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Varenicline enhances the survival of doxorubicin-treated mice

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Abstract. – OBJECTIVE: Doxorubicin (DOX) is widely used to treat various types of cancer. However, DOX treatment increases oxidative stress and causes other undesirable effects, such as cardiotoxicity, cognitive impairment, and death. Nicotine has been shown to inhibit DOX-induced cytotoxicity in vitro by activating nicotinic acetylcholine receptors. This study aimed to investigate whether combined treatment with varenicline, a partial agonist of nicotinic acetylcholine receptors, increased the survival rate of DOX-treated mice.

MATERIALS AND METHODS: Forty male albino mice were divided into four groups of 10. Control-group mice received a single intraperitoneal (i.p.) injection of 0.9% saline. The DOX group received a single dose of DOX (20 mg/kg body weight, i.p.). The varenicline group received varenicline daily in their drinking water at 0.1 mg/mL. The DOX+varenicline group received a single dose of DOX (20 mg/kg body weight, i.p.) and daily administration of varenicline in their drinking water (0.1 mg/mL). Mice were observed daily to evaluate the survival rate, and their body weight was recorded on alternate days.

RESULTS: All mice treated only with DOX died within 8 days. Co-administration of varenicline with DOX slightly improved the survival time and rate compared with the DOX-only group.

CONCLUSIONS: Combined treatment with varenicline and DOX may be useful for improving survival relative to treatment with DOX alone. This may be because varenicline is an α7-nicotinic acetylcholine receptor agonist; however, further research into its precise mechanism of action is required.

Key Words:
Varenicline, Doxorubicin, Survival rate, Bod weight, Mice.

Introduction

Chemotherapy can prolong survival in some cancer patients[1]; however, there are limitations pertaining to the use of some anticancer drugs[2-4]. Doxorubicin (DOX) is a frequently used chemotherapy drug; it inhibits DNA replication and promotes the apoptosis of tumor cells. However, DOX also increases oxidative stress and causes other undesirable effects, such as cardiotoxicity, hepatotoxicity, nephrotoxicity, cognitive impairment, and even death[2,3,5-8]. Myocardial damage is particularly common with DOX therapy[9], with an incidence of 7% to 65% for cumulative doses of 150 to 550 mg/m^2. It is proposed that DOX increases the oxidation of a variety of cellular molecules, including phospholipids, which are associated with increased cell death[10,11]; however, the underlying mechanisms by which DOX induces cytotoxicity are not completely understood.

The co-administration of some drugs with DOX may reduce its cytotoxicity; for instance, the antioxidant drug dexrazoxane can reduce the generation of free radicals caused by DOX use[12]. Additionally, the antidiabetic drug metformin was shown to prolong the survival rate of mice with acute DOX cardiotoxicity[13]. On the other hand, nicotine was found to inhibit DOX-induced cognitive impairment by activating the α7-nicotinic acetylcholine receptor (α7-nAChR) and the α4β2-nAChR[14]. At least nine α and three β subunits of the nAChR have been identified in the brain, with the α7 homometric and α4β2 heterometric nAChRs being the major two subtypes. The mechanism by which nicotine provides neuroprotection has been attributed to both the α4β2 and α7 subtypes. In fact, studies have indicated that activation of the α7-nAChRs activates the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, which, in turn, upregulates anti-apoptotic proteins (e.g., B-cell lymphoma 2 [Bcl-2] and Bcl) to provide neuroprotection[15]. Therefore, activation of specific nAChRs in the brain may help alleviate some of the detrimental effects observed with DOX.

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Varenicline is a heterocyclic compound used to combat nicotine-withdrawal symptoms[16,17], as it interacts with the mesolimbic and mesocortical pathways in the brain by acting as a partial α4β2-nAChR agonist and a full α7-nAChR agonist[18,19]. It also has partial agonistic activity on the α3β4 and α6β2 nAChR subtypes. When activated by nicotine in the nucleus accumbens, α4β2-nAChRs release dopamine, which is a neurotransmitter known to regulate conditioned learning and reward-motivated behavior. Consequently, nicotine addiction can result as the α4β2-nAChRs are upregulated, meaning compulsive use of nicotine is required to maintain homeostasis. In this way, partial agonists of the α4β2-nAChR, such as varenicline, can be used to reduce cravings in smoking cessation as they allow the release of dopamine, but to a less significant degree than nicotine. Varenicline's agonism of the α4β2-nAChR results in a dopamine release that is approximately 50% of that released by nicotine, and it does not cause α4β2-nAChR upregulation[16].

The α7-nAChR is involved in inflammatory responses (*via* the cholinergic anti-inflammatory pathway) and cancer (proliferation, angiogenesis, metastasis, and inhibition of apoptosis)[20]. Furthermore, based on the anti-apoptotic effect of nicotine, another α7-nAChR agonist (PNU-282987) was shown to exert an anti-apoptotic effect in astrocytes[21]. Due to its activity as a partial α4β2-nAChR agonist and a full α7-nAChR agonist, varenicline may help protect against DOX-induced toxicity in the same way as nicotine and other nAChR agonists. Indeed, clinical and preclinical studies[22,23] have reported that varenicline improves cognitive function (e.g., in schizophrenia and Huntington's disease) by acting through nAChRs. Furthermore, varenicline is generally well tolerated, although it commonly causes mild-to-moderate nausea in users, and it has no known clinically relevant drug-drug interactions[17]. It should be noted, however, that although several reviews have shown no increased risk of neuropsychiatric side effects or serious adverse cardiovascular events, it is not advised for those with a history of cardiovascular disease or psychiatric illness due to some reports of detrimental effects, including suicidal thoughts[16]. In addition, as varenicline is mostly excreted unchanged in urine by the kidneys, it is not recommended for those with severe kidney failure.

Despite emerging evidence of the role of the α7-nAChR in cell survival, the effect of varenicline on DOX-induced cell death has not yet been reported. Therefore, this study aimed to assess whether varenicline could mitigate the toxic effects of DOX treatment in mice and increase the survival rate of non-nicotine-addicted animals. The secondary aim was to identify a new co-treatment that may reduce the toxic effects of DOX.

Materials and Methods

Ethical Approval of the Study Protocol

Ethical approval of the study protocol was obtained from the research unit of the College of Pharmacy, Qassim University (2020 - CP – 5) in Al Qassim, Kingdom of Saudi Arabia.

Chemicals

DOX (ADRIM®) was obtained from Fresenius Kabi Oncology (India). Varenicline (CHAMPIX®) was purchased from Pfizer (New York, NY, USA).

Animals

Forty male albino mice (8-10 weeks old, weight) were housed individually in cages. They were exposed to a 12-h light/dark cycle (lights on at 6 am) and had unlimited access to water and food. Animals were observed daily to check survival, and their body weight was measured on alternate days.

Grouping and Drug Administration

Mice were divided into four groups of 10. Control-group mice received a single intraperitoneal (i.p.) injection of physiologic (0.9%) saline. Mice in the DOX group received a single dose of DOX (20 mg/kg body weight, i.p.). Animals in the varenicline group received varenicline (administered daily by dissolving in drinking water at a concentration of 0.1 mg/mL. Mice in the DOX+varenicline group received a single dose of DOX (20 mg/kg body weight, i.p.) and daily administration of varenicline (at 0.1 mg/mL in drinking water).

Statistical Analysis

Data were collected and analyzed using one-way analysis of variance. Results are the mean ± the standard error of the mean (SEM). A p-value of less than 0.05 was considered significant.

Results

Varenicline Improves the Overall Survival Rate of DOX-Treated Mice

Mice treated with DOX+varenicline showed a significantly increased survival rate compared to DOX-treated mice ($p < 0.05$; Figure 1). For example, all mice treated with DOX+varenicline survived for at least 4 days, while 20% of mice treated only with DOX had died by day 4. Furthermore, at the end of the study period (9 days), 100% of mice in the DOX-only group had died, while 20% of mice in the DOX+varenicline group survived. All mice in the control and varenicline-only groups survived until the end of the study period. Thus, combining DOX with varenicline appears to reduce the toxic effect of DOX in mice.

Varenicline Does Not Improve DOX-Induced Weight Loss

The body weights of mice in the DOX and DOX+vareni cline groups were significantly lower than those of mice in the control and varenicline groups (Figure 2). The body weight of mice in the varenicline-only group was also lower than the body weight of mice in the control group.

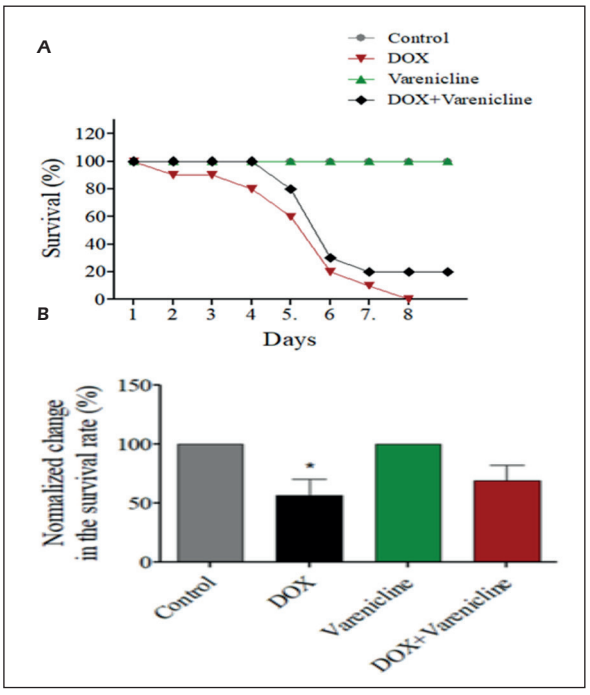

Figure 1. Varenicline increased the survival rate of DOX-treated mice. A single dose of DOX (20 mg/kg bodyweight) was administered intraperitoneally and varenicline was administered orally daily by dissolving in drinking water (0.1 mg/mL). **A,** Shows the survival rate over 9 days; **B,** shows the survival rate for the last 3 days of the study (*$p <$ 0.05 compared with control animals).

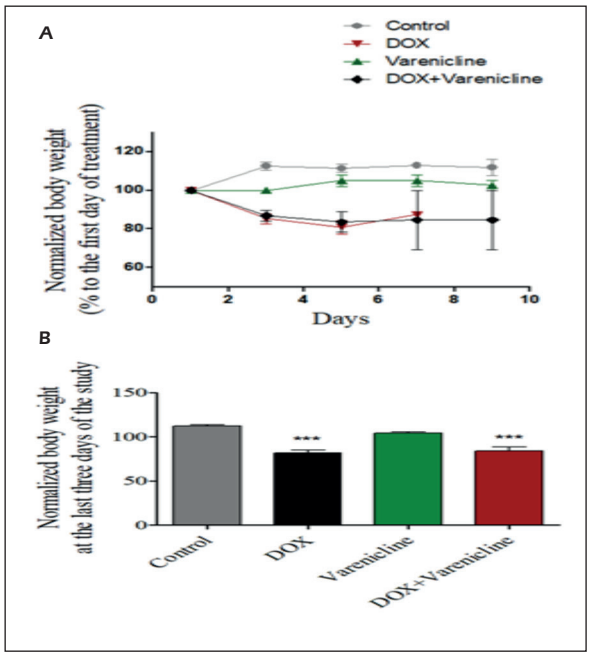

Figure 2. Effects of DOX and varenicline on the bodyweight of mice. A single dose of DOX (20 mg/kg bodyweight) was administered intraperitoneally and varenicline was administered orally daily by dissolving in drinking water (0.1 mg/mL). Bodyweight was monitored every 2 days. Data are the mean ± SEM (n = 10 per group) and were normalized to the mean bodyweight on the first day of treatment. **A,** Illustrates the bodyweight changes over 9 days. **B,** Shows the bodyweight changes for the last 3 days of the study (***$p <$ 0.05 compared to control animals).

Discussion

This study is the first to demonstrate mitigation of the toxic effects of DOX in mice by varenicline co-treatment by evaluating the survival rate of animals. Although the DOX-treated mice had a lower survival rate than those treated with DOX+varenicline, the combined treatment did not entirely prevent death. As one of the major adverse effects of DOX is cardiotoxicity[2], this was likely the cause of death in DOX-treated mice. Therefore, additional experiments using a sublethal endpoint, such as the development of cardiomyopathy, are warranted. Although the dose used in the present study was clinically relevant to the dose used in cancer patients

undergoing chemotherapy[24], the death rates in mice were significantly higher in DOX-treated mice *vs.* those receiving a combined treatment with varenicline. Therefore, using a lower dose of DOX that produces the same toxic endpoint in mice, as is observed in the clinic, may help ascertain the benefits of the combined therapy on DOX-induced cytotoxicity. Despite these limitations, the mice used in the study were of the same strain and similar age, so any variation in study outcomes due to age and strain was minimized.

DOX-treated mice survived for up to 7 days in this study, during which they showed a generally continuous reduction in body weight. In the varenicline-only group, the body weight of the mice remained the same as day 1 but, by the end of the study, it was significantly lower than that of the control group. This result is not surprising as one of the adverse effects of varenicline is reduced appetite[25]. Weight loss in the DOX+varenicline group appeared similar to that of the DOX-only group. Thus, from the point of view of (alleviation of) DOX toxicity, co-treatment with varenicline had no effect on DOX-induced weight loss. The mechanisms by which varenicline may interfere with DOX toxicity to increase the survival time are not well understood, and additional research is required. Furthermore, the mechanisms for DOX-induced toxicity are complex and several have been proposed, including the influence of DOX on the mitochondrial electron transport chain, redox cycling, oxidative stress, calcium dysregulation, and apoptosis pathways[26]. However, based on structural analyses it may be hypothesized that varenicline exerts its effect in a similar manner to nicotine through activation of the nAChRs. In this regard, it is important to note that DOX enhances the activities of pro-apoptotic proteins like Bcl-2-associated X (Bax) and caspase-3[27]. Bax is a mitochondrial protein that regulates cytochrome *c* release from mitochondria, which ultimately activates caspase-3 and subsequent apoptosis processes[28]. In contrast, activation of the α7-nAChR with an agonist such as varenicline can reduce the H_2O_2-induced expression of Bax and caspase-3, and increase Bcl-2 expression, thereby preventing apoptosis[21] and potentially ameliorating the overly toxic effects of DOX. In addition, nicotine has been shown to induce the phosphorylation of Bcl-2-associated agonist of cell death (Bad) and Bax through the PI3K/AKT pathway, resulting in reduced apoptosis, and varenicline may act through this pathway in a similar way. Other researches[29,30] have shown α7-nAChR agonism can reduce the production of the pro-apoptotic proteins glycogen synthase kinase 3 beta (GSK-3β) and caspase-3 through the PI3K/AKT signaling pathway. α7-nAChR activation was also shown to reduce neuroinflammation and oxidative stress in mice with ischemic stroke and bone fracture[31]. Therefore, it may be hypothesized that α7-nAChR activation by varenicline may help reduce the oxidative stress associated to DOX, thus extending survival time, although this conjecture requires further research. Evidence also indicates that α7-nAChR agonists may promote the survival of acutely isolated or cultured retinal ganglion cells through modulation of GABAergic synaptic transmission. This suggests that nAChR agonists may confer neuroprotection during DOX treatment by suppressing excitotoxic processes[32].

It should be noted that research has suggested that nicotine interacts with DOX in several other ways, and varenicline may also act through these pathways. For example, nicotine is known to affect cellular ATP production, upon which apoptosis is dependent[33,34]. In addition, nicotine inhibits iron uptake and iron transport, which may affect the interaction between DOX and iron that is required for free-radical generation[35]. Thus, nicotine may confer protection against the oxidative stress associated to DOX through this pathway also. On the other hand, it has been well established that nicotine can increase the production of reactive oxygen species (ROS) and itself lead to oxidative stress. It has consequently been hypothesized through structural analyses that varenicline may also increase the production of ROS, although evidence of this is lacking[36]. Notably, varenicline has been shown to increase Mn superoxide dismutase (MnSOD), which is a mitochondrial antioxidant enzyme, and this increase may be sufficient to prevent oxidative damage, thus ameliorating oxidative stress and potentially enhancing the survival time of DOX-treated animals[36]. Given the complexity of the mechanisms involved, the mitigating effects of α7-nAChR agonists like varenicline on toxicity during chemotherapy should be further investigated.

There are some limitations to this study. Wild-type, cancer-free mice were used to evaluate the direct effects of DOX and varenicline treatment without the compounding effect of cancer itself. The end point was death, rather than a sublethal effect, such as the development of cardiotoxicity. The DOX dose used here meant that, even in the DOX+varenicline group, 80% of the mice died (compared with 100% in the DOX-only group).

Moreover, it is worth mentioning that, as α7-nAChR activation promotes cell viability during DOX therapy, it may also contribute to the development of chemoresistance[37]. Indeed, nicotine treatment combined with DOX therapy was found to reduce DOX-mediated cytotoxicity in human breast cancer cells[38]. Therefore, the optimal doses and formulations for a combined drug regimen that provides an appropriate tumor-killing effect while reducing the off-target cytotoxicity must be determined in the future.

Conclusions

Varenicline has been established as a safe therapeutic to assist smoking cessation and nicotine-withdrawal symptoms. However, its potential as an adjunct in the treatment of cancer with DOX to reduce the toxic side effects and increase survival has not been studied previously. The results presented here show for the first time that varenicline provides some mitigation of the toxic effect of DOX in mice and increases the survival time; however, further research into the underlying pharmacology is required to determine its potential clinical use.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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