

## CHAPTER 1

### Introduction

Neuronal cells are susceptible to oxidative damage. One reason is the brain's high O<sub>2</sub> consumption: it consumes 20% of the body's oxygen, but only comprises a few percent of the body's weight. The high rate of O<sub>2</sub> consumption can lead to a high level of O<sub>2</sub><sup>-</sup> generation that increases with the age of brain mitochondria. The adult brain contains about 10<sup>11</sup>-10<sup>12</sup> neuronal cells which are supported by at least twice that number of neuroglial cells, including microglia, astrocytes, and oligodendrocytes. Glial cells, such as microglia and astrocytes, are capable of secreting cytokines, including tumor necrotic factor alpha (TNF), which can prime microglia to produce reactive oxygen species/reactive nitrogen species (ROS/RNS) upon activation. Cytokine-dependent microglia activation may involve induction of inducible nitric oxide synthase (iNOS) in microglia and astrocytes.

It is well documented that oxidative stress plays an important role in neurologic diseases, including Alzheimer's disease (AD). Neuronal injury, especially loss of neurons, may lead to dementia. Chemo-therapeutic drugs, such as adriamycin (ADR), a prominent member of the anthracyclin family, exhibit therapeutic activity against a wide spectrum of human and experimental animal tumors (Hitchcock-Bryan et al. 1986; Fisher et al. 1989). It is well established that ADR leads to generation of free radicals (Olson and Mushlin 1990). It also has the potential to bind nitric oxide (NO) (Chaiswing et al. 2004), or to induce cytokine production (Ujhazy et al. 2003; Usta et al. 2004). The clinical use of ADR is compromised by an unusual and potentially lethal cardiac toxicity (Singal et al. 2000). However, its potential toxicity to other normal tissues, including the central nervous system (CNS), has only recently been reported (Joshi et al. 2005) and has generated considerable discussion. Recent studies in breast cancer survivors have shown persistent changes in cognitive function, including memory loss, distractibility, and difficulty in performing multiple tasks, following treatment with chemotherapy including ADR (Ahles and Saykin 2002; Ahles et al. 2002) suggesting

function, including memory loss, distractibility, and difficulty in performing multiple tasks, following treatment with chemotherapy including ADR (Ahles and Saykin 2002; Ahles et al. 2002) suggesting that ADR may induce transient symptoms resembling those of AD. The goal of this dissertation is to investigate the mechanisms by which chemotherapeutics induce CNS toxicity. The literature reviews will focus on the mechanism of oxidative stress in neurological diseases and chemotherapy. Understanding the biochemical mechanisms of ADR-induced neuronal dysfunction is important for the development of preventive strategies.

## **Oxidative stress and neuronal dysfunction**

### **Generation of ROS/RNS in brain tissues**

Reactive oxygen species (ROS) are a radical and non-radical derived  $O_2$  species, such as  $O_2^{\cdot-}$ , OH $\cdot$ ,  $H_2O_2$ , hypochlorous acid (HOCl, an oxidizing and chlorinating agent produced by activated phagocytes). Several enzymes are capable of reducing  $O_2$  to  $O_2^{\cdot-}$ , such as xanthine oxidase, NADH cytochrome P-450, and nitric oxide synthase (NOS). The most important source of  $O_2^{\cdot-}$  *in vivo* in many aerobic cells is the electron transport chain. It has been suggested that under physiological conditions about 1-3% of  $O_2$  reduced in mitochondria may generate  $O_2^{\cdot-}$  (Turrens 1997).

Nitric oxide (NO) is formed *in vivo* from the amino acid L-arginine by nitric oxide synthases (NOSs). The activity of NOS is carefully regulated in healthy tissues. There are three types of well-characterized NOSs. Neuronal NOS (nNOS) was originally identified in both central and peripheral nervous system tissues and is present all the time in cells. Endothelial NOS (eNOS) is also expressed constitutively in endothelial cells to produce the NO that is needed for blood pressure regulation. Both nNOS and eNOS require  $Ca_2^+$  and calmodulin proteins for their action. Inducible NOS (iNOS) was first identified in macrophages after treatment with endotoxin and cytokines. iNOS binds calmodulin extremely tightly and iNOS activity is essentially  $Ca_2^+$ -independent. iNOS catalyzes rapid NO generation and increases concentration of NO in the micromolar range (Endoh et al. 1994; Bredt et al. 1999). NO is a colorless gas and can rapidly diffuse intracellularly and from cell to cell which might result from cell injury or exposure to other activators such as TNF in the local

environment (Kim et al. 1999). Excess NO production, often involving iNOS, may occur in multiple neurodegenerative diseases, such as AD, and has been suspected as a major contributor to the disease's pathology.

### **The physiological role of ROS/RNS in brain tissue**

ROS and RNS can cause oxidative damage in brain tissues. The excess production of  $O_2^{\cdot-}$ ,  $H_2O_2$ , and NO, an inflammatory response of microglial cells, has been reported in AD (Wisniewski et al. 1998). NO rapidly reacts with  $O_2^{\cdot-}$  to form  $ONOO^-$  at the rate of  $k=7 \times 10^9 M^{-1} S^{-1}$ , which is comparable to the rate at which  $O_2^{\cdot-}$  reacts with SOD enzymes (Li and Shah 2004). Addition of  $ONOO^-$  to cells or tissues leads to oxidation of lipids (Graham et al. 1993), DNA strand breakage, nitration and decontamination of DNA bases (Yermilo et al. 1995), and protein nitration (Ischiropoulos et al. 1992; Beckman et al. 1996). The most studied nitration of proteins is the conversion of tyrosine to 3-nitrotyrosine (Beckman et al. 1996). Nitrotyrosine adducted protein *in vivo* is widely used as a bioassay indicative of  $ONOO^-$  generation. Nitration of tyrosine residues leads to enzyme inactivation (Beckman et al. 1996; McMillan-Crow et al. 1997).  $ONOO^-$  is a potent inhibitor of mitochondrial respiration chain enzymes, including Fe-S containing enzymes (Hausladen and Fridovich 1994; Castro et al. 1994).

### **Mitochondrial dysfunction and neurodegeneration**

Mitochondrial alterations have been reported in neurodegenerative diseases, such as AD, which also have inflammatory components involving activation of brain astrocytes and microglia to express iNOS and high levels of NO (Smith et al. 1997; Castegna et al. 2003; Calabesse et al. 2004; Sultana et al. 2006). Exogenous NO can directly diffuse to mitochondria and reversibly inhibit cytochrome oxidase, leading to increased  $O_2^{\cdot-}$  in mitochondria (Brown and Cooper 1994; Brown 1997). NO rapidly reacts with  $O_2^{\cdot-}$  to form  $ONOO^-$ , a potent inhibitor of mitochondrial respiration enzymes, and enhances nitration of proteins, including MnSOD (Cassina and Radi 1996; MacMillan-Crow et al. 1996). MnSOD nitration amplifies the increase of  $O_2^{\cdot-}$  and  $ONOO^-$  production leading to

increased oxidative stress in mitochondria (MacMillan-Crow et al. 1996). Similarly, impaired complex IV, cytochrome oxidase, activity has been demonstrated in AD (Davis et al.1996).

### **Adriamycin, a potent anticancer drug**

Adriamycin (ADR, common name doxorubicin) is a potent anthracycline antibiotic which is isolated from cultures of *Streptomyces peucetius* var. *caesius* (Arcarmone et al. 2000). The cytotoxic effect of ADR on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and the cell membrane lipid binding activities of ADR. Intercalation inhibits nucleotide replication and the action of DNA and RNA polymerases. The interaction of ADR with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of ADR cytotoxic activity. However, the side effects of ADR can be toxic to several organs, including heart, liver and kidney (Singal et al., 2000; Meredith et al., 1983; Burtani et al. 1986). ADR binding to cellular membrane may affect a variety of cellular functions. Special cautions of ADR are cumulative across membranes of the anthracycline and the generation of highly reactive species of the quinone moiety of anthracyclines via enzymatic electron reduction, oxidases, reductases and dehydrogenases (Powis et al. 1989). The biochemical basis for ADR-induced CNS injury is not well documented because ADR does not cross the blood brain barrier (BBB).

### **Adriamycin and somnolence syndrome**

Recent studies in breast cancer survivors have shown persistent changes in cognitive function, including memory loss, distractibility, and difficulty in performing multiple tasks, following treatment with chemotherapy including ADR (Ahles and Saykin 2002; Ahles et al. 2002). Studies in groups of breast cancer survivors report patients experiencing persistent changes in cognitive function following treatment with chemotherapy (Schagen et al. 1999; Brezden et al. 2000). These studies support the hypothesis that cognitive deficits, particularly in the areas of memory and concentration, are associated with ADR-based chemotherapy regimens during and shortly after treatment. These results also support the neuropsychologic impact of ADR-based chemotherapy regimens in long-term

survivors of breast cancer and lymphoma, more than 5 years after diagnosis (Ahles et al. 2002). Previous studies in mouse model demonstrate a significant increase in levels of protein oxidation, lipid peroxidation and increased expression of MRP1 in brain isolated from mice, 72 h post intraperitoneal injection of ADR (Joshi et al. 2005). These results suggest a link between chemotherapy with a redox cycling agent and side effects in brain tissues.

### **Oxidative stress and Adriamycin**

ADR is a quinone-containing anthracycline chemo-therapeutic and is well known to produce ROS via redox-cycling of ADR catalyzed by NADH cytochrome P-450 reductase, NADH dehydrogenase, xanthine oxidase, and nitric oxide synthase (Goodman and Hochstein 1997; Doroshow 1983; Pan and Bachur 1980; Vasquez-Vivar et al. 1997). A free radical-mediated mechanism has been proposed to be responsible for ADR-induced toxicity in heart. Cardiomyocytes treated with ADR show significant protein oxidation and lipid peroxidation (DeAtley et al. 1998). Antioxidants prevent oxidative damage of cardiomyocytes (DeAtley et al. 1999). ADR also disrupts the mitochondrial electron transport system in heart tissues (Yen et al. 1999). Increased expression of MnSOD protects complex I against ADR-induced cardiomyopathy (Yen et al. 1996). The structure of ADR contains a quinone, and, as is well known, quinones undergo a one-electron reduction to form semiquinone radicals (Handa and Sato 1975; Gutteridge 1984). ADR increases superoxide production and enhances formation of NO (Vasquez-Vivar et al. 1997). As a consequence, the imbalance between  $O_2^{\cdot -}$  and NO can lead to formation of  $ONOO^{\cdot -}$ , a potent free radical (Vasquez-Vivar et al. 1999). Although it is well documented that ADR does not cross the blood brain barrier, it has been reported that, apart from its known cardiotoxic effects, ADR can cause side effects in various non-involved tissues, including brain (Steinherz et al 1997).

When 10 mg/kg of ADR was divided into four equal injections and injected i.p. over a period of 2 weeks, a significant increase in malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) and decrease in reduced glutathione (GSH) were observed in brain tissue (Hanan et al. 2004). The thiobarbituric acid reductants and the antioxidant enzymes SOD, catalase

(CAT) and GSH-Px decreased upon exposure to ADR (1 mg/kg for a period of 7 days) (Julka et al. 1993).

### **Alzheimer's disease and somnolence syndrome**

Alzheimer's is a progressive, degenerative condition of the brain, usually associated with advanced age. Although up to 5% of people aged 65 and over suffer from dementia and about half of these have AD, AD also can develop at a younger age. At whatever age a person is affected, the condition is always progressive and degenerative. Formerly self-reliant people eventually become dependent upon others for routine daily activities. The first indications of AD are subtle changes in behavior, and difficulty with short-term memory then becomes apparent; moreover, adjustments to new places or situations may prove to be stressful. Learning, making decisions, or executing tasks becomes problematic. Eventually, emotional control becomes more and more difficult (Thomas et al. 2002).

There is strong evidence implicating cholinergic neurons as the mediators of memory loss in AD. The illness results from selective damage of specific neuronal circuits in the neocortex, hippocampus, and basal forebrain cholinergic system (Hyman et al. 1984; Gosch et al. 2002). At the onset of clinical AD, the hippocampus is affected with or without neocortical neuronal lesions. Loss of hippocampal synapses rather than neuronal loss in the temporal cortex or hippocampus may be the major correlate of memory deficits in human AD (Ball et al. 1985).

### **Alzheimer's disease and genetic defects**

Amyloid  $\beta$ -peptide ( $A\beta$ ), a major constituent of senile plaques (SPs) and hallmarks of AD, is normally secreted by neurons as part of the processing of amyloid precursor protein (APP) and can be found in low concentrations in cerebrospinal fluid (CSF) and plasma (Selkoe et al. 1996, 1998). AD is characterized by tissue deposition of  $A\beta$  in the brain (Mark et al. 1996). Apolipoprotein E  $\epsilon 4$  allele, a risk factor significantly correlates with the rate of hippocampal atrophy for the development of AD and is reportedly associated with a greater accumulation of  $A\beta$  (Mark et al. 2002;

Morri et al. 2002; Bennette 2003). Hyperphosphorylated tau (PHFtau) accumulates as neurofibrillary tangles (NFTs) and neurophil threads (NTs) in the brain of affected individuals (Manish et al. 2002; Nagy et al. 1995,; Mitchell et al. 2002). The hallmark neuropathological lesions of AD are NFTs and NTs, both of which are composed of PHFtau arranged in paired helical filaments, as well as SPs composed of A $\beta$ .

A $\beta$  is a 39-43 amino acid peptide that is derived from the transmembrane APP by two proteolytic cleavages caused by  $\beta$ - and  $\gamma$ -secretases (Holsinger et al. 2002). At the lowest concentration measured in biological fluids (0.1-1.0 nM), A $\beta$  is an antioxidant and therefore can induce the antioxidant defense system. At low nanomolar concentrations of A $\beta$  (1-40), the physiologically predominant form inhibits auto-oxidation of CSF lipoproteins and plasma low density lipoproteins (Kontol et al. 2001).

Genetic evidence supports the hypothesis that mutations in genes for the Alzheimer APP on chromosome 21 and in the presenilin-1 (PS-1) and presenilin-2 (PS-2) genes on chromosomes 14 and 1, respectively. All APP, PS-1, and PS-2 mutations analyzed so far have been shown to increase the production of the A $\beta$ , a cleavage product of the APP gene and a major component of the amyloid plaques found in AD. The increase of soluble A $\beta$ , especially the longer form, A $\beta$  (1-42), is toxic to neurons and leads to the concept of the centrality of A $\beta$  (1-42) to the pathogenesis of AD (Kontol et al. 2001; Jensen et al. 1999; Ulberto et al. 1999; Lodish et al. 1999).

### **Oxidative stress and Alzheimer's disease**

A free radical is an atom or molecule that contains one or more unpaired electrons, a state that makes it highly unstable and reactive. Free radicals are formed during normal metabolism, and free radical injury occurs within living cells when the generation of ROS and RNS exceeds intrinsic antioxidant ability. The situation is also referred to as oxidative stress. The brain may be particularly vulnerable to oxidative damage because it has high energy requirements and a high oxygen consumption rate, which may catalyze the formation of ROS and RNS (Pratico et al. 2000; Beal et al. 1995).

An accumulation of extracellular deposits of A $\beta$  and NPTs in the brain stimulate microglia and monocytes, leading to TNF-dependent expression of iNOS, which is responsible for NO and ONOO<sup>-</sup> production and subsequent apoptosis in primary mouse neuronal cultures (Combs et al. 2001; Lam et al. 2001). On the other hand, A $\beta$  induces oligodendrocyte death by nuclear DNA fragmentation, mitochondrial dysfunction, and cytoskeletal disintegration (Xu et al. 2001a, b). The activation of A $\beta$  on oligodendrocytes produces chemokines, as well as microglia and astrocytes, to synthesize inflammatory mediators, including cytokines, iNOS, and cyclooxygenase (COX2).

A $\beta$  can induce NOS and the resulting NO can readily cross biological membranes and increase in mitochondria (Smith et al. 1997; Canevari et al. 1999). NO can bind cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain. The reports that low nanomolar concentrations of NO inhibit cytochrome oxidase reversibly and competitively with molecular O<sub>2</sub> indicate that these interactions have a potential physiological role in the control of cell respiration, and also that the inhibitory effect might be involved in the inception of pathology (Cleeter et al. 1994; Brown et al. 1994; Torres et al. 1995; Giuffire et al. 1996). Inhibition of cytochrome oxidase enhances the formation of O<sub>2</sub><sup>-</sup> (Poderoso et al. 1996). The increase in O<sub>2</sub><sup>-</sup> generation by the electron transport chain in the presence of NO might also lead, under some circumstances, to the local formation of ONOO<sup>-</sup> and hence to further pathophysiology (Lizasoin et al. 1996; Packer et al. 1996; Lopez-Figueroa et al. 2000). Low doses of ONOO<sup>-</sup> have been shown to cause programmed cell death and large doses result in necrosis. ONOO<sup>-</sup> can cause damage to macromolecules, including DNA oxidation (Inoue et al. 1995), lipid peroxidation (Radi et al. 1991), and protein oxidation (Smith et al. 1997). More specifically, ONOO<sup>-</sup> can nitrate tyrosine residues to form 3-nitrotyrosine (3-NT) and 0-0'-dityrosine (Hensley et al. 1998) in the hippocampus and cortex of AD. The available information is that the mechanism of ONOO<sup>-</sup> induced apoptosis is accompanied by the production of other oxidative species in the ONOO<sup>-</sup> treated cells and that Bcl-2 confers protection against ONOO<sup>-</sup> induced apoptosis (Lin et al. 1997; Lee et al. 2001).



### **Tumor necrotic factor alpha (TNF), Adriamycin and neuronal dysfunction**

TNF has been isolated as a soluble factor released by host cells that caused necrosis of a transplanted tumor (Carswell et al. 1975). TNF is a protein of 185 amino acids glycosylated at positions 73 and 172. It is synthesized as a precursor protein of 212 amino acids. Monocytes express at least five different molecular forms of TNF with molecular masses of 21.5-28 kDa. They mainly differ in post-translational alterations such as glycosylation and phosphorylation. The human TNF gene has a length of approximately 5 kb and contains five exons. It maps to human chromosome 7p21-p14. The murine gene maps to chromosome 5. TNF is produced by many different cell types. The main sources *in vivo* are stimulated monocytes, fibroblasts, and endothelial cells. Macrophages, T-cells and B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells, and keratinocytes also produce TNF after stimulation. TNF is an important cytokine participating in damage and inflammation in both animal and human renal diseases (Gomaz-Chiarri et al. 1994). TNF affects differentiation of macrophages into inflammatory cells and primes neutrophils to increase secretory responses and generation of ROS and RNS. TNF also triggers cell death by apoptosis and necrosis. TNF is known to increase NO production through iNOS (Poljakovic et al. 2003).

The term tumor necrosis factor originates from the observation that this protein plays a crucial role in the killing of tumor cells, and it can induce both necrotic and apoptotic forms of cell death (Laster et al. 1988; Locksley et al. 2001). TNF itself induces the synthesis of other cytokines and chemokines such as IL-1, IL-6, IL-8 and platelet-activating factor (Nawroth et al. 1986; Tracey and Cerami 1993). Its biosynthesis is induced by many inflammatory stimuli such as endotoxins, exotoxins, enterotoxins, and ionizing radiation, and regulation occurs at the transcriptional and post-transcriptional levels (Aggarwal et al. 2003; Mohan et al. 1994). Current evidence suggests that these stimuli use ROS as signaling messengers to activate transcription factors, such as nuclear factor kappa B (NF- $\kappa$ B) and activator protein-1 (AP-1), and induce the expression of a number of genes including TNF (Janssen-Heininger et al. 2000; Schulze-Osthoff et al. 1992, 1995).

ADR, an anthracycline anti-tumor drug, is one of the most widely used antitumor agents and is a key drug for treatment of malignant lymphoma. It is readily activated to the

semiquinone radical and generates ROS. ADR-induced increased TNF-mediated NO production and apoptosis have been reported in nephropathy in ADR-treated rats (Usta et al. 2004). It has also been shown that serum levels of TNF are increased in breast cancer patients following chemotherapy (Berberoglu et al. 2004).

TNF generally increases oxidative stress both in its target cells and in surrounding cells to promote phagocyte ROS production. Increased ROS production appears to involve the effects of TNF on mitochondria (Richter et al. 1995). In addition, it has been demonstrated in L929 cells that TNF leads to early degeneration of the mitochondrial ultrastructure without any pronounced damage to other cellular organelles (Schulze-Osthoff et al. 1992)

### **TNF-alpha and neurodegeneration**

TNF expression is elevated in a variety of neuropathologies, including AD (McCusker et al. 2001; De Luigi et al. 1995), multiple sclerosis (Sharief and Hentges 1991), cerebral malaria (Brown et al. 1999), and HIV encephalitis (Achim et al. 1993; Nuovo et al. 1994). TNF levels have been quantified in post-mortem tissue from the brains of patients with both acute and chronic neuropathologies, indicating local production of the cytokine. TNF expression has also been detected in post-mortem brain tissue from patients with bacterial meningitis (Waage et al., 1989), a condition in which intrathecal levels of TNF correlate positively with the degree of BBB breakdown, disease severity and indices of meningeal inflammation (Sharief et al. 1992). Furthermore, TNF expression is associated with demyelinating multiple sclerosis lesions (Woodroffe and Cuzner 1993), and the presence of TNF in cerebrospinal fluid from multiple sclerosis patients correlates with disease severity (Hauser et al. 1990).

### **Antioxidant prevention and neuroprotective system**

Oxidative stress has been implicated in neurodegenerative disease, such as AD. There are several indicators suggesting that enhanced oxidative stress plays an important role in the pathogenesis or progression of AD (Markesbery 1997; Markesbery and Carney 1999; Butterfield

et al. 2001; Butterfield and Lauderback 2002). Increased production of ROS and RNS, such as  $O_2^{\cdot -}$  and NO, coinciding with a depletion of antioxidant defenses is observed in neuronal systems after A $\beta$  (1–42) treatment (Butterfield 1997; Yatin et al. 2000). Involvement of oxidative stress has been reported in either AD brain samples or in experimental models of AD (Butterfield et al. 2001), including protein oxidation (Castegna et al. 2003), lipid peroxidation (Butterfield and Lauderback 2002; Picklo et al. 2002; Lovell et al. 1995) and 3-nitrotyrosine (3-NT) formation (Hensley et al. 1998).

MnSOD is an important primary antioxidant defense in the mitochondria. Inactivation of the MnSOD gene in mammals yields detrimental effects. Lack of MnSOD activity can severely impair mitochondria and affect the brain, which has high demands for oxidative metabolism. In addition to being essential for survival in an oxygen-rich environment, overexpression of MnSOD protects against numerous agents and conditions that cause oxidative stress and/or neuronal injury (Gonzalez-Zulueta et al. 1998; Keller et al. 1998). The transcription factor NF- $\kappa$ B can prevent neuronal cell death in experimental models of neurodegenerative disorders by inducing the expression of anti-apoptotic proteins including Bcl-2 and MnSOD (Camandola et al. 2000; Mattson et al. 1997). In ADR treatment, increased expression of MnSOD protects complex I against ADR-induced cardiomyopathy (Yen et al. 1996). Overexpression of catalase has been shown to increase resistance to ADR-induced cardiac injury (Kang et al. 1996). MnSOD mimetic is the most frequently used antioxidant to prevent ADR-induced cardiotoxicity (Sawyer et al. 1999). The effects of oxidative stress in the mitochondria can be eliminated by direct scavenging of radicals, enhancing intracellular defense mechanisms such as antioxidant enzymes, or by using certain antioxidant mimetics to prevent mitochondrial dysfunction and toxic effects in both neurodegenerative diseases and chemotherapy.

Therapeutic use of nutritional antioxidants, such as Vitamin E, has been reported to protect cortical synaptosomal membranes from A $\beta$  (25-35) (Subramaniam et al. 1998) and to prevent ADR-induced cardiotoxicity (Mimnaugh et al. 1979). Curcumin, a phenolic compound, can inhibit lipid peroxidation and neutralize reactive oxygen and NO-based free radicals (Calabrese et al. 2003). Ethyl-4-hydroxy-3-methoxy-cinnamic acid (FAEE), a phenolic compound, shows antioxidant and anti-inflammatory activity on A $\beta$  (1–42)-induced oxidative stress and neurotoxicity. The large amount of NO produced by iNOS has been closely correlated with pathophysiology in a variety of

diseases and inflammation (Bredt 1999). iNOS induction may reflect the degree of inflammation and may provide a measure to assess the effects of drugs on the inflammatory process. Treatment of neurons with FAEE significantly reduces the levels of iNOS and nitrated proteins induced by A $\beta$  (1–42) (Sultana et al. 2005). Tea flavonoids (catechins) have been reported to possess divalent metal chelating, antioxidant and anti-inflammatory activities, to penetrate the blood brain barrier and to protect against neuronal death in a wide array of cellular and animal models of neurodegenerative disorders (Weinreb et al. 2004), suggesting that natural products can minimize pathological and toxic effects associated with oxidative stress (Butterfield et al. 2002).

### **Somnolence syndrome (Cognitive dysfunction)**

Cognitive decline is a well recognized symptom of AD and identification of the early diagnostic indices of dementia is most helpful for patients (D’Introno et al. 2004; Bond et al. 2002). Several studies have suggested that most patients who meet mild cognitive impairment (MCI) criteria will progress to AD (Panza et al. 2005). MCI is best characterized from a diagnostic standpoint, and is often a pathology-based condition with progression to AD (Peterson et al. 1999, 2001; Morris et al. 2001).

Somnolence syndrome has been reported in many cancer patients experiencing impairment of neurocognitive function, including memory loss, distractibility, and difficulty in performing multiple tasks (Meyers 2000; Schagen et al. 1999; van Dam et al. 1998; Wieneke and Dienst 1995). Somnolence syndrome is a condition characterized by excessive daytime sleepiness or prolonged drowsiness, including long periods of sleep (10 hours or more at a time), excessive amounts of deep sleep, and difficulty staying awake during the day. Somnolence syndrome has been reported in children with acute lymphocytic leukemia treated with cranial irradiation, and has been identified as a possible precursor of later cognitive dysfunction (Berg et al. 1983). Somnolence syndrome can be caused by both cancer and cancer treatments. Possible causes of somnolence syndrome include brain cancers in adults, childhood central nervous system (CNS) cancers, secondary brain tumors (brain metastases), chemotherapy drugs, and side effects of cancer treatments (van Dam et al. 1998). Proinflammatory cytokines, including TNF, have profound effects on brain

function and are known to cause cognitive and mood dysfunction. TNF is elevated in acute leukemia patients whose diagnosis includes neurodegenerative symptoms (Bruserud et al. 1995; Meyers et al. 1992). Neurocognitive dysfunction has a negative impact on life. Improving the quality of life of those afflicted with cancer can be possible with the use of early diagnosis of neurocognitive dysfunction and the interventions available for maintaining or improving neuro-cognitive status (Cohen et al. 1999).

### **Summary and Experimental approaches**

Accumulating evidence strongly suggests that oxidative stress may be the common cause underlying some of the cognitive problems associated with ADR treatment and A $\beta$  deposits. Neurotoxicity in AD brain caused by free radical-based oxidative stress serves as an important model with which to compare the neurological mechanisms in brain associated with the oxidative stress of ADR. I hypothesize that TNF- mediated NO production, mitochondrial dysfunction, and consequent MnSOD nitration/inactivation are the mechanisms underlying some chemotherapeutic-induced neuronal dysfunction. In the present study, as demonstrated and discussed in the following chapters, a APP/PS-1 double knock-in mouse model of Alzheimer's disease was used to investigate how ADR given systemically induced CNS toxicity.