CHAPTER 19

Chemo Brain (Chemo Fog) as a Potential Side Effect of Doxorubicin Administration: Role of Cytokine-Induced, Oxidative/Nitrosative Stress in Cognitive Dysfunction

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Abstract

Doxorubicin (ADRIAMYCIN, RUBEX) is a chemotherapeutic agent that is commonly administered to breast cancer patients in standard chemotherapy regimens. As true of all such therapeutic cytotoxic agents, it can damage normal, noncancerous cells and might affect biochemical processes in a manner that might lead to, or contribute to, chemotherapy-induced cognitive deficits when administered either alone or in combination with other agents.

Introduction

In 1980, Dr. Peter Silberfarb and colleagues reported cognitive changes in patients undergoing chemotherapy treatments. Twenty-two men and twenty-eight women were included in this study with malignancies such as respiratory, digestive, Hodgkin's disease, leukemia and multiple myeloma. It was reported that, overall, patients scored significantly worse on various tests of cognition and recall after undergoing chemotherapy.1 Interestingly, cognitive decline was evident in patients not receiving chemotherapy directed at the central nervous system (CNS); this was surprising, due to the fact that a majority of the drugs administered in this study are known to not cross the blood brain barrier (BBB). This report was the first to observe that drug penetration of the brain parenchyma is seemingly not a requirement for cognitive dysfunction resulting from non-CNS-directed chemotherapy.1

Memory impairment as a result of brain radiation or CNS-directed chemotherapy is a well-established and universally accepted consequence of these treatment options. However, cognitive defects resulting from chemotherapeutic agents known specifically not to cross the BBB is a less understood phenomenon. As a result, there is still some debate over the validity of declining brain function as a direct side effect of chemotherapy. Emotional factors, such as anxiety and depression, that are consequences of cancer diagnosis and treatment, are likely to contribute to deficits in memory and cognition. Nevertheless, multiple reports find significant association of chemotherapy with instances of memory impairment, even after methodological or statistical

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DOX and Central Nervous System Oxidative Stress

Although the aforementioned pathways of ROS/RNS interplay (downstream of $O_2^-$ formation by DOX) are valid for any biological system, the introduction of free radicals in brain as a result of DOX is different in the CNS compared to the periphery, due to the purported inability of DOX to cross the BBB, although it may be possible for DOX to enter the brain area outside BBB. Therefore, the potential exists for an indirect mechanism of CNS oxidative toxicity as a result of DOX, one that does not necessarily involve redox cycling within the CNS. Although presently unclear, recent studies suggest that the mitigator of brain toxicity as a result of DOX
may be cytokine related (see next section). Oxidative stress has been detected in brains of mice treated i.p. with DOX and will be reviewed here.

Increased levels of protein oxidation, protein nitration and lipid peroxidation have been observed in brains from DOX-treated mice, indicative of oxidative stress that we hypothesize is related to chemo fog in humans.46,51-53 Because oxidative stress is largely considered a cellular imbalance of oxidants and antioxidants, studies have shown that DOX also leads to depletion of CNS antioxidants, rendering cells vulnerable to these deleterious modifications.51,53 Cardoso et al found that subchronic subcutaneous injections of DOX to Wistar rats lowered reduced glutathione (GSH) content in brain but increased vitamin E, possibly reflecting an oxidative stress defense response.13 Co-administration of N-acetylcysteine (NAC), a GSH precursor capable of crossing the BBB, with DOX resulted in improvements in behavior relative to DOX-treated rats alone.94 Another GSH precursor, gamma glutamylcysteine ethyl ester (GCEE), prevented DOX-induced oxidative stress in brain, further supporting our hypothesis that increasing endogenous brain-resident GSH may prevent chemo fog symptoms.51

DOX has also been observed to cause mitochondria-localized changes in brain. DOX lowered activity of brain aconitase, a mitochondrial tricarboxylic acid cycle enzyme, possibly via oxidative damage of this protein.55 Alterations in mitochondrial levels of nitrated proteins were also observed with DOX treatment.52 In the same study, MnSOD, another mitochondrial-localized enzyme that converts O$_2^-$ to H$_2$O$_2$, was observed to be nitrated with i.p. administration of DOX.54 As a result, the activity of MnSOD was observed to be decreased in brain mitochondria with DOX treatment.52 Dysfunction of this protein could have disastrous consequences leading to buildup of mitochondrial O$_2^-$ that could eventually culminate in cell death. Interestingly, changes in mitochondrial nitration were not observed in inducible nitric oxide synthase (iNOS) knockout mice with DOX treatment, implicating this enzyme in DOX-related oxidative damage to brain mitochondria.52

**Role of Cytokines on Doxorubicin-Induced CNS Toxicity**

Chemotherapy and chemotherapy-related neurotoxicity are associated with the release of proinflammatory cytokines. Cytokines are signaling molecules activated in response to infection or injury that trigger inflammation. In the CNS, cytokines also have roles in dopamine and serotonergic metabolism, neural repair and neuronal/glial cell modulation.52 Although inflammation and cytokine release is the body's primary defense against pathogen invasion, prolonged activation of these pathways can have adverse effects on the brain, resulting in fatigue, lack of motivation and appetite, as well as disturbances in sleep and concentration. It is generally accepted that cytokines in the blood can cross the BBB,54,56 so that modulation of the levels of cytokines in the periphery, in principle, can mitigate the aforementioned brain effects.43,56,57

Cancer and chemotherapy are known to cause increases in circulating cytokine levels, which may be one mechanism by which cognitive impairment is manifested in these patients.43,57,58 Meyers et al 2005 found that patients with acute leukemia had elevated levels of circulating cytokines before treatment, which correlated with the extent of cognitive impairment and fatigue.54 Disruptions in cytokine levels have also been observed in neurodegenerative diseases such as Alzheimer disease (AD), multiple sclerosis and Parkinson disease (PD).59 Clear associations between cytokines and cognitive dysfunction have been reported with immunotherapy administration, which resulted in depression, weakness and fatigue, in addition to cognitive decline.60

As noted, DOX cannot cross the BBB, as it has not been detected in areas protected by the BBB such as the cortex and the hippocampus.67,68 However, DOX administration causes increases in levels of peripheral cytokines that are able to cross the BBB and stimulate local cytokine production,69,70 inflammation and oxidative stress, leading to CNS toxicity. Increased levels of circulating tumor necrosis factor alpha (TNF-α) and TNF-α in the cortex and the hippocampus have been detected in mice treated intraperitoneally (i.p.) with DOX.71-73 TNF-α in brain can activate glial cells to initiate local production of TNF-α,74 which in turn induces nitric oxide synthase, leading to the overproduction of RNS.44 Co-administration of DOX with an antibody against TNF-α quenches the aforementioned effects, further implicating this particular cytokine in DOX-related CNS toxicity.57
Evidence of Cell Death in Brain with DOX

Cell loss is intimately related to neurodegenerative disorders such as AD, a condition that in its earliest stages may share commonalities of pathology and symptoms with chemo fog. Because neurons are postmitotic cells, neuronal apoptosis is generally an irreversible event and could heavily contribute to a chemo fog-like condition in patients. The latter point is still debatable, because chemo fog symptoms are possibly transient,12 while AD is an irreversible condition; however, cancer survivors are more predisposed to AD later in life,67 so the possibility of neuronal death with a compensatory response of other neurons is also feasible. Magnetic resonance imaging studies demonstrated that chemotherapy for breast cancer led to lower white and grey matter volumes.68,69 Administration of DOX is reported to affect levels of brain-localized apoptotic markers in vivo, further supporting the role of cell death in chemo fog.57

Disturbances in mitochondrial respiration can lead to apoptotic cell death. Tangpong et al reported decreased mitochondrial respiration 3 hours after i.p. administration with DOX.57 Treatment of mice with DOX increased levels of pro-apoptotic proteins Bax and p53, as well as the levels of anti-apoptotic Bcl-XL, in brain mitochondria.61 Bax is capable of forming complexes with p53 and inducing permeability of the outer mitochondrial membrane, leading to cytochrome c release;62 this complex was detected in brains of DOX-treated mice, along with elevated cytosolic levels of cytochrome c.60 DOX has also been shown to increase susceptibility of brain mitochondria to permeability transition pore (PTP) opening induced by Ca2+.60 Brain changes as a result of DOX are likely due to TNF action, as co-administration of DOX with TNF antibody abrogated TNF levels in brain and mitochondrial toxicity.61 During apoptosis, cytochrome c release to the cytosol leads to a series of reactions that activate caspase 3 to initiate programmed cell death; increased caspase 3 activity was detected as early as 3 h and as long as 72 h in brain after i.p. treatment of mice with DOX. Increased levels of TUNEL positive cell death were also observed in brains of DOX-treated mice, consistent with results discussed above.62

Description of Chemo Fog in Context of DOX

The terms “chemo fog” or “chemo brain” have been currently adopted to describe the cognitive decline experienced by some patients after cessation of chemotherapy. Such symptoms can last for at least 10 years following cessation of therapy.16 After treatment, noticeable differences in memory, executive function, attention/concentration and processing speed are commonly described.12 Of these symptoms, memory changes are the most frequently documented, particularly in studies of breast cancer patients.10,11 Coincidentally, breast cancer patients are commonly treated with anthracyclines such as DOX to suppress tumor growth. Rodents treated with DOX (in addition to cyclophosphamide) displayed deficits in hippocampal-related learning and memory.17 Memory impairment has also been demonstrated in rats treated with DOX as evidenced by passive avoidance testing.18 Patients treated with DOX and cyclophosphamide displayed lower overall cognitive scores and visuospatial skill, although this report found increases in executive function after chemotherapy.72 In general, the cognitive changes resulting from chemotherapy are relatively mild compared to other memory impairments, such as AD. Also unlike AD, data suggest that this side effect may not be permanent. Nevertheless, even temporary cognitive alterations are capable of negatively affecting patient quality of life.73

As mentioned previously, peripheral administration of DOX causes biochemical changes such as increases in peripheral inflammatory cytokines (TNF-α) and oxidative stress,47,51,57 brain oxidative damage,40,51,54 mitochondrial impairment51 and depletion of CNS antioxidants,51 potentially leading to neuronal death and observed defects in memory.15,54 Furthermore, cotreatment of DOX with brain accessible antioxidants has resulted in improvements in memory,14 correlating with preservation of the oxidative status of the periphery and CNS.47,51,54 Therefore, the presence of oxidative damage in brains of subjects treated with DOX may mimic the early stages of AD, which has overwhelming evidence of brain-resident oxidative stress and impairments in working memory. However, co-administration of antioxidants with DOX in cancer patients has been met with some resistance in the oncology community, as ROS generation is one of several hypothesized
mechanisms by which DOX is lethal to tumors. However, Wang et al found that DOX induces apoptosis differently in tumor cells than normal cells; detoxification of \( \text{H}_2\text{O}_2 \) in tumor cells does not affect DOX-induced apoptosis, in stark contrast to normal epithelial cells.\(^7\)

Chemo fog patients complain of having to exert more cognitive effort for everyday tasks after chemotherapy compared to before treatment.\(^6\) In correlation with this statement, breast cancer patients 5-10 years after cessation of chemotherapy were observed to have lower resting brain glucose metabolism, along with a greater modulation of blood flow in the frontal cortex and cerebellum during a short-term memory recall test (compared to healthy controls);\(^9\) results of this study imply that affected areas of the brain must work harder to function normally during testing, in turn utilizing more glucose, compared to control subjects. Because glycolytic, TCA and electron transport enzymes are susceptible to oxidative damage in the presence of increased free radicals,\(^10\) the possibility exists for free radical damage to enzymes involved in glucose metabolism as an indirect result of DOX, eventually leading to clinical observations of memory impairment.

Because ATP is the end product of glucose metabolism, oxidative damage to glycolysis-related pathways would decrease metabolic efficiency, resulting in higher amounts of glucose needed to maintain basal ATP levels. Decreased cellular ATP could disrupt ion channels, namely the Na+/K+ ATPase and Ca\(^{2+}\) in neurons, resulting in cognitive dysfunction; this is purely speculative, however and requires more study to be decisively concluded. Nevertheless, oxidative damage and changes in glycolytic metabolism can both result in cell death, either in concert or independently, which would heavily contribute to symptoms of chemo fog.

Conclusion

Five-year survival rates for the treatment of breast cancer are approximately 80% in the United States\(^7\) and much of this demographic group were at one point treated with anthracyclines such as DOX. Although the primary objective of chemotherapy is improved survival, it is imperative to also preserve the quality of life of the patient as best as possible. While the efficacy of DOX cannot be ignored, this drug has been linked to toxicity in several organs including heart and brain, the latter described in this chapter. Recent research shows that chemo fog experienced by a fraction of cancer survivors treated with drugs such as DOX may be a result of cytokine elevation in the periphery which crosses across the BBB to induce inflammation/oxidative stress leading to cell death. Figure 1 illustrates our model. This mechanism of toxicity is somewhat different from the DOX-related toxicity of other organs that are not protected by the BBB. This side effect of chemotherapy is receiving more attention as the number of cancer survivors continues to rise. Ultimately, any alteration to chemotherapy regimens to address the issue of chemo fog will have to be rigorously tested to ensure that these precautions do not compromise drug efficacy against tumors. Studies to further elucidate DOX-induced chemo fog are currently in progress in our laboratories.

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References


