Agomelatine protects hippocampal neurons against doxorubicin induced toxicity

Betul Danisman 1,* and Betul Cicek 2

1 Department of Biophysics, Faculty of Medicine, Ataturk University, Erzurum, Turkey.
2 Department of Physiology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey.

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Abstract

Doxorubicin (DOX) is a powerful chemotherapy drug used in numerous cancer treatments, known to causes neurotoxicity and impair cognitive function in cancer patients. The main mechanism thought to be responsible for the neuronal damage related to chemotherapy is oxidative stress. Agomelatine (AGM) has recently been identified as a neuroprotective agent against toxicity and the effect of AGM on DOX-induced neurotoxicity was investigated in this study. In order to evaluate the effect of AGM on oxidative stress and apoptosis, malondialdehyde (MDA), lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT), and Caspase-3 expression were analyzed in the HT-22 cell line. AGM considerably reduced oxidative damage due to DOX in vitro. MDA and Caspase-3 were considerably higher in the DOX group. These values were dramatically decreased by AGM. AGM greatly increased the CAT and SOD levels when compared to the DOX group. These results suggest that DOX therapy alters antioxidant status in hippocampus, a critical cognition-related region. So if we are aware of the functional effects of DOX, it is possible to avoid some of the disabling side effects of chemotherapy in cancer patients and may prevent these effects with antioxidant agents such as AGM.

Keywords: Doxorubicin; Agomelatine; HT-22 cell line; Antioxidant; Apoptosis

1. Introduction

Chemotherapeutic treatments widely induce toxicity and can develop cognitive problems by causing anatomical and functional changes in the brain so patients’ quality of life is negatively affected [1]. DOX, a chemotherapeutic drug, belongs to the anthracycline family and is used to treat a variety of cancers such as lung, sarcoma, gastric, breast, thyroid etc. [2]. By blocking the topoisomerase II enzyme, DOX remove histone protein from chromatin and stops DNA replication [3]. DOX causes cell damage by breaking the DNA chain, inhibiting macromolecule production, and producing hydroxyl radicals. Also, it causes production of reactive oxygen species (ROS) [4]. In DOX neurotoxicity, ROS cause oxidative stress and impair the antioxidant system. DOX-induced damage has been demonstrated to MTT and LDH. MDA expression in the brain has been shown to be dramatically increased by oxidative stress [5].

Cognitive problems are seen in the majority of DOX-treated cancer patients [6]. The majority of patients receiving chemotheraphy drugs like DOX may experience cognitive deficiencies that show up as difficulties with memory, attention, learning, and thinking. Chemo-brain or chemotherapy-related cognitive impairment is the term used to describe the phenomena of cognitive decline brought on by chemotherapy. DNA damage, oxidative stress, inflammation, mitochondrial dysfunction, apoptosis are the consequences of DOX-mediated neurotoxicity. The hippocampus has a pivotal role in memory and its changes in pathological conditions are still unknown [7]. In these study, we have used HT-22, a sub-line generated from parent HT4 cells that were first immortalized from primary mouse hippocampal neuronal culture, a commonly utilized hippocampal neuronal cell line.

* Corresponding author: Betul Danisman

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Chemotherapy caused a decrease in tasks in the hippocampus and other areas of the brain, as well as increased cell death, according to animal research. White matter damage has also been recorded [8, 9]. It has also been shown that DOX treatment cause oxidative stress, inflammation, and endoplasmic reticulum stress in brain and hippocampus [10]. All these are well-defined dose-limiting side effects limiting clinical usefulness.

AGM has anticancer, antioxidant, anti-inflammatory, and anti-apoptotic effects [11]. It also has neuroprotective, hepatoprotective, cardioprotective, and testicular-protective properties [11-13]. It is a melatonin analog that is now used to treat depression and sleep disorders. Melatonin is a well-known antioxidant and anti-inflammatory substance. AGM has been shown in previous research to reduce neuroinflammation and neurodegeneration [14]. It was discovered in another study that it has a neuroprotective impact by lowering oxidative stress and enhancing antioxidant capacity [11]. The epilepsy investigation discovered a protective effect on the cerebral cortex and hippocampus [15]. It may also play a significant neuropharmacological role in the treatment of neurological diseases [11]. AGM’s potential neuropharmacological effect has been observed in a variety of disorders including Alzheimer’s, depression, epilepsy, and Parkinson’s [16].

In light of the foregoing, we set out to investigate the effects of AGM on oxidative stress, inflammation, and apoptosis in DOX-induced hippocampal damage. The effects of DOX on hippocampus structure and metabolic changes are still poorly understood, despite several studies suggesting reduced cognitive performance and memory impairments following DOX treatment. Therefore, the purpose of the current study was to look into how DOX affected the indicators of oxidative stress and apoptosis in hippocampus cells.

2. Material and methods

2.1. Cell culture

The mouse hippocampal neuronal cell line HT-22 was kindly provided by Asst. Prof. Caner Gunaydin (Samsun University, Samsun). Cells were seeded into 96-well plates at density of 1 × 10^4 cells/well and left to attach overnight. DOX (1 µM) was applied to each well for half an hour [17]. Then, different concentrations of AGM (5, 10 and 20 µM) were applied to HT-22 cells and left for 24 hours of incubation (5% CO₂, 95% humidity, and 37 °C), and the effect of the active ingredient against DOX toxicity was evaluated. The media was withdrawn after drug treatments, and cells were then exposed to MTT (1 mg/ml) solution for 4 hours in the dark [18]. Cell viability was measured after purple formazan crystals were dissolved in DMSO (100 µl) and standardized to control values applied [19, 20]. Cell viability (%) was calculated by optical density read at 570nm using the Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific, Canada, USA) [21]. On the other hand, LDH produced from cells with broken membranes was used to measure cell death in accordance with the manufacturer’s instructions (Elabscience, USA). Using a spectrophotometer plate reader (Multiskan GO, Thermo Scientific, USA), the optical density was calculated at 450 nm.

2.2. Oxidative stress and apoptotic parameters

MDA, SOD, CAT and Caspase-3 were determined with a commercial kit (Elabscience, USA) with respect to manufacturer directions as described before [21, 22]. Optical density was evaluated at the wavelength of 450nm.

2.3. Quantitative Reverse Transcription Polymerase Chain Reaction for Gene Expression

Table 1 Primers for qPCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Caspase-3-F</td>
<td>5’-TGGGACTGATGAGGAGA-3’</td>
</tr>
<tr>
<td>Caspase-3-R</td>
<td>5’-ACTGGATGAACCACGAC-3’</td>
</tr>
<tr>
<td>GAPDH-F</td>
<td>5’- CCTTCCCGTGTTCCTAC-3’</td>
</tr>
<tr>
<td>GAPDH-R</td>
<td>5’- GACAACCTGGTGCCCTCA-3’</td>
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The same procedures for seeding and treating HT-22 cells were used. Following that, mRNA was used for the cDNA synthesis kit (Thermo Scientific, USA) as previously mentioned [22], and total RNA was extracted using an RNAseasy kit (Thermo Scientific, USA) in accordance with the manufacturer’s instructions. Rotor-Gene 6000 (Corbett Life Science, Mortlake, Australia) was used to determine the cells’ levels of Caspase-3 mRNA expression. GAPDH was used as the
reference gene. The expressions of Caspase-3 were normalized to GAPDH using the 2−ΔΔCt technique. Table 1 lists the primer sequences.

2.4. Statistical analysis
All analyzes were performed using one-way analysis of variance (ANOVA) with Tukey post hoc test (IBM SPSS 22.0, Corp., Armonk, NY, USA) (p < 0.05). Data are shown as mean ± SD.

3. Results

3.1. Effect of Agomelatin on DOX-induced reduction of cell viability
Hippocampal neuronal cells treated with 1 µM DOX caused a 47 % reduction in cell viability, as seen in Figure 1A (p<0.001). AGM protected cell viability between 10-20 µM dose (p<0.001). As seen in Figure 1A, treatment with 10 and 20 µM AGM significantly increased cell viability when compared to the DOX group. On the other hand, 5 µM AGM demonstrated partial protection from the deleterious effects of DOX on HT22 cells; however; this was not statistically significant. Additionally, compared to the control group, DOX induced notable LDH release from the cells (p<0.0001). LDH release was significantly reduced in 10 and 20 µM AGM compared to DOX (p<0.01 and p<0.05, respectively; Figure 1B). It indicates that the protection of the impairment to membrane integrity induced by DOX. Similar to the MTT results, the 5 M AGM concentration was not significantly different from DOX in LDH levels.

3.2. Effect of Agomelatin on DOX-induced oxidative damage
The DOX group had the greatest MDA level, as seen in Figure 2 (p < 0.001). In all concentrations of AGM treatment, the DOX-induced increment in MDA levels decreased. In comparison to the DOX group, the reduction in MDA levels in the 10 and 20 µM AGM groups was statistically significant (p<0.05, p<0.01, and p<0.01 respectively). According to Figure 2, the ethanol group had the lowest levels of SOD and GSH (p<0.001). The administration of all concentrations of AGM also exacerbated the reduction in SOD and CAT levels caused by DOX.

As shown below in Figure 2A, the highest MDA level was observed in the MDA group (p < 0.001). Same time the increase of MDA level caused by DOX decreased in all concentrations of AGM treatment groups. The reduction in the MDA levels in the 10 and 20 µM AGM groups were statistically significant compared DOX group (p < 0.01, and p < 0.05; respectively). As shown in Figure 2B and 2C, the SOD and CAT levels was the lowest in DOX group (p < 0.001). In addition, the decrease in SOD and CAT levels caused by DOX increased as a result of the application of all concentrations of AGM.
Figure 2 Agomelatin effects on oxidative stress marker MDA (A) and the antioxidant enzymes SOD (B) and CAT (C). Data are expressed as the means ± SD. *** p < .001 vs. control group, # p < 0.05, ## p < 0.01 vs. DOX group. AGM, agomelatin; DOX, Doxorubicin.; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

3.3. Effect of Agomelatin on DOX-induced Apoptotic Marker

Caspase-3 expression was markedly up-regulated in the DOX group compared with the control group (p<0.001). At all concentrations of AGM were significantly decreased in neuronal culture compared with that of DOX (Figure 3).

Figure 3 Agomelatin effects on apoptotic marker Caspase 3 expression. Data are expressed as the means ± SD. *** p < .001 vs. control group, # p < 0.05, ## p < 0.01 vs. DOX group. AGM, agomelatin; DOX, Doxorubicin

4. Discussion

Chemotherapy has been linked to toxicity in the brain and many regions of the brain [4]. DOX toxicity mechanisms involve cellular toxicity, disruption of cell metabolism, oxidative stress as well as eventual degradation of brain function [23]. This neurodegenerative effect is caused by DOX’s potential cellular toxicity. Understanding molecular and cellular processes relevant to a disease pathogenesis can require studies employing in vitro cellular models, which can be a crucial and sometimes indispensable part of this process. Memory is greatly influenced by the hippocampus. Among the well-known neuronal cell lines, HT-22 is one of the often utilized hippocampus neuronal cell lines that is employed in a variety of memory-related investigations [18, 24]. In this study, DOX-induced hippocampal damage was minimized by
AGM. AGM also reduced oxidative stress in the hippocampus neuronal cell line HT-22 and prevented DOX-induced apoptosis. AGM was found to be protective against DOX-induced neurotoxicity in our investigation. Our results suggest that it may be necessary to use DOX in conjunction with AGM to minimize neurotoxicity.

LDH is released into the extracellular space as a result of neuron damage [22]. In HT-22 cells treated with DOX, we evaluated the amount of LDH present. We discovered that pretreatment with 10 and 20 μM of AGM significantly boosted metabolic activity and decreased LDH release. Therefore, these results show that AGM may suppress oxidative damage and apoptosis linked to DOX-toxicity. We also looked at how pretreatment with AGM affected the DOX-induced changes in antioxidant enzymes and the production of lipid peroxidation in HT-22 cells. In this work, DOX-induced oxidative damage appeared as an increased MDA level and caspase 3 expression, also a noticeably decreased CAT and SOD level.

Oxidative stress is induced by an imbalance of oxidants and antioxidants in the tissue. It is also linked to the production of excessive free radicals [25]. NADPH-cytochrome P450 reductase causes free radical production by DOX. This occurs as a result of single-electron activation [10]. DOX's harmful action is caused by the production of free radicals. Cells suffer oxidative damage as a result of DOX treatment [25]. The generation of free radicals and oxidative damage as a result of anticancer drug treatment causes side effects in the brain and other healthy tissues [26]. Previous research has shown that free radicals produced by DOX increase oxidative stress in the brain and raise apoptosis [27, 28]. The generation of ROS and the depolarization of the mitochondrial membrane in neurons are two processes that doxorubicin promotes to produce neurotoxicity [29]. DOX causes mitochondrial degradation and dysfunction in the neurons by raising the mitochondrial outer membrane permeabilization and Bax/Bcl-2 ratio [30]. Hydrogen peroxide can cause neuronal degeneration, both endogenously and ectopically, according to prior research [31]. By increasing the Bax/Bcl-2 ratio on mitochondrial membranes, DNA fragmentation following DOX treatment is a potent inducer of intrinsic or mitochondrial apoptotic pathways [29, 31]. Additionally, it can cause the release of cytochrome C and an increase in mitochondrial outer membrane permeability, which increases the caspase-dependent intrinsic apoptotic pathways. The DOX-induced impairment of mitochondrial activity in the hippocampus was discovered to be associated with increased levels of mitochondrial ROS and calcium dysregulation [32]. One of the main causes of cognitive impairment is oxidative stress and neuron damage brought on by aberrant mitochondria.

It has been discovered that DOX-induced apoptosis in primary cortical neurons depends on the exogenous route. DOX is a direct apoptotic mechanism that is mediated via the exogenous apoptotic pathway. The integration of mature neurons in the circuit is crucial to hippocampal neurogenesis because the hippocampus is one of the brain regions directly linked to spatial processing and memory formation [33]. DOX-treated animals demonstrated a clear reduction in neurogenesis. Our study showed that an important apoptotic marker increased in DOX-treated group and these effects were reversed with AGM.

When we investigated the effect of DOX on oxidative parameters, we discovered that SOD and CAT decreased dramatically in the DOX group and increased when AGM was administered. Other investigations have found that AGM lowers the elevated MDA caused by DOX and has an anti-oxidant impact. AGM was found to attenuate apoptosis in hippocampus after injury in another study [34, 35]. AGM inhibits DOX-induced inflammation in the hippocampus, indicating that it may have neuroprotective properties. In our investigation, caspase-3 expression dramatically increased in the DOX group. In the AGM-treated groups, this rise was dramatically reduced.

Oxidative stress caused by free radicals produces significant neurological damage. When DOX is administered, the brain produces ROS and so oxidative stress. Consequently, production of ROS interferes with the antioxidant system. DOX-induced neurotoxicity reduced antioxidant enzymes in the brain [23]. The antioxidant capacity in the brain was found to be reduced in the current investigation as a result of DOX treatment. The antioxidant capacity lowered by DOX was shown to increase again in the group treated with AGM. Furthermore, we have convincingly proven that DOX enhanced the level of MDA in the brain while decreasing the level of SOD and CAT. AGM was reported to reduce inflammation and boost antioxidant capacity in motor neurons in the ischemia-reperfusion model [36]. AGM was found to boost antioxidant capacity in psychosis-relevant behavior model [37]. AGM has been found to specifically boost antioxidant capacity in various areas of the brain [18, 36]. AGM has been proven to improve antioxidant capacity in the hippocampus in the LPS-induced neuro-inflammation [38].

Lipid peroxidation, apoptosis and oxidative stress were shown to be higher in the HT-22 hippocampal cell line in our study. In keeping with these findings, we found that DOX affects peroxidation, apoptosis and oxidative stress in hippocampus and that AGM blocks the pathways that produce this damage in neurons.
5. Conclusion

In conclusion, we investigated the effect of AGM on DOX-induced neurotoxicity. We discovered that DOX increased oxidative stress in the hippocampal cell line. AGM is neuroprotective, as evidenced by its antioxidant, anti-inflammatory, and anti-apoptotic effects in several areas of the brain. In addition to demonstrating the importance of AGM as a new therapy agent in minimizing brain damage induced by the use of chemotherapeutic medicines. Our study highlights the necessity for more research to determine alternative mechanisms to investigate AGM’s potential neuroprotective properties. We believe that our research will be useful in providing light on further studies and determining the role of AGM in neurodegenerative illnesses.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

References


