RESEARCH ARTICLE

Association between Praziquantel and Cholangiocarcinoma in Patients Infected with *Opisthorchis viverrini*: A Systematic Review and Meta-Analysis

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Abstract

Background: The liver fluke, *Opisthorchis viverrini*, and the associated incidence of subsequent cholangiocarcinoma (CCA) are still a public health problem in Thailand, and praziquantel (PZQ) remains the antihelminthic drug of choice for treatment. Evidence in hamsters shows that repeated infection and PZQ treatments could increase the risk of CCA. However, the existing evidence in humans is inconclusive regarding increased risk of CCA with frequency of PZQ intake. Objectives: To investigate the relationship between number of repeated PZQ treatments and CCA in patients with *O viverrini* infection. Materials and Methods: The reviewed studies were searched in EMBASE, MEDLINE, ProQuest, PubMed and SCOPUS from inception to October, 2012 using prespecified keywords. The risk of bias (ROB) of included studies was independently assessed by two reviewers using a quality scale from the Newcastle-Ottawa Scale (NOS). Risk effect of PZQ was estimated as a pooled odds ratio (OR) with its 95% confidence interval (95%CI) in the random-effects model using DerSimonian and Laird's estimator. Results: Three studies involving 637 patients were included. Based on the random effects model performed in two included studies of 237 patients, the association between PZQ treatments and CCA was not statistical significant with a pooled OR of 1.8 (95%CI; 0.81 to 4.16). Conclusions: The present systematic review and meta-analysis provides inconclusive evidence of risk effect of PZQ on increasing the risk of CCA and significant methodological limitations. Further research is urgently needed to address the shortcomings found in this review, especially the requirement for histological confirmation.

Keywords: Cholangiocarcinoma - *Opisthorchis viverrini* - repeated praziquantel - treatments - systematic review

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Introduction

Parasitic diseases associated with cancer remain common and widespread in tropical countries. In Thailand, liver fluke infection with *Opisthorchis viverrini* remains an important public health problem due to the traditional human habit of eating raw fish. The eating of raw fish and cultural popular dishes made with partially cooked or raw fish, which may contain the infective stage (metacercariae) of *O viverrini*, has been practised in the northeastern region where, as a consequence, the highest prevalence of *O viverrini* infection and incidence of subsequent cholangiocarcinoma have been observed (Sripa et al., 2007; Kaewpitoon et al., 2008; Grundy et al., 2012). The problem is endemic in the Mekong region. In Thailand and the Lao People's Democratic Republic (Laos PDR), many people are infected with the liver fluke, *O viverrini*, namely, eight million Thai and two million in Laos PDR. Approximately 80.0% of Thai cases occur in the northern and northeastern regions of Thailand (Sithithaworn and Haswell-Elkins, 2003; Sripa et al., 2011).

For Thai people, the age-standardized rate (ASR) of liver and bile duct cancers is between 67.6 and 94.8 per 100,000 people in males and between 27.3 and 39.4 per 100,000 in females. CCA has been detected as the most common histological type, accounting for between 82.0% and 89.0% of all primary liver cancers (Vatanasapt et al., 1993, Deerasamee et al., 1999, Sriplung et al., 2003, Khuhaprema et al., 2007; 2010; 2012). The average prevalence of liver fluke infection caused by *O viverrini* has been reported as 9.6% of the Thai population (Jongsuksuntigul, 2002), but it was distributed predominantly in the north and northeast (Jongsuksuntigul, 2002; Sithithaworn and Haswell-Elkins, 2003).

Little evidence of the risk factors for CCA is available, and most studies have focused on the role of *O viverrini* infection in the subsequent development of CCA; that is, the areas with a high incidence of CCA show high OV intensities. The odds ratios of *O viverrini* infection have

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ranged from 1.7 to 27.1 (Parkin et al., 1991; Honjo et al., 2005; Poomphakwaen et al., 2009). In a previous study reporting prevalence odds ratios (POR), the light infection group was approximately 1.7 times more likely to have CCA than the non-infection group (95%CI: 0.2 to 16.3) while the moderate infection group was approximately 3.2 times more likely to have CCA than the non-infection group (95%CI: 0.4 to 30.0), and the heavy infection group was approximately 14.1 times more likely to have CCA than the non-infection group (95%CI: 1.7 to 119.0) (Haswell-Elkins et al., 1994). In addition to human epidemiological studies, experiments in hamsters have also shown that *O viverrini* infection is associated with CCA (Bhamarapravati and Virranuvatti, 1966; Thamavit et al., 1978; Sripa et al., 2005; Sithithaworn, 2005).

Praziquantel (PZQ) is known as the antihelminthic drug of choice to treat *O viverrini* infection, and it provides effective chemotherapy (WHO, 1995). However, a previous study has found that rapid re-infection can occur after successful PZQ treatments (Upatham et al., 1988). People enjoy eating raw, undercooked, or inadequately fermented freshwater fish and, because they are aware that PZQ is an effective treatment, they return to eating cultural dishes which are likely to lead to a further *O viverrini* infection. Many know that if they are re-infected, they can again obtain treatment with PZQ, and so the cycle is perpetuated. Previous studies in hamsters infected with *O viverrini* reported that repeated infection and PZQ treatments can increase the risk of CCA (Pinlaor et al., 2004; 2009; Charoensuk et al., 2011). However, the few available studies in humans have shown unclear evidence of an increased risk of CCA with increased frequency of PZQ intake (Chernrungroj, 2000). A systematic review of the currently available evidence was performed to investigate the relationship between number of repeated PZQ treatments and CCA in patients with *O viverrini* infections.

Materials and Methods

Types of studies

Studies were selected if they used any analytical design, such as case-control, matched case-control, nested case-control, cohort and cross-sectional designs, to investigate the association between PZQ treatments and CCA.

Selection of studied

Screens showing the title, abstract, and finally the full text of publications were independently evaluated by two researchers, Supot Kamsa-ard (SK) and Malinee Laopaiboon (ML). Disagreement was resolved through discussion with a third researcher, Vajarabhongsa Bhudhisawasdi (VB).

Exposure

The exposure of interest was repeated treatment with PZQ. The frequency of PZQ treatment was categorized into four different groups: never used, used once, used twice, and used more than twice (Chernrungroj, 2000).

Outcome

The outcome was CCA. Cholangiocarcinoma (CCA) is a primary liver cancer, which arises in the epithelial cells lining the intrahepatic and extrahepatic bile ducts, but which does not include malignancies of the gallbladder or ampulla of vater (Bhudhisawasdi et al., 2012).

Literature research

In addition to hand searching, the following databases were searched without language restriction: EMBASE, MEDLINE, ProQuest, PubMed, and SCOPUS.

Search terms were used in combination and covered the names and synonyms of "praziquantel", "cholangiocarcinoma", "cancer", "risk factor", and "*Opisthorchis viverrini*". The more detailed terms used were as follows: for "praziquantel", these were Opticide, Praquantel, Prasikon, Prazite and Wormicide; for "cholangiocarcinoma", intrahepatic bile ducts, intrahepatic bile duct carcinoma, intrahepatic bile duct cancers, bile duct adenocarcinoma, bile duct cystadenocarcinoma, bile duct cancer, Klatskin's tumor, perihilar bile duct cancers, hilar bile duct cancers and distal bile duct cancers; for "cancer", tumor(s), malignancy and carcinoma; for "risk factor", risk, association, relationship, relation, correlation, connection and link; and for "*Opisthorchis viverrini*", liver fluke and *O viverrini*. Figure 1 is a flow diagram showing the article selection process.

Study inclusion/exclusion criteria

The inclusion criteria for the selection of primary studies were as follows: *i*) the article investigated the association between frequency of PZQ treatments and risk of CCA; and *ii*) the diagnosis of CCA was based on histological examination and the appropriate imaging characteristics. The exclusion criteria were: *i*) duplicate articles; where duplication occurred, only one of the

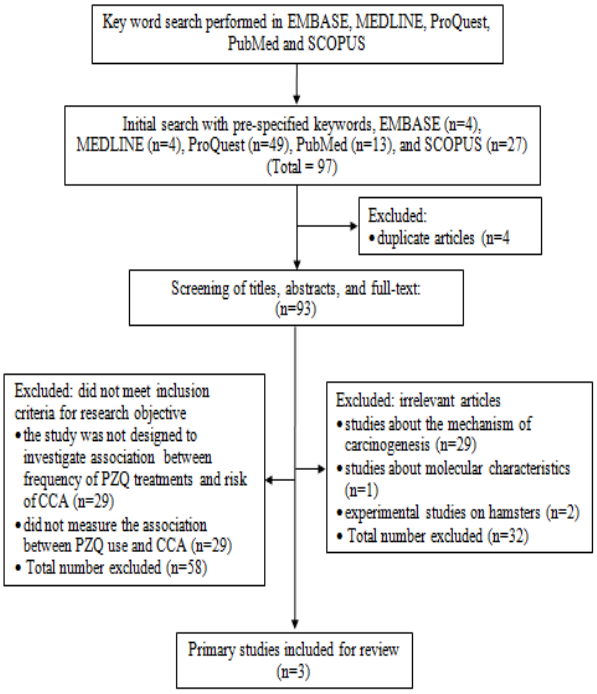

Figure 1. Flow Diagram Showing the Selection Process for Articles to be Included in the Systematic Review

articles was included; and *ii*) non-human research.

Quality assessment of primary studies

Assessment of Risk of Bias (ROB): the assessment of ROB in the included studies, all of which used a non-randomized research design, was based on the Cochrane risk of bias tool developed by the Non-Randomized Studies Methods Group (NRSMG). This tool examines selection bias, attribution bias, detection bias and reporting bias with the addition of the influence of confounding variables (Non-Randomised Studies Methods Group, 2013). The particular instrument chosen for evaluating the studies according to the ROB tool was the Newcastle-Ottawa Scale (NOS) for assessing the quality of case-control studies (Wells et al., 2012).

Data extraction and management: this is an 8-item scale which covers the assessment of case selection and control selection, study comparability, and exposure. For the purpose of the present review, the scale was adapted so that each primary study was rated in terms of a 'low', 'high' or 'unclear' risk of bias on each of the eight items. The adapted scale is reproduced in Table 1. Independent ratings were made by SK and ML, and any disagreements were resolved through discussion with the third researcher (VB). Review Manager Software was used to double enter all the data (RevMan, 2008).

Data analysis

Measures of association: the magnitude of the associations between PZQ treatments and CCA in terms of PZQ use (categorized into two different groups: 'yes' or 'no') were presented in the form of odds ratios (ORs) and their 95% confidence intervals (95%CI).

Assessment of heterogeneity: heterogeneity of the results across studies was assessed using forest plots, the Cochran's Q test, and I^2. An unacceptable degree of inconsistency across the study outcomes was considered to have occurred when the p-value of the Cochran's Q test statistic was <0.10 and I^2 was >50% (Higgins et al., 2003).

Pooling association: a random effects model was used to pool the association effects across the studies when there was evidence of an unacceptable degree of heterogeneity which could not be explained.

Ethnical consideration

This study was met the criteria for exempt review of the Khon Kaen University Ethics Committee for Human Research.

Results

Characteristics of included studies

A total of 97 studies were indentified using the search strategies described above. After reading the titles, abstracts, and full-text articles, three relevant studies were selected for detailed evaluation. A summary of these studies, including details of quantification of PZQ use, is provided in Table 2.

Risk of bias (ROB)

The review authors assessed the quality of the three studies using the ROB scale. One study was judged as having "unclear risk of bias" regarding its definition of controls. No detailed definition was provided, and controls were simply described as randomly selected subjects who had not developed CCA. Furthermore, two studies were assessed as having "unclear risk of bias" concerning a lack of information about non-response rates (Figure 2; Figure 3).

Table 1. Risk of Bias (ROB) Items, Adapted from Newcastle-Ottawa Scale (NOS)s

SELECTION	1. Is the Case Definition Adequate?	(a) yes, low risk of bias (e.g. eligibility criteria/operational definition)
		(b) yes, high risk of bias (e.g. not stated in part (a))
		(c) no description, unclear risk of bias
	2.Representativeness of the Cases	(a) yes, low risk of bias (e.g. consecutive representative series of cases)
		(b) yes, high risk of bias (e.g. not satisfying requirements in part (a), or not stated)
		(c) no description, unclear risk of bias
	3.Selection of Controls	a) yes, low risk of bias (e.g. community controls)
		b) yes, high risk of bias (e.g. hospital controls)
		c) no description, unclear risk of bias
	4.Definition of Controls	a) yes, low risk of bias (e.g. no history of CCA)
		b) yes, high risk of bias (e.g. no mention of history of CCA)
		c) no description of source, unclear risk of bias
COMPARABILITY	1.Comparability of cases and controls on the basis of the design or analysis	
		a) yes, low risk of bias
		(e.g. matching consideration by age, multiple analysis with age adjusted)
		b) yes, high risk of bias (e.g. not stated in part (a))
		c) no description of source, unclear risk of bias
EXPOSURE	1.Ascertainment of exposure	a) yes, low risk of bias
		(secure record e.g. surgical records; structured interview where blind to case/control status)
		b) yes, high risk of bias
		(e.g. interviewer not blinded to case/control status; written self report or medical record only)
		c) no description of source, unclear risk of bias
	2.Same method of ascertainment for cases and controls	
		a) yes, low risk of bias (e.g. use of a structured questionnaire)
		b) yes, high risk of bias (e.g. not stated in part (a))
		c) no description of source, unclear risk of bias
	3.Non-Response rate	a) yes, low risk of bias (e.g. same rate for both groups)
		b) yes, high risk of bias (e.g. non-respondents described; rate different and no designation)
		c) no description of source, unclear risk of bias

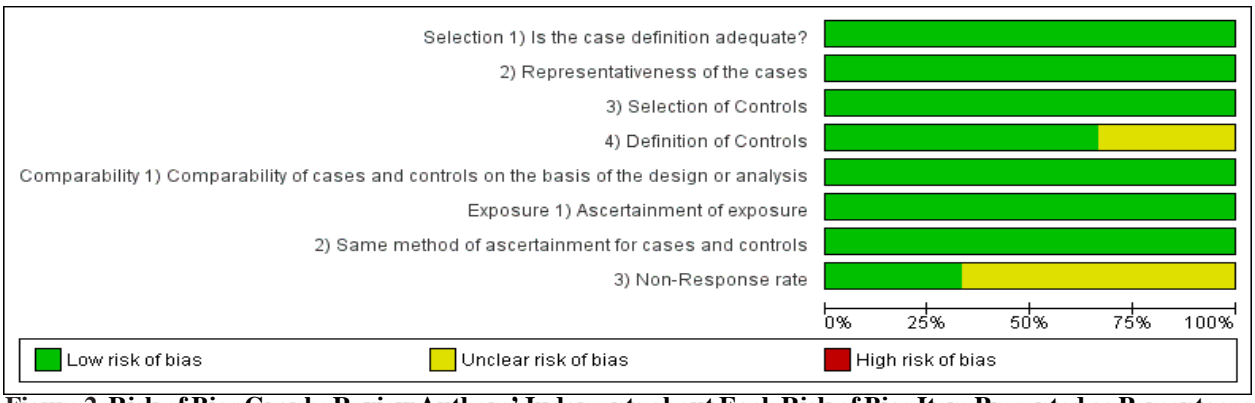

Figure 2. Risk of Bias Graph: Review Authors' Judgments about Each Risk of Bias Item Presented as Percentages Across All Included Studies

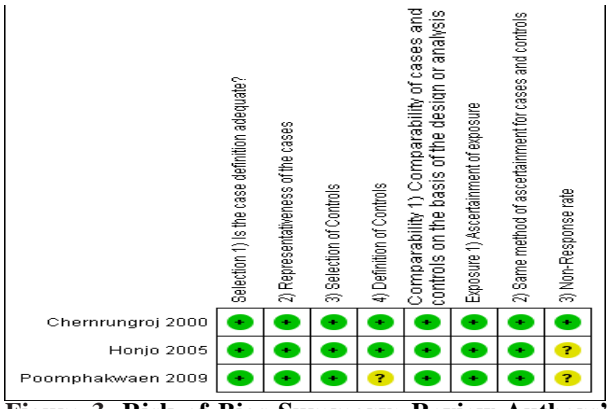

Figure 3. Risk of Bias Summary: Review Authors' Judgments about Each Risk of Bias Item for Each of the Included Studies

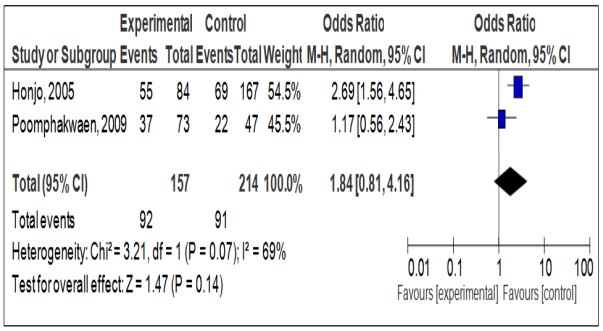

Figure 4. Meta-Analysis Forest Plots of the Relationship between PZQ Treatments and CCA

Main finding

One study categorized PZQ use into four different groups (never used, used once, used twice, and used more than twice) (Chernrungroj, 2000) and, for this reason, was excluded from the meta-analysis. The primary aim of the study had been to measure the independent association between use of alcoholic beverages and CCA, while use of PZQ was investigated as a potential risk factor. In addition, although the study did provide a clear case definition in terms of the ROB scale, the method of diagnosis for inclusion of many of the cases could be considered unsatisfactory. In the selection of cases using information from medical records, the diagnosis was histologically confirmed in only 28.0% (56 out of 200) subjects. For the remaining 72.0%, the diagnosis was made on the basis of a combination of ultra sound, CT and clinical examinations.

The other two studies categorized PZQ use into two groups ('never used' and 'used') and were able to be included in the meta-analysis (Honjo et al., 2005; Poomphakwaen et al., 2009). However, the outcome of the meta-analysis showed that the association between PZQ treatments and CCA was inconclusive. When the results of the two studies were pooled using a random effects model, the overall OR was 1.8 with a 95%CI of 0.81 to 4.16 (Figure 4).

Discussion

The aim of this systematic review was to investigate the relationship between PZQ treatments and CCA in patients with *O viverrini* infections. Three epidemiological studies were found which met the eligibility requirements for inclusion in the review (Chernrungroj, 2000; Honjo et al., 2005; Poomphakwaen et al., 2009). All were case-control studies. Two of the studies (Chernrungroj, 2000; Honjo et al., 2005) found a statistically significant association between PZQ treatments and CCA, whilst the third (Poomphakwaen et al., 2009) did not. Unfortunately, only two (Honjo et al., 2005; Poomphakwaen et al., 2009) were able to included in the meta-analysis, and the overall result was a non-significant relationship between PZQ treatments and CCA.

A histologically confirmed diagnosis should be required for most cancers. Isabel (1999), but all three studies failed to do this. In the study by Chernrungroj (2000) histological confirmation of CCA was obtained for 28.0% of the cases, in Honjo et al. (2005) only 7.0% of the case diagnoses were histologically confirmed, and in Poomphakwaen et al. (2009) the percentage is only 7.4 %. Without histological evidence some cases may be incorrectly diagnosed with the result that real exposure differences between cases and controls are diluted, and the chances of demonstrating an effect are reduced. This may at least partly explain the absence of statistically significant outcome in the meta-analysis. In addition, there appeared to be a high degree of heterogeneity or inconsistency of effects across the two studies included in the meta-analysis, and this may have contributed to the non-significant result. Regrettably, it was not possible to pinpoint the sources of this lack of consistency due the limited number of studies.

In presenting the results of the assessment of ROB in the three included studies, two figures were generated

Table 2. Summary of Study Characteristics According to PZQ Treatments

Authors	Study design	Setting	CCA n	CCA Exposure	Control n	Control Exposure	Comments
Chern-rungroj, 2000	Population-based case–control study Frequency matched by sex and age	Khon Kaen, Thailand, 1998	200	79	200	37	PZQ use: four different groups (never used, used once, used twice, and used more than twice)
Honjo et al., 2005	Population-based case–control study Individuals matched by sex, age and place of residence	Nakhon Pahnom provincial hospital, Thailand,1999–2001	124	55	127	29	Treatment with PZQ: two different groups (No/ Yes)
Poom-phakwaen et al., 2009	Nested case-control study Individuals matched by sex, age (± 3 years) and same period of recruitment to the cohort (±3 months)	Khon Kaen, Thailand,1990-2001	59	37	61	36	History of PZQ use: two different groups (No/ Yes)

using RevMan (RevMan, 2008). A ROB graph figures illustrates the proportion of studies with each of the authors' judgments ('yes', 'no', 'unclear') for each entry in the tool. In one of the two studies included in the meta-analysis, there was an "unclear risk of bias" arising from the lack of definition of controls. In both studies included in the meta-analysis there was an "unclear risk of bias" due to the absences of information about response rates. Taken together, these risks of bias raise further doubts about the validity of the outcome of the meta-analysis. Nevertheless, the risk of bias was assessed as "low" on six of the eights items of the ROB scale for all three studies included in the review.

There was a total 383 CCA patients with infected *O viverrini* in the three Thailand studies. We did not included studies from other countries such as Lao People's Democratic Republic (Laos PDR), Cambodia, or Vietnam. This was because, as our search of databases indicated, there were no studies in these neighboring countries which specifically investigated a relationship between number of PZQ treatments and CCA, although there were a few studies which reported descriptive data about *O viverrini* and CCA (Chai et al., 1998; Lee et al., 2002; De et al., 2003). However, as is generally known, infection with *O viverrini* is endemic in the Lower Mekong region. We therefore need to be cautious about the representativeness of the published articles.

There were two important strengths of this study. Firstly, this is an appropriate design. This is the systematic review of the currently available evidence which was performed to investigate whether PZQ treatments are positively related to an increased risk of CCA in patients with *O viverrini*. Secondly, the literature review included a search of the EMBASE, MEDLINE, ProQuest, PubMed and SCOPUS databases, and the method of identifying and selecting studies for inclusion in the review was explicit. This limited any bias in the choice of studies for review and increased the reliability and accuracy of the conclusions.

This systematic review was limited by the scarcity of studies which were eligible for inclusion in the review, and only one study explored frequency of PZQ use by categorising subjects into four group (never used, used once, used twice, and used more than twice), while the other two studies simply categorized subjects into two groups (never used and used). The number of studies included in the review and the total sample size of CCA patients infected with *O viverrini* included in the studies were not large enough, and this may have weakened the power, and hence the validity, of the analysis. The outcome may also have been weakened by the absence of histologically confirmed diagnoses.

In conclusion, the findings provide no firm evidence as to whether PZQ treatments could increase the risk of CCA. Further research is needed to focus on the primary aim of assessing the relationship between number of PZQ treatments and CCA, using definitions of cases and controls which are adequate for the satisfactory measurement of outcome, and providing information about response rates. In particular, a histologically confirmed diagnosis of CCA should be a requirement in future studies.

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