# EFFICACY OF PRAZIQUANTEL FOR TREATMENT OF REPEAT INFECTION OF *Opisthorchis viverrini* IN HAMSTER

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*Received: July 24, 2015; Revised: September 25, 2105; Accepted: February 16, 2016*

## Abstract

**The human liver fluke, *Opisthorchis viverrini* infects several million people in Thailand. Chronic opisthorchiasis combined with nitrosamine ingested with food frequently leads bile duct cancer, cholangiocarcinoma (CCA). Repeated infection by *O. viverrini* increases hepatobiliary disease as well as increases the risk of CCA. Praziquantel (PZQ) is the drug of choice for treatment of opisthorchiasis. Whereas PZQ is considered safe to use, recent reports have cautioned that repeated use might influence the risk of liver fluke infection-induced CCA. Therefore, the efficacy of repeated treatment with PZQ warranted evaluation. Here the efficacy of PZQ treatment was investigated in hamsters repeatedly infected with *O. viverrini* and in hamsters both infected with *O. viverrini* and exposed to the carcinogen dimethylnitrosamine (DMN). Experimental hamsters were infected with *O. viverrini* and treated two times with 300 mg/kg of PZQ. Other hamsters were infected with *O. viverrini* as well as provided with drinking water that contained 12.5 ppm DMN, following by two treatments with PZQ. As controls, uninfected hamsters, uninfected hamsters treated with PZQ, and uninfected hamsters treated with both PZQ with DMN were included. Histopathology of hepatobiliary tissues was examined, specifically for infiltration of white blood cells, proliferation of cholangiocytes and fibrosis. The pathology including inflammation, bile duct cell proliferation and liver fibrosis decreased**

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**significantly by one week following treatment with PZQ. Following repeated infection with *O. viverrini* and exposure to DMN, and treatment with PZQ, disease was less marked compared to hamsters infected twice with *O. viverrini* but without treatment with PZQ. Severe disease manifested in hamsters with repeated liver fluke infection without PZQ treatment. Differences in pathology were not evident among the control hamsters – non-infected hamsters, uninfected hamster receiving PZQ only and uninfected hamsters treated with both PZQ with DMN. These findings confirmed the efficacy of PZQ for treatment of opisthorchiasis.**

**Keywords:** ***Opisthorchis viverrini,*** **praziquantel, cholangiocarcinoma, hamster, hepatobiliary**

## Introduction

The human liver fluke *Opisthorchis viverrini* is a major pathogen that causes serious public health problems in East Asia (IARC,1994 and Sripa, 2003).The prevalence of *O. viverrini* infection is significantly higher in the Northeast of Thailand compared to other regions of the country (Sithithaworn, 2012). The major factor of high transmission rate was from the local people habit of eating raw or undercooked cyprinid fish (Sripa, 2011).The pathology of *O. viverrini* infection occurs in the bile ducts in the liver and gall bladder. The histopathological changes include hyperplasia of bile duct epithelial, biliary periductal fibrosis, inflammation of the gallbladder including mucosal atrophy, goblet cell metaplasia, mucous gland hyperplasia, dysplasia and fibrosis (Riganti, 1989 and Sripa, 2003). Chronic infection with *O. viverrini* can lead to severe hepatobiliary diseases that, in turn, associate with the cholangiocarcinogenesis (IARC, 1994; Watanapa, 2002; Mairiang, 2003; Sripa, 2003 and Honjo, 2005).

The mechanisms of cholangiocarcinogenesis are long processes and associated with several key factors (Sripa, 2012). The mechanical damage cause by worms resident in the bile ducts combine with excretions-secretions from the parasites to cause bile duct inflammation and periductal fibrosis (Sripa, 2007 and Sripa, 2012). Nitrosamines are carcinogens formed from nitrosating agents particularly nitrites, nitrates and nitro stable secondary or tertiary amine. Nitrosamines in fermented fish products, which are popular in the diet of the population of northeastern Thailand, have been assigned a contributory role in the elevated incidence of liver fluke infection-induced cholangiocarcinoma (CCA) (Thamavit, 1978 and Sripa, 2005).

At present, praziquantel (PZQ) is the most effective drug for treatment of opisthorchiasis. PZQ at a single dose of 40 and 50 mg/kg provide clearance rates of *O. viverrini* of 91 and 97%, respectively. PZQ at 25 mg/kg for each of three divided doses gave cure rates of 80-100% (Bunnag, 1980 and Mairiang, 2003). There are reports showing that repeated infection with *O. viverrini* and PZQ re-treatment induces a host immune response that increases inflammatory cells, which in turn leads to oxidative stress and apoptosis related gene expression in the short term (Boonmars, 2007 and Pinlaor, 2008). A recent meta-analysis did not conclude a risk for PZQ treatment of opisthorchiasis in development of CCA (Kamsa-ard, 2013).

This study was undertaken to evaluate the efficacy of PZQ in repeated treatment of opisthorchiasis in a hamster model of chronic opisthorchiasis.

## Meterials and Methods

### Preparation of *O. viverrini* Samples

Metacercariae of *O. viverrini* were isolated from naturally infected cyprinid fish purchased from local food markets in Khon Kaen Province. Fishes were digested with 0.25% pepsin-HCl, after which the lysate was sieved, sedimented and washed several times with

**Table 1. Experimental design and treatment groups of hamsters to investigate the effects of repeated treatment of opisthorchiasis with praziquantel**

| Group | *O. viverrini* infection | PZQ | DMN | N |
|---|---|---|---|---|
| 1 | + | + | – | 5 |
| 2 | + | + | + | 5 |
| 3 | – | + | + | 5 |
| 4 | – | – | – | 5 |
| 5 | + | – | – | 5 |
| 6 | – | + | – | 5 |
| 7 | – | – | + | 5 |

*O. viverrini* infection = infected 50 *O. viverrini* metacercariae twice
PZQ = praziquantel 300 mg/kg at 4 weeks after *O viverrini* infection
DMN = 12.5 ppm DMN supplemented drink for 8 weeks

normal saline as described (Sithithaworn, 1997). *O. viverrini* metacercariae were identified and collected under a dissecting microscope. Hamsters were infected with 50 viable metacercariae delivered through an orogastic tube.

## Animal and Experimental Design

Thirty-five male golden Syrian hamsters aged 6-8 weeks were purchased from the animal facilities of the Faculty of Medicine, Khon Kaen University. These rodents were housed under conventional conditions and feed a standard diet. Protocols used for animal experimentation were approved by the Animal Ethics Committee of Khon Kaen University based on the Ethics of Animal experimentation of the National Research Council of Thailand (Approval number AEKKU55/2554). The hamsters were assigned into one of seven groups of five hamsters each: Group 1) hamsters repeatedly infected with *O. viverrini* and treated with PZQ (50 metacercariae twice + PZQ 300 mg/kg bodyweight (BW) single dose at 30 days after infection); Group 2) hamsters infected with *O. viverrini* plus DMN supplement and treated with PZQ (50 metacercariae twice +12.5 ppm DMN in drinking water for eight weeks + PZQ 300 mg/kg bodyweight twice at 30 days after infection); Group 3) hamsters received DMN and PZQ(12.5 ppm DMN in drinking water for eight weeks and PZQ 300 mg/kg bodyweight at day 30 and at day 67); Group 4) not treated (control) hamsters; Group 5) hamsters reinfected with *O. viverrini* (infected with 50 metacercariae twice by re-infection at five weeks after first infection); Group 6) hamsters treated twice with PZQ (PZQ 300 mg/kg bodyweight)at weeks 4 and 8 of the experiment; and Group 7) hamsters received DMN only (DMN 12.5 ppm supplemented in drinking water for 8 weeks) (Table 1).

## Specimen Collection and Histopathological Study

Hamsters were euthanized by inhalation of diethyl ether one week after the final treatment (day 74 for control group). After necropsy, liver tissues were randomly dissected from each hepatic lobe and fixed in 10% buffered formalin for 24 h, after which they were processed and embedded in paraffin. Five μm thickness sections were cut from paraffin blocks, and stained with conventional hematoxylin and eosin (H&E). The thin sections were examined by compound light microscopy (X100) to investigate histopathological changes. Bile duct inflammatory cells, bile duct cell proliferation and periductal fibrosis were evaluated using ocular grid micrometer (10×10 mm grids). The percentage of inflammatory cells was calculated from 100 grid of ocular grid micrometer. Bile duct proliferation and periductal fibrosis areas were measured as percentage of 100 grid of ocular grid micrometer (10×10 mm grids) under microscope.

### Data Analysis

The mean ± SD was calculated by the average of percentage in each group. One-way ANOVA analysis was used to compare between

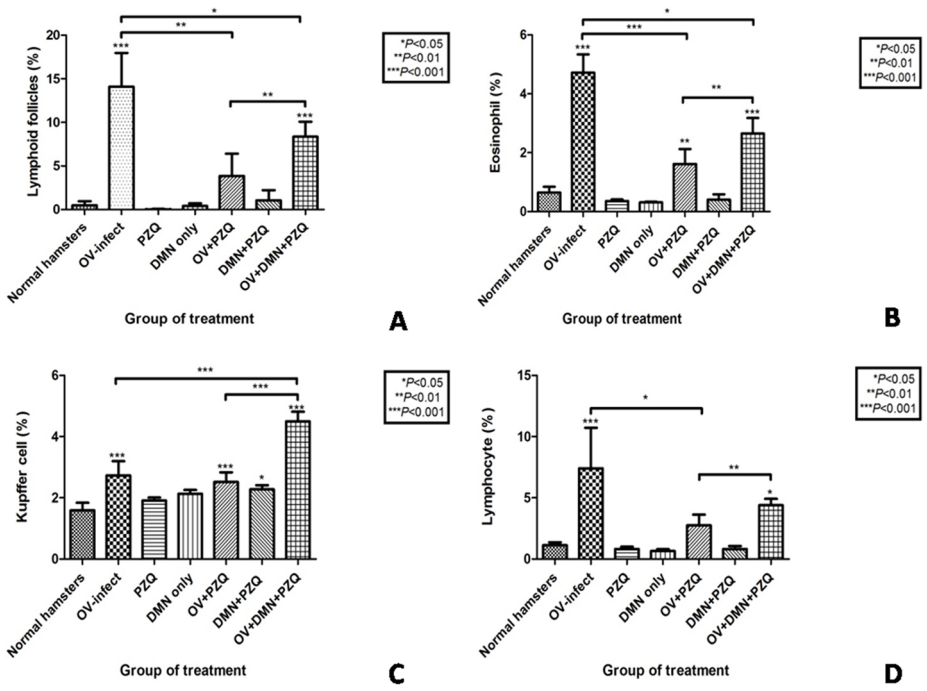


**Figure1. Percentage of white blood cells infiltration in the hamster livers: percentage of lymphoid follicles (A), eosinophil (B), Kupffer cells (C) and lymphocyte (D). OV-infect = hamsters infected with *O. viverrini* repeatedly, PZQ = hamsters received PZQ 300 mg/kgBW only for 2 times, DMN only = hamsters received 12.5 ppm DMN supplemented drink for 8 weeks, OV+PZQ = hamsters repeatedly infected with *O. viverrini* + PZQ 300 mg/kgBW and OV+DMN+PZQ = hamsters infected with *O. viverrini* +12.5 ppm DMN supplemented drink for 8 weeks + PZQ 300 mg/kgBW**

treated groups and control group. The *t*-test was used for comparing between all of treated groups. A $p$ value < 0.05 was considered statistically significant. GraphPad Prism version 5.3 software was used for statistics analysis.

## Results

### Fewer Bile Duct Inflammatory Cells After PZQ Treatment

The percentage of lymphoid follicles, eosinophils, and lymphocytes in liver tissue were significantly lower in the group of *O. viverrini*-repeated infection treated with PZQ groups than the hamsters infected with *O. viverrini* alone ($P$< 0.01, 0.001 and 0.05 respectively) (Figure 1(a-d)). The percentage of lymphoid follicles, eosinophils and lymphocytes in hamster liver tissue was higher in the *O. viverrini* infection group when compared to other treatment groups $P$< 0.001, 0.001 and 0.001 respectively) (Figure 1(a-d)). The percentage of Kupffer cell in *O. viverrini* infection plus DMN with PZQ treatment was higher than other groups (Figure 1(c)).

### Bile Duct Cell Proliferation Decreased in Infected Hamsters After PZQ Treatment

The percentage of bile duct cell proliferation in the *O. viverrini* repeatedly infected hamsters and treated with PZQ groups were significantly lower ($P$ <0.01) compared to *O. viverrini* infection alone group (Figure 2). None of control groups (normal hamsters, hamsters received PZQ only and hamsters

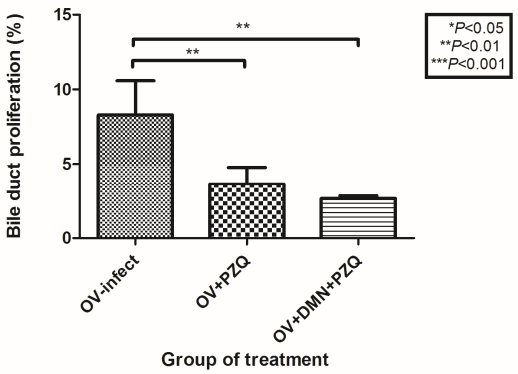


**Figure 2. Percentage of bile duct cell proliferation in hamster livers. OV-infect = hamsters infected *O. viverrini* repeatedly, OV+PZQ = hamsters repeatedly infected with *O. viverrini* + PZQ 300 mg/kgBW and OV+DMN+PZQ = hamsters infected with *O. viverrini* +12.5 ppm DMN supplemented drink for 8 weeks + PZQ 300 mg/kgBW.**

received DMN only) showed proliferation of bile duct cells. Micrographs representative of histopathological changes in bile duct epithelia of each treatment group are presented in Figure 3.

### Periductal Fibrosis Decreased in *O. viverrini* Infected Hamsters After PZQ Treatment

The percentage of periductal fibrosis in the hamster infected *O. viverrini* and hamsters infected *O. viverrini* plus exposure to DMN with PZQ treatment decreased significantly ($P<0.05$) compared to hamsters infected *O. viverrini* without PZQ treatment (Figure 4). Periductal fibrosis was not observed in any of the control groups (normal hamster, hamster received PZQ only and hamster received DMN only). The histopathological features of periductal fibrosis are shown in Figure 5, panels A-G.

## Discussion and Conclusions

This study showed that the pathology of hamsters with repeated *O. viverrini* after treatment with PZQ was less prominent that in non-PZQ treated infected hamsters. The pathology of *O. viverrini* infection in hamsters included acute inflammation of the bile ducts and portal connective tissue, eosinophilic infiltration of the portal areas (Bhamarapravati, 1978), the inflammatory cell infiltration such as eosinophil, Kupffer cell and lymphocyte occurred in *O. viverrini*-infection hamsters. In the chronic phase, from about 30 days after infection onwards, hyperplasia and adenomatous formations of the bile duct epithelium (Bhamarapravati, 1978), bile duct proliferation and periductal fibrosis occurred in the liver fluke infected animals (Bhamarapravati, 1978).

The pyrazinoisoquinolone compound praziquantel was discovered in the 1970s, was subsequently introduced for the treatment of schistosomiasis. It is active also against many other trematodes and cestodes (Greenberg, 2005). The chemo-activity of the medication damages the tegument of worms leading to vacuolization, swelling, disruption and detachment of the tegument and leading to parasite death (Day, 1992 and Harnett, 1988). In some previous studies, PZQ seemed to induce an inflammatory cell infiltration through *O. viverrini* antigen-mediated inflammation, and showed increasing of oxidative stress within 24 h of treatment (Pinlaor, 2006 and Pinlaor, 2008). Here, the level of inflammatory cell infiltration in *O. viverrini*-infected hamsters treated with praziquantel was less marked than that in hamsters infected with *O. viverrini* without

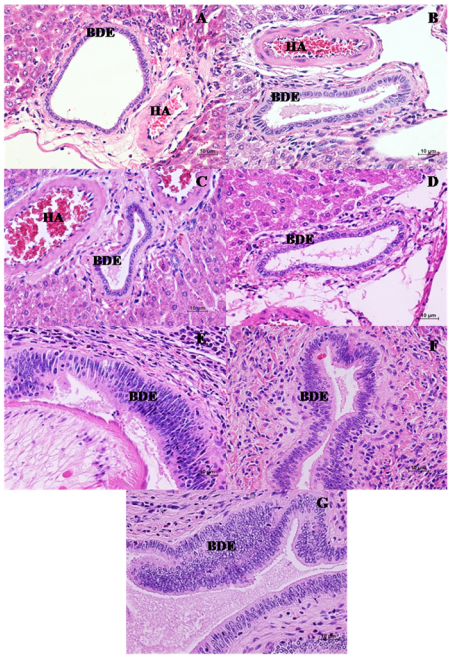


**Figure 3. Histopathology of bile duct cell proliferation in livers of hamsters: A) Control (normal) hamsters; B) hamsters received PZQ 300 mg/kgBW only, twice; C) hamsters received 12.5 ppm DMN supplemented drink for 8 weeks; D) hamsters received 12.5 ppm DMN supplemented drink for 8 weeks + PZQ 300 mg/kgBWtwice; E) hamsters infected with *O. viverrini* repeatedly; F) hamsters repeatedly infected with *O. viverrini* + PZQ 300 mg/kgBW;and G) hamsters infected with *O. viverrini* +12.5 ppm DMN supplemented drink for 8 weeks + PZQ 300 mg/kgBW. (40X) (HA: Hepatic artery; BDE: Bile duct epithelium; OV: *O. viverrini*)**

praziquantel treatment, when observed one week after PZQ treatment.

To summarize and conclude, hamsters infected *O. viverrini* repeatedly and hamsters infected *O. viverrini* plus exposure to exogenous nitrosamine in addition to treatment with PZQ exhibited reduced disease compared to the pathology induced in hamsters not treated with PZQ treatment when observed at one week after treatment with PZQ. The findings confirmed the efficacy of treatment with PZQ for infection with *O. viverrini* in either single or repeated infection.

## Acknowledgements

The authors thank laboratory staffs of the Department of Parasitology, and the Animal Experimental Unit, Faculty of Medicine, Khon Kaen University for facilities and technical supports.

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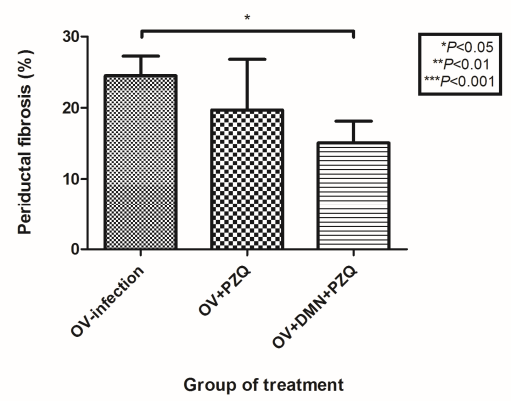


**Figure 4.The percentage of periductal fibrosis in liver of hamsters.**

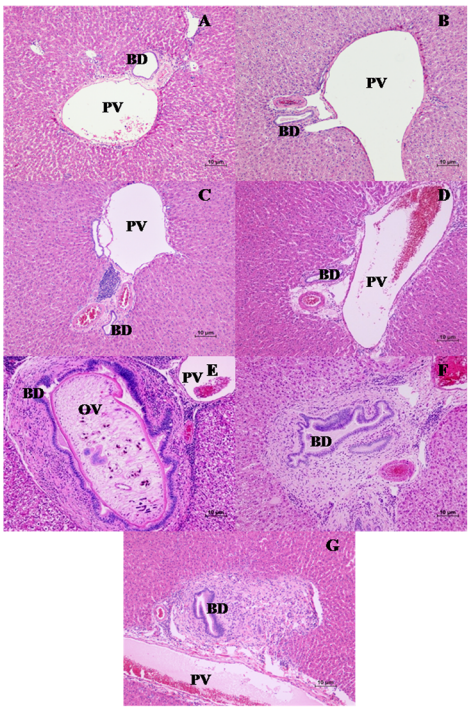


**Figure 5. Histopathology of periductal fibrosis in liver of hamsters: A) normal hamsters; B) hamsters received PZQ 300 mg/kgBW only, twice;C), hamsters received 12.5 ppm DMN supplemented drink for 8 weeks; D) hamsters received 12.5 ppm DMN supplemented drink for 8 weeks + PZQ 300 mg/kgBW, twice; E), hamsters infected with *O. viverrini* repeatedly; F) hamsters infected with *O. viverrini* repeatedly + PZQ 300 mg/kgBW; and G) hamsters repeatedly infected with *O. viverrini* +12.5 ppm DMN supplemented drink for 8 weeks + PZQ 300 mg/kgBW.(10X) (PV; Portal vein; HA: Hepatic artery; BD; Bile duct; OV: *O. viverrini)***

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