CHAPTER 5

Summary and Future Studies

Adriamycin is an anthracycline antibiotic which is widely used as a chemotherapeutic agent. However, its clinical effectiveness is limited by dose-related cardiotoxicity and congestive heart failure (Singal and lliskovic 1998). However, no dose limiting effect of ADR in CNS toxicity associated with forgetfulness, lack of concentration, dizziness, distractibility, and difficulty in performing multiple tasks, following treatment with chemotherapy including ADR (Ahles and Saykin 2002, Ahles et al. 2002), has been well documented. Extant reports elucidate that the side effects of cancer therapy to CNS significantly reduce the quality of life for patients during and after cancer chemotherapy. Thus, determining the mechanisms of ADR-induced CNS toxicity and preventing the damaging effects of ADR to the brain without inhibiting its anti-tumor effects are essential for the improvement of chemotherapy.

Recently, it has been reported that systemic administration of a high dose of ADR is associated with an increase in oxidative stress in brain tissues (Joshi et al. 2005). The present study intended to demonstrate that ADR-induced oxidative stress and mitochondrial dysfunction in brain tissues are mediated by TNF. The study confirms that ADR accumulated only in areas outside the blood brain barrier, but increased TNF levels were found in serum and both the hippocampal and cortical regions of the brain. The TNF led to generation of reactive oxygen and nitrogen species (RONS) in the brain tissue and subsequently to mitochondrial respiration dysfunction, cytochrome c release, caspase 3 activation, and TUNEL positive cells. Although ADR-induced circulating levels of TNF increased in both wild-type mice and iNOSKO mice, the decline in mitochondrial respiration after ADR treatment was observed only in wild-type mice, suggesting a role for NO in ADR-induced, TNF-mediated mitochondrial dysfunction. Consistent with this finding, ADR treatment led to brain protein nitration and MnSOD nitration/inactivation in the wild-type mice but not in the iNOSKO

mice. These results resemble the mitochondrial dysfunction and MnSOD nitration found in the homozygous knock-in APP/PS-1 AD mouse model.

Because ADR does not directly cross the BBB, it is unlikely that ADR is directly catalyzed by mitochondrial electron transport chain complex I (NADH dehydrogenase), or that it can be the major source of ADR-induced ROS production in brain tissues. However, ADR-induced circulating TNF mediates TNF increases in the neurons and is a possible source of ADR-toxicity. TNF is an inflammatory cytokine that is known to induce neuronal damage (Campbell 2004). It has been demonstrated that TNF causes rapid inhibition of mitochondrial respiration leading to ROS production, induction of mitochondrial permeability transition pore and tissue injury (Higuchi et al. 1998; Lancaster 1989; Szewczyk and Wojtczak; 2002; Goossens et al. 1995). Yen et al. have previously demonstrated that overexpression of MnSOD in transgenic mice protected against ADR-induced cardiotoxicity (1996). Thus, it is possible that an increase in TNF levels may be the link between ADR-induced oxidative stress (Joshi et al. 2005) and CNS injury.

It is well documented that oxidative stress is associated with Alzheimer's disease (Markesbery 1997; Butterfield et al. 2001, 2002). In addition to increased levels of lipid peroxidation (Markesbery and Lovell 1998), protein oxidation (Hensley et al. 1998; Smith et al. 1996), 3-nitrotyrosine (Smith et al. 1997; Castegna et al. 2003), advanced glycation end products (Butterfield et al. 2001), and RNA/DNA oxidation products (Mecoci et al. 1994; Nuhomura et al. 1999), mitochondrial dysfunction associated with inflammatory components have been reported in AD. Activation of brain microglia and astrocytes to express iNOS and high levels of NO (Smith et al. 1997; Castegna et al. 2003; Calabesse et al. 2004; Sultana et al. 2006) may be the cause of peroxynitrite-induced MnSOD nitration in AD brain. Thus, neurotoxicity in AD brain caused by free radical-based oxidative stress serves as an important model for the neurotoxic mechanisms in brain associated with the oxidative stress of ADR. Prevention of MnSOD inactivation by neutralizing elevated TNF levels or removal of NO production conceivably could be effective means for the prevention of ADR- or Aβ-induced CNS toxicity.

In this dissertation report, I have shown in both chemotherapeutic drug, ADR, and increased Aβ in APP/PS-1 AD mouse models that oxidative stress induced a decline in brain

mitochondrial respiration complex I. This result is consistent with the possibility that the (4Fe-4S) cluster protein in complex I is inactivated by ROS generated in mitochondria, since the (4Fe-4S) protein of complex I extends into the inner membrane where superoxide is generated. This possibility is strongly supported by previous studies that demonstrated that transgenic mice overexpressing MnSOD are protected from ADR-induced complex I inactivation in cardiac tissues (Yen et al. 1996, 1999). Mitochondrial dysfunction may be one of the signals initiating the mitochondrial apoptosis pathway by recruiting the translocation of pro-apoptotic proteins, p53 and Bax, to mitochondria, which, in turn, leads to cytochrome c release and subsequent induction of caspase 3 cleavage and apoptotic cell death. The increase of these pro-apoptotic proteins coincides with the increase in mitochondrial dysfunction, suggesting mitochondrial dependent tissue injury. The finding that p53 interacted with Bcl-xL in mitochondria further supports the role of mitochondria in ADR-induced CNS injury. These data are consistent with recent reports demonstrating that Bax is required for p53 translocation to mitochondria (Chipuk et al. 2003, 2004).

p53 protein localizes to the mitochondria and is associated with p53-dependent apoptosis but not p53-mediated cell cycle arrest (Marchenko et al. 2000; Mihara et al. 2003). Within 1 hour after cellular stress activates p53, p53 rapidly accumulates in mitochondria, and this leads to changes in mitochondrial membrane potential, cytochrome c release, and procaspase-3 activation (Marchenko et al. 2000). p53 accumulates in the cytoplasm, where it directly regulates the pro-apoptotic protein Bax to promote mitochondrial outer membrane permeabilization and Bax translocated p53 to mitochondria (Mihara et al. 2003; Chipuk et al. 2004). Pro-apoptotic p53 also can directly induce permeabilization of outer mitochondrial membrane by forming a complex with Bcl-xL, resulting in cytochrome c release (Mihara et al. 2003). Overexpression of anti-apoptotic protein, Bcl-xL, abrogates stress signal-mediated mitochondrial p53 accumulation and apoptosis (Machenko et al. 2003).

The mechanism by which p53-mediates apoptosis in neurodegenerative diseases, such as AD, is not completely understood. Neuronal apoptosis has been reported to involve mitochondrial membrane where pro-apoptotic protein either promotes (p53, Bax, Bid and Bad) or prevents (Bcl-2, Bcl-xL) membrane permeability changes, cytochrome c release, caspase 3

activation, and condensation and degradation of nuclear DNA (Hong et al. 2004; Polster et al. 2004). Treatment with β-amyloid peptide has been shown to induce TUNEL-positive cell death in primary hippocampal neuronal cell culture (Loo et al. 1993). Thus, p53 translocation to mitochondria in AD may be initiated by genetic factors, leading to mitochondrial impairment and increased oxidative stress (Culmsee et al. 2001; Gilman et al. 2003).

In conclusion, systemic ADR treatment led to an increased circulating level of TNF in wild-type mice and in iNOSKO mice. Circulating TNF, in turn, mediated iNOS induction to produce NO in brain tissues that subsequently led to a decline in mitochondrial respiration. ADR treatment led to MnSOD nitration, which was inactivated in wild-type mice. Nitration/inactivation of MnSOD after ADR treatment resembled that found in APP/PS-1 mice. These results are consistent with the notion that nitric oxide is a mediator coupling the effect of ADR or Aβ with cytokine production and subsequent inactivation of MnSOD in the brain. Thus, prevention of MnSOD inactivation by neutralizing elevated systemic TNF levels or removal of NO production could be effective means for the prevention of ADR- or Aβ-induced CNS toxicity. It is tempting to speculate that ADR-induced cognitive decline may be a transient effect resembling early AD.

Figure 5.1: A hypothetical model of Nitric oxide-mediated Manganese superoxide dismutase inactivation: An insight into the mechanisms of chemotherapeutic-induced neuronal dysfunction.