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SHORT COMMUNICATION

Turmeric reduces inflammatory cells in hamster opisthorchiasis

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Abstract The curcumin compound from turmeric is effective in the treatment of many inflammatory diseases. The aim of our present study was to evaluate the efficacy of turmeric on reducing the histopathological changes of hamster opisthorchiasis. Hamsters were infected with *Opisthorchis viverrini* and then administered turmeric. Using light microscopic observation, liver function tests for alanine transaminase (ALT), alkaline phosphatase, and direct bilirubin were investigated. The resulting histopathological changes show that turmeric has anti-inflammatory properties—during both *N*-nitrosodimethylamine administration and *O. viverrini* infection—by reducing the aggregation of inflammatory cells surrounding the hepatic bile ducts, which correlates with a decreased serum ALT level. The decrease in direct bilirubin levels in the hamsters treated with turmeric suggests that turmeric may enhance biliary contraction. The present study found that turmeric clearly reduces the inflammatory cells in hamster opisthorchiasis at an early stage. This finding may be connected with a reduction in the risk factors of cholangiocarcinoma development.

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Introduction

Chronic infection with *Opisthorchis viverrini* is one of the risk factors for cholangiocarcinoma development in Southeast Asia, including Thailand (IARC 1994; Sripa et al. 2007). Humans are infected by ingestion of raw cyprinoid fish, which contain the infective stage known as metacercaria. After *O. viverrini* metacercariae ingestion, the excysted juveniles migrate to the bile canal at the duodenum and grow to adulthood at the common bile duct or gallbladder, an area which is suitable for their survival. At the early stages of infection, liver changes are due to the inflammatory response (eosinophils, monocytes, and neutrophils) around the juvenile flukes in the intrahepatic bile ducts. The severity of inflammation gradually increases and reaches a maximum at about 3–4 weeks post-infection, as evidenced by the accumulation of mononuclear cells and eosinophils that infiltrate the intrahepatic bile ducts. The virulence of the disease also involves the host's own immune response, such as cytokine expression and resultant free radicals (Pinlaor et al. 2004). Thus, chronic infection with *O. viverrini* for many years has been associated with several hepatobiliary diseases (Sripa 2003), which are in

turn associated with the development of hepatobiliary cancer and cholangiocarcinoma (CCA). Because of the prevalence of the behavior of eating raw fish, the infection rate of this parasite remains high. Therefore, reducing the pathogenesis from an *O. viverrini* infection may be one of the choices to reduce the risk of CCA development. Thus, the present study was performed to evaluate the efficacy of a traditional medicine, turmeric, whose active compound is curcumin. Turmeric is widely used for treatment of many inflammatory diseases (Motterlini et al. 2000; Menon and Sudheer 2007; Singh and Singh 2009; Uddin et al. 2009), as well as in animal models with opisthorchiasis. In the present study, histopathological changes and liver function tests—alanine transaminase (ALT), alkaline phosphatase (ALP), and direct bilirubin—were observed.

Materials and methods

Parasite preparation

O. viverrini metacercariae were obtained from naturally infected cyprinoid fish in an endemic area of Khon Kaen, northeast Thailand. Fresh fishes were digested in 1% pepsin/HCl and incubated at 37°C for 1 h, then filtered and precipitated with normal saline in a sedimentation jar. Afterwards, the metacercariae were identified under a dissecting microscope.

Turmeric diet

Turmeric, acquired from a farm in Loei province, Thailand, was sliced into small pieces, dried, and ground into powder. The turmeric was checked for quality by thin layer chromatography (Wagner et al. 1983; Department of Medical Sciences, Ministry of Public Health, Thailand 1998) and prepared as 0.25% curcumin in animal food (Charoen Pokphand, Thailand) as described in a previous report (Kaewsamut et al. 2007).

Infected with *Opisthorchis viverrini*

Twenty of the hamsters were given 50 *O. viverrini* metacercariae by oral intragastric intubation, as in the previous protocol (Boonmars et al. 2007; 2008), and fed with the assigned diet.

Induced inflammation by administration of *N*-nitrosodimethylamine

Twenty of the hamsters were administered 12.5 ppm *N*-nitrosodimethylamine (Wako, Japan) daily in their water until the animals were killed.

Animal groups

Sixty Syrian hamsters were divided into six groups: (1) uninfected control (N); (2) administered a turmeric diet alone (TUR; treated control); (3) administered *N*-nitrosodimethylamine alone (NDMA); (4) administered *N*-nitrosodimethylamine and a turmeric diet (NDMA+TUR); (5) infected with *O. viverrini* alone (OV); and (6) infected with *O. viverrini* and administered a turmeric diet (OV+TUR). The hamsters were treated as the designed groups and killed on days 30 and 60, for collection of their whole liver tissues for the observation of histopathological changes and their sera for liver function tests. The Animal Ethics Committee of the Faculty of Medicine, Khon Kaen University, Thailand (ethical clearance no. AEKKU30/2551) approved the protocol.

Light microscopic observation

Liver tissues from each group were analyzed by light microscopic observation, as in previous reports (Boonmars et al. 2007, 2008).

Biochemical estimation

Measurement of serum liver enzymes

Serum was obtained to determine liver damage by evaluation of ALT, ALP, and direct bilirubin analysis at the Chemistry Room, Community Laboratory, Faculty of Associated Medical Sciences, Khon Kaen University.

Statistical analysis

The data of histopathological changes, serum ALT, ALP, and direct bilirubin levels were analyzed and presented as means ± SD. Statistics were analyzed from five hamsters using one-way analysis of variance (SPSS version 13.0, USA). Values were considered statistically significant when $p<0.05$.

Results

The effect of turmeric on the liver histopathology of *O. viverrini* infection and NDMA administration in hamster models

Analysis of histopathological changes focused on the aggregation of inflammatory cells surrounding the hepatic bile ducts. Histopathological changes in the uninfected group or normal control (Fig. 1a, d) were similar to those observed in the TUR group (Fig. 1g, j) at both time points

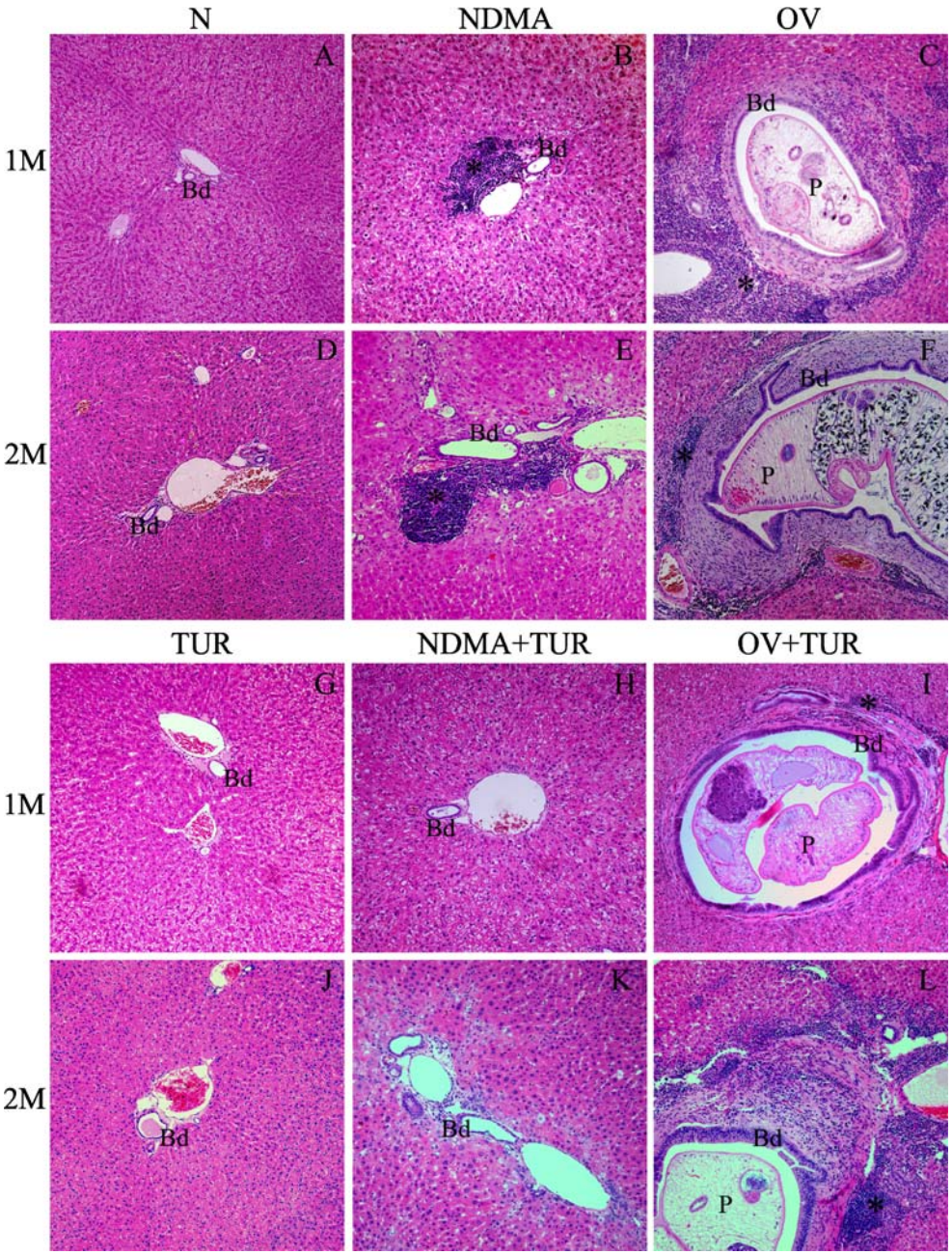

Fig. 1 Histopathological changes in the group of administration of turmeric alone (*TUR*; **g**, **j**), administration of NDMA alone (*NDMA*; **b**, **e**), administration of NDMA plus turmeric (*NDMA+TUR*; **h**, **k**), Infected with OV (*OV*; **c**, **f**), infected with OV plus TUR (*OV+TUR*; i, l) compare with normal control (*N*; **a**, **d**). *Bd* bile duct, *P* parasite, *asterisk* inflammatory cell (magnification ×10)

(1 and 2 months). The aggregation of inflammatory cells surrounding the hepatic bile ducts was observed at both time points in groups receiving NDMA administration (Fig. 1b, e) and *O. viverrini* infection groups (Fig. 1c, f). The NDMA+TUR group at both time points showed only few or no inflammatory cells surrounding the intrahepatic bile ducts (Fig. 1h, k) as was the case with *O. viverrini* infection at 1 month (Fig. 1i). However, a decrease in inflammatory cells was observed in the group of NDMA+TUR at 2 months. Hence, the reduction of inflammatory cells was not clear at 2 months post-infection (Fig. 1l).

Turmeric diet effect on liver enzymes

Table 1 shows the activities of serum ALT, ALP, and concentration of direct bilirubin, which correlate with histopathological changes (Fig. 1). The serum markers (ALT, ALP, and direct bilirubin) in the TUR group remained within normal levels. Serum ALT levels increased about five- to sixfold after hamsters were administered NDMA and two- to threefold after infection when compared with values in the uninfected control and in those administered TUR alone. There was a significant decrease in serum ALT in the group of NDMA+TUR at 1 month and in the OV group at 2 months.

Table 1 Liver function tests Serum alanine transaminase, alkaline phosphatase, and direct bilirubin levels in the group of administration of turmeric alone (TUR), administration of NDMA alone (NDMA), administration of NDMA plus turmeric (NDMA+TUR), infected with OV (OV), infected with OV plus TUR (OV+TUR) compare with normal control (N). Mean with different superscripts are significantly different ($P<0.05$)

Experimental group	Alanine transaminase (U/L)		Alkaline phosphatase (U/L)	Direct bilirubin (mg/dL)
	30days	60days	60days	60days
N	157.33±52.00[a]	69.67±22.19[a]	55.33±11.06[a]	0.50±0.17[a]
TUR	199.00±32.14[a]	93.00±36.64[a]	59.33±8.96[a]	0.33±0.35[a]
NDMA	899.00±456.99[e]	271.50±16.26[b]	72.67±30.44[a]	0.20±0.35[a]
NDMA+TUR	461.33±145.24[b]	311.00±82.02[c]	170.67±61.60[b]	0.13±0.15[a]
OV	550.00±231.06[d]	230.00±7.07[b]	51.67±15.82[a]	0.87±0.35[b]
OV+TUR	502.67±65.99[d]	107.33±23.16[a]	79.67±6.51[a]	0.10±0.00[a]

Serum ALP levels in the TUR, NDMA, OV, and OV+TUR groups remained within normal levels. However in the NDMA+TUR group, the value increased about two- to threefold compared with the others. However, all of the groups that were administered turmeric seemed to show an increased ALP level. The serum direct bilirubin level (which was lower in groups given a turmeric diet than in the untreated groups) shows that turmeric enhanced bile flow and biliary contraction. Serum direct bilirubin levels in the TUR, NDMA, NDMA+TUR, and OV+TUR groups remained within normal levels. Serum direct bilirubin levels increased about two- to eightfold after infection when compared with values in the OV+TUR, NDMA+TUR, NDMA, TUR, and the uninfected control groups.

Discussion

The present study clearly shows that turmeric has anti-inflammatory and biliary contraction-enhancing properties. This was evident in both the hamster model administered with NDMA and the model infected with *O. viverrini* (at an early stage of infection). The pathological changes evidenced by the reduction in the inflammatory cells surrounding the hepatic bile ducts correlated with a decrease in the results of the liver function tests ALT and direct bilirubin, and an increase in ALP due to decreased liver cell damage.

Examination of pathological changes in the livers revealed the anti-inflammatory property of turmeric in hamsters infected with *O. viverrini*. The positive control for inducing inflammatory cells was NDMA administration. The histopathological results showed that turmeric has no toxic side effects in normal hamsters; this was supported by the liver function tests results and agreed with a previous report that turmeric has no toxic effect in hamster models (Kaewsamut et al. 2007). The histopathology was similar to previous reports (Thamavit et al. 1987; Boonmars et al. 2007; Boonmars et al. 2008). The administration of NDMA was directly toxic on liver tissue and induced the infiltration of inflammatory cells surrounding the biliary tree and hepatic tissue at days 30 and 60. These results agreed with Thamavit et al. (1987) and Thamavit et al. (1993). Moreover, NDMA administration in rats induced chronic inflammation and led to liver tumors (Peto et al. 1991). Bile duct proliferation and liver fibrosis was also observed (George et al. 2001). The anti-inflammatory property of turmeric was obviously demonstrated by the reduction of inflammatory cells in hepatic tissue compared with the untreated group at both 30- and 60-day time periods (Fig. 1h, k). Decreased inflammatory cells (Fig. 1) led to decreased serum in ALT and decreased direct bilirubin levels (Table 1) in all groups treated with a turmeric diet. This result agrees with previous reports that the curcumin in turmeric reduces inflammation in many types of diseases (Yadav et al. 2009), including liver diseases and toxicity from CCl_4 (Reyes-Gordillo et al. 2008) by inhibiting cyclooxygenase-2, lipoxygenase, NO production, inducible nitric oxide synthase expression, and nuclear factor kappa B activation in lipopolysaccharide-activated macrophages (Unnikrishnan and Rao 1995; Sreejayan and Rao 1994; Menon and Sudheer 2007; Pae et al. 2008). Significantly reduced inflammatory cells in NDMA+TUR at 2 months was observed, as well as formation of new bile ducts. This was correlated with increased ALP levels, which may be produced from bile duct epithelial cells (Roncoroni et al. 2008). The pathological changes from *O. viverrini* infection alone were observed from the inflammatory cells (mononuclear cells and eosinophils) surrounding the hepatic bile ducts, as well as epithelial hyperplasia, goblet cell metaplasia, adenomatous metaplasia, and thickened periductal fibrosis (Fig. 1c, f). These results agreed with Sripa (2003). A slight decrease in inflammatory cells during *O. viverrini* infection was observed when a turmeric diet was administered. However, this result was different from that observed during the administration of NDMA and turmeric together. One possible explanation is that different inflammatory inducers may have different host immune response mechanisms. The metabolized product from NDMA is directly toxic to the liver and subsequently enhances inflammatory cell response, whereas *O. viverrini* induces a host immune response, which enhances inflammatory cells surrounding

the hepatic bile duct. In addition, turmeric decreases liver detoxification (cytochrome P450), leading to a reduction of toxic metabolite products from NDMA (Sugiyama et al. 2006; Surh and Chun 2007; Choi et al. 2008). Moreover, the present study shows the property of turmeric to enhance bile flow and gall bladder contraction through decreased direct bilirubin level, a result which is supported by Deters et al. (2000).

The present study demonstrates the advantages of turmeric as an anti-inflammatory in hamster opisthorchiasis at an early stage of infection, leading to a reduction in liver pathology.

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