CHAPTER II
LITERATURE REVIEW

1. Leber's hereditary optic neuropathy (LHON)

Leber's hereditary optic neuropathy (LHON) is a mitochondria genetic disease that preferentially causes blindness in young adult males, with a mean age of onset between 18 and 35 years. It is characterized by bilateral subacute loss of central vision owing to focal degeneration of the retinal ganglion cell layer and optic nerve. Over 95% of LHON cases are primarily the result of one of three mitochondrial DNA (mtDNA) point mutations G11778A, G3460A and T14484C, which all involve genes encoding complex I subunits of the respiratory chain (Man et al., 2002).

1.1 Clinical Features

1.1.1 Acute Phase

LHON carriers remain asymptomatic until they experience blurring or clouding of vision in one eye. In the vast majority of cases, visual dysfunction is bilateral, the follow eye becoming affected either simultaneously (25%) or sequentially (75%), with a median inter-eye delay of eight weeks. The characteristic field defect in LHON is centrocaecal scotoma. Other clinical features include the early impairment of colour perception but more importantly, papillary reflexes are preserved and patients usually report no pain on eye movement. Fundoscopy provides other diagnostic clues and in classical cases the following abnormalities can be observed: vascular tortousity of the central retinal vessels, circumpapillary telangiectatic microangiopathy and swelling of the retinal nerve fibre layer. However, in 20% of LHON cases, the optic disc looks entirely normal in the acute phase (Figure 2.1).

1.1.2 Chronic Phases

The retinal nerve fiber layer gradually degenerates and after six months optic atrophy is a universal feature of LHON (Huopone et al., 2002). In addition to visual loss, patients and their maternal relatives have a variety of ancillary symptoms, such as cardiac conduction defects (Nikoskelainen et al., 1994), various minor neurological problems, including ataxia and sensory neuropathy, have been reported in patients

without other neurological finding (Mondelli et al., 1991; Paulus et al., 1991) (Figure2.1).

1.2 Genetics

During fertilization, the human sperm cytoplasm with its few mitochondria does not contribute significantly to the zygote, and virtually all of the zygote's mitochondria come from the mother. Mitochondrial and hence mitochondrial DNA (mtDNA), are maternally inherited. Every offspring, male or female, will inherit the mother's mitochondrial genotype, but only the daughters will pass on this genotype to the subsequent generation. Although proteins encoded by mtDNA are necessary for normal mitochondrial production, they are not sufficient. Indeed, most of the protein found in the mitochondrial are encoded by nuclear genes, synthesized in the cytoplasm and transported into the mitochondria. The cell is dependent on the mitochondria for energy production and the mitochondria are dependent on the cell for the majority of its structural enzymatic proteins necessary for adequate function. The nuclear genome is also involved in the regulation of mitochondrial activities, including the expression and replication of mtDNA. Defects in either the mtDNA or nuclear DNA could result in abnormalities in the oxidative phosphorylation system (Man et al., 2002).

These are three most common type of point mutation in LHON mtDNA. The first, most common is the substitution of adenosine for guanine at position 11778 in the mtDNA. The second is a mutation at position 14484 changes the coding for subunit 6 of complex I. The third is a mutation at position 3460 changes the coding for subunit 1 of complex I. In most LHON pedigrees, the primary mutation is homoplasmic. By contrast, 10-15% of LHON carriers are thought to be heteroplasmic, with one mtDNA subpopulation carrying the wild type allele. It has been suggested that heteroplasmy might influence the expression and inheritance pattern of LHON (Newman et al., 1991; Smith et al., 1993).

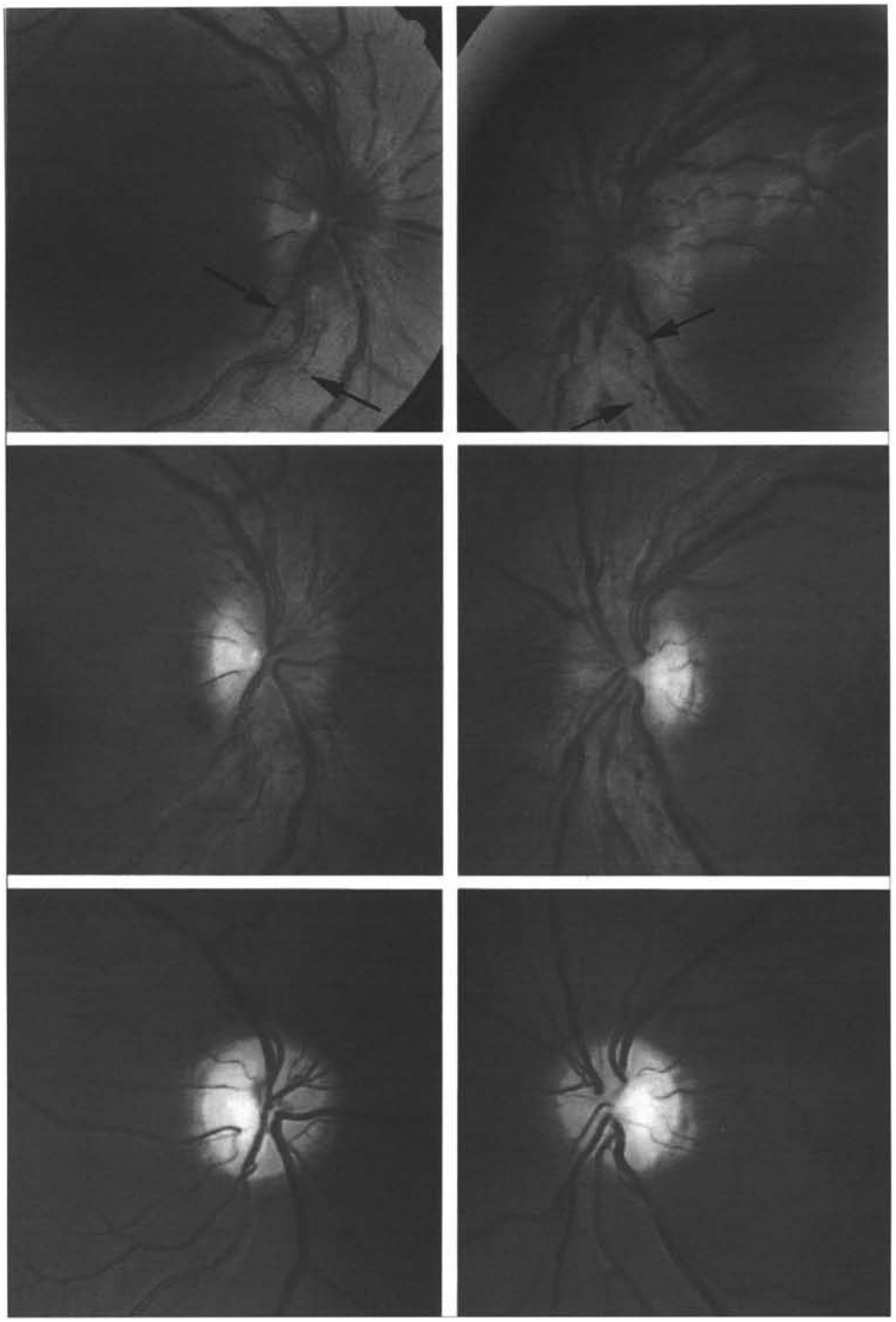


Figure 2.1 Serial fundus pictures of the proband on admission (top), two months (center) and four years later (bottom). Top, in addition to disc elevation and hyperemia, peripapillary microangiopathy (see arrows) is visible in early stage of the disease. Center, intermediate stage of optic atrophy. Bottom, late atrophic stage with attenuated arterioles and severe nerve fiber loss. Microangiopathy is no longer visible (Huoponen et al., 2002).

The 11778, 3460, 14484 mutations are designated "primary mutations". The three primary mutations account for approximately 85-90% of LHON cases world wide (Table 2.1). Other point mutations, "secondary mutations" are polymorphisms without deleterious effect. However, in some combinations, they were found in a synergistic manner with pathogenic LHON mutations to increase the risk of disease expression or to cause a more severe clinical outcome (Man et al., 2002). Among the primary mutations, the LHON clinical phenotype is remarkably similar. The only consistent differentiating feature is the better prognosis for visual outcome in those patients with the 14484 mutation. Up to 60% of patients with the 14484 mutation will have some degree of visual improvement compared with only 5% of patients with the 11778 mutation. Patient with the 3460 mutation may have a better chance of visual recovery than those with the 11778 mutation. Patients with a younger age of onset of visual loss, especially less than 15 years, have a much better visual prognosis, regardless of which mtDNA mutation they habor (Carelli et al., 2004).

Table2.1 Reported pathogenic primary mtDNA mutations in LHON

	Mutation	Protein	Prevalence
Common			>95%
	G3460A	ND1	13%
	G11778A	ND4	69%
	T14484C	ND6	14%
Rare			<5%
	G13730A	ND5	
	G14459A	ND6	
	C14482G	ND6	
	A14495G	ND6	
	C14498T	ND6	
	C14568T	ND6	
	T14596A	ND6	

1.3 Biochemical features

The formation of the respiratory chain is under the control of two separate genetic systems, the nuclear genome, and the mitochondrial genome [mitochondrial DNA (mtDNA)]. In particular, four of the five respiratory chain complexes (I, III, IV and V) contain both nuclear-encoded and mtDNA-encoded polypeptides. In terms of function, the first two linked events of respiration, i.e. electron transfer and proton pumping are carried out by the mitochondrial electron transport chain, a functional supramolecular structure located in the lipid bilayer of the membrane and composed of four complexes (complex I–IV) (Figure 2.2). In human, complex I or NADH-ubiquinone oxidoreductase, is the entry point for electrons into the respiratory chains of mitochondria of most eukaryotes. It couple electron transfer with the translocation of protons across the membrane, thus contributing to the proton motive force essential for energy consuming processes. The current knowledge about human complex I indicates that it is built up by the co-ordinate expression and assemblage of 46 different subunit proteins, 39 being encoded by the nuclear DNA and 7 by mtDNA. Complex I is an L-shape molecule with a long water soluble peripheral arm protruding into the mitochondrial matrix and a short water-insoluble hydrophobic arm embedded in the inner mitochondrial membrane. The seven mitochondrial-encoded subunits of complex I all locate in the water-insoluble short arm. The common pathogenic LHON mutations at nucleotide positions 11778, 3460 and 14484, affecting ND4, ND1 and ND6 subunits genes of complex I of the respiratory chain respectively (Figure 2.3), may cause complex I dysfunction (Carelli et al., 2004).

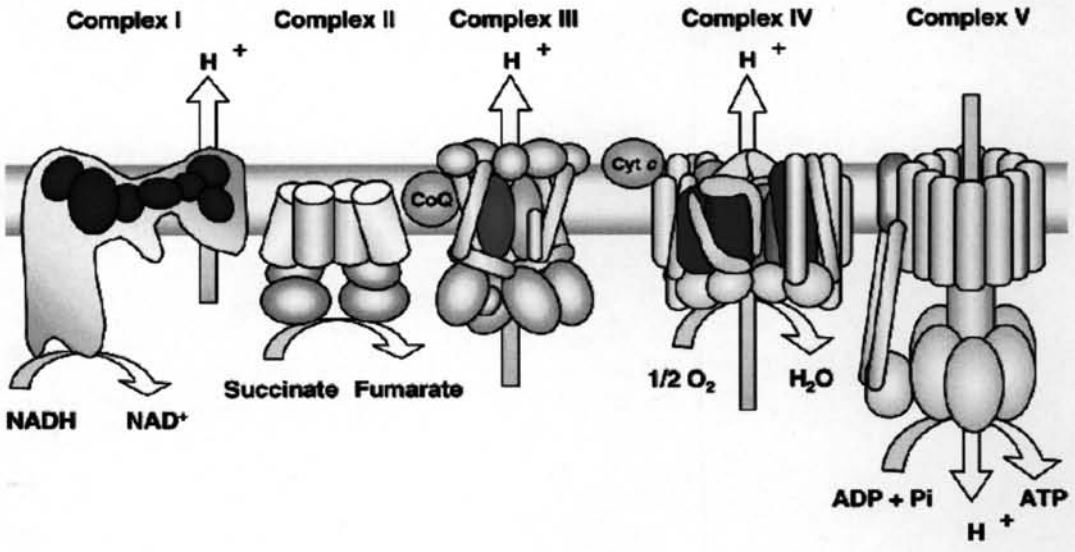


Figure 2.2 Respiratory chain complexes. Mitochondrially encoded subunits, embedded in the midst of nuclear-encoded subunits, Pi = inorganic posphate; Cyt c = cytochrome c; CoQ = coenzyme Q (Zeviani and Di Donato, 2004).

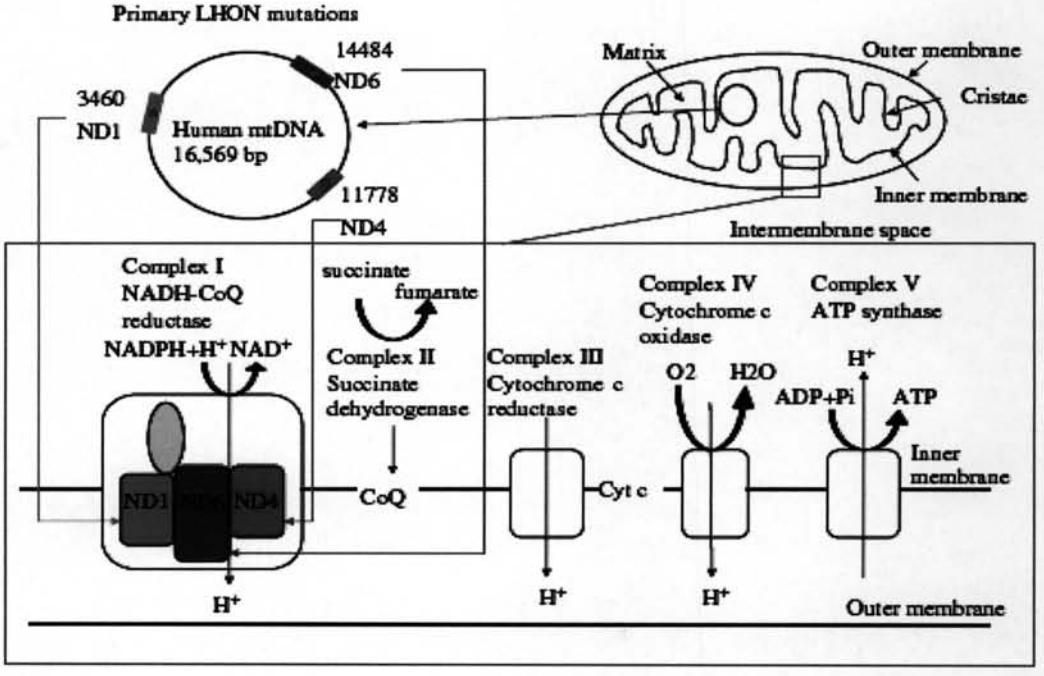


Figure 2.3 The three primary mutations at nucleotide position 11778, 3460 and 14484, affect ND4, ND1 and ND6 subunits genes of complex I of the respiratory chain respectively (Yen et al., 2006).

Biochemical investigation of three most frequent mutation revealed only the 3460/ND1 mutation showed a consistent reduction in complex I electron transfer activity (60-80%). While both 11778/ND4 and 14484/ND6 mutation had normal or slightly reduced activities (Man et al., 2002) (Table 2.2).

Table 2.2 Complex I dysfunction in LHON

Point mutation	In vitro	
	Complex I activity	Respiratory rate
G3460A	60-80 %	30-35%
G11778A	0-50%	30-50%
T14484C	0-65%	10-20%

Three major consequences may derive from the complex I dysfunction reported in LHON: first, respiratory function may be disturbed at the level of quinol product release because of the impaired electron flow, leading to decreased total respiratory activity; in addition, due to alteration of the hydrophobic quinone binding site, proton pumping through complex I may defective thereby affecting energy conservation; finally, an increase of ROS generation may occur as a consequence of altered electron flow (Lenaz et al., 2004).

1.4 LHON mutations and ROS

It has been found that a major site of ROS production at the respiratory chain level is complex I (Kudin et al., 2004). Superoxide is rapidly converted to H_2O_2 by superoxide dismutase (SOD) and H_2O_2 in the presence of reduced transition metals, such as Fe^{2+}, may either undergo further a very reactive and toxic hydroxyl radical (Carelli et al., 2004). Wong and colleages and Ghelli et al, study in two different experiments, showed that cybrids with LHON mutation have increased production of ROS (Wong et al., 2002; Ghelli et al., 2003). Qi and colleages induced the reduction of mitochondrial superoxide dismutase in an animal model system for study of oxidative injury to the optic nerve. They observed similar histopathological findings to those seen in the retinal ganglion cells (RGCs) of patients with LHON (Qi et al., 2003). Battist and colleages induced apoptotic response of peripheral blood

lymphocytes from LHON found that cells of patients with LHON had a higher rate of apoptosis than control (Battisti et al., 2004). Floreani and colleagues investigated enzymatic antioxidant defenses in LHON cybrids grow either in glucose rich medium, a condition in which cybrids rely mostly on anaerobic metabolism for energy production, or in galactose medium, where cybrids are forced to rely upon the respiratory chain for ATP synthesis. In glucose medium, the soluble antioxidant defenses of cybrids are not different between LHON and controls whereas glutathione reductase and glutathione peroxidase activities were lower in LHON cybrids carrying the 3460 and 11778 mutations. In galactose medium, GSSG concentrations increased significantly in all cells, indicating severe oxidative stress (Floreani et al., 2005).

1.5 Treatment

In clinical management, there is no treatment available for improvement the final visual outcome in LHON (Man et al., 2002). Although different substances, such as thiamine, coenzyme Q10, vitamin B12, vitamin K, vitamin C have been tested, to date there is not any effective treatment for LHON (Orssaud, 2003). Mashima et al. have reported that oral administration of Idebenone combined with vitamin B2 and vitamin C can speed up visual recovery but do not necessary promote visual improvement (Mashima et al., 2000). It is thus highly recommended to avoid all risk factors such as alcohol or tobacco, which might play a role in the occurrence of LHON (Man et al., 2000).

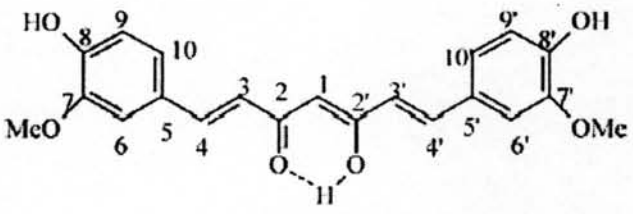
2. Curcuminoids

The dried ground rhizome of the perennial herb *Curcuma longa* Linn., called tumeric has been used in traditional medicine for the treatment of jaundice, ulcers, parasitic infections, inflammation of the joints, cold and flu symptoms. It is also used for preserving food as antimicrobial. *Curcuma* spp. Contains turmerin, an essential oil and curcuminoid. Curcuminoid refer to a group of phenolic compounds present in turmeric consist of curcumin and two minor constituents is demethoxycurcumin, bisdemethoxycurcumin (Figure 2.4). Numerous experimental studies have demonstrated that curcuminoid has various biological activities, such as antitumor activity, antivenom activity, antiinflammatory and antioxidant activity (Jayaprakasha et al., 2005). In clinical study about pharmacokinetic in human found that

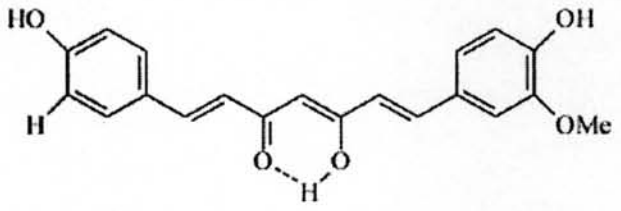
curcuminoid has a low bioavailability of orally administered. Shoba and colleagues administered 2 g of pure curcumin powder to fasting volunteers resulting in low curcumin concentrations detected in plasma (less than10 ng/ml) 1 h post-dose (Shoba et al., 1998). In a study of high dose oral curcumin performed in Taiwan, Cheng and colleagues administered 0.5–8 g daily of curcumin for 3 months to patients with pre-invasive malignant or high risk pre-malignant conditions of the bladder, skin, cervix, stomach or oral mucosa. Serum curcumin concentrations were found to peak 1–2 h after oral intake and gradually decline within 12 h. The 8 g/day dose resulted in a peak serum concentration of 1.75 ± 0.80 μM (Cheng et al., 2001).

2.1 Chemical properties

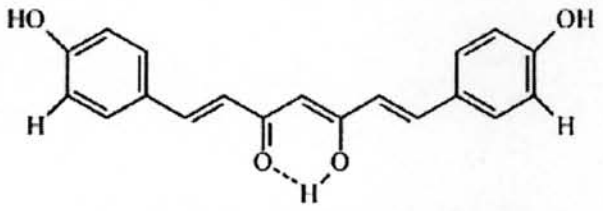
Curcumin is a bis-a, β-unsaturated β-diketone. Curcumin exists in equilibrium with its enol tautomer. The bis-keto form predominates in acidic and neutral aqueous solutions and in the cell membrane. At pH 3–7, curcumin acts as an extraordinarily potent H-atom donor. This is because, in the keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, and the C–H carbon bonds on this carbon are very weak due to delocalization of the unpaired electron on the adjacent oxygen. In contrast, above pH 8, the enolate form of the heptadienone chain predominates and curcumin acts mainly as an electron donor, a mechanism more typical for the scavenging activity of phenolic antioxidants (Figure 2.5). Curcumin is relatively insoluble in water, but dissolves in acetone, dimethylsulphoxide and ethanol. Curcumin has a molecular weight of 368.37 and a melting point of 183^{0}C. Commercial grade curcumin contains the curcuminoids desmethoxycurcumin (MW 338; typically 10–20%) and bisdesmethoxycurcumin (MW 308; typically less than 5%) (Sharma et al., 2005).



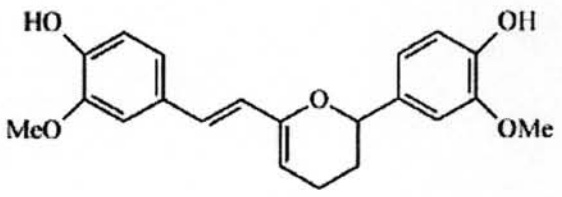
Curcumin (1)



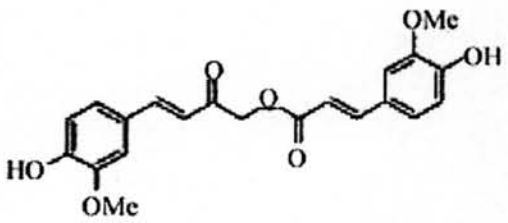
Demethoxycurcumin (2)



Bisdemethoxycurcumin (3)



Cyclo curcumin (4)



Calebin (5)

Figure 2.4 Structures of curcuminoids from *C. longa* (Jayaprakasha et al., 2005).

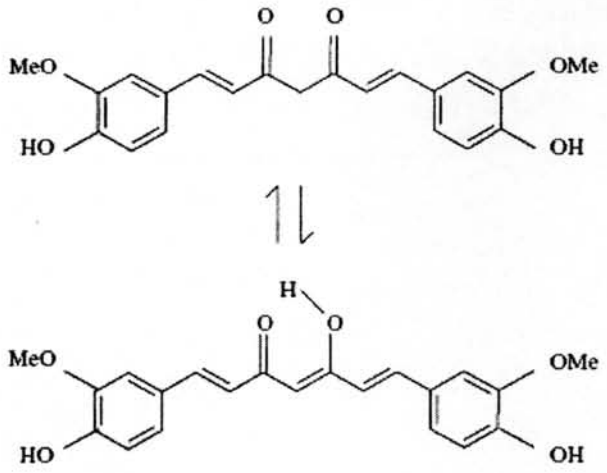


Figure 2.5 Tautomerism of curcumin under physiological conditions. Under acidic and neutral conditions, the bis-keto form (top) predominates, whereas the enolate form is found above pH 8 (Sharma et al., 2005).

2.2 Safety

Studies of curcumin in animals have confirmed a lack of significant toxicity. Systematic preclinical studies funded by the Prevention Division of the US National Cancer Institute did not discover adverse effects in rats, dogs or monkeys of doses up to 3.5 g/kg body weight (BW) administered for up to 3 months. In more recent preclinical studies, no toxicity has been observed from 2% dietary curcumin (approximately 1.2 g/kg BW) administered to rats for 14 days (Sharma et al., 2001) or from 0.2% dietary curcumin (approximately 300 mg/kg BW) administered to mice for 14 weeks (Perkin et al., 2002). However, have some study reported gastrointestinal adverse events: one patient consuming 0.45 g daily and one patient consuming 3.6 g daily developed diarrhea after one month and four months into treatment, respectively. One patient consumes 0.9 g curcumin daily experienced nausea (Sharma et al., 2005).

2.3 Antioxidant activities of curcuminoid

Pharmacological actions of curcumin as antioxidant agent have been examined. In vitro, Deng et al. used oxidative hemolysis of human red blood cell (RBCs) as a model to study the free radical-induced damage of biological membranes

and the protective effects of curcumin and analogues. They found that curcumin could effectively inhibit the free radical induced oxidative hemolysis of RBCs (Deng et al., 2005). The study for ability to inhibit the stimulated lipid peroxidation in rat brain homogenate and rat liver microsome found that curcumin is more potent than alpha-tocopheral (Sreejayan et al., 1994).

In vivo, curcumin 2% w/v was added to male ddY mice for 30 days. They show significantly increased in the activities of glutathione peroxidase, glutathione reductase, and catalase in liver (Iqbal et al., 2003). In other study, curcuminoid 1.0 g/100 g diet was supplement in rat that fed a moderately high-fat diet for 2 weeks. The liver's thiacylglycerol and cholesterol concentration were significantly lower than control (Miyazawa, 2001). In Thailand, the study in thalassemia patients who received curcumin 500 mg daily for 6 months, found that MDA was reduced 30 – 37%, while the activities of enzyme superoxide dismutase and glutathione peroxidase (GPx) were declined 15.30% and 18.91% respectively. The level of reduced glutathione was increased 19.48% (Praphaiphit, 2004).

3. Oxidative stress

The oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense systems of an organism (Halliwell and Gutteridge, 1999). Oxidants compass reactive oxygen species (ROS), reactive nitrogen species (RNS), sulfur-centered radicals and various others. Oxidant can be generated in numerous ways, such as by ionizing radiation, by chemical reactions, metal ions bound to enzymes. Important cellular source of oxidative stress are the formation of reactive oxygen species by incomplete reduction of oxygen in the respiratory chain of mitochondria and the oxidative burst mediated by NADPH oxidase, producing superoxide radical (Abuja and Albertini, 2001).

4. Antioxidant defense systems

The substances that neutralize the potential harmful effects of reactive oxygen species are antioxidant defense systems. Antioxidant present at low concentration, compare with those of oxidizable substrate, considerably delays or inhibits oxidation of substrate.

The antioxidant defense systems are divided into two groups: enzymatic antioxidants and nonenzymatic antioxidants (Table 2.3).

Table2.3 The antioxidant defense systems

	Mode of action
Enzymatic antioxidants	
Superoxide dismutases	Catalytic removal from cell of $O_2^{\bullet-}$
Catalase	Catalytic removal of H_2O_2 at high concentration (catalatic activity). Has a peroxidatic activity when methanol, ethanol, formate and nitrite are electron donors.
Glutathione peroxidase	Catalytic removal of H_2O_2 and lipid hydroperoxides. Can effectively remove low steady-state levels of H_2O_2.
Nonenzymatic antioxidants	
Vitamin E	Lipid soluble, chain-breaking antioxidant. May also protect lipoprotein lipids in the plasma.
β-Carotene	Singlet oxygen and $OH^{\bullet}$ radical scavenger; inhibitor of lipid peroxidation under certain conditions.
Vitamin C	Free radical scavenger, singlet oxygen quencher, regeration of vitamin E
Glutathione	Catalytic removal of hydrogen peroxide, hydroxyl radical quencher, singlet oxygen quencher, Regeneration of vitamin E and vitamin C.
Transferrin	Binds ferric ions.
Lactoferrin	Secreted by phagocytic cells, binds ferric ions and retains them at low pH.

4.1 Enzymatic antioxidants

Antioxidant enzymes or scavenging enzymes that are directly involved in the detoxification of reactive oxygen species are catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

4.1.1 Catalase (CAT)

Catalase is present in virtually mammalian cell types. It is located in the cytosol and in peroxisomes of liver and kidney. Catalase exerts a dual function: (1) It show the catalytic activity by decomposition of H_2O_2 to give H_2O and O_2 and (2) It show the peroxidic activity by oxidation of H donors, example, methanol, ethanol, formic acid, phenols, with the consumption of 1 mol of peroxide (Aebi, 1984).

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

4.1.2 Superoxide dismutase (SOD)

The role of superoxide dismutase is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet-}$), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. There are four types of SOD, Copper, and Zinc containing superoxide dismutase (CuZnSOD), manganese containing superoxide dismutase (MnSOD), iron containing superoxide dismutase (FeSOD) and extracellular superoxide dismutase (ECSOD). In mammalian cells two types of superoxide dismutase are dimeric CuZnSOD and a tetrameric MnSOD. The CuZnSOD is located in the cytosal. The MnSOD is mainly located in mitochondria. Both enymes catalyze the reation of dismutation with the same efficiency (Pitkänen and Robinson, 1996).

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

4.1.3 Glutathione Peroxidase (GPx)

Glutathione peroxidase is mainly found in the mitochondrial and cytosol of animal liver, lung and human erythrocytes. GPx acts on lipid hydroperoxide substrates that are released from membrane phospholipids by phospolipase A2 (Van Kuijik et al., 1987). It can utilize cholesterol hydroperoxide (Thomas et al., 1990) and hydrolyzes H_2O_2 at low concentration (Grisham, 1992). Glutathione peroxidase catalyzes the reaction of hydroperoxide and organic hydroperoxide (ROOH) with reduced glutathione (GSH) to form oxidized glutathione disulfide (GSSG) and the reduction product of the hydrogen peroxide (Bompat et al., 1990).

$$H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSSG$$

$$ROOH + 2GSH \xrightarrow{GPx} ROH + H_2O + GSSG$$

$$GSSG + NADPH + H^+ \xrightarrow{GSH\ reductase} 2GSH + NADP^+$$

4.2 Nonenzymatic antioxidant

4.2.1 Glutathione (GSH)

Reduced glutathione (GSH), a tripeptide with a free thiol group, is a major antioxidant in human tissue. There is variability in different organs depending on their function of oxidative capacity. The roles of GSH include: (1) to serve as a cosubstrate for GSH peroxidase (GPx). Whereas GSH is used as a hydrogen donor to reduce hydrogen peroxide and organic peroxide to water and alcohol, respectively; (2) to conjugate exogenous and endogenous toxic compounds, catalyzed by GSH sulfur-transferase; (3) to reduce protein disulfide and GSH-protein mixed disulfide bonds, maintaining the sulfhydryl residues of certain proteins and enzymes in the reduced state; (4) to store cysteine in a nontoxic form; and (5) to assume a vital role in keeping vitamin E and vitamin C in the reduced states (Satu et al., 1997). During the process of an antioxidant GSH become oxidized glutathione (GSSG). The GSSG is then recycled into GSH by glutathione reductase and a nicotinamide adenine dinucleotide

phosphate (NADPH) (Figure 2.6). When mammalian cells are exposed to increased oxidative stress, the ratio of GSH/ GSSG will decrease as a consequence of GSSG accumulation.

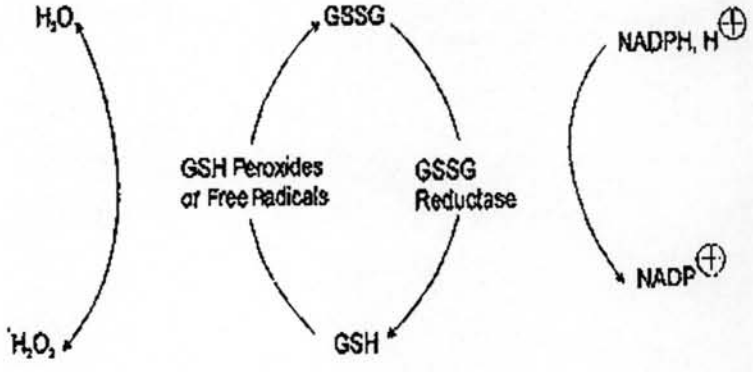


Figure 2.6 Recycling method of glutathione (Anderson, 1985).