect as a useful drug against T. gondii tachyzoites, also it be supposed to be examined as an in vitro antitoxoplasmosis drug. Spiramycin has proved to be helpful drug used at present and it may be practical if its dose was higher than those used in this study.
Table (1): Reduction of numbers of T. gondii tachyzoites in peritoneal fluid by treatment with myrrh aqueous extract and spiramycin after starting infection in mice by $2 \times 10^2$ tachyzoites / ml / mice

<table>
<thead>
<tr>
<th>Drug used</th>
<th>Dose of drug</th>
<th>No. of mice used</th>
<th>No. of tachyzoites</th>
<th>Mean of tachyzoites</th>
<th>Standard deviation</th>
<th>% growth inhibition of tachyzoites*</th>
<th>% survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myrrh water extract</td>
<td>100</td>
<td>3</td>
<td>200</td>
<td>66.7</td>
<td>115.5</td>
<td>96.6 %</td>
<td>3.4 %</td>
</tr>
<tr>
<td>Myrrh water extract</td>
<td>200</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>100 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>100</td>
<td>3</td>
<td>200</td>
<td>66.7</td>
<td>115.5</td>
<td>96.6 %</td>
<td>3.4 %</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>200</td>
<td>3</td>
<td>1400</td>
<td>466.7</td>
<td>416.3</td>
<td>75.9 %</td>
<td>24.1 %</td>
</tr>
<tr>
<td>Water only</td>
<td>100</td>
<td>2</td>
<td>1600</td>
<td>800</td>
<td>0.00</td>
<td>72.4 %</td>
<td>27.6 %</td>
</tr>
<tr>
<td>Water only</td>
<td>200</td>
<td>2</td>
<td>800</td>
<td>400</td>
<td>0.00</td>
<td>86.2 %</td>
<td>13.8 %</td>
</tr>
<tr>
<td>Infected control</td>
<td>-</td>
<td>2</td>
<td>5800</td>
<td>2900</td>
<td>141.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

% growth inhibition = (1 - No. of tachyzoites after treatment / No. of tachyzoites in infected control) x 100

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**Fig. (1):** Mean number of Toxoplasma gondii tachyzoites.

**Fig. (2):** % growth inhibition and % survival rate of Toxoplasma gondii tachyzoites.
REFERENCES


RH parasites in mice at treatment with atovaquone and pyrrolidine dithiovarbamate. Microbes and Infection, 7 (1) : 49 - 54.


Al-Zanbagi, N.A.


فعالية الماء والسبيروميرسين كمُثبطات للأطوار النشطة لطفيل النوكسيولاوا جوانغاي في حيوانات التجربة

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لقد قدر نشاط كلاً من نبات الماء وعقار السبيروميرسين ضد طفيل النوكسيولاوا في حيوانات التجربة لعدة داخل الغشاء البريتوني باستخدام السلالة الضارة لطفيل النوكسيولاوا. لقد أظهرت هذه الأدوية تأثيراً جيداً عند إعطائها باعداد 100 و 200 ملجم/كجم/يوم في الأطراف المعدية بعد من الأطوار النشطة بـ 200 كل مليلتر. إن إعطاء هذه الأدوية الخفيفة داخل الغشاء البريتوني في متوسط عدد الأطوار النشطة الموجودة في السائل البريتوني للأطراف المعدية لعدة 4 أيام، بعد العدوى باستخدام الماء بجرعة 100 و 200 ملجم/كلجم/يوم كانت النسبة المئوية تشฤط الدمى هي 96.6 و 100% على التوالي. كما حدث باستخدام العقار الرجع وهو السبيروميرسين (النسبة المئوية تشฤط الدمى 96.6 و 75.9% بالنتيجة) مقارنة بالفترات المعدية غير المعالجة.