Biochemical metabolism of brain and Neuregulin-1/ErbB signaling in the hippocampus after doxorubicin administration

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Abstract

Background: The metabolic fate of glucose in the brain depends upon the cell type and the selective expression of metabolic enzymes. Neurons are predominantly oxidative, while astrocytes are mostly glycolytic. In addition to the production of adenosine- 5′- triphosphate (ATP), glucose is also used to generate metabolic intermediates for the synthesis of fatty acids and other lipids required for membrane and myelin synthesis ; amino acids for protein synthesis and neurotransmitter production; and 5- carbon sugars for the synthesis of nucleotides); and to produce glycogen in astrocytes. Doxorubicin (Dox) is an effective anthracycline chemotherapeutic developed for the treatment of solid tumors and hematologic malignancies. NRG1 is suggested as a susceptibility gene for several psychiatric disorders, including schizophrenia, bipolar disorder, and depression. NRG1 is well known to play an essential role in neuronal development as well as in maintaining normal function in the mature nervous system. Recent biochemical studies have shown that NRG1 can be neuroprotective for cortical neurons, motor neurons, dopaminergic neurons, cochlear sensory neurons, and PC12 cells. Previous research showed that aberrant changes in these NRG1-related pathways are tightly linked to the pathogenesis of depression. It is tempting to hypothesize that Dox may inhibit neural NRG1/ErbB signaling, thereby triggering the neurotoxicity and behavioral changes

Keywords: Neuregulin-1/ErbB signaling, Biochemical metabolism, doxorubicin

Introduction

The metabolic fate of glucose in the brain depends upon the cell type and the selective expression of metabolic enzymes. Neurons are predominantly oxidative, while astrocytes are mostly glycolytic. In addition to the production of adenosine- 5′- triphosphate (ATP), glucose is also used to generate metabolic intermediates for the synthesis of fatty acids and other lipids required for membrane and myelin synthesis ; amino acids for protein synthesis and neurotransmitter production; and 5- carbon sugars for the synthesis of nucleotides); and to produce glycogen in astrocytes. **(1).**

In neurons, each molecule of glucose is oxidized via glycolysis, the pentose phosphate pathway (PPP), the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation, with the production of carbon dioxide, water, and 30–36 molecules of ATP depending upon the rates of proton leakage in the mitochondria. The glycolytic process metabolizes glucose to pyruvate, which can be actively transported into the mitochondria where it is converted to acetyl coenzyme A (acetyl- CoA). **(1).**

Acetyl- CoA is complexed with citrate which undergoes a series of regenerative enzymatic reactions producing reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) in

the TCA cycle. The NADH and FADH2 produced during glycolysis and the TCA cycle are subsequently re- oxidized in the electron transport chain (ETC). **(2).**

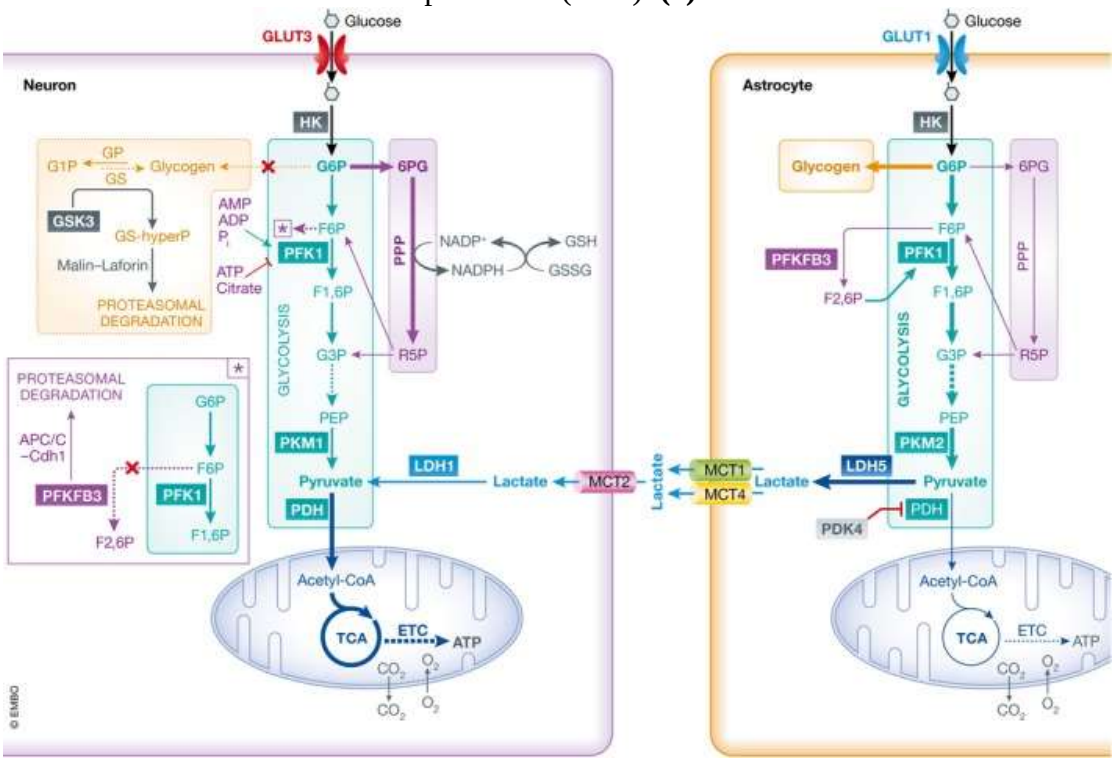

Figure (1): **Metabolic pathways of glucose utilization in neurons and astrocytes**. In neurons after entering the cell via glucose transporter 3 (GLUT3), glucose is phosphorylated by hexokinase to glucose-6- phosphate (G6P), which is subsequently routed in the glycolytic pathway and the pentose phosphate pathway (PPP). The end product of glycolysis is pyruvate that enters the mitochondria where it is metabolized through the TCA cycle and oxidative phosphorylation in the ETC, generating ATP and CO2 while consuming oxygen (O2). Pyruvate can also be generated from lactate dehydrogenase 1 (LDH1)-dependent conversion of lactate. In the PPP, G6P is converted to 6- phosphogluconate (6PG) that is transformed in ribulose- 5- phosphate (R5P), with the concomitant production of reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is utilized to regenerate oxidized glutathione (GSH) and thioredoxin. Neurons are not able to store glucose in the form of glycogen due to constitutive degradation of glycogen synthase (GS) via glycogen synthase kinase 3 (GSK3) phosphorylation, and subsequent ubiquitin- dependent proteasomal digestion mediated by the malin–laforin complex. In astrocytes, glucose is imported trough glucose transporter 1 (GLUT1) and stored as glycogen, or metabolized via glycolysis. The pyruvate generated is converted to lactate due to the expression of lactate dehydrogenase 5 (LDH5), and pyruvate dehydrogenase kinase 4 (PDK4)- dependent inhibition of pyruvate dehydrogenase (PDH). The presence of 6- phosphofructo- 2- kinase/fructose- 2,6- bisphosphatase 3 (Pfkfb3) allows astrocytes to generate fructose- 2,6- bisphosphate (F2,6P) that acts as an allosteric modulator of PKF1 boosting glycolysis. Glycogen represents the largest energy reserve in the brain. Glycogen metabolism is regulated by two key enzymes, glycogen synthase (GS) and glycogen phosphorylase (GP). The reason why glycogen is produced and stored exclusively in astrocytes. **(3).**

The balance between glycolysis and PPP rates in neurons is very important, and diversion of glucose utilization toward exclusive glycolysis can result in decreased availability of NADPH, increased oxidative stress and cell death. The preferential use of G6P in the PPP in neurons, as well as their inability to up-regulate glycolysis, is due to the selective expression of enzymes favoring such a metabolic route coupled with the absence of specific glycolysis modulators. The expression in neurons of the low- pyruvate-affinity isoform of lactate dehydrogenase (LDH1), prevents pyruvate conversion to lactate and favors its entrance into the TCA cycle. Further metabolic bias toward the TCA cycle results from the lower levels of

expression in neurons of pyruvate dehydrogenase kinase 4 (PDK4) which controls the activity of pyruvate dehydrogenase (PDH), and therefore the decarboxylation of pyruvate to acetyl- CoA. **(4)**

Astrocyte utilization of glucose is complementary to that of neurons. A portion of G6P is channeled into glycogen synthesis and PPP, but its predominant metabolism occurs via glycolysis with production of lactate and very low rates of mitochondrial oxidation. This metabolic phenotype of astrocytes is the result of their unique expression of various enzymes and transporters. In contrast to neurons, astrocytes express very high levels of Pfkfb3 which favors glycolysis via allosteric activation of PFK by F2,6P. Furthermore, under basal conditions the levels of PDH phosphorylation are high thanks to elevated expression of PDK4, efficiently limiting the conversion of pyruvate to acetyl- CoA. Astrocytes also express low levels of mitochondrial aspartate/glutamate carrier (AGC) decreasing the import of reduced equivalents (NADH) from the cytosol. The expression of LDH5, which has a high affinity for pyruvate, rather than LDH1, ensures its conversion to lactate with concomitant oxidation of NADH to NAD^+ thus maintaining high rates of NAD^+/NADH that further favor aerobic glycolysis. The presence of PKM2 instead of PKM1 also enables astrocytes to easily up- regulate the rate of glycolysis to increase the production of lactate, if needed. **(5).**

Neurotransmitters

Neurotransmitters are endogenous chemicals that allow neurons to communicate with each other throughout the body. They enable the brain to provide a variety of functions, through the process of chemical synaptic transmission. These endogenous chemicals are integral in shaping everyday life and functions. Chemical synaptic transmission primarily through the release of neurotransmitters from presynaptic neural cells to postsynaptic receptors. Alterations in the levels of specific neurotransmitters have been observed in various neurological disorders, including Parkinson disease, schizophrenia, depression, and Alzheimer disease. **(6).**

There are a number of neurotransmitters used by the body for different functions, including acetylcholine, glutamate, GABA, glycine, dopamine, norepinephrine, and serotonin. Glutamate is the principal excitatory neurotransmitter used in the brain. It is also the primary mediator of nervous system plasticity. Glutamate has been implicated in modifiable synapses, which researchers suspect are the memory-storage elements of the brain **(7).**

Gamma-aminobutyric acid (GABA) and glycine, conversely, serve as the major inhibitory neurotransmitters. GABA, for example, can account for approximately 40% of the inhibitory processing in the brain. Glycine is found primarily in the spinal cord. Dopamine, another major neurotransmitter, plays an essential role in several brain functions, including learning, motor control, reward, emotion, and executive functions. Dopamine has also been implicated in psychiatric and neurological disorders. Serotonin is a neurotransmitter that modulates multiple neuropsychological processes and neural activity. Serotonin also affects GIT processes like bowel motility, bladder control, and cardiovascular function. Norepinephrine is a monoamine that is synthesized in the central nervous system and sympathetic nerves. The locus coeruleus of the brain plays a vital role in the signaling of norepinephrine. The release of norepinephrine in the brain exerts effects on a variety of processes, including stress, sleep, attention, focus, and inflammation. It also plays a role in modulating the responses of the autonomic nervous system . **(8).**

Histamine is another neurotransmitter that mediates homeostatic functions in the body, promotes wakefulness, modulates feeding behavior, and controls motivational behavior. Neurotransmission medication occurs via the vesicular release of neurotransmitters at presynaptic nerve terminals. Specifically, calcium-evoked exocytosis of the presynaptic vesicles is what enables the release of neurotransmitters into the synapse. Active zones, specialized areas on the presynaptic plasma membranes, tether the neurotransmitter-containing vesicles to the plasma membrane. Once an action potential triggers calcium influx into the presynaptic cleft, active zones undergo fusion with the vesicles, allowing neurotransmitter release **(9)**.

There are multiple proteins involved in the fusion of neurotransmitter-containing vesicles and the active zone. The soluble N-ethyl maleimide sensitive factor attachment protein receptors (SNAREs) syntaxin-1, SNAP-25, and synaptobrevin-2 together form a SNARE complex, a key component in membrane fusion

and ultimately exocytosis. A number of the proteins involved in this process may act as inhibitors and activators of the exocytosis of neurotransmitters from the presynapse. **(10).**

Doxorubicin

Uses

Doxorubicin (Dox) is an effective anthracycline chemotherapeutic developed for the treatment of solid tumors and hematologic malignancies.The anti-tumor activity of Dox has been reported to be mediated through the inhibition of the nuclear enzyme DNA topoisomerase II, causing DNA breakage, and generation of superoxide, hydrogen peroxide, and hydroxyl radicals. It is assumed that oxidative stress and free radical formation play a crucial role in the mechanism of Dox-induced organ toxicity **(11)**.

Doxorubicin pegylated liposomal (as Caelyx) is indicated to treat breast cancer, ovarian cancer, and AIDS-related Kaposi's sarcoma. It is indicated to treat multiple myeloma in combination with bortezomib. Doxorubicin hydrochloride is indicated to treat breast cancer in combination with cyclophosphamide. Doxorubicin is commonly used to treat some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others. Commonly used doxorubicin-containing regimens are AC (Adriamycin, cyclophosphamide), TAC (taxotere, AC), ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), BEACOPP, CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone) and FAC (5-fluorouracil, adriamycin, cyclophosphamide). **(12).**

Toxicity

Dox treatment can increase the risk of secondary malignancies based on postmarketing reports. Doxorubicin hydrochloride was mutagenic in the in vitro Ames assay, and clastogenic in multiple in vitro assays (CHO cell, V79 hamster cell, human lymphoblast, and SCE assays) and the in vivo mouse micronucleus assay. Dox decreases fertility in female rats, Dox may cause infertility and result in amenorrhea. Premature menopause can occur. Recovery of menses and ovulation is related to age at treatment. In animal studies,Dox produces testicular atrophy, diffuse degeneration of the seminiferous tubules, and oligospermia/hypospermia in rats. Dox induces DNA damage in rabbit spermatozoa and dominant lethal mutations in mice. **(12).**

Based on findings in animals and its mechanism of action, Dox Injection can cause fetal harm when administered to a pregnant woman; avoid the use of Dox Injection during the 1st trimester. Available human data do not establish the presence or absence of major birth defects and miscarriage related to the use of doxorubicin hydrochloride during the 2nd and 3rd trimesters. Pediatric patients treated with doxorubicin hydrochloride are at risk for developing cardiovascular dysfunction. Risk factors include young age at treatment (especially < 5 years), high cumulative doses and receipt of combined modality therapy. Long-term periodic cardiovascular monitoring is recommended for all pediatric patients who have received doxorubicin hydrochloride. Dox may contribute to prepubertal growth failure and may also contribute to temporary gonadal impairment. **(14).**

Doxorubicin-Induced neurotoxicity

It was generally believed that doxorubicin has a limited capacity to penetrate the blood-brain barrier and thus the brain is protected from its damage. However, several studies have shown that doxorubicin has potential antitumor effects on brain cancer. Clinical as well as animal studies have shown that doxorubicin was detected in the brain after peripheral administration of the drug. Recently, it was reported that doxorubicin could cross the blood-brain barrier through vascular-associated apical projections of neural stem cells (which are about 30 nm in diameter), can establish direct membrane-membrane contacts with the endothelial cells in specific regions of the irregular endothelial basement membrane, and have abundant vesicular activity. **(15).**

DNA Damage

An important mechanism by which doxorubicin kills cancer cells is its ability to effectively cross-link with DNA, resulting in disruption of the cell cycle and subsequent death of cancer cells. However, doxorubicin can also damage normal and non-cancerous cells. Manchon et al. proved that doxorubicin accumulated in the nucleus of neurons, leading to DNA double-strand breaks (DSBs) and DNA cross-linking. Furthermore, breast cancer type 1 susceptibility protein (BRCA1), which is responsible for DNA repair, was downregulated in primary cortical neurons after doxorubicin treatment. **(16)**

DNA fragmentation following doxorubicin treatment is a strong stimulus to mitochondrial apoptotic pathways *via* increasing the Bax/Bcl-2 ratio; resulting in increased mitochondrial outer membrane permeability (MOMP) and the release of cytochrome C, and activating the caspase-dependent intrinsic apoptotic pathways. Also, it was showed that doxorubicin remarkably increased neuronal cell death in the early and late days. In a human neuroblastoma model, doxorubicin prevented cell cycle progression in the G2/M and S phases. The death of neurons seriously affects the normal activities of the brain, which is manifested by impaired cognitive functions such as learning and memory. **(17).**

Increased Oxidative Stress

Doxorubicin causes neurotoxicity by facilitating ROS production and mitochondrial membrane depolarization in neurons. Doxorubicin increases the MOMP and Bax/Bcl-2 ratio, leading to mitochondrial degeneration and neuronal dysfunction. Previous studies have shown that both endogenic and ectogenic hydrogen peroxide can induce neural degeneration. Mitochondria are the main organelles which adjust calcium absorption and redox signaling under physiological conditions. It was found that doxorubicin damaged mitochondrial function in the hippocampus, resulting in elevated mitochondrial ROS levels and calcium disorder. In addition, glucose metabolism was declined in both the hippocampus and bilateral cortex after intra-thecal injection of doxorubicin. **(18).**

This damage may be generated by the opening of the mitochondrial permeability transition pore (mPTP), which is assembled between the mitochondrial membranes by three protein subunits including cyclophilin D (CyP-D), adenine nucleotide translocase (ANT), and VDAC. ROS initiates the activation of glycogen synthase kinase-3, which phosphorylates CyP-D into its active form. In addition, Calcium dysregulation contributes to mitochondrial membrane depolarization and ANT conformational changes. All these contribute to the mPTP formation, leading to abnormal mitochondrial swelling. Interestingly, injection of the antibodies against TNF-α or iNOS totally prevented the damage of mitochondrial oxidative reaction in mice, suggesting that doxorubicin reduces the mitochondrial function *via* inflammatory reaction and NO• production. **(18).**

Effect on Autophagic Lysosomal System

Doxorubicin can cause damage to the progenitor neuronal degradation pathways, impair progenitor neuronal lysosomes, promote the formation of pre-autophagic complexes, up-regulate autophagy, and affect the clearance of the autophagic marker p62 protein. Under electron microscopy, an accumulation of vacuolar structures, autophagosomes, mitochondria, and lipid droplets was observed in doxorubicin-exposed neurons. **(16)**

Degradation disorders seriously affect the function of neurons, resulting in cognitive impairment after chemotherapy. **(16)**

Activation of Apoptosis

It has been noted that doxorubicin-induced apoptosis is dependent on the exogenous pathway in primary cortical neurons (death receptor-mediated" apoptosis). Doxorubicin increases Fas-Fas ligand (FasL) interactions, leading to the recruitment of Fas-associated protein with death domain (FADD) by connecting to the death domain, which initiates exogenous apoptotic pathways. Endogenous apoptotic pathways are activated by cellular stress, DNA damage, developmental signaling, and loss of survival factors. This pathway is regulated by Bcl-2 family proteins, and is related to the mechanism of mitochondrial oxidative stress, which has been described in detail above. Abnormal apoptosis greatly reduces the number of neurons, thus leading to cognitive impairment. **(19).**

Damaged Neurogenesis

Animals treated with doxorubicin showed an obvious decrease in neurogenesis, as manifested by a distinct reduction in the number of neuro-specific nuclear antigen bromodeoxyuridine (BrdUrd)-labeled cells. Others also found that DOX in combination with cyclophosphamide reduced cell survival in the subgranular areas and dentate gyrus of rats. A large number of studies have shown that activation of astrocytes and subsequent release of inflammatory mediators caused by doxorubicin render the nerve non-viable. TNF-α was reported to be anti-neurogenic, and can cause a decrease of the BrdUrd-labeled cells in the sub-granular zone following injection. Besides, mice deficient in TNF-α-receptor-1 (TNFR1) had an increased proliferation of BrdUrd-labeled cells in the sub-granular compartment, suggesting that TNFR1 mediates the anti-neurogenic effects of TNF-α. Neuro-inflammation not only affects the proliferation, differentiation, and survival of hippocampal cells, but also prevents the incorporation of new neurons into existing neural networks. **(20).**

Down-Regulation of Neurotransmitters

Many animal researches have manifested that doxorubicin can cause dysregulation of neurotransmitter production and release in the brain. Acetylcholine (ACH) is a significant neurotransmitter in the cholinergic nervous system that supports brain functions through long-term potentiation (LTP). During acetylcholine composition, phosphatidylcholine (PtdCho) is disintegrated by phospholipase D (PLD) and this releases choline, which is acetylated by choline acetyltransferase (ChAT) to form acetylcholine. In mice, the levels of PLD, ChAT activity, and choline-containing compounds in the hippocampal region were significantly declined after doxorubicin treatment, reflecting the exhaustion of ACH production. Moreover, doxorubicin-induced oxidative stress increased ROS-mediated acetylcholinesterase (AChE) activity. Changes in the choline-containing substances are thought to be related to membrane turnover (synthesis and degradation of phospholipids), and have been attributed to myelin injury following chemotherapy. **(21).**

Elevated TNF-α may decrease PLD activity, thereby inhibiting PtdCho synthesis . In addition, TNF-α is thought to be associated with decreases of phosphatidic acid levels, suggesting an interdependence between phospholipase and TNF-α expression. Inhibition of PLD leads to decreased production of cytokines, including TNF-α. Phosphatidic acid, an intermediate product of the PLD pathway, stimulates Ca2+ mobilization and displays growth factor-like activity, which helps reduce doxorubicin-induced mitochondrial dysfunction in the mouse brain. The enzymatic activity of PLD is critical for cell survival, and structural damage to PLD and reduced PLD activity may activate apoptotic pathways. In addition to regulating acetylcholine metabolism, doxorubicin can alter glutamate levels in the synaptic gap. Doxorubicin reduced glutamate clearance, as showed by a decline in the rate of uptake of glutamate in the frontal cortex of mice. In this context, it was suggested that the decreased glutamate clearance is due to decreased expression of glial transport proteins or increased glutamate production from neurogliocyte, particularly in astrocytes. As mentioned earlier, TNF-α-induced activation of astrocytes triggers substantial glutamate release. **(22).**

When glutamate concentrations are high in the synapse, glutamate can diffuse to and combine with NMDA acceptors. Activation of extrasynaptic NMDA acceptors causes increased calcium-dependent excitability and suppression of BDNF composition, leading to loss of synaptic plasticity and increased neuronal apoptosis. This may explain how doxorubicin decreased the expression of BDNF and its receptor tropomyosin receptor kinase B (TrkB). **(22).**

In addition, doxorubicin injection distinctly reduced the levels of two monoamines that are closely related to cognitive function: serotonin (5-HT) and dopamine (DA). 5-HTergic neurons play a significant role in regulating hippocampal synaptic plasticity through 5-HT1A receptor-mediated inhibitory control. Depletion of 5-HT negatively affects hippocampus-dependent declarative memory and performs poorly in a new object recognition task. During encoding, doxorubicin mediates the acquisition of long-lasting, long-term memory in the hippocampus by activating the D1/D5 receptor. **(18).**

Synaptic Dysplasia

Abnormal synaptic plasticity in the brain is an important cause of cognitive impairment. Synaptic plasticity is associated with synapse-associated proteins such as synapsin protein (SYP) and postsynaptic dense protein 95 (PSD95). In addition, the expression of brain-derived neurotrophic factor (BDNF)-synuclein

(SYP)-microtubule-associated protein 2 (MAP2) pathway-related proteins in the hippocampus is also involved in the development of synaptic plasticity. Dox decreases synapsin expression, resulting in cognitive impairment. **(23).**

Altered Protein Kinase Signaling Pathways

Doxorubicin can affect some key memory-related kinase systems.It activates p38 MAPK and extracellular signal-regulated kinase (ERK), two kinases that have opposite roles: while the former mediates synaptic inhibition, the latter promotes synaptic facilitation. In hippocampal sensory neurons, doxorubicin can inhibit serotonin-induced long-term facilitation (LTF) and promote Phe-Met-Arg-Phe-NH2 (FMRFa)-mediated long-term depression (LTD), suggesting that doxorubicin may block learning-related changes in hippocampal excitability. These studies mean that long-term memory damage may be the result of doxorubicin action, partially due to the dominant activation of p38 MAPK. In addition, doxorubicin inhibited the phosphorylation of the downstream transcriptional repressor cAMP response element binding protein 2 (CREB2), which promoted LTD. The ERK pathway is essential for neuronal survival and is required for the synthesis of Arc, a protein that plays a crucial part in long-term memory formation, neuronal activity and synaptic plasticity. **(24).**

A recent study found an increase in Arc staining after doxorubicin treatment, suggesting that doxorubicin induces neuronal activity. On the contrary, inhibition of neural activity with n -methyl-d-aspartate (NMDA) receptor antagonists or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists partly eliminated the induction of DNA DSBs by doxorubicin, suggesting that doxorubicin-induced neurotoxicity is dependent on neuronal activity . **(24).**

Epigenetic Alterations

Homozygous mice exposed to chemotherapeutic agents show more pronounced disruptions in post-transcriptional regulation of gene expression, mainly miRNA changes in the prefrontal cortices. miRNA dysregulation is associated with the altered levels of brain-derived neurotrophic factor (BDNF) that plays a key role in cognition and memory. **(25)**

Neuregulin 1 and ErbB genes

Neuregulin 1

Neuregulin 1, or **NRG1**, is a gene of the epidermal growth factor family that in humans is encoded by the *NRG1* gene. NRG1 is one of four proteins in the neuregulin family that act on the EGFR family of receptors. Neuregulin 1 is produced in numerous isoforms by alternative splicing, which allows it to perform a wide variety of functions. It is essential for the normal development of the nervous system and the heart. **(26).**

Function

Neuregulin 1 is thought to play a role in synaptic plasticity. It has been shown that a loss of Neuregulin 1 within cortical projection neurons results in increased inhibitory connections and reduced synaptic plasticity. Similarly, overexpression of Neuregulin 1 results in disrupted excitatory-inhibitory connections, reduced synaptic plasticity, and abnormal dendritic spine growth. Nrg's involvement in regulating dendritic pruning in ddaC neurons in a Rab5/ESCRT-mediated endocytic pathway. Thus, careful regulation of the amount of Neuregulin 1 must be maintained in order to preserve balance between excitatory and inhibitory connections within the central nervous system (CNS). Any disruption in this inhibitory system may contribute to impaired synaptic plasticity, a symptom endemic in schizophrenic patients. **(5).**

ErbB

The ErbB family of proteins contains four receptor tyrosine kinases, structurally related to the epidermal growth factor receptor (EGFR), its first discovered member. In humans, the family includes Her1 (EGFR, ErbB1), Her2 (ErbB2), Her3 (ErbB3), and Her4 (ErbB4). The gene symbol, ErbB, is derived from the name of a viral oncogene to which these receptors are homologous: erythroblastic leukemia viral oncogene. Insufficient ErbB signaling in humans is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer's disease,[1] while excessive ErbB signaling is associated with the development of a wide variety of types of solid tumor. **(26).**

ErbB protein family signaling is important for development. For example, ErbB-2 and ErbB-4 knockout mice die at midgestation leads to deficient cardiac function associated with a lack of myocardial ventricular trabeculation and display abnormal development of the peripheral nervous system. In ErbB-3 receptor mutant mice, they have less severe defects in the heart and thus are able to survive longer throughout embryogenesis. Lack of Schwann cell maturation leads to degeneration of motor and sensory neurons. ErbB-1 and ErbB-2 are found in many human cancers, and their excessive signaling may be critical factors in the development and malignancy of these tumors. **(27).**

Neuregulin-1/ErbB signaling in the hippocampus of rats after administration of doxorubicin

Our previous study demonstrated that prolonged use of Dox caused neural apoptosis and successfully induced depression-like behaviors in rats. However, the underlying mechanisms of Dox-induced neurotoxicity remain elusive. **(28).**

NRG1 is suggested as a susceptibility gene for several psychiatric disorders, including schizophrenia, bipolar disorder, and depression. NRG1 is well known to play an essential role in neuronal development as well as in maintaining normal function in the mature nervous system. Recent biochemical studies have shown that NRG1 can be neuroprotective for cortical neurons, motor neurons, dopaminergic neurons, cochlear sensory neurons, and PC12 cells. Previous research showed that aberrant changes in these NRG1-related pathways are tightly linked to the pathogenesis of depression **(28).**

It is well known that the NRG1/ErbB signal system plays a critical role in the development of the cardiovascular system and the maintenance of adult heart function. Interestingly, the NRG1/ErbB system is also involved in the Dox-induced cardiotoxicity. It has been reported that Dox treatment pronouncedly inhibits NRG1 expression and suppresses ErbB4 activation, whereas NRG1 treatment alleviates Dox-induced myocardial apoptosis and improves cardiac function **(25).**

it is tempting to hypothesize that Dox may inhibit neural NRG1/ErbB signaling, thereby triggering the neurotoxicity and behavioral changes **(25).**

Wen et al also reported that NRG1 is a common susceptibility gene for major depressive disorder in Han population. Similarly, Bi et al also found that the endogenous NRG1-ErbB4 signaling pathway in the basolateral amygdala is critical for the stress-induced behavioral changes, including major depression, and the administration of NRG1 into the basolateral amygdala of high-anxiety mice alleviates their anxiety and enhances GABAergic neurotransmission **(29)**.

Dox-induced cardiotoxicity and neurotoxicity are dose-related and essentially irreversible, and the long-term use of Dox tends to induce neurotoxicity and may cause neuropsychiatric diseases including depression. In vivo study has shown that Dox-induced cardiovascular disease is tightly associated with the suppression of NRG1/ErbB signaling, whereas the activation of NRG1/ErbB signaling promotes cardiac protection during acute cardiac injury and chronic ventricular remodeling. However, the potential link between Dox-induced neurotoxicity and NRG1/ErbB pathway remains largely unknown**.** Salas-Ramirez et al also found that the rats treated for three times with a combination of cyclophosphamide and Dox show significantly activated ERK and Akt signaling pathways. The results of the previous behavioral testing also demonstrated that chemotherapy can impair cognitive function, without affecting anxiety **(30).**

Horie et al have found that the acute cardiotoxicity induced by Dox is associated with the inhibition of the NRG1-ErbB pathway, and studies also showed that the supplementation of NRG1 is effective in attenuating Dox-induced cardiac dysfunction in mouse hearts. Thus, as a potential therapeutic agent for the treatment of heart failure and nerve damage, the NRG1/ErbB signaling pathway might be the underlying mechanisms of Dox-induced tissue damage in both heart and brain. **(31).**

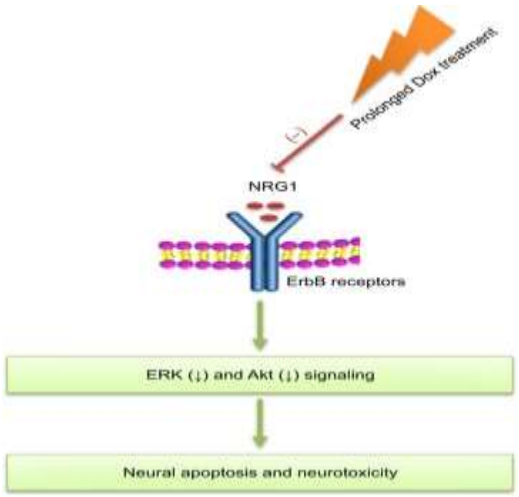

Figure (2): Proposed mechanisms of the Dox-induced neurotoxicity. **(32).**

References

1. **Grimm, A., & Eckert, A. (2017).** Brain aging and neurodegeneration: from a mitochondrial point of view. Journal of neurochemistry, 143(4), 418-431.
2. **Daniele, S., Giacomelli, C., & Martini, C. (2018).** Brain ageing and neurodegenerative disease: The role of cellular waste management. Biochemical pharmacology, 158, 207-216.
3. **Vilchez, D., Ros, S., Cifuentes, D., Pujadas, L., Vallès, J., García-Fojeda, B., ... & Guinovart, J. J. (2007).** Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. Nature neuroscience, 10(11), 1407-1413.
4. **Boumezbeur, F., Mason, G. F., De Graaf, R. A., Behar, K. L., Cline, G. W., Shulman, G. I., ... & Petersen, K. F. (2010).** Altered brain mitochondrial metabolism in healthy aging as assessed by in vivo magnetic resonance spectroscopy. Journal of Cerebral Blood Flow & Metabolism, 30(1), 211-221.
5. **Zhang, Y., Chen, K., Sloan, S. A., Bennett, M. L., Scholze, A. R., O'Keeffe, S., ... & Wu, J. Q. (2014).** An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. Journal of Neuroscience, 34(36), 11929-11947.
6. **Sheffler, Z. M., Reddy, V., & Pillarisetty, L. S. (2019).** Physiology, neurotransmitters ,Journal of Neuroscience.1-15.
7. **Zhou, Y., & Danbolt, N. C. (2014).** Glutamate as a neurotransmitter in the healthy brain. Journal of neural transmission, 121, 799-817.
8. **O'Donnell, J., Zeppenfeld, D., McConnell, E., Pena, S., & Nedergaard, M. (2012).** Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance. Neurochemical research, 37, 2496-2512.
9. **Rizo, J. (2018).** Mechanism of neurotransmitter release coming into focus. Protein Science, 27(8), 1364-1391.
10. **Südhof, T. C. (2013).** Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. Neuron, 80(3), 675-690.
11. **Kumari, S., Sharma, S., Advani, D., Khosla, A., Kumar, P., & Ambasta, R. K. (2021).** Unboxing the molecular modalities of mutagens in cancer. Environmental Science and Pollution Research, 1-49.
12. **Brayfield, A. (2014).** Martindale: The Complete Drug Reference, Pharmaceutical Press. United

Kingdom.
13. **Arcamone, F. (2012).** Doxorubicin: anticancer antibiotics. Elsevier.148-153.
14. **Chatterjee, K., Zhang, J., Honbo, N., & Karliner, J. S. (2010).** Doxorubicin cardiomyopathy. Cardiology, 115(2), 155-162.
15. **Licht, T., Sasson, E., Bell, B., Grunewald, M., Kumar, S., Kreisel, T., ... & Keshet, E. (2020).** Hippocampal neural stem cells facilitate access from circulation via apical cytoplasmic processes. Elife, 9, e52134.
16. **Manchon, J. F. M., Dabaghian, Y., Uzor, N. E., Kesler, S. R., Wefel, J. S., & Tsvetkov, A. S. (2016).** Levetiracetam mitigates doxorubicin-induced DNA and synaptic damage in neurons. Scientific reports, 6(1), 25705.
17. **Du, J., Zhang, A., Li, J., Liu, X., Wu, S., Wang, B., ... & Jia, H. (2021).** Doxorubicin-induced cognitive impairment: the mechanistic insights. Frontiers in oncology, 11, 673340.
18. **Park, H. S., Kim, C. J., Kwak, H. B., No, M. H., Heo, J. W., & Kim, T. W. (2018).** Physical exercise prevents cognitive impairment by enhancing hippocampal neuroplasticity and mitochondrial function in doxorubicin-induced chemobrain. Neuropharmacology, 133, 451-461.
19. **Walczak, H. (2013).** Death receptor–ligand systems in cancer, cell death, and inflammation. Cold Spring Harbor perspectives in biology, 5(5), a008698.
20. **Kohman, R. A., & Rhodes, J. S. (2013).** Neurogenesis, inflammation and behavior. Brain, behavior, and immunity, 27, 22-32.
21. **Keeney, J. T., Ren, X., Warrier, G., Noel, T., Powell, D. K., Brelsfoard, J. M., ... & Butterfield, D. A. (2018).** Doxorubicin-induced elevated oxidative stress and neurochemical alterations in brain and cognitive decline: protection by MESNA and insights into mechanisms of chemotherapy-induced cognitive impairment ("chemobrain"). Oncotarget, 9(54), 30324.
22. **Tanguy, E., Tran Nguyen, A. P., Kassas, N., Bader, M. F., Grant, N. J., & Vitale, N. (2019).** Regulation of phospholipase D by Arf6 during FcγR-mediated phagocytosis. The Journal of Immunology, 202(10), 2971-2981.
23. **Fernandez, S. P., Muzerelle, A., Scotto-Lomassese, S., Barik, J., Gruart, A., Delgado-García, J. M., & Gaspar, P. (2017).** Constitutive and acquired serotonin deficiency alters memory and hippocampal synaptic plasticity. Neuropsychopharmacology, 42(2), 512-523.
24. **Liu, S., Liu, C., Xiong, L., Xie, J., Huang, C., Pi, R., ... & Li, L. (2021).** Icaritin alleviates glutamate-induced neuronal damage by inactivating GluN2B-containing NMDARs through the ERK/DAPK1 pathway. Frontiers in Neuroscience, 15, 525615.
25. **Kovalchuk, A., Ilnytskyy, Y., Rodriguez-Juarez, R., Katz, A., Sidransky, D., Kolb, B., & Kovalchuk, O. (2017).** Growth of malignant extracranial tumors alters microRNAome in the prefrontal cortex of TumorGraft mice. Oncotarget, 8(51), 88276.
26. **Kataria, H., Alizadeh, A., & Karimi-Abdolrezaee, S. (2019).** Neuregulin-1/ErbB network: An emerging modulator of nervous system injury and repair. Progress in neurobiology, 180, 101643.
27. **Fantauzzo, K. A., & Soriano, P. (2015).** Receptor tyrosine kinase signaling: regulating neural crest development one phosphate at a time. Current topics in developmental biology, 111, 135-182.
28. **Wu, Y. Q., Dang, R. L., Tang, M. M., Cai, H. L., Li, H. D., Liao, D. H., ... & Jiang, P. (2016).** Long chain omega-3 polyunsaturated fatty acid supplementation alleviates doxorubicin-induced depressive-like behaviors and neurotoxicity in rats: involvement of oxidative stress and neuroinflammation. Nutrients, 8(4), 243.
29. **Bi, L. L., Sun, X. D., Zhang, J., Lu, Y. S., Chen, Y. H., Wang, J., ... & Gao, T. M. (2015). Amygdala** NRG1–ErbB4 is Critical for the Modulation of anxiety-like behaviors. Neuropsychopharmacology, 40(4), 974-986.
30. **Salas-Ramirez, K. Y., Bagnall, C., Frias, L., Abdali, S. A., Ahles, T. A., & Hubbard, K. (2015).** Doxorubicin

and cyclophosphamide induce cognitive dysfunction and activate the ERK and AKT signaling pathways. Behavioural brain research, 292, 133-141.

31. **Horie, T., Ono, K., Nishi, H., Nagao, K., Kinoshita, M., Watanabe, S., ... & Kimura, T. (2010).** Acute doxorubicin cardiotoxicity is associated with miR-146a-induced inhibition of the neuregulin-ErbB pathway. Cardiovascular research, 87(4), 656-664.
32. **Liao, D., Guo, Y., Xiang, D., Dang, R., Xu, P., Cai, H., ... & Jiang, P. (2018).** Dysregulation of Neuregulin-1/ErbB signaling in the hippocampus of rats after administration of doxorubicin. Drug design, development and therapy, 231-239.