CHAPTER V
DISCUSSION

This study clearly indicated that curcumin induced Nrf2-driven antioxidants against oxidative/nitrative stress in *O. viverrini*-infected hamsters treated with praziquantel. Curcumin not only increased the expression of cytoplasmic and nuclear protein levels of Nrf2, but also induced transcriptional regulation of Nrf2, HO-1, Keap1, NQO1, GCL, ATF3, Prdx3, Prdx6, SOD2, and CAT, resulting in an increase in the level of antioxidant capacity in the plasma. In contrast, this nutraceutical agent suppressed oxidant genes including NF-κB, iNOS, COX2, and pro-inflammatory cytokines (IL-1β and TNF-α) leading to a decrease in oxidative/nitrative stress markers (8-oxodG, nitrate/nitrite, and MDA) and reduction in liver injury. Therefore, we hypothesize that curcumin has an ability to increase Nrf2-regulated stress response, resulting in cell protection against oxidative/nitrative stress and prevention of liver injury as chemotherapy-related adverse effect.

Previously demonstrated that a short-term praziquantel treatment induces dispersion of *O. viverrini* antigens and recruits mast cell and eosinophil infiltration, resulting in increased oxidative/nitrative stress (Pinlaor et al., 2008). In this study, we showed that curcumin inhibited eosinophil infiltration and modulated lymphocyte infiltration. This nutraceutical agent also showed an increase in the expression of Nrf2 at the nucleus of inflammatory cells, mainly lymphocytes, and epithelial bile duct, suggesting that cell type-dependent immunomodulatory effects occur (Varalakshmi et al., 2008). Dietary curcumin led to an increase in Nrf2 expression at the transcriptional and translational levels in the cytoplasm and nucleus, suggesting that Nrf2-Keap1 interaction enables Nrf2 to translocate to the nucleus. This result is supported by the finding that Keap1 expression was increased by curcumin treatment. Activated Nrf2 binds to the antioxidant-responsive element (ARE) and initiates the transcription of genes coding detoxifying enzymes and cytoprotective proteins (Wakabayashi et al., 2004). An increase in TGF-β expression may lead to Nrf2-mediated response (Churchman et al., 2009). Moreover, our result indicates that curcumin is an extremely potent inducer of not only Keap1, but also the phase 2

response [e.g., elevation of NQO1, HO-1, GCL], which is a major protector of cells against oxidative and electrophile stress. Induction of HO-1 by curcumin requires the activation of the Nrf2/ARE pathway (Balogun et al., 2003; Farombi et al., 2008), which is dependent on cell type and cellular microenvironment (Prawan et al., 2005). Also, the results of this study add to the growing evidences that curcumin has an ability to induce HO-1 (Balogun et al., 2003; Pae et al., 2007), Keap1 (Balogun et al., 2003; Lee, Surh, 2005), NQO1 (Rushworth et al., 2006), and GCL (Lee, Surh, 2005) expression, presumably through Nrf2 activation (Balogun et al., 2003; Dinkova-Kostova, Talalay, 2008)). Taken together, curcumin induces Nrf2-driven stress response against oxidative/nitrative stress induced by praziquantel treatment in *O. viverrini*-infected hamsters via cell type-dependent immunomodulatory effects.

ATF3 is a novel repressor of the Nrf2-regulated stress response (Brown et al., 2008). Unexpectedly, we showed that curcumin induced Nrf2-regulated ATF3 expression, suggesting that up-regulation of ATF3 is a negative feedback of Nrf2. Relevantly, curcumin-treated MDA-1986 cells showed a rapid, dose-dependent increase in the expression of ATF3 mRNA and protein as a novel contributor to the proapoptotic effect of curcumin (Yan et al., 2005).

Peroxiredoxins (Prdx) are a family of antioxidants that protect cells from metabolically produced reactive oxygen species by host immune effector cells. Recently, we have identified Prdx6 in *O. viverrini*-infected hamster and suggested it plays an important role for host defense (Khoontawad et al., 2010). Here, we firstly demonstrated that curcumin treatment increased mRNA expression of antioxidant genes including not only Prdx6, but also Prdx3 and antioxidant enzymes (SOD2, and CAT). Induction of Prdx3 (Bae et al., 2009) and Prdx6 (Chowdhury et al., 2009) are activated by Nrf2 activation. In agreement, we found that curcumin enhanced the expression of Prdx3, Prdx6, SOD2 and CAT in association with the activation of Nrf2. Increase in antioxidative stress response led to an increase in ferric-reducing antioxidant power in the plasma and decreased ALT activity.

Thanan et al. demonstrated that the level of 8-oxodG, an oxidative DNA lesion, in the urine significantly increases in the order of CCA>OV-infected patients > healthy subjects, suggesting that 8-oxodG is a useful biomarker to monitor not only infection but also carcinogenesis. In this study, we demonstrated the protective effect

of curcumin on 8-oxodG level in *O. viverrini*-infected hamsters treated with praziquantel. Curcumin effectively suppressed oxidative/nitrative stress and inhibited the expression of oxidant genes (iNOS, NF-κB, and COX-2), and proinflammatory cytokines (IL-1β, and TNF-α), in relation to the decrease in MDA and NOx levels in the plasma. Suppression of iNOS and COX-2 may be explained by inhibiting of the activity of its regulator, NF-κB (Surh et al., 2001), presumably due to suppression of IκB degradation and p65 translocation to the nucleus (Singh, Aggarwal, 1995). Therefore, the present results indicated that curcumin reduces oxidative/nitrative stress after praziquantel treatment by inhibiting NF-κB-mediated pathway in addition to inhibition of iNOS-dependent DNA damage (Pinlaor et al., 2009), resulting in the reduction of liver fibrosis (Pinlaor et al., 2010).

Formation of 8-oxodG in DNA is known to cause mutation, leading to carcinogenesis. 8-oxodG formation results in G:C to T:A transversion, which is frequently found in tumor relevant genes (Bruner et al., 2000). This DNA lesion is significantly decreased by curcumin treatment. In addition, we firstly reported curcumin induced not only Keap1 gene, but also genes encoding phase 2 detoxifying enzymes and antioxidants in *O. viverrini*-infected hamsters treated with praziquantel. Its immunomodulatory effect on Nrf2 expression and related stress response may be associated with the accumulation of mononuclear cell infiltration.

In conclusion, curcumin can inhibit oxidative/nitrative stress via induction of Nrf2 and suppression of NF-κB-mediated pathway. These findings provide an insight into the new approach for prevention of chemotherapy-related adverse effects and Nrf2 may be a novel therapeutic target for not only parasitic infection but also other types of inflammation-mediated diseases. Clinical trials are needed to determine whether this nutraceutical can be applied to prevention of chemotherapy-related adverse effects in *O. viverrini*-infected patients.