

CHAPTER V

DISCUSSION AND CONCLUSION

Leber's hereditary optic neuropathy (LHON) typically presents in young adults as painless subacute bilateral visual failure. Males are more commonly affected than females. Women tend to develop the disorder slightly later in life and may be more severely affected (Chinnery, 2005). LHON discovered with mean age of onset between 18 and 35 years. In our study, the age of LHON patients were in a range of 12-67 years. The positive correlation between MDA level and age ($r = 0.407, p < 0.05$) were found and indicated that the older patients had higher oxidative stress status than the younger ones. The association between the generation of lipid peroxidation products and aging has also been reported (Janero, 1990). We also found that MDA level in female was higher than male. So we suggested that the higher level of MDA at baseline in curcuminoid group might be due to the higher age and higher number of female compared to placebo group. In addition, patients in curcuminoid group had other disease such as allergy, hypertension and liver function impairment that have been reported higher MDA level (Mates et al., 1999; Sela et al., 2004).

MDA level was commonly used as a parameter of oxidative stress. LHON was an optic nerves degenerative disease. The pathogenesis of LHON was selectively on optic nerve degeneration. In our study, MDA levels from plasma of 11778 LHON mutation were not significantly different from normal individuals, indicating that oxidative stress was not present in plasma of LHON patients and can not refer to oxidative stress in the optic nerve and pathogenesis of LHON. However, we cannot directly measure the oxidative stress in optic nerve.

The investigation of endogenous activities of antioxidant enzymes in red blood cells of LHON patients found that the activities of CAT, SOD and GPx were not significantly different from normal individuals, indicating that endogenous enzyme activities were in normal. However, we found some effects of curcuminoid extracts capsule on antioxidant enzyme activities.

The superoxide dismutase is an enzyme that catalytic removal of superoxide anion from cell. The activities of SOD in red blood cell were decline 12.6% from baseline when treated with curcuminoid extracts capsule for 3 month. There was evidenced that curcuminoid have a radical scavenging activity due to phenolic group

and β -diketone moiety on the structure of curcuminoid (Masuda et al., 1999). So our results indicated that curcuminoid might be used to scavenge superoxide anion generated in red blood cells. Then the activities of antioxidant enzyme defense result to decrease.

Hydrogen peroxide is another ROS found in biological systems. It is catalyzed by catalase and glutathione peroxidase enzyme. The activities of catalase and glutathione peroxidase vary from tissue to tissue. For example, both enzymes are extremely active in the liver (where considerable H_2O_2 is formed) but only glutathione peroxidase is active in the brain (where is H_2O_2 product is much less). In human erythrocytes, the principal antioxidant enzyme for the detoxification of H_2O_2 is GPx, as CAT has much lower affinity for H_2O_2 than GPx (Izawa et al., 1996). Our study, the catalase activities in erythrocyte of curcuminoid treated group were not significantly difference from placebo but GPx activities in curcuminoid group were declined significantly 24% from baseline. These effects of curcuminoid on GPx enzyme activities were also decreased 18.9% in twenty-four thalassemia patients received curcuminoid capsule 500 mg daily for 6 months (Praphaiphit, 2004).

GSH is a major antioxidant in human tissue that provides reducing equivalents for the glutathione peroxidase catalyzed reduction of hydrogen peroxide and lipid hydroperoxides to water and the respective alcohol. During this process GSH become GSSG. Determination of percentage of GSSG/GSH was a useful indicator of oxidative stress. GSSG/GSH balance was important to homeostasis, facilitating cellular performance and survival (Meister and Anderson, 1983). In this study, the percentage of GSSG/GSH in both group of LHON patients were not significant difference and were in normal range (2-4) (Floreani et al., 2005). Our result indicated that percentages of GSSG/GSH in both groups were in balance and oxidative stress was not occurred in red blood cells of LHON patients.

In this study we found the positive correlation between antioxidant enzyme and MDA level only in placebo group. The positive correlation between SOD and CAT were found since SOD and CAT enzymes work in sequence to scavenge the superoxide anion and hydrogen peroxide, respectively. We also found the positive correlation between GSH and CAT since GSH work as a hydrogen donor to hydrogen peroxide or lipid peroxide by glutathione peroxidase then GSH were convert to GSSG. While CAT enzyme work to decompose hydrogen peroxide to oxygen and

water. In addition, we found the positive correlation between MDA level and GSSG level, indicating that high level of lipid peroxidation resulting high level of GSSG. These correlations were found only in placebo group because of the normal antioxidant defense system. However these correlations were disappeared in the determined group. We suggested that the scavenging oxidants property of curcuminoid extracts capsule might be changed normal antioxidant defense.

Conclusion and Perspective

The MDA levels and antioxidant enzyme activities in circulation were in normal range, indicating that oxidative stress was not present in circulation of LHON patients. However, curcuminoid was able to reduce SOD and GPx activities by its antioxidative activity.

In this study, the measurement of the oxidative stress and antioxidant enzymes activities in red blood cells might not truly represent the oxidative status in neuron of the involved optic nerve. In the further study to clarify this situation, the effect of curcuminoid in the mitochondrial cell derived from LHON patients should be investigated. Because curcuminoid exhibited low oral bioavailability and rapid first-pass metabolism (Ireson et al., 2002; Sharma et al., 2001), the higher dose of curcuminoid capsule should be further studied in LHON patients as well as the level of curcuminoid and its metabolites in circulation should be investigated.