Doxorubicin-induced Toxicity Through the p38 MAPK Protein Kinase Pathway

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Abstract. While exhibiting great value in treating multiple cancers, the chemotherapy drug, Doxorubicin, also manifests many side effects that significantly affect the post-chemotherapy life of patients. In the cardiac system, Doxorubicin causes oxidative stress due to increasing amount of Reactive oxygen species (ROS), and it promotes production of inflammatory cytokines. Oxidative stress and inflammatory cytokines then activate p38 mitogen-activated protein kinase (p38 MAPK), which can stimulate cardiomyocyte apoptosis. In the nervous system, Doxorubicin activates both extracellular signal-regulated kinase (ERK) and p38 MAPK. p38 MAPK predominately determines the result, leading to an overall reduction in Long-term Potentiation (LTP), or an analogous process of Long-term Facilitation (LTF). Moreover, neuroinflammatory effect achieved through the p38 MAPK pathway contributes to memory deficits by killing neurons excessively. Various inhibitors of p38 MAPK have shown promising results in lessening the effects of p38 MAPK, indicating future possibilities of using those inhibitors to ensure a safer application of Doxorubicin, while preserving the pharmacological values and properties of Doxorubicin.

Keywords: p38 MAPK, Doxorubicin, cardiomyocyte apoptosis, neurotoxicity.

1. Introduction

Cancer has been considered as one of the most dangerous and life-threatening diseases, causing over 500,000 deaths in the United States in a single year [1]. It usually happens when gene mutations occur on a couple of proto-oncogenes and tumor-suppressor genes, causing abnormal and excessive cell growth that eventually invade normal, functional human tissues [2, 3]. Cancer has been under extensive research by scientists, who are looking for novel ways aiming to reduce pain, arrest the development, and ultimately curing the cancer.

Chemotherapy treatments have been an area of research that's being paid significant attention to by the scientists. Among the chemotherapy treatments, Doxorubicin is a widely used drug in chemotherapy that is used to regress or degenerate the disseminated conditions in cancers such as breast cancer, ovarian cancer, and leukemia [4].

Despite the wide usage in the cancer treatment, this review focuses on the side effects of Doxorubicin in human cardiac and nervous systems and explores specific mechanisms that are related to the p38 MAPK signaling pathway.

2. The structure of Doxorubicin and its main mechanisms of actions

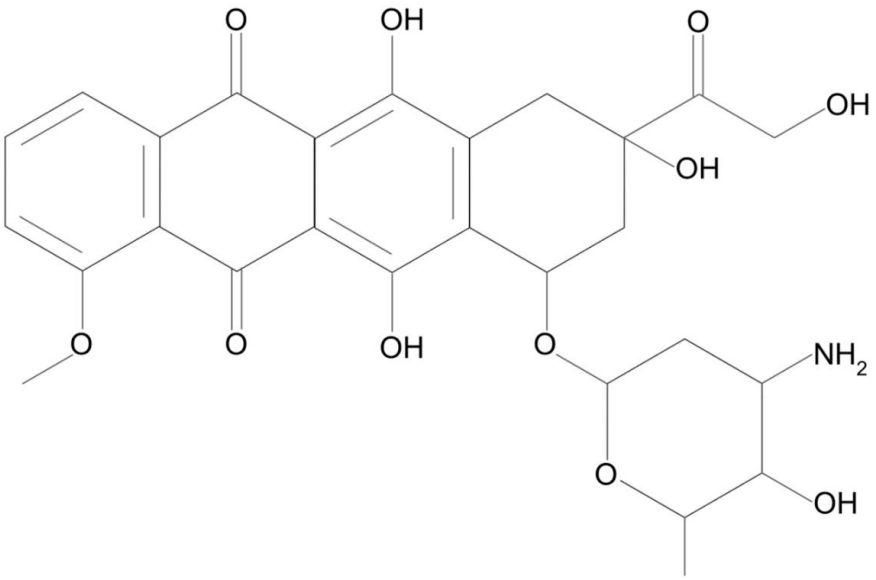

Figure 1. Structure of Doxorubicin.

Doxorubicin or Adriamycin, 23214-92-8, has a UNII of 80168379AG and a DSSTox Substance ID of DTXSID8021480. The molecular formula of Doxorubicin is $C_{27}H_{29}NO_{11}$ (Fig. 1) and the molecular weight is 543.5. Being among the most common anthracycline agents, Doxorubicin functions primarily by interrupting RNA production and blocking DNA synthesis via DNA intercalation and, as a result, inducing apoptosis in the tumor cells [5]. Another way in which Doxorubicin exerts its effects on the tumor cells is the inhibition of the functions of topoisomerase type II, an enzyme important in DNA replication, transcription, and recombination [5]. DNA intercalation, together with the inhibited topoisomerase type II, leads to the disruption of the DNA double helix, and therefore results in the reduction in mitosis of cancer cells [5].

3. Pharmacokinetics of Doxorubicin

Doxorubicin is mainly administered by direct intravenous and will be distributed in plasma and tissues [6]. Doxorubicin is rapidly metabolized where its first pass effect occurs in the liver, and the biospecimen of Doxorubicin can be found in blood and urine [7]. Doxorubicin can go through three metabolic pathways: one-electron reduction, two-electron reduction, and deglycosidation [4]. Doxorubicinol, which is thought to be the primary metabolite, is the result of the pathway of two electron reduction [7]. Thus, it is thought to manifest cardiotoxic properties [8].

Doxorubicin does not have the ability to pass the blood-brain barrier [6]. However, chemotherapy with Doxorubicin can facilitate and yield more output of ROS, which in turn increase lipid peroxidation and promote the disruption of cross blood-brain barrier [9].

4. Cardiotoxicity

4.1. p38 MAPK is associated with heart malfunction

p38 MAPK is a key factor in many signaling pathways and physiological processes within the human body. Many stress factors can activate p38 MAPK. For instance, osmotic shock, heat, biomechanical stress, radiation stress, and oxidative stress can all induce p38 MAPK to be activated [10, 11].

The organ heart consists mainly of cardiomyocytes. In addition, other types of cells, such as endothelial cells making up the lining of blood vessels, and vascular smooth muscle cells (SMCs) building up the vessel wall are also integral parts of the normal function of the heart.

Over-expression of the p38 branch of MAPK was found to be an enhancer of cardiomyocyte apoptosis *in vitro* [10]. In addition, in the mouse heart with dominant transgenesis which could induce more and stronger p38 MAPK activation, more myocardial cell deaths were found *in vivo* [10]. It was explained that in vascular SMCs, p38 MAPK, once activated by stresses, could directly phosphorylate p53, a tumor-suppressor protein possessing the functions of recognizing and repairing DNA damage, removing the potentially mutagenized cells [12], and put it on a pre-activated state. As a result, p53 became more sensitive to DNA mutants and enhanced cell apoptosis mode was turned on [12].

Similar to cell apoptosis, cardiac hypertrophy is initially a biological result of hemodynamic stimuli in a compensatory way, but, if it lasts, contributes to heart diseases [11]. When induced by stress, p38 MAPK and similar signaling pathways can directly result in stress-hypertrophy/apoptosis responses induction [13]. Though apoptosis is a necessary function to maintain, when outside stress carries on, the initial balance would be interrupted between the hypertrophic responses and apoptotic responses, therefore the apoptosis would be escalated [13].

4.2. Doxorubicin's effect of activating p38 MAPK in heart cells leads to excessive apoptosis

Studies and trials showed that Doxorubicin could lead to excessive apoptosis through the p38 MARK pathway.

Scientists have discovered specific mechanisms that may lead to heart failure induced by Doxorubicin, among which the increase in cardiac oxidative stress have been thought to be a major one (Table 1) [14]. ROS is the natural product of molecules' usage of oxygen. Under normal circumstances, organisms can achieve a balance between the formation of ROS and detoxification capacity. When this balance is broken, oxidative stress occurs. A lot of cellular and animal models demonstrated the increasing level of ROS and oxidative stress after being treated by Doxorubicin [14]. Increased level of ROS induced by Doxorubicin administration led to oxidative stress, which in turn activated p38 MAPK and then extra apoptosis [14].

Some animal studies showed that antioxidants such as Vitamin E alone could not reduce the cardiotoxic effects caused by Doxorubicin at a statistically significant level [15]. These findings suggested that Doxorubicin may cause cardiomyopathy through pathways other than "ROS production - oxidative stress - p38 MAPK - apoptosis". The p38 MAPK pathway could also be induced by TNF-α and IL-1, two types of inflammatory cytokines [16]. Doxorubicin has been found to be able to stimulate the production of inflammatory cytokines. Upon activation, p38 MAPK would further inhibit some proteins like Bcl-xl that normally acts to mitigate apoptosis, which escalate apoptosis extra more [10, 11].

4.3. Potential therapeutic methods of Doxorubicin-induced cardiac toxicity

The cardiomyocyte proliferation was shown to be regulated and regenerated by inhibiting p38 MAPK activities [11]. *In vivo* experiment showed that transgenic mice who have a dominant allele of negative p38 MAPK could be protected from the injury caused by ischemia-reperfusion [10].

After finding out the mediating functions of p38 MAPK in the Doxorubicin's cardiotoxicity, efforts have been put into inhibiting p38 MAPK, and in turn attenuating the toxicity caused by Doxorubicin in the heart without compromising its killing effects on cancer cells.

Animal studies demonstrated the protective effects of Irbesartan (IRB), an angiotensin II receptor blocker against cardiotoxicity induced by Doxorubicin in rats by inhibiting p38 MAPK [17].

In clinical trials, a p38 MAPK inhibitor, losmapimod, was found to have potential protective effects in reducing inflammation in cardiac patients [18].

5. Neurotoxicity

5.1. Chemotherapy-related neurotoxicity

Despite chemotherapy's valuable advantages in treating cancer, many may suffer from long-term cognitive damage. Symptoms such as memory deficits, poor speed in processing, loss of concentration, and deficiency in language learning are common and can greatly affect a patient's post-cancer and post-chemotherapy life (Table 1) [19].

5.2. Roles of ERK and p38 MAPK in long term memory formation

Long-term depression (LTD) and long-term potentiation (LTP) are two kinds of long-term plasticity that commonly occurs in the hippocampus. And these are well-studied mechanisms that are found to be a main factor in forming memories [20]. LTP is induced in the synapse when a neuron rapidly activates another neuron, resulting in modifications such as more dendritic spine formation, calcium channel regulation, or changes in NMDA and AMPA receptors [21]. This mechanism strengthens the firing of neurons, allowing easier transfer of signal from one neuron to another, helping a specific memory to be established and more easily retrieved. Conversely, when a set of neurons are rarely activated, LTD occurs, modifying the synapse so that their efficiency of neuron firing is decreased.

Mechanisms underlying the achievement of LTP and LTD include the ERK and the p38 MAPK cellular signaling [22]. Interestingly, ERK and p38 MAPK are two MAPK isoforms that have been shown to manifest inverse effects in long term memory formation. ERK is an upstream effector that phosphorylates cAMP response element binding protein 2 (CREB2) and CCAAT enhancer-binding protein (C/EBP), two key proteins that are shown to be required for Long-term facilitation, a process analogous to vertebrate LTP [23]. Conversely, inhibiting p38 MAPK with a pharmacological inhibitor and antibodies both induce LTF, suggesting that p38 MAPK exhibits opposite effects as ERK [23].

5.3. Doxorubicin decreases LTF through ERK and p38 MAPK activation

Doxorubicin has been demonstrated to be able to activate both of the two MAPK isoforms. In an experiment involving sensory neurons in Aplysia, it was observed that Doxorubicin induces both an increase in the level of activated ERK (pERK) and activated p38 MAPK (p-p38 MAPK) [22]. Similar results were obtained with rat cortical neurons, reinforcing that the result can be generalized to other types of neurons [22].

A possible explanation of the result is that Doxorubicin decreases the expression of some isoforms of MAPK phosphatase, and in turn increases MAPK activities [22]. Indeed, some treatments with Doxorubicin that increase pERK level are accompanied with a decrease in MAPK phosphatase 1 (MKP1), a phosphatase specific for ERK [22]. However, there may be other more direct mechanisms that account for the short-term pERK level increase, since a Doxorubicin treatment that is sufficient to cause increased levels of pERK isn't necessarily accompanied with a significant change in the level of MKP1 [22]. The mechanism behind increasing levels of pERK and p-p38 MAPK induced by Doxorubicin is still a remaining question for future research.

It has been found that the result of the application of Doxorubicin depends on the cellular function of p38 MAPK. Lower overall amplitude of excitatory postsynaptic potential (EPSP) in neurons suggests that p38 MAPK predominates the result. Though not completely understood, a possible explanation is that p38 MAPK enhances the function of some phosphatase, leading to ERK dephosphorylation. As a result of the predominantly activated p38 MAPK, deficits in LTF or LTP are induced through activating other crucial proteins such as IL-1β, a protein inhibiting various forms of LTP [24]. As a result of the signaling pathway following the activation of p38 MAPK, the establishment of new synaptic relationships are repressed, and long-term memory is hindered.

5.4. Neuroinflammation may lead to memory impairment

The decrease in LTF may not only account for events associated with chemobrian. The phosphorylation of p38 MAPK is an essential step leading to neuroinflammation [25]. Neuroinflammation has been shown to be related to diseases linked with memory and cognitive disorders [25]. Underlying the induction of neuroinflammation, p38 MAPK is shown to participate in the production of proinflammatory transcription factor NF-κB and mRNA stability and translation [24]. Persistent inflammation functioning through the p38 MAPK signaling pathway can cause some glial cells, specifically microglia and astrocytes, to change to an activated state. These activated glial cells drive ROS creation and neurotoxic molecules which can lead to the death of neuron cells. Extending from this relationship between p38 MAPK and neuroinflammation, the memory and cognitive deficits associated with Doxorubicin may be attributable to the neuronal cell death driven by ROS and neurotoxic molecules.

5.5. Resistance to brain deficits carried out by p38 MAPK

In an *in vivo* study, the attenuation of LTF induced by the predominating p-p38 MAPK is relieved when the p38 MAPK inhibitor SB203580 is preexposed [22]. Some scientists have also applied p38 MAPK inhibitors, like BIRB796, to clinical trials. However, possibly due to cross-reactivity with other proteins, these inhibitors brought about side effects in the liver and the CNS [25].

Scientists are now discovering more and experimenting with different p38 MAPK inhibitors to minimize the side effects implemented through the p38 MAPK signaling pathways. For example, SB681323, GW856553X, and SCIO-469 are currently in phase II clinical trials, preparing for treating consequences such as depressive disorder [26].

Table 1. Doxorubicin toxicity: mechanisms, symptoms and drugs.

Toxicity	Related Mechanism	Symptoms	Potential Inhibitors
Cardiotoxicity	Cardiac oxidative stress, increased production of inflammatory cytokines [14, 16]	Left ventricular dysfunction, dilated cardiomyopathy, heart failure [27]	IRB, losmapimod [17, 18]
Neurotoxicity	Decreased LTP and induced LTD, neuroinflammation [22, 25]	Cognitive deficits, long-term memory deficits [22]	SB203580, BIRB796, SB 681323, GW856553X, SCIO-469 [22, 25, 26]

6. Relationship between isoforms of p38 MAPK and Doxorubicin toxicity

In fact, p38 MAPK is not a single type of kinase, but a family of several isoforms. Previous studies have found that there are four isoforms, p38α, p38β, p38γ, and p38δ [11]. Though they share more than 60% of amino acid sequences [11], sometimes, they function in different ways, even in opposite ways [13]. Isoforms are also differently localized or expressed in different organs. For example, p38α is the most common one found to be present in many cell types, while p38β is found to be mainly in the brain and the lung [11]. Therefore, more studies about the different ways in which p38 MAPK isoforms mediate the DOX's toxicity in different human organs are necessary.

7. Conclusion

In the cardiac system, specifically in the cardiomyocyte cells, the application of Doxorubicin is accompanied with increasing level of ROS, leading to oxidative stress, which is a stimulation of p38 MAPK, and in turn inducing excessive apoptosis as the response of the signaling pathway. Doxorubicin can also promote increasing amounts of inflammatory cytokines, activating p38 MAPK and stimulating more apoptosis. Additionally, patients receiving Doxorubicin treatments also

experienced cognitive and memory related deficits. Doxorubicin activates both ERK and p38 MAPK proteins in several types of neurons, with p38 MAPK predominating the result. Incorporating other key players in the signaling pathway such as the IL-1β, LTF/LTP is decreased. Moreover, p38 MAPK is also associated with neuroinflammatory effects, creating ROS and neurotoxic molecules that can kill the neurons. Recently, some inhibitors of p38 MAPK have been demonstrated functional and successful at attenuating the toxicity of Doxorubicin. Many clinical trials testing the function and safety of the inhibitors are also currently taking place. Together with studies about the different isoforms of p38 MAPK that are in progress, future research can focus on finding more efficient ways to relieve the toxicity of Doxorubicin in human organs, and at the same time maintain and enhancing its functionalities in treating cancers.

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