Optimization of Route of Melatonin Administration in Reducing Inflammation and Oxidative/Nitrative Stress in *Opisthorchis viverrini*-infected Hamsters

วิธีการให้เมลาโทนินที่เหมาะสมในการลดการอักเสบและภาวะออกซิเดทีฟและไนเตรทีฟสเตสในหนูแฮมสเตอร์ที่ติดพยาธิใบไม้ตับ (*Opisthorchis viverrini*)

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ABSTRACT

The liver fluke *Opisthorchis viverrini* infection is a major health problem in northeastern Thailand. We have previous indicated the short-term treatment with praziquantel induces inflammation and oxidative/nitrative stress. This study, we evaluated the effect of melatonin and optimized route of administration (oral and intraperitoneal (i.p.) injection) on inflammation and oxidative/nitrative stress induced by short-term treatment with praziquantel in *O. viverrini*-infected hamsters. The result showed that melatonin treatment dose-dependently decreased the inflammatory cells and the levels of oxidative/nitrative stress markers. In contrast, melatonin increased the level of antioxidant power. Oral administration had more effective on these parameters than the i.p. injection groups. Therefore, oral administration of melatonin may be used as a chemopreventive agent to reduce the severity of the disease during the short-term praziquantel treatment.

บทคัดย่อ

การติดเชื้อพยาธิใบไม้ตับ (*Opisthorchis viverrini*) มีการระบาดมากในภาคตะวันออกเฉียงเหนือของไทย การศึกษาก่อนหน้านี้ชี้ให้เห็นว่าภายหลังการรักษาด้วยยาพราซิควอนเทลในระยะเวลาสั้น ๆ เหนี่ยวนำให้เกิดการอักเสบและภาวะออกซิเดทีฟและไนเตรทีฟสเตสการศึกษาครั้งนี้จึงทดสอบประสิทธิภาพของเมลาโทนินและหาวิธีการที่เหมาะสมในการให้สาร(กินและฉีดเข้าหน้าท้อง) ต่อการอักเสบและภาวะออกซิเดทีฟและ

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ไนเตรทีฟสเตสที่เหนี่ยวนำโดยการรักษาด้วยยาพราซิควอนเทลในระยะเวลาสั้น ๆ ในหนูที่ติดพยาธิใบไม้ตับ ผลการทดลองพบว่าการให้เมลาโทนินลดเซลล์อักเสบและระดับของค่าชี้วัดภาวะออกซิเดทีฟและไนเตรทีฟสเตสตามความเข้มข้น ในทางตรงข้ามเมลาโทนินเพิ่มความสามารถรีดิวซ์สาร อนุมูลอิสระ การให้โดยการกินมีผลต่อค่าต่าง ๆ เหล่านี้มากกว่าการให้โดยการฉีด ดังนั้นการให้เมลาโทนินโดยการกินน่าจะนำมาใช้เป็นสารเคมีป้องกันรักษาเพื่อลดความรุนแรงของโรคระหว่างการรักษาด้วยยาพราซิควอนเทลในระยะเวลาสั้น ๆ

Key Words : *Opisthorchis viverrini*, Melatonin, Oxidative/Nitrative
คำสำคัญ : พยาธิใบไม้ตับ *Opisthorchis viverrini* เมลาโทนิน ออกซิเดทีฟและไนเตรทีฟสเตส

Introduction

Opisthorchiasis caused by infection with liver fluke, *Opisthorchis viverrini*, is endemic in Southeast Asian countries including Thailand, Lao People's Democratic Republic, Vietnam and Cambodia (IARC, 1994; Sithithaworn & Haswell-Elkins, 2003). The highest prevalence of *O. viverrini* infection has been found in the Northeast Thailand and is associated with the high incidence rate of cholangiocarcinoma (CCA). The availability of antihelminthic drug praziquantel provides effective chemotherapy for the treatment of Opisthorchis parasite infection (Jongsuksuntigul & Imsomboon, 2003). The cure rate of a single dose of praziquantel for liver fluke treatment is more than 90%. However, short-term treatment with praziquantel induces dispersion of parasite antigens, which induces recruitment of inflammatory cells and results in expression of inducible nitric oxide synthase (iNOS), nuclear factor-kappaB (NF-κB), and antioxidant enzymes related to oxidative and nitrative stress (Pinlaor *et al.*, 2008).

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced in the brain by the pineal gland. Melatonin is effective agent against pathological states characterized by an increase in basal rate of reactive oxygen species (ROS) / reactive nitrogen species (RNS) production. Melatonin has the capability of scavenging both oxygen- and nitrogen based reactants, and blocking transcriptional factors, which induces pro-inflammatory cytokines (Acuna *et al.*, 2002). Additionally, melatonin is capable of increasing the activity and expression of antioxidant enzymes (Rodriguez *et al.*, 2004). Besides being a highly effective direct free radical scavenger and indirect antioxidant, melatonin has attracted clinical interest. However, route of administration of melatonin in reducing oxidative and nitrative damages caused by *O. viverrini* infection during short-term treatment with praziquantel in *O. viverrini*-infected hamsters has not been evaluated. This study was evaluated and optimized route of administration of melatonin against oxidative/nitrative stress and inflammation induced by short-term treatment with praziquantel in *O. viverrini*-infected hamsters.

Materials and Methods

Parasites

Opisthorchis viverrini metacercariae were isolated from naturally infected cyprinid fishes obtained from Khon Kaen province, Thailand. After fishes were digested in 0.25% pepsin-HCl, *O. viverrini* metacercariae were collected and counted under dissecting microscope, and viable cysts were used for hamster infection.

Animals

The Animal Ethics Committee of Khon Kaen University (AEKKU 20/2552) approved this study. Forty-five male Syrian golden hamsters (*Mesocricetus auratus*) (obtained from the Animal Unit, Faculty of Medicine, Khon Kaen University) aged between 4-6 weeks were used. All of animals were housed under conventional conditions and fed a stock diet and given water *ad libitum*.

Chemicals

Melatonin (Huanggang Innovation Biochemicals Co. Ltd., China) was dissolved in a small amount of ethanol before being diluted with saline. The final concentration of ethanol in the melatonin solution was < 1%. Praziquantel (Biltricide®, single dose of 400 mg/kg of body weight; Bayer, Pittsburgh, PA) was suspended in 2% Cremophor EI (Sigma, St. Louis, MO).

Experimental design

Forty-five hamsters were infected with *O. viverrini* (50 metacercariae/ animal). After 3 months post-infection, the animals were divided into two major groups (oral and intraperitoneal (i.p.) injection administration of melatonin). Each group was divided into 5 sub-groups; group I *O. viverrini*-infected hamsters were treated with vehicle (2% cremophor and diluent of melatonin), group II *O. viverrini*-infected hamsters were treated with praziquantel, group III, IV and V *O. viverrini*-infected hamsters were treated with praziquantel and melatonin 25, 50 and 100 mg/kg body weight respectively. Melatonin (or vehicle) was orally or i.p. injection administered for 1 week and praziquantel (or cremophor) was then orally administered for 12 h before sacrificed. At the end of the experiment, animals were anesthetized with diethyl ether, EDTA blood and livers were collected. The liver sections were taken from the middle lobe in the part of hilar region and adjacent areas including the secondary order bile duct lumens, where the worms are usually found. Liver tissues were fixed in 10% buffered formalin for histopathological study by using hematoxylin and eosin staining. EDTA blood was collected from the heart and centrifuged to separate plasma for measure the levels of biochemical parameters.

Biochemical assays

The level of malondialdehyde (MDA) was measured by using the thiobarbituric acid (TBA) assay. The level of nitrate/nitrite (NOx) was determined by the vanadium-based simple spectrophotometric method using the Griess reaction (Miranda *et al.*, 2001). The level of ferric reducing antioxidant power (FRAP) was measured by spectrophotometer according to the method of Benzie and Strain (1996) (Benzie and Strain, 1996). The activity of liver function enzyme, alanine aminotransferase (ALT), was analyzed by spectrophotometer using automate analyzer (automate RA100).

Statistical analysis

The data are expressed as means $\pm$ SD. Parametric and non-parametric data were determined using one-way analysis of variance (ANOVA) and Mann-Whitney U test, respectively, with SPSS version 11.5. Student t-test was used to compare between oral and i.p. injection. *P* values less than 0.05 were considered statistically significant.

Results

Effect of melatonin on histopathological changes

Hematoxylin and eosin staining was performed in the liver section of *O. viverrini*-infected hamsters following short-term treatment with praziquantel and given melatonin. Accumulation of inflammatory cells was observed in inflamed areas surrounding the worm after praziquantel treatment (Figure 1C and D) compared with in the *O. viverrini*-infected hamsters and treated with vehicle (Figure 1A and B). Accumulation of inflammatory cells were dose-dependently decreased by treatment with melatonin 25 (Figure 1E, F), 50 (G, H), and 100 mg/kg body weight (I and J), respectively, both oral and i.p. injection.

Effect of melatonin on biochemical parameters

Short-term treatment with praziquantel in *O. viverrini*-infected hamsters increased plasma levels of nitrate/nitrite (NOx) and malondialdehyde (MDA) and the activity of alanine aminotransferase (ALT), and decreased the level of ferric reducing antioxidant power (FRAP) compared with the *O. viverrini*-infected hamsters treated with 2% cremophor and diluent.

Both oral and i.p. injection with melatonin dose-dependently decreased the levels of NOx and MDA and the activity of ALT compared with untreated group. In contrast, melatonin dose-dependently increased the plasma level of FRAP. The alterations of these parameters were more effective in the oral administration than in the i.p. injection groups (Figure 2).

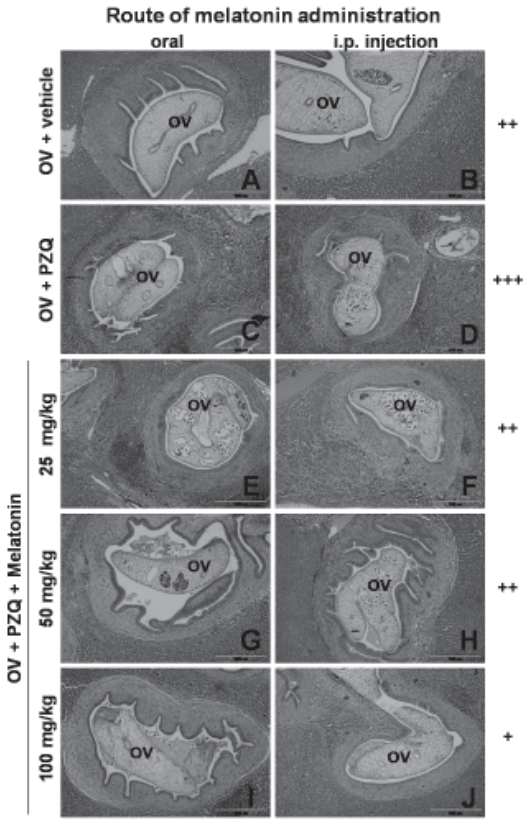

Figure 1 Effect of melatonin and route of administration on histopathological changes in the liver of *O. viverrini*-infected hamsters. Hematoxylin and eosin staining was performed in the liver section of oral (panel A-I) and i.p. (panel B-J) administration. *O.viverrini*-infected hamsters were given 2% cremophor and diluent of melatonin (A and B), *O. viverrini*-infected hamsters were given praziquantel (C and D), and supplement with melatonin 25 (E and F), 50 (G and H) and 100 (I and J) mg/kg body weight. Original magnification is 100x. OV = *O. viverrini*, PZQ = praziquantel, + = mild inflammatory cells, ++ = moderate inflammatory cells, +++ = heavy inflammatory cells

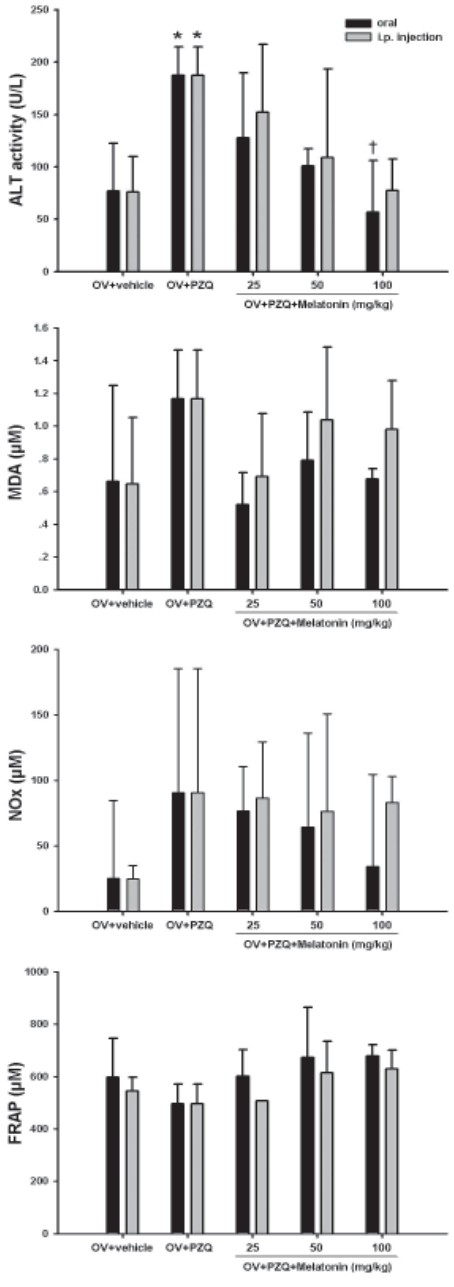

Figure 2 Biochemical parameters in the plasma of *O. viverrini*-infected hamsters. Data are means ± SD. * significantly different from OV + vehicle group ($P < 0.05$). † significantly different from OV + praziquantel group ($P < 0.05$). For each group, n=5. Groups of data were analyzed with ANOVA.

Discussion

In the present study, we have evaluated the effect of melatonin and route of administration on oxidative/nitrative stress and inflammation induced by short-term treatment with praziquantel in hamsters infected with *O. viverrini*. We have shown that the increases in inflammation, oxidative and nitrative damage, and liver injury as well as the decrease in antioxidant power induced by treatment with praziquantel in the short-term were prevented by melatonin administration both oral and i.p. injection.

A recent report suggested that short-term praziquantel treatment in *O. viverrini*-infected hamsters induces inflammation and oxidative and nitrative stress through *O. viverrini* antigen release (Pinlaor *et al.*, 2008). In agreement with this report, in the current study there were increased in inflammation, as indicated by increased the numbers of inflammatory cells around the bile ducts in the liver section of *O. viverrini*-infected hamsters not receiving melatonin. Such events were protected by melatonin treatment both oral and i.p. injection with dose dependent when performed by using hematoxylin and eosin staining.

Also, we observed that oral and i.p. melatonin administration to *O. viverrini*-infected hamsters decreased oxidative and nitrative damage. This consumption based on our data showed that melatonin decreased the plasma levels of NOx and MDA, which is an oxidative/nitrative stress marker as well as increased in FRAP level, an antioxidant power, compared to *O. viverrini*-infected hamsters not receiving melatonin. Moreover, melatonin treatment reduced liver damage, as demonstrated by the reduction of ALT activity, an indicator of liver injury, in the plasma of *O. viverrini*-infected hamsters. It is probable that short-term praziquantel

treatment generate oxygen/nitrogen free radical and contribute to oxidative and nitrative stress, resulting in biomolecules damage and impair cellular function. Melatonin may be protective against short-term praziquantel treatment-induced oxidative/nitrative injury by balancing oxidant-antioxidant status, due to its antioxidant and free radical scavenging activity (Reiter *et al.*, 1997; Korkmaz *et al.*, 2009).

Conclusions

Melatonin reduced inflammation, oxidative/ nitrative stress, and liver injury induced by short-term treatment with praziquantel in *O. viverrini*-infected hamsters both oral and i.p. injection, especially in oral route. Therefore, the data in this study provide a basis for melatonin utilizing as a chemopreventive agent to reduce the severity of the disease following short-term praziquantel treatment in the patient.

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References

Acuna, CD., Escames, G., Carazo, A., Leon, J., Khaldy, H., and Reiter, R.J. 2002. Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. Curr Top Med Chem, 2(2): 133-151.

Benzie, IF., and Strain, JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem, 239(1): 70-76.

IARC. 1994. Infection with liver flukes (*Opisthorchis viverrini*, *Opisthorchis felineus*, *Clornorchis sinensis*). IARC monographs on the Evaluation of Carcinogenic Risks to Human. International Agency for Research on Cancer, Lyon, 61: 121-175.

Jongsuksuntigul, P., and Imsomboon, T. 2003. Opisthorchiasis control in Thailand. Acta Trop, 88(3): 229-232.

Korkmaz, A., Reiter, RJ., Topal, T., Manchester, LC., Oter, S., and Tan, DX. 2009. Melatonin: an established antioxidant worthy of use in clinical trials. Mol Med, 15(1-2): 43-50.

Miranda, KM., Espey, MG., and Wink, DA. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5(1): 62-71.

Pinlaor, S., Prakobwong, S., Hiraku, Y., Kaewsamut, B., Dechakhamphu, S., Boonmars, T. *et al.* 2008. Oxidative and nitrative stress in *Opisthorchis viverrini*-infected hamsters: an indirect effect after praziquantel treatment. Am J Trop Med Hyg, 78(4): 564-573.

Reiter, R., Tang, L., Garcia, JJ., and Munoz-Hoyos, A. 1997. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci, 60(25): 2255-2271.

Rodriguez, C., Mayo, JC., Sainz, RM., Antolin, I., Herrera, F., Martin, V. *et al.* 2004. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res, 36(1): 1-9.

Sithithaworn, P., and Haswell-Elkins, M. 2003. Epidemiology of *Opisthorchis viverrini*. Acta Trop, 88(3): 187-194.