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Roles of some antioxidants in modulation of cardiac myopathy induced by sodium nitrite via down-regulation of mRNA expression of NF-κB, Bax, and flt-1 and suppressing DNA damage

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ABSTRACT

The underlying pathology of cardiac damage involves various molecular and signaling pathways. Therefore, this study aimed to explore the role of Quercetin (Querc), alone or in combination with Melatonin (Melat) against cardiac damage induced by sodium nitrite (Sod nit), as well as to elucidate different signaling pathways. Querc and Melat were injected intraperitoneally (i.p.), followed by induction of hypoxia in rats by using a single dose of Sod nit (60 mg/kg, s.c.). Treatment with Sod nit significantly decreased hemoglobin (Hb) levels in blood. Pretreatment of hypoxic rats with Querc and/or Melat elevated the declined Hb concentration. The forementioned antioxidants also successfully ameliorated the alteration of heat shock protein 70 (HSP-70) and markers of cardiac injury, including troponin T (Trop. T), creatine kinase-MB (CK-MB), tumor necrosis factor-α (TNF α), and C-reactive protein (CRP) in the rats serum. Furthermore, RT-PCR revealed that these antioxidants successfully modulated mRNA expression of NF-κB, Bax, Bcl-2, and flt-1. They also regulated vascular endothelial growth factor (VEGF), the apoptosis marker caspase 3, and oxidative DNA damage in cardiac tissue, compared to Sod nit-intoxicated rats. The present biochemical results are reinforced by histopathological examination. In Conclusion: The results reflected that treatment with Querc in combination with Melat was most effective in improving Sod nit-toxicity induced cardiac damage, thus confirming the promising role of this combination as an effective treatment for cardiac damage induced by other cardio-toxic agents.

1. Introduction

Sodium nitrite (Sod nit) is a widely used as a medication for different human and animal diseases. It acts as a vasodilator, a bronchodilator, an intestinal relaxant, and an antidote for cyanide poisoning (Kroupova et al., 2005). However, overdose of Sod nit could be life threatening (Gonchar et al., 2006). The interaction between Sod nit and red blood cells (RBC) oxidizes Fe^{+2} to Fe^{+3}. This reaction results in formation of methemoglobin causing loss of the capability of RBC to carry O_2, thus ending in hypoxia (Fraser and Mays, 1986).

Al-Gayyar et al. (2014) reported that Sod nit administration causes dysregulation of inflammation, hypoxia, ischemia, oxidative stress, and impaired energy metabolism, resulting in organ damage. In many tissues, hypoxia induces expression of vascular endothelial growth factor (VEGF) mRNA by activating hypoxia inducible factor 1-α (HIF-1α), which is a major regulator of VEGF (Clerici and Planes, 2009). VEGF induces the expression of anti-apoptotic proteins in human endothelial cells, thus promoting the survival of these cells. VEGF mRNA transcripts and proteins

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are also expressed by other cell types, such as hepatocytes (Mochida et al., 1996). VEGF has two primary receptors: VEGFR1 (flt-1) and VEGFR2.

Heat shock proteins (HSPs) induced by stress play an important role in defense against cellular injury (Barral et al., 2004). Caspases initiate cell death programming caspase-3(CASP-3) acts as an initiator of the intrinsic pathway (Riedl and Shi, 2004).

Ghosh et al. (2014) reported that many conditions, including DNA damage, are mediated by oxidative stress. Bcl-2 family proteins, including Bcl-2 and Bax, play an important role in the regulation of apoptosis, and Bax/Bcl-2 ratio can influence the susceptibility of cells to apoptosis (Yao et al., 2012).

Flavonoids play vital biological effects; they act as antioxidants and scavenge free radicals (Gautam et al., 2007). Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one; Querc) and other flavonoids show anti-prostanoid and anti-inflammatory responses, protect low-density lipoprotein from oxidation, prevent platelet aggregation, and promote relaxation of cardiovascular smooth muscles. Querc protects liver cells from carbon tetrachloride toxicity and cyclosporin-induced nephrotoxicity (Pavanato et al., 2003).

Melatonin (N-acetyl-5-methoxytryptamine; Melat) is a neuro hormone produced by the pineal gland, which is involved in the regulation of natural circadian rhythm (Lundmark et al., 2006). It is used as a therapeutic agent to ameliorate molecular and organ/tissue damage and serves as a free radical scavenger and excitotoxicity mitigator (Ramos et al., 2017). Furthermore, it prevents cancer by maintaining DNA integrity (Gitto et al., 2012).

The aim of the present study was to evaluate protective effects of Querc and Melat on cardiac myopathy induced by Sod nit, as well as to elucidate different signaling pathways.

2. Material and methods

2.1. Chemicals

All chemicals used in the study were products of Sigma and Merck companies of high analytical grade.

2.2. Experimental animals

Fifty Wistar adult male albino rats weighing 170–200 g were obtained from the Experimental Animal House, Faculty of Pharmacy, King Saud University, Saudi Arabia. Procedures outlined by the Experimental Animal Ethics Committee were followed. Animals were maintained under standard conditions of temperature and humidity. Rats were fed with standard rat pellet chow with free access to tap water ad libitum for 1 week before the experiment to allow acclimatization. Rats were divided into five groups of ten rats each: Group 1, control; Group 2, Sod nit-treated animals (60 mg/kg; Cigerci et al., 2009); Group 3, Sod nit-treated animals pre-injected with Querc (200 mg/kg, i.p.; Gautam et al., 2007); Group 4, Sod nit-treated animals pre-injected with Melat (200 mg/kg, i.p.; Rao et al., 2000); Group 5, Sod nit-treated animals intraperitoneally injected with a combination of Querc (200 mg/kg) and Melat (200 mg/kg).

A single dose of Sod nit was treated (60 mg/kg) subcutaneously. Querc and Melat were administered 24 h and 1 h before Sod nit injection. One hour after Sod nit injection, the rats were killed, and blood samples were collected for determining ***hemoglobin (Hb)*** and another portion for serum separation via centrifugation at 3000*g* for 10 min, and the supernatants and the serum were stored at −80°C and used for further biochemical analysis. The heart samples were collected, washed, minced, and homogenized in phosphate buffer to yield 20% homogenates. These were centrifuged at 4042*g*. Four hearts from each group were maintained in 4% formalin for histopathological examination.

2.3. Biochemical blood analysis

2.3.1. Determination of hemoglobin (Hb)

Hb was determined colorimetrically using Drabkin's reagent, according to the method described by Kjeldsberg (1993).

2.3.2. Biochemical serum analysis

2.3.2.1 Determination of heat chock protein-70 (HSP-70), troponin T (Trop. T), creatine kinase-MB (CK-MB), tumor necrosis factor-α (TNFα) and C-reactive protein (CRP). For the quantitative determination of HSP-70, a sandwich rat HSP-70 ELISA Kit (Kamiya Biomedical, Washington) was used. Troponin T (Trop. T) concentration was determined using a Siemens Dimension Xpand Plus instrument (IL, USA). CK-MB was estimated spectrophotometrically using a standard enzyme kit supplied by Spinreact, S.A.-Spain (Cat. No. 1001055). TNF α was measured using a high sensitive rat (ELISA) kit (IBL International GmbH, Flughafenstr, Hamburg, Germany) following instructions of the manufacturer. CRP was estimated using immunonephelometric assay (Dade Behring N Latex High Sensitivity CRPTM mono assay) on a Behring Nephelometer II analyzer.

2.4. Biochemical cardiac tissue analysis

2.4.1. Assay of caspase 3 activity

Caspase 3 activity was assayed according to the method described by Vaculova and Zhivotovsky (2008).

2.4.2. Vascular endothelial growth factor (VEGF)

The level of VEGF was determined using kits (ELISA; R&D Systems, UK) in accordance with the manufacturer's instructions.

2.5. Quantitative Real-Time Polymerase Chain Reaction (Qrt-Pcr) for analysis of hepatic NF-κB, Bax, Bcl-2 and flt-1 mRNA expression

2.5.1. Total RNA extraction

Total RNA was isolated from cardiac tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA), according to manufacturer's instructions. RNA quality was confirmed by gel electrophoresis on a 1% agarose gel stained with ethidium bromide.

2.5.2. cDNA synthesis and qRT-PCR

First-strand cDNA was synthesized from 4 μg of total RNA using an Oligo(dT) 12–18 primer and Superscript™ II RNase Reverse Transcriptase.

Equal amounts of RNA (2 μg) were reverse transcribed into cDNA using Superscript Choice systems (Life Technologies, Breda, Netherlands), according to the manufacturer's instructions. To assess the mRNA expression of NF-κB, Bax, Bcl-2, and flt-1, quantitative real-time PCR was performed using SYBR green PCR Master mix (Applied Biosystems, CA, USA), as described by the manufacturer. Briefly, in a 25 μL reaction volume, the following components were added: 5 μL of cDNA, 12.5 μL of 2× SYBR green Master Mix, 200 ng of each primer, and 5 μL RNase-free water. The sequences of primers are given in Table 1. The relative expression was calculated using $2^{-\Delta\Delta CT}$ formula (Livak and Schmittgen, 2001).

2.6. Comet assay

It is single cell gel electrophoresis, described by Singh et al. (1988),

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