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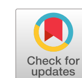
Protective potential of thymoquinone against peroxynitrite induced modifications in histone H2A: In vitro studies



Zafar Rasheed [a,*], Abdullah A. Altorbag [a], Abdulilah S. Al-Bossier [a], Nasser A. Alnasser [a], Omar S. Alkharraz [a], Khalid M. Altuwayjiri [a], Abdulaziz S. Alobaid [a], Ahmad K. Alsaif [a], Yazeed H. Alanazi [a], Bassam A. Alghidani [a], Muath A. Alduayji [a], Ali A. Bu Mozah [a], Sultan A. Alsuhaibani [b]

[a] *College of Medicine, Qassim University, Buraidah, Saudi Arabia*
[b] *Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraidah, Saudi Arabia*

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ABSTRACT

Peroxynitrite ($ONOO^-$) is a reactive oxidant involved in numerous pathological conditions. Thymoquinone (TQ) is an active constituent of *Nigella sativa* and is reported to have anti-disease activities, but its role on $ONOO^-$ has never been investigated. This study was undertaken to investigate the role of TQ on $ONOO^-$-induced damage of histone-H2A. Our novel data showed TQ significantly inhibited $ONOO^-$-induced oxidative damage in histone-H2A. $ONOO^-$ induces UV-hypochromicity of histone-H2A, whereas TQ reversed this effect to hyperchromicity. Tyrosine fluorescence was significantly reduced by $ONOO^-$ and was significantly increased upon TQ treatment. TQ reduces $ONOO^-$-induced hydrophobicity in histone-H2A and also reduces thermal stability of $ONOO^-$-histone H2A complex. SDS-PAGE of native histone-H2A showed a single band, which disappeared when treated with $ONOO^-$ alone. This changed was retained when protein samples were treated with TQ. Similar protective effects of TQ were found when protein carbonyl contents were estimated. In conclusion, this is the first study that shows the potential of TQ against $ONOO^-$-induced damaged of histone-H2A. TQ inhibits oxidative modification of tyrosine, lysine, arginine, proline and threonine in histone-H2A. These results have importance for the development of novel therapeutic strategies for the treatment of disorders, where $ONOO^-$ plays a role.

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1. Introduction

Thymoquinone (TQ) is the most active and most abundant constituent of black seed oil (*Nigella sativa*) [1]. Several reports have shown that consumption of back seed has health benefits against many disorders including hypertension, headache, gastrointestinal problems, eczema, obesity, dysentery, bronchial asthma, etc. [2]. Studies undoubtedly suggested that major anti-disease activity of black seed is derived from TQ [1–3]. Now it is well documented that TQ plays an important roles in suppression of oxidative damage and also it ameliorates inflammation under several pathological conditions [1–4]. Its anti-oxidant potential either directly or indirectly linked with its potential to alter "redox state" of biological systems and its inhibitory capacity against reactive oxygen species (ROS) through modulation of hepatic and extra hepatic antioxidant enzymatic system has been well defined [3,4]. Moreover, TQ is also known to suppress the cellular oxidative damage by modulating glutathione and other anti-oxidants [4]. Furthermore, TQ also provides kidney protection against toxicity induced by mercuric chloride, ifosfamide and cisplatin [3]. Not only have these, TQ also inhibits formation of lipid peroxide by reducing the malonaldehyde content in liver [3,4]. TQ also proved to be a powerful chemopreventive agent as it improves anti-oxidant enzymatic system and reduces the onset of hepatic lipid peroxides [1–4]. Now it is well established that TQ has anti-cancer and anti-inflammatory effects as it slowdown proliferation of tumor cell via modulating of apoptotic/necrotic cell signaling events and to monitor angiogenesis [1–4].

Peroxynitrite is a reactive oxygen and nitrogen species (RONS) with an anion formula $ONOO^-$. It is highly unstable oxidizing/nitrating agent which is involved in a wide range of biomolecular damage in almost all biological systems [5–7]. In human, it is generated in response to endogenous/exogenous toxins, stress, ultraviolet light and many other stimuli under various pathological conditions [5–7]. It is now well known that most of the cytotoxic effects of nitric oxide are mainly due to peroxynitrite, which is more reactive and frequent to react with almost all biomolecules including proteins, lipids and nucleic acids [6]. Peroxynitrite not only causing oxidative biomolecular damage but also triggers number of cell signaling events which further inducing oxidative injury and committing cells to necrosis/apoptosis [5–7]. Importantly, peroxynitrite is also involved in the abnormal induction of many molecular pathways which directly associated with pathology of

* Corresponding author at: Department of Medical Biochemistry, College of Medicine, Qassim University, P.O. Box 6655, Buraidah 51452, Saudi Arabia.
E-mail address: zafarrasheed@qumed.edu.sa (Z. Rasheed).

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stroke, myocardial infarction, chronic heart failure, diabetes, inflammation, neurodegenerative disorders, cancer, etc. [5–7]. Not only have these, studies have also shown that peroxynitrite and others RONS are involved in the initiation and progression of various autoimmune responses [8–11].

Histones are highly conserved cationic proteins which bind DNA and remain confined in the nucleus [12]. There are five families of histones H1/H5, H2A, H2B, H3, and H4. Histone H2A is an important constituent of histone core that has tripartite union with central (H3-H4)2 flanked by two H2A-H2B dimmers, making histone H2A more prone to oxidative damage [13]. Recently, we have demonstrated that oxidative modifications of histone H2A lead to the generation of neoepitopes for the induction of autoantibodies in patients with systemic lupus erythematosus (SLE) [14]. More importantly, several studies have shown deleterious effects of peroxynitrite on histone H2A, causing extensive structural modifications, which directly or indirectly associated with the pathophysiology of several autoimmune disorders [15]. As we know studies revealed that TQ has the anti-oxidant activity [1–4], but the effects of TQ on peroxynitrite-induced oxidative modification of histone H2A have never been investigated. Here, we have addressed the question for the first time of a possible inhibitory effect of TQ on peroxynitirite-induced damage of histone H2A. Our novel data may have importance for the development of novel therapeutic strategies for the treatment of disorders, where peroxynitrite plays a role.

2. Methods

2.1. Treatment of histone H2A by thymoquinone and peroxynitrite

Histone H2A (catalog # H9250, Sigma-Aldrich, St Louis, MO, USA) was treated simultaneously with TQ (catalog # 274666, Sigma-Aldrich) and peroxynitrite anion ($ONOO^-$) in phosphate buffer saline (10 mM sodium phosphate buffer containing 150 mM NaCl, pH 7.4) as described previously [9,16] with some modifications. Briefly, histone H2A (1 mg) was treated with TQ (0.1–3.0 μM), and a mixture of SNP (0.5 mM; catalog # 13451, Sigma-Aldrich), pyrogallol (0.5 mM; catalog # P0381, Sigma-Aldrich) and DTPA (0.5 mM; catalog # D1133, Sigma-Aldrich) at 37 °C for 24 h. Reaction mixture was extensively dialyzed against PBS (pH 7.4) to remove excess salts. Histone H2A without TQ, SNP or pyrogallol served as negative controls, whereas histone H2A without TQ but with SNP and pyrogallol served positive controls.

2.2. Ultra-violet spectroscopy

Treated and non-treated protein samples were characterized by ultraviolet (UV) absorption spectroscopy as described previously [17,18]. UV absorption spectra of native and modified histone H2A samples were taken by PerkinElmer Lamda Spectrophotometer (Model # 7110190; Lamda XLS, PerkinElmer Ltd., Beaconsfield, UK). Percentage of hypochromicity at 280 nm was calculated using the following equation as described previously [19].

$$\%\text{Hypochromicity} = [(\text{Native H2A OD}_{280\text{ nm}} - \text{Modified H2A OD}_{280\text{ nm}})/\text{Native H2A OD}_{280\text{ nm}}] \times 100.$$

2.3. Tyrosine fluorescence studies

Modification of tyrosine residue in the protein samples were studied by exciting the protein samples at 280 nm as described previously [20]. Fluorescence intensities (FI) were measurements by Multimode Detector (Anthos Zenyth 3100, Salzburg, Austria). Percent changed in the FI was calculated using the following equation as described previously [21].

$$\%\text{Decrease of FI} = [(\text{FI}_{\text{native H2A}} - \text{FI}_{\text{modified H2A}})/\text{FI}_{\text{native H2A}}] \times 100$$

$$\text{Or}\%\text{Increase of FI} = [(\text{FI}_{\text{modified H2A}} - \text{FI}_{\text{native H2A}})/\text{FI}_{\text{modified H2A}}] \times 100$$

2.4. Protein hydrophobicity studies

The protein hydrophobicity was studied in all histone H2A samples (1 mg/ml) by probing with 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid (*bis*-ANS) (10 μM) at 25 °C in 67 mM sodium phosphate buffer (pH 7.4). The ANS-protein complexes were excited at 380 nm and FI were measurements as described previously [20–22].

2.5. SDS-polyacrylamide gel electrophoresis

Protective effect of TQ against peroxynitrite on histone H2A was visually detected by SDS-PAGE under denaturing conditions using sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) as described previously [22]. Briefly, the protein samples (30 μg) and protein standards (catalog # 161-0398, Precision Plus Protein Standard, Life Technologies Corporation, Carlsbad, CA, USA) were electrophoresed on 10% resolving gel with 2.5% stacking at room temperature for 3 h at 80 V and the gel was stained with comassie brilliant blue R-250.

2.6. Thermal denaturation studies

Stability of native and modified histone H2A samples were determined by thermal denaturation profiling at 280 nm and percent denaturation of the protein samples was measured as described previously [23].

2.7. Protein carbonylation assays

Levels of oxidation in the native and modified histone H2A samples were determined by Protein Carbonyls Colorimetric Assay Kit (catalog # 10005020; Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturers' instructions. The estimated levels of protein carbonyl contents were presented in nmol/mg protein as described previously [23,24].

3. Results

Histone H2A was treated with peroxynitrite or TQ and the induced changes were analyzed by UV absorption spectroscopy. The UV absorption spectra of peroxynitrite histone H2A revealed 43.5% hypochromicity at 280 nm (Fig. 1A&B). Importantly, addition of TQ (0.1–3.0 μM) in the reaction mixture significantly decreased the peroxynitrite-induced hypochromicity in a dose-dependent manner ($p < 0.05$; Fig. 1C,D&E). The protective effect of TQ against peroxynitrite induced damage of histone H2A has been shown by overlapping of UV spectra of all tested protein samples in Fig. 1F. The complete hypochromatic changes in the spectra of native and treated histone H2A have been summarized in Table 1. These structural modifications were further confirmed by tyrosine fluorescence studies. Fig. 2 shows that treatment of histone H2A by peroxynitrite alone caused extensive structural protein damage as tyrosine fluorescence intensity was significantly decreased ($p < 0.001$). However, addition of TQ in the reaction mixture significantly increased tyrosine fluorescence intensity in a dose-dependent manner ($p < 0.05$; Fig. 2). These changes in the tyrosine fluorescence intensities are summarized in Table 2. The protective effect of TQ against peroxynitrite induced hydrophobicity in histone H2A has been demonstrated by hydrophobic probe bis-ANS binding to histone H2A samples. As shown in Fig. 3, treatment of histone H2A with peroxynitrite significantly increased the hydrophobicity ($p < 0.001$).

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