

# Neurotoxicity of cancer chemotherapy<sup>☆</sup>

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## Abstract

There is accumulating clinical evidence that chemotherapeutic agents induce neurological side effects, including memory deficits and mood disorders, in cancer patients who have undergone chemotherapeutic treatments. This review focuses on chemotherapy-induced neurodegeneration and hippocampal dysfunctions and related mechanisms as measured by *in vivo* and *in vitro* approaches. These investigations are helpful in determining how best to further explore the causal mechanisms of chemotherapy-induced neurological side effects and in providing direction for the future development of novel optimized chemotherapeutic agents.

## Key Words

neural regeneration; reviews; neurogenesis; behavioral dysfunction; chemotherapy; hippocampal dysfunction; memory deficit; mood disorder; neurodegeneration; neuron; neuroplasticity; neurotoxicity; grants-supported paper; neuroregeneration

## Research Highlights

- (1) There is increasing clinical evidence that chemotherapeutic agents induce neurological side effects, including memory deficits and mood disorders, in cancer patients.
- (2) This article reviews *in vivo* and *in vitro* studies of chemotherapy-induced neurodegeneration and hippocampal dysfunctions, and related mechanisms.

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## INTRODUCTION

Chemotherapy is one of the primary treatments for cancer. However, one of the most disturbing findings of recent studies of cancer survivors is the apparent prevalence of chemotherapy-associated adverse neurological effects, including vascular complications, seizures, mood disorders, cognitive dysfunctions, and peripheral neuropathies<sup>[1-3]</sup>. Epidemiological studies have revealed chemotherapy-induced cognitive impairments and psychological distress in diverse types of cancer patients with peripheral tumors, including breast cancer, colorectal cancer, and lymphoma, as well as brain tumors such as glioma,

glioblastoma, and primary central nervous system lymphoma<sup>[4-6]</sup>. In addition, chemotherapy triggers changes in ion channels on dorsal root ganglia and dorsal horn neurons that generate secondary changes resulting in neuropathic pains<sup>[2]</sup>. There is clear evidence of the neurotoxicity of at least some forms of chemotherapy, although factors related to the use of specific diagnostic techniques and cancer treatments may also contribute to the side effects that have been identified<sup>[1, 3]</sup>.

Among various neurological side effects, cognitive impairment and mental depression can occur in a subset of cancer survivors, although these are generally subtle. A number of factors may either protect against

cognitive impairments or place individuals at an increased risk of impaired cognitive function. These factors include the concomitant effects of the cancer and its treatment (e.g., medications, fatigue, depression, or anxiety), indirect and direct effects of chemotherapy (e.g., chemotherapy-induced anemia or menopause), factors particular to the individual patient (e.g., age, intelligence, educational level, or menopausal status)<sup>[7]</sup>. Thus, a number of factors complicate assessments of correlations between the side effects of chemotherapy and brain dysfunctions in patients, particularly memory deficits and mental depression. Despite intensive efforts to manage the neurological side effects of chemotherapy in patients, and the development of chemoprotective agents, there is no therapy generally accepted to not produce neurological side effects<sup>[8]</sup>.

It is well established that the cytostatic effects of chemotherapeutic agents inhibit the process of cancer cell division. Chemotherapy may induce related changes, particularly in neurogenesis and synaptic plasticity, which are closely linked to the hippocampus. The hippocampus is a major structure of the limbic system that has been studied extensively in individuals with difficulties in learning and memory and the regulation of emotions, which can be manifested in symptoms such as depression<sup>[9]</sup>. However, further studies on experimental animals are needed to verify these correlations. Furthermore, epidemiological studies of cancer survivors exhibiting cognitive impairments are required to clarify the side effects of chemotherapeutic agents, and thus allow the development of new therapeutic agents without such side effects.

Recently, various groups using experimental *in vivo* and *in vitro* models have reported that chemotherapy induces neurodegeneration and hippocampal dysfunctions, such as memory deficits (Table 1). This review provides an overview of *in vivo* and *in vitro* studies investigating the neurological effects of chemotherapeutic agents on the hippocampus and its functions, including cognition and depression, as well as the neurotoxicity of these agents.

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## ROLES OF HIPPOCAMPAL NEUROGENESIS AND PLASTICITY IN HIPPOCAMPAL FUNCTIONS

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In the adult brain, progenitor/stem cells give rise to new neural cells in two active germinal zones called the subgranular zone in the dentate gyrus, which generates

new granular cells in the adult hippocampus, and the forebrain subventricular zone, which gives rise to granular cells in the olfactory bulb<sup>[37-38]</sup>. The hippocampus is a limbic structure that plays a critical role in the formation and recovery of certain types of memory and the regulation of emotion, such as in depression. New neurons in the adult hippocampus are derived from neural progenitor/stem cells located at the border between the hilus and granular cell layer of the dentate gyrus, a region called the subgranular zone, during the process of hippocampal neurogenesis<sup>[9]</sup>. Factors such as age, genetics, excitatory input, ionizing radiation exposure, physiological stimuli, and environmental conditions<sup>[39-48]</sup> regulate the rate of cell proliferation in the hippocampus, which in turn influences behavior<sup>[49]</sup>. Mental depression and cognitive impairment may be due not only to changes in neurotransmitter concentrations and receptor activity levels but also to the impairment of brain plasticity, tissue remodeling, and alterations in adult hippocampal neurogenesis<sup>[49-53]</sup>. Manipulations that decrease neurogenesis, such as radiation exposure, impair an animal's performance in hippocampal-dependent learning and memory tasks. Conversely, factors that increase neurogenesis, such as exercise, and improve hippocampal performance<sup>[40, 54]</sup>. In addition, animal models of depression have also been reported to result in a decline in adult hippocampal neurogenesis, through either the proliferation of hippocampal progenitors or short-term survival of daughter cells<sup>[55]</sup>. Thus, reduced rates of hippocampal neurogenesis lead to cognitive impairments and/or depression-like effects in experimental models<sup>[40, 47]</sup>. Therefore, neurotoxic agents affecting hippocampal neurogenesis may be useful tools to clarify the relationship between hippocampal neurogenesis and hippocampal dysfunction.

Neuronal synaptic plasticity in the adult brain, which is closely linked with neurogenesis, is manifested at the cellular level by modifications in dendritic growth, axonal sprouting, synaptic remodeling, and the creation of new synapses<sup>[56]</sup>. Hippocampal principal synapses are especially plastic, as they have been shown to be strengthened or weakened by activity<sup>[57]</sup>. The ability of a synapse to change the efficacy of its synaptic transmission, a process known as synaptic plasticity, is crucial to brain functions, and therefore, hippocampal plasticity plays an important role in hippocampal functions. In the mammalian brain, two of the best-characterized forms of synaptic transmission in the hippocampus are long-term potentiation and long-term depression, which have been identified as cellular substrates that are clearly related to learning and memory<sup>[58]</sup>.

Table 1 Summary of *in vivo* and *in vitro* studies evaluating the effects of various chemotherapeutic agents on behavioral dysfunctions and neurobiology

Anticancer drug	Animal or cell type	Cognition & histological and biochemical evaluations	Comment	Reference
5-FU	Lister-hooded rat	Spatial working memory impairment (OLR) BDNF, DCX level ↓	No change of hippocampal proliferation	[10]
5-FU	Lister-hooded rat	Hippocampal proliferation ↓ Memory impairment (OLR)		[11]
5-FU, CYP	Fischer-344 rat (7 or 18 months)	No memory impairment (MWM, Stone 14-unit T-maze); no induced LTP (acute)	Transient memory & LTP ↑ (recovery)	[12]
5-FU, thioTEPA	C57BL/6 mouse (6 weeks)	No change of hippocampal cell proliferation (5-FU); dose-dependent hippocampal cell proliferation ↓ (thioTEPA)		[13]
5-FU&CYP &MTX	Wistar rat (4 months)	Hippocampal cell proliferation ↓; memory impairment (MWM)	Histone acetylation ↑; histone deacetylase activity ↓	[14]
5-FU&MTX	BALB/C mouse (2 months)	Memory impairment (MWM, NMTS, delayed NMTS)	No impairment (cued memory, discrimination learning)	[15]
CIS, CYP, MTX, Vinblastin, thiotepa, Ifosfamide	Primary cortical cells (7 DIV) Wistar rat (7 days)	Dose-dependent cell death Apoptosis ↑; neuronal degeneration—dendritic swelling	Excitotoxic mechanism, caspase-mediated cell death; age-dependent neurotoxicity	[16]
CIS, MTX, Taxol	Primary cortical neurons (7, 10, or 14 DIV)	Neuronal degeneration—neurite degeneration; changes in the actin cytoskeleton	Rho signaling pathway	[17]
CYP	Male ICR mouse (8–10 weeks)	Hippocampal neurogenesis ↓; no induced apoptosis	Cognitive impairment (PA, ORT)	[18]
CYP	Cortical neurons (4 DIV)	Prevention of cell death after serum deprivation	PI3K/Akt&MAPK/ERK activation ↑; Bcl-2&BDNF ↑	[19]
CYP, DOX	Athymic nude rats (2 months)	Memory impairment (NPR, CFC); hippocampal neurogenesis (DCX) ↓	Microglia activation (CYP) ↑	[20]
DOX	Primary cortical cells (8 DIV)	Neuronal cell death, cytotoxicity ↑ (under 0.5 μmol/L); no effect (higher concentration)	Different pathway (caspase-dependent/-independent apoptosis)	[21]
DOX	Wistar rat (2–3 months)	Memory impairment (IA)—acquisition (not consolidation)	Exploratory behavior ↓	[22]
DOX	Primary cortical cells (10 DIV)	ROS/RNS, NF-κB activation ↑; GSH level, ATP ↓, cell death		[23]
Ifosfamide	Mature cortical neurons (12 or 20 DIV)	Cellular acidification	AMPA/kainite receptor activation ↑	[24]
MTX	Cerebellar explant culture (5 weeks DIV)	Loss of axon; empty myelin sheaths		[25]
MTX	Wistar rat (4 months)	Brain amines concentration ↓; memory impairment (dark-bright arena test, conditioned avoidance task)	No effect on anxiety behavior	[26]
MTX	Primary cortical cells (12 DIV)	Tau phosphorylation ↑; PP2A methylation ↓	Folate deficiency	[27]
MTX	Wistar rat (3 months)	Hippocampal cell proliferation ↓; memory impairment (MWM, ORT)		[28]
MTX	C57BL/6 mouse (10–18 weeks)	Memory impairment (CFC)	Ability to gate incoming auditory stimuli ↓; no memory impairment (ORT)	[29]
MTX	Wistar rat (3 months)	Hippocampal cell proliferation ↓; white matter density ↓; memory impairment (MWM, CFC)	No induced hippocampal cell death	[30]
MTX	Long Evans rat (10 weeks)	Spatial memory deficit; no induced deficits (ORT)	Serum and CSF folate ↓	[31]
MTX	Wistar rat (3 months)	Hippocampal blood vessel density ↓; Microglia activation	No change of hippocampal cytokine levels	[32]
MTX	Buffalo rat (Hepatoma 7777 - 3 months)	Hippocampal cell proliferation ↓	No differences between animals with or without a tumor	[33]
MTX	C57BL/6 mice (8–9 weeks) Primary hippocampal neurons (0 DIV)	Cognitive impairment (ORT), depressive behavior (TST), neurogenesis ↓ Cytotoxicity ↑; cell viability ↓	Folate deficiency Caspase-dependent cell death	[34]
MTX	C57BL/6 mouse (8–9 weeks)	Changes in synaptic plasticity-related signals (biphasic-early and late phase ↑); changes in neurotrophic factors (late phase ↑)	Related to neuronal survival and plasticity-related cellular remodeling	[35]

Table 1 Continued

Anticancer drug	Animal or cell type	Cognition&histological and biochemical evaluations	Comment	Reference
MTX	C3H/HeN mouse (FM3A breast cancer cell-6 weeks)	Cognitive impairment (PA); depressive behavior (TST), hippocampal neurogenesis (DCX)↓; proinflammatory enzymes (COX2, iNOS) ↑	Tumor formation; depressive behavior ↑; no effect on cognition; trend of neurogenesis ↓; trend of proinflammatory enzymes ↑	[36]

5-FU: 5-Fluorouracil; OLR: object location recognition; BDNF: brain-derived neurotrophic factor; DCX: doublecortin; CYP: cyclophosphamide; MWM: Morris water maze task; LTP: long-term potentiation; MTX: methotrexate; NMTS: non-matching to sample learning test; CIS: cisplatin; DIV: days *in vitro*; PA: passive avoidance; ORT: object recognition memory test; DOX: doxorubicin; PI3K/Akt: phosphoinositide 3-kinase/Akt; MAPK/ERK: mitogen-activated protein kinase/extracellular signal-regulated kinase; NPR: novel place recognition task; CFC: contextual fear conditioning task; IA: inhibitory avoidance conditioning; ROS/RNS: reactive oxygen species/reactive nitrogen species; NF-κB: nuclear factor-kappa B; GSH: glutathione; CSF: cerebrospinal fluid; TST: tail suspension test; COX2: cyclooxygenase-2; iNOS: inducible nitric oxide synthase.

Several studies have shown that neurotoxic agents and neurodegenerative diseases induce changes in synaptic plasticity and neurogenesis in the adult hippocampus<sup>[59-60]</sup>. Therefore, further research on changes in plasticity-related signals under various conditions should provide a greater understanding of the specific relationships between plasticity and brain functions.

Other limbic structures, including the amygdala, hypothalamus, and fornix, are also involved in cognition and emotional regulation. The amygdala, mammillary body, and hypothalamus play pivotal roles in memory and mental depression<sup>[61]</sup>. In addition to the limbic system, the striatum and cortex are also thought to be associated with some types of memory<sup>[62]</sup>. The frontal and temporal cortices of the forebrain, the basal nuclei, and pituitary gland have been implicated in mental depression<sup>[63]</sup>. The structure and function of the hippocampus, which is closely associated with both cognition and emotional regulation, have been studied under various conditions. Therefore, this review will focus mainly on the detrimental effects of chemotherapy in the hippocampus of experimental animals.

## CHEMOTHERAPY-INDUCED NEUROTOXICITY *IN VIVO*

Chemotherapy is one of the primary treatments for cancer, and has been applied successfully to extend patients' life spans. However, there is increasing clinical interest in its adverse neurotoxic symptoms, including leukoencephalopathy, seizures, cerebral infarctions, and cognitive impairments<sup>[64]</sup>. There is now a growing body of evidence that cancer therapy-associated cognitive dysfunction occurs in many adults undergoing treatment for cancer and cannot simply be attributed to stress,

fatigue, or depression<sup>[1]</sup>. Therefore, behavioral evaluations of hippocampal dysfunction induced by chemotherapy in experimental models are necessary to gain further insights into the genuine phenomena of chemotherapy-induced cognitive impairments and depression.

Several studies using animal models have shown that various chemotherapeutic agents impair performance in one or more cognitive tests (Table 1). However, the outcomes of tests of cognitive impairment after treatment with chemotherapeutic agents are highly dependent on the protocols used<sup>[12, 22]</sup> and the specific learning tasks applied<sup>[15, 29]</sup>. Furthermore, some chemotherapy agents that can easily cross the blood-brain barrier (e.g., 5-fluorouracil and methotrexate) directly or indirectly have detrimental effects on brain functions such as memory and depression<sup>[10, 34]</sup>. However, several antineoplastic agents that do not cross blood-brain barrier (e.g., cyclophosphamide) also affect cognitive functions<sup>[18]</sup>. Hence, we do not yet mechanistically understand the effects of chemotherapy on cognition and emotional regulation.

To date, various groups have focused on the hippocampus, which has a critical role in memory processing, and they suggested that most cognitive deficits induced by chemotherapeutic agents in diverse paradigms are associated with the inhibition of hippocampal neurogenesis<sup>[11, 14, 20, 28, 30]</sup>. In addition, although the influence of chemotherapeutic agents on mood disorders has been less well studied than their effects on cognition, Yang *et al.*<sup>[34]</sup> reported recently that methotrexate increases depression-like behaviors in mice both 1 and 7 days after treatment, which was related to reduced hippocampal neurogenesis and cell viability and increased cytotoxicity and apoptosis. However, some studies have indicated that

chemotherapeutic agents induce memory impairments and mood disorders, but do not affect hippocampal cell proliferation<sup>[10, 13]</sup>.

Several possible pathways that may contribute to the neurotoxicity observed after chemotherapy in breast cancer patients have been elucidated, including indirect chemical toxicity and oxidative damage, direct neuronal injury, inflammation, or a type of autoimmune response<sup>[4]</sup>. Inflammatory mechanisms within the central nervous system contribute to cognitive impairments through interactions between neurons and glial cells<sup>[65]</sup>. Several agents, including cyclophosphamide and methotrexate, activate microglia in a manner that is characterized by thicker and longer processes and persistent activation<sup>[20, 32]</sup>, and these increased inflammatory responses may contribute to cognitive impairment. Folate is also critical to optimal cognitive function within the mature brain, neurotoxic amino acid metabolism, and neurotransmitter synthesis<sup>[31]</sup>. Folate deficiency induced by methotrexate treatment is associated with mild cognitive dysfunction, depression, and a reduction in proliferating cells in the hippocampal dentate gyrus of adult mice<sup>[66-68]</sup>. In addition, chemotherapy-induced memory impairments are also involved in diverse factors including reductions of brain amine concentrations and increases in histone acetylation and apoptosis<sup>[14, 26, 34]</sup>. Moreover, chemotherapeutic agents (e.g., methotrexate) affect synaptic plasticity-related signals, so such changes are associated with neuronal survival and plasticity-related cellular remodeling in the hippocampus<sup>[35]</sup>. Thus, these factors may affect brain function, especially in the hippocampus, and thereby possibly impair cognition and induce depression.

In addition, tumor formation itself may affect hippocampal functions, possibly through inflammatory responses<sup>[36, 69-70]</sup>. In murine cancer models, tumor formation induces depression-like behavior and memory deficits<sup>[69-70]</sup>, and upregulates proinflammatory enzymes, including cyclooxygenase-2 and inducible nitric oxide synthase, in the hippocampus of tumor-bearing mice<sup>[36]</sup>. In a mouse model of breast cancer, methotrexate treatment further leads to mental depression and memory deficits and inhibition of hippocampal neurogenesis<sup>[36]</sup>. However, compared with tumor-free mice, tumor-bearing mice do exhibit a slight, non-significant decrease in hippocampal neurogenesis<sup>[33, 36]</sup>. In addition to cognitive impairment and mental depression, alterations in consciousness, seizures, cerebral infarctions, paralysis, peripheral neuropathy, leukoencephalopathy, and ototoxicity are common and

often dose-limiting complications of chemotherapy treatment<sup>[2, 64, 71]</sup>. These drugs induce such neurotoxicity *via* diverse mechanisms, including decreased hippocampal cell proliferation and hippocampal blood vessel density, cell death and neuronal degeneration, and the activation of microglia without changes in hippocampal cytokine levels *in vivo*<sup>[16, 32, 34]</sup>. Additional studies of chemotherapy-induced neurotoxicity may assist in the development of strategies for ameliorating these serious side effects in cancer survivors.

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## EFFECTS OF CHEMOTHERAPY ON NEURONS *IN VITRO*

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The prevalence of neurotoxicity induced by chemotherapy markedly increases when the blood-brain barrier is either overwhelmed or bypassed<sup>[72]</sup>. Several chemotherapeutic agents are used to treat brain tumors because they readily cross the blood-brain barrier (e.g., methotrexate, an antimetabolite and folic acid antagonist; carmustine and lomustine, alkylating agents; cisplatin, a heavy metal; ifosfamide, an alkylating prodrug)<sup>[73]</sup>. As some chemotherapeutic drugs, including the anthracycline anticancer drug doxorubicin, do not cross the blood-brain barrier<sup>[74]</sup>, several modified delivery systems have recently been developed to circumvent the limited access of such drugs to the brain, to allow these compounds to be used against brain tumors<sup>[75-76]</sup>.

In addition to passage across the blood-brain barrier, other factors may contribute to the neurotoxicity of chemotherapy, including genetic predispositions, nutritional deficiencies, metabolic abnormalities, accompanying medications, depression, anxiety, or fatigue<sup>[77]</sup>. While there have been many *in vivo* studies associated with chemotherapy-induced memory deficits, there are complications in the direct extrapolation of specific data from *in vivo* studies. This is because the damage seen *in vivo* following radiation exposure and other treatments affects not only neurons but also surrounding cells, such as glial and vascular endothelial cells<sup>[78-79]</sup>. Therefore, to exclude these secondary effects of chemotherapy-induced damage to glial or vascular endothelial cells or neurons, it is important to clarify the precise negative effects of chemotherapy on neurons *in vitro*.

Despite intensive efforts to determine ways to successfully manage the neurological side effects of chemotherapy, and the development of chemoprotective

agents, there is no generally accepted therapeutic protocol that precludes these untoward effects<sup>[6]</sup>. Therefore, various studies have focused on identification and characterization of the mechanisms underlying chemotherapy-induced neurotoxicity. Although few studies have suggested that pretreatment with some chemotherapeutic agents (e.g., cyclophosphamide) prevents neuronal cell death caused by serum deprivation<sup>[19]</sup>, most research has shown that diverse chemotherapeutic agents have detrimental effects on neuronal cells due to the resultant changes in neuronal morphology<sup>[17, 25, 33]</sup>. Furthermore, it has been reported that potential toxic effects in mature neuronal cells exposed to anticancer drugs can be alleviated through inhibition of the Rho signaling pathway<sup>[17]</sup>, the generation of neuronal nitric oxide synthase, and subsequent reactive oxygen species/reactive nitrogen species<sup>[23]</sup>, as well as activation of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainite receptors<sup>[24]</sup>. In addition, folate deficiency caused by chemotherapy induces abnormal Tau phosphorylation and amyloid precursor protein upregulation, which can contribute to the pathogenesis of Alzheimer's disease. This indicates that folate deficiency is related to neurotoxicity, and its effects are comparable to the results of a previous *in vivo* study<sup>[27]</sup>. Moreover, excitotoxic mechanisms and caspase-mediated cell death contribute to the neurotoxicity of some compounds in mature neuronal cultures<sup>[21, 33]</sup>.

However, as mature neurons have stopped differentiating and are less sensitive than differentiating cells<sup>[80]</sup>, further investigations related to chemotherapy-induced effects on immature neurons are required. In immature neurons, methotrexate induces increases in cytotoxicity and reduction of cell viability by a caspase-dependent pathway<sup>[34]</sup>. The expression of active caspase-3 and caspase-specific poly(ADP-ribose) polymerase cleavage are increased markedly in immature neurons following methotrexate treatment, and the caspase-3 inhibitor, Z-DEVD, significantly blocks the methotrexate-induced cytotoxicity, supporting the suggestion that the neurotoxicity of methotrexate in immature cells depends on the proapoptotic caspase pathway, particularly the caspase-3 related pathway. However, mature hippocampal neurons are resistant to the toxicity of methotrexate, suggesting that immature hippocampal cells are significantly more sensitive to methotrexate treatment than mature cells, and that the susceptibility of such hippocampal cells is consequentially dependent on cell differentiation<sup>[34]</sup>. Therefore, these *in vitro* systems may be useful in the

study of the diverse neurotoxic effects of cancer chemotherapy.

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## CONCLUSION

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Despite the abundance of data regarding chemotherapy-induced neurotoxicity in cancer patients, there is still considerable uncertainty regarding the underlying mechanisms. Moreover, many clinical studies are limited by diverse factors that complicate identification of the causes of such neurotoxic effects and resultant symptoms<sup>[7]</sup>. Thus, there have been many cellular and molecular studies of chemotherapy-induced neurotoxicity, especially of cognitive impairment and mental depression, and its underlying mechanisms using experimental *in vivo* and *in vitro* approaches, such as animal behavioral tests. Studies using animal behavioral tests have demonstrated that treatment with various chemotherapeutic agents engenders memory deficits and depression, which have been linked to hippocampal neurogenesis. Several mechanisms of chemotherapy-induced neurotoxicity have been elucidated from *in vivo* models, including inflammatory responses and changes in levels of brain amines, folate, and histones. However, it is difficult to determine which direct pathways are linked to chemotherapeutic-agent-induced neurotoxicity *in vivo* because of secondary effects of diverse factors, such as changes in glial cells and endothelial cells and various other factors. Using *in vitro* approaches, most anticancer drugs have been shown to induce morphological changes and various toxic effects in neurons through several pathways. Although far from complete, these investigations using *in vivo* and *in vitro* approaches are helpful in determining how best to further explore the causal mechanisms of chemotherapy-induced neurological side effects, and also in providing direction for the future development of optimized chemotherapeutic agents.

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