Antioxidant effects of dexmedetomidine against hydrogen peroxide-induced DNA damage in-vitro by alkaline Comet assay

Abstract

Background/Aim: Dexmedetomidine (DEX) is a alpha-2 adrenergic agonist that is commonly used as a sedative and an anesthetic. DEX’s protective effects against oxidative damage in both in-vitro and in vivo conditions have been demonstrated. It was aimed to evaluate and compare the protective effects of DEX and Vitamin C (Vit C) on DNA against H2O2-induced DNA damage in human lymphocyte cell cultures in vitro by alkaline Comet assay.

Materials and Methods: Lymphocyte cell cultures were divided into five groups as negative control; solvent control; positive control; H2O2(150μM) + DEX(1μM;2.5μM;5μM); H2O2(150μM) + Vit C (1μM;2.5μM;5μM) and incubated at 37°C for 1 hour. Cell viability was measured by Trypan Blue test. DNA damage was measured by Alkali Comet Technique and % tail intensity was evaluated. Statistical analysis was performed by one-way analysis of variance and Tukey’s multiple comparison test.

Results: It was observed that H2O2 significantly induced DNA damage in lymphocytes and this damage is decreased significantly with Vit C and DEX. It was observed that Vit C at 1μM and 2.5μM doses had a significantly stronger antioxidant effect, but there was no significant difference between the antioxidant effects of Vit C and DEX with 5μM doses. The concentration of 5μM DEX was found to be most effective in reducing oxidative DNA damage.
Conclusion: There is limited data on protective effects of DEX against oxidative DNA damage. The primary effect might be cytoprotection. Our results showed that dexmedetomidine is protective against H2O2 induced in vitro oxidative DNA damage in lymphocyte cell cultures in a dose-dependent manner. DEX might have a potential therapeutic value in the prevention of oxidative DNA damage in patients.

Keywords: Antioxidant, dexmedetomidine, DNA damage
1. **Introduction**

Dexmedetomidine (DEX) is a highly selective alpha-2 adrenergic receptor agonist that is commonly used in clinical practice as a sedative and an anesthetic agent due to its sedative, analgesic, hemodynamic stabilizing and diuretic effects [1,2]. In addition to its sedative and anesthetic effects, anti-inflammatory and antioxidant effects on vital organs such as heart [3,4], lung [5,6,7], kidney [8], spinal cord [9] and brain [10] have been demonstrated. DEX has anti-inflammatory and protective effects against oxidative damage shown in both in-vitro and in-vivo conditions [11,12]. It shows these effects probably by inhibiting the toll-like receptor (TLR) [4,13], suppressing high mobility group box 1 (HMGB1) factor [14], inhibiting the nuclear factor (NF)-κB and phosphoinositide-3 kinase (PI3K-) signaling pathway [3,15].

Oxidative stress; that is induced by ischemia, mechanical stress or toxins; is a condition that results from the imbalance between production of reactive oxygen species (ROS) and free radicals as well as inappropriate antioxidant functions. The ROS induced oxidative stress in cells triggers a mechanism that, through the release of cytochrome c and activation of caspase-3, leads to intrinsic apoptosis. ROS plays a critical role in maintaining homeostasis and cell signaling [16]. Hydrogen peroxide (H2O2), a reactive ROS derivative, is considered to be the most responsible radical for oxidative damage. It is widely used to mimic in vitro oxidative stress in many different cell types [17].

ROS can lead to DNA-strand breaks by loss of DNA bases, known as apurinic/apyrimidinic sites, and inhibit transcription. Moreover, the DNA strand break, an indicator of increased oxidative stress, is a complicated process and it is more likely that the body will tend to make mistakes when attempt to repair [18]. There are many
antioxidant and DNA repair systems that protect the organism from undesirable consequences of DNA damage. Although these systems work perfectly throughout life, there may be conditions such as disease or aging that lead to increased levels of DNA damage and some external protections such as vitamin administration would be needed [19]. Therefore, some medications such as DEX, which may also have some protective effects besides their crucial effects, are worth focusing on in terms of patient health.

In the present study we used alkaline Comet assay to detect DNA damage in lymphocytes. The alkaline Comet assay, which detects single-strand breaks (SSBs) as well as alkali-labile sites, is one of the most popular techniques to detect DNA damage for the last decades. The Comet assay may be conducted in vitro using single cells from immortalized cell lines or in vivo for any tissue that can be dispersed to a single cell suspension. In the Comet assay, the damaged DNA migrates away from the undamaged DNA-containing nucleoid body, resembling the structure of a Comet during electrophoresis. The percentage of DNA in the tail is directly proportional to the percentage of DNA damage and therefore could be measured [20]. In this study, it was aimed to evaluate the antioxidant capability of DEX against H2O2-induced DNA damage in human lymphocyte cell cultures in vitro by alkaline Comet assay. On the other hand this effect was compared with Vitamin C (Vit C) which is one of the best known antioxidants against DNA damage.
2. Materials and Methods

2.1. Chemicals

Vitamin C and DEX were obtained from Redox-C 100mg/ml, (Bayer, Turkey) and Hipnodex 200mcg/2 ml (Haver Farma, Turkey), respectively. Other chemicals and reagents used in experiments were provided by Sigma-Aldrich Chemical Co. (St. Louis, USA).

2.2. Sample collection and lymphocyte isolation

The study was approved by the Ethics Committee of University of Health Sciences, Ankara Health Training and Research Hospital (Date: 01.08.2018, Approval No: 052). Peripheral blood from three healthy donors (nonsmokers, 29, 33 and 49 years old) was collected after all the subjects signed an informed consent form and filled out the questionnaire. The questionnaire contains certain information about their demographic characteristics and general health status. Subsequently, lymphocytes were isolated using the density gradient centrifugation technique [21].

2.3. Viability test

Prior to initiating experiments, exposure to H₂O₂, Vit C, and DEX was assessed individually on lymphocytes in terms of cell viability. Final concentration levels of treated lymphocytes were 50, 100, 150 μM for H₂O₂ and 1, 2.5, 5 μM for Vit C and DEX. Compounds were dissolved in 0.9% NaCl solution to prepare stock solutions and their diluted solutions. The treated lymphocytes were incubated for 1 h at 37 °C and then the trypan blue exclusion test was performed [22]. The number of unstained/total cells were determined using a hemocytometer under a light microscope.
2.4. Treatments

For determining DNA damage, lymphocyte cultures were performed in 5 groups including negative control (water), vehicle control (0.9% NaCl), positive controls (150 µM H$_2$O$_2$) as well as 1, 2.5, 5 µM of DEX or Vit C together with 150 µM of H$_2$O$_2$ for 1 h at 37 °C. The final concentration of compounds in the medium was adjusted to 1% v/v. Three independent experiments with the samples from three donors in duplicate were performed. After the incubation process, the lymphocytes were centrifuged for 3 min at 200 x g and isolated.

2.5. Comet assay

For the detection of DNA damage, the alkaline version of the Comet assay was performed as described by Singh et al. (1988) with minor modifications [23]. Briefly, 100 µL of treated lymphocytes were mixed with 100 µL of 1% low melting point agarose (LMPA) at 37 °C and was spread to a slide pre-coated with 1% normal melting point agarose (NMPA) and immediately covered with a coverslip. Duplicate slides were made for each sample. After gel solidification, the slides were immersed in lysis solution (2.5 M NaCl, 0.1 M Na$_2$EDTA, 10 mM Tris HCl, pH 10, 1% Triton X-100) and kept at 4 °C overnight. The slides were incubated in a cold electrophoresis solution (0.3 M NaOH, 1 mM EDTA, pH > 13) for 20 min to allow DNA unwinding. Then, electrophoresis was performed at 4 °C, 25 V and 300 mA for 20 min. After that, the slides were washed with neutralization buffer (0.4 M Tris, pH = 7.5) and stained with ethidium bromide (20 µg/ml). A total of 300 cells randomly selected per treatment were examined using the fluorescence microscope (Zeiss AxioScope, Germany) at 400 × magnification for image analysis (Comet Assay III image analysis system...
(Perceptive Instruments, UK). To determine the DNA damage, the percent tail intensity
(also known as % tail DNA) was used.

2.6. Statistical analysis

Data on viability were displayed as a percentage of the control which was not exposed to H$_2$O$_2$, Vit C or DEX. Values are mean ± SEM of three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparisons test using the GraphPad Prism version 7 (demo version). p < 0.05 was considered as significant.
3. Results

3.1. Viability Test

Figure 1 shows the effects of H$_2$O$_2$, Vit C and DEX on lymphocyte viability. In the trypan blue test performed to determine whether Vit C and DEX substances cause a cytotoxic effect on lymphocytes, viability was found to be above 90% in all tested concentrations.

3.2. Detection of DNA damage

It was observed that H$_2$O$_2$ significantly induced DNA damage in lymphocytes and this damage decreased significantly with Vit C and DEX. When H$_2$O$_2$ alone was assessed in 1-hour treatments, H$_2$O$_2$-induced DNA damage exhibited a dose-dependent response (data not shown). Thus, the 150 μM H$_2$O$_2$ which is not cytotoxic but inducing significantly DNA damage, was adopted for the main experiments.

The effect of dexmedetomidine and Vit C in lymphocytes cells was demonstrated over various concentrations (1, 2.5, and 5 μM). Lymphocytes incubated with H$_2$O$_2$ + Vit C and H$_2$O$_2$ + DEX in different concentrations showed significantly decreased DNA damage up to 50% compared to H$_2$O$_2$ treatment alone (p<0.05) (Table 1). Although Vit C and DEX caused an antigenotoxic effect on DNA damage, they were not dose-dependent. It was observed that 1 and 2.5 μM concentrations of Vit C treatments were more effective than DEX treatments at the same concentrations. On the other hand, DEX showed a similar antigenotoxic effect on lymphocytes at 5 μM concentration compared to 5 μM Vit C treatment (p=0.946) (Figure 2).
4. Discussion

In general, oxidative DNA damage induce cytotoxicity leading to multiple organ damages, which may result in multisystem organ failure. Oxidative DNA damage is known to directly induce cytotoxicity and can also alter cell signaling pathways. Interestingly, the source of oxidative injury may be a key to the extent of cellular cytotoxicity [24]. The overwhelming production of oxidative injury threatens the integrity of protein oxidation and leading to DNA strand breaks resulting in tissue damage [25]. The aim of this study was to investigate the effects of the alpha-2 adrenoceptor agonist, dexmedetomidine, against H2O2-induced oxidative DNA damage in-vitro.

Mechanistically, it was previously reported that DEX acts as an anti-inflammatory agent and provides cell protection by increasing expression of cell survival proteins and reducing apoptosis. In addition, DEX has a structure similar to imidazoline and its antiapoptotic effect is enhanced by the activation of imidazoline receptors [26]. It has been demonstrated that TLR4 and NF-κB signaling is involved in the DEX-mediated protection against oxidative injury. Gao et al. used TLR4 knockdown by TLR4-RNA transfection and overexpression by TLR4-DNA transfection in-vitro approaches to explore the mechanisms underlying dexmedetomidine mediated protection [4]. Expression of TLR4 has been shown to be triggered through endogenous ligands, including damage-associated molecular patterns and cytokines. Terminal deoxynucleotidyl transferase-mediated digoxigenin deoxyuridine nick-end labeling which is increased oxidative damage staining to detect dead cells [5]. As a result, dexmedetomidine may have prevented the increased expression of TLR4 by attenuating tissue injury and pre-treatment of dexmedetomine resulted in almost complete
attenuation of TLR4 expression associated with decreased cell death of epithelial cells [4,5].

It has been suggested that human fetal osteoblast cells pretreated with DEX could be protected against H2O2-induced oxidative stress [27]. Cui et al. demonstrated that DEX attenuated the bilirubin-induced injury of epithelial alveolar cells both in vitro and in vivo. In this condition, it is described by reducing the alveolar damage and epithelial cell proliferation by its inhibitory effect on bilirubin induced cell cycle arrest [7]. Moreover, in a study, astrocytes were treated with DEX had significantly increased neurotrophic factor production and shown to preserve cell viability via release of neurotrophic factors compared with control [9]. Although the anti-apoptotic and anti-inflammatory effects of DEX have been reported to be associated with phosphoinositide kinase and extracellularly signal-regulated kinase signaling pathways, [4,5,9,12,28] there is limited data on its protective effects against oxidative DNA damage. In the present study we investigated the antioxidant potential of DEX and compared this effect with Vit C in an in vitro model by Comet assay. Our results showed that DEX might be a protective agent against H2O2-induced oxidative DNA damage in lymphocyte cell cultures in vitro. Therefore the primary effect of DEX might be cytoprotection. This allows DEX to act as an antioxidant against oxidative DNA damage following H2O2 administration. 5µM dexmedetomidine was found to be the most effective concentration in reducing the oxidative DNA damage. Although it starts at higher doses this effect was comparable with Vit C.

The protective effects of DEX which is comparative to a highly effective antioxidant as Vit C might be associated either with phosphoinositide kinase and extracellularly signal-regulated kinase signaling pathways or alteration in transcription
factor control. The mechanisms for altered transcription factor control could be either
via decreased binding to promoter regions via oxidative damage to DNA or more direct
by redox regulation of transcription factor activation [29] and/or altered DNA-binding
due to redox-induced modification of the transcription factor protein [30].

On the other hand, HMGB1, which is strong damage-associated molecular patterns
released from dying cells during oxidative damage, acts by binding to TLR-4 to initiate
downstream NF-B signaling cascade that greatly increases the synthesis of
proinflammatory cytokines [31]. HMGB1 proteins are targeted to particular DNA sites
in chromatin by either protein–protein interactions or recognition of specific DNA
structures. Furthermore, the accumulation of HMGB1 protein is found at sites of
oxidative DNA damage in live cells, thus defining HMGB1 as a component of an early
DNA damage response [14]. As shown in a previous study dexmedetomidine caused
reduced translocation of HMGB1 from the nucleus to the cytoplasm. HMGB1 levels in
the nucleus were significantly reduced after oxidative damage, but addition of
dexmedetomidine significantly increased HMGB1 protein levels in the cytoplasm [14].

In conclusion, our results showed that in vitro oxidative DNA damage in
lymphocyte cell cultures can be prevented by DEX administration. DEX showed
protective effect against H2O2-induced DNA damage in vitro and this effect was
comparable with Vit C which is a known antioxidant. We suggest that DEX might have
a potential therapeutic value in the prevention of oxidative DNA damage in patients.
References


cell death in patients with sepsis, shock and multiple organ dysfunctions. Critical

31. O’Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in
   doi: 10.1038/nri2079.
<table>
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<th>Treatments</th>
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<td>NC</td>
<td>1.75 ± 0.43</td>
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<tr>
<td>VC</td>
<td>1.38 ± 0.35</td>
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<tr>
<td>H₂O₂</td>
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<td>0.0003</td>
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<tr>
<td>H₂O₂+DEX 5 µM</td>
<td>19.84 ± 2.53</td>
<td>&lt;0.0001</td>
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Table. Protective effects of Vit C and DEX on H₂O₂-induced DNA damage on human lymphocytes

NC=negative control; VC= vehicle Control

*compared to VC    #compared to H₂O₂
Figure Legends

Figure 1. Effects of H$_2$O$_2$, Vit C and DEX treatments for 1 hour on viability % of human lymphocytes

Figure 2. Vit C and DEX antigenotoxic effects on lymphocytes incubated with H$_2$O$_2$
Figure 1. Effects of H$_2$O$_2$, Vit C and DEX treatments for 1 hour on viability % of human lymphocytes.

Figure 2. Vit C and DEX antigenotoxic effects on lymphocytes incubated with H$_2$O$_2$. 

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**Figure 1.**

![Graph showing viability percentage vs. concentration of H$_2$O$_2$. The graph compares different treatments: Vit-C and DEX.](image)

**Figure 2.**

![Bar graph showing tail DNA% for different conditions: NC, VC, H$_2$O$_2$, H$_2$O$_2$+Vit C 1 μM, H$_2$O$_2$+Vit C 2.5 μM, H$_2$O$_2$+Vit C 5 μM, H$_2$O$_2$+DEX 1 μM, H$_2$O$_2$+DEX 2.5 μM, H$_2$O$_2$+DEX 5 μM.](image)