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Effect of treatment with xanthohumol on cardiological alterations secondary to ageing

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ABSTRACT

This study investigated the effect of xanthohumol (XN), a flavonoid present in hops (*Humulus lupulus* L.), against ageing-related heart alterations in male senescence-accelerated prone mice (SAMP8). Male senescence-accelerated resistant mice (SAMR1) were used as controls. Animals were divided into four experimental groups of each strain: non-treated young mice, non-treated old mice and old mice treated either with 1 mg/kg/day or 5 mg/kg/day XN. After 30 days of treatment, animals were sacrificed and their hearts were collected and immediately frozen. mRNA and protein expressions of TNF-α, IL-1β, NFκB p65, HO-1, HO-2, BAX, caspase 3, AIF and SIRT1 were measured by RT-PCR and Western blot, respectively. Our results showed an increase in inflammatory, oxidative stress and apoptosis markers in heart of old SAMP8 mice, in contrast to their younger counterparts. After treatment with XN, these age-related changes were reversed suggesting that XN could prevent the appearance of cardiovascular alterations related to ageing.

1. Introduction

Ageing is a biological process associated with several characteristic morphological, histological and biochemical changes. The fact that the world population presents an increasing life expectancy turns ageing into a major risk factor of prevalent diseases, including cancer, cardiovascular diseases (CVDs) and neurodegeneration (Niccoli & Partridge, 2012). CVDs are the most common cause of mortality in developed countries and increase exponentially with advancing age. Therefore, several studies have attempted to understand completely the alterations related to ageing (Paneni, Diaz Cañestro, Libby, Lüscher, & Camici, 2017; Pugh & Wei, 2001; Steenman & Lande, 2017).

In terms of morphological alterations common to the ageing cardiovascular system, loss of myocytes, amyloid deposition, left ventricle hypertrophy and dilation of aorta are some of the changes of the aged heart. Consequently, alterations of cardiac functions such as diastolic dysfunction, reduction in the maximum heart rate or in the cardiac output and increase in the blood pressure or in the vascular resistance occur. There is emerging evidence that these processes may be due to the accumulation of oxidative damage, inflammation and apoptosis (Wadley, Veldhuijzen van Zanten, & Aldred, 2013; Wu, Xia, Kalionis, Wan, & Sun, 2014). Apoptosis is a highly regulated process resulting in cell death. Inflammation, oxidative stress, endoplasmic reticulum stress and mitochondrial dysfunction are likely contributors to start the apoptotic cascade.

Increased inflammation in the ageing process results in higher levels

Abbreviations: AIF, apoptosis-inducing factor; BAX, B-cell lymphoma-2-associated X protein; Bcl-2, B-cell lymphoma-2; CVDs, cardiovascular diseases; HO, heme oxygenase; IκB, inhibitor kappa B; IL-1β, interleukin 1 beta; MOM, mitochondrial outer membrane; NFκB, nuclear factor kappa B; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SAMP, senescence-accelerated prone mice; SAMR, senescence-accelerated resistant mice; SIRT1, situin 1; TNF-α, tumor necrosis factor alpha; XN, xanthohumol

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of inflammatory mediators. On the other hand, ageing is accompanied by mitochondrial dysfunction and increased production of reactive oxygen species (ROS) (Wickens, 2001) leading to apoptosis. Furthermore, mitochondrial dysfunction is associated with changes in calcium signaling that are involved in relaxation and contraction processes of the cardiac muscle. Finally, several recent studies evidence that shortening of telomeres (Bär & Blasco, 2016), accumulation of genome alterations and epigenetic alterations are implicated in cellular senescence (Paneni, et al., 2017).

Hops (*Humulus lupulus* L.), used in the brewing industry to preserve beer and to give beer its characteristic aroma and flavor, have been studied in relation to their possible health benefits because they are an important source of prenylated flavonoids (Nikolic & van Breemen, 2013). Xanthohumol (XN) is the most abundant prenylated flavonoid in hops and it has been reported to possess multiple therapeutic effects (Liu et al., 2015). For example, XN and its metabolites isoxanthohumol and 8-prenylnaringenin may be used as botanical dietary supplements for menopausal symptoms (Krause et al., 2014; van Breemen et al., 2014). In recent studies, it has been shown that XN may be useful in preventing and treating cancers such as glioblastoma (Festa et al., 2011), prostate cancer (Klósek et al., 2016), breast cancer (Vanhoecke et al., 2005) and lung adenocarcinoma (Slawińska-Brych et al., 2016). In addition, XN seems to prevent cancerous cell growth through inhibition of cytochrome P450 enzymes (Yuan et al., 2014) and it shows anti-angiogenesis properties (Albini et al., 2006; Negrão, Incio, Lopes, Azevedo, & Soares, 2007). Furthermore, safety of XN has been demonstrated and animal studies have revealed that oral administration of XN exhibits no adverse effects on major organ function and homeostasis (Dorn, Bataille, Gaebele, Heilmann, & Hellerbrand, 2010). However, additional studies may be helpful to demonstrate the good tolerance of XN so to allow its use in human studies.

Our group has recently studied the effects of XN in ageing using different tissues including brain (Rancan et al., 2017) and liver (Fernández-García et al., 2018). In addition, a recent study proved the effects of XN on calcium signaling pathways in isolated rat ventricular myocytes suggesting that XN may be a candidate for treatment of arrhythmia (Arnaiz-Cot, Cleemann, & Morad, 2017).

The senescence-accelerated mice model was first described in 1981 by Takeda and colleagues (Takeda et al., 1981). It consists of 14 strains of senescence-accelerated prone mice (SAMP) with accelerated senescence and age-associated pathologies and 4 strains of senescence-accelerated resistant mice (SAMR) with normal ageing (Takeda, 1999). Thus, studies of ageing have compared SAMP phenotype to that of SAMR, which can be used as their controls. SAMP8 mice, a substrain of SAMP, are widely used to study accelerated senescence and neurodegenerative disorders due to their pathological phenotype that includes deficits in learning and memory, emotional disorders, impaired immune response and altered circadian rhythms (Takeda, 2009). In addition, studies suggest that SAMP8 animals are a suitable model for the study of ageing related to vascular and cardiological alterations (Karuppagounder et al., 2017).

Therefore, the aim of the present study was to investigate the possible protective effect of XN against age-related inflammatory, oxidative stress, and apoptotic heart damage in SAMP8 compared to SAMR1 as controls.

2. Materials and methods

2.1. Animal model and study groups

This study was approved by the Research and Animal Experimentation Committee of the Complutense University of Madrid. All experiments complied with the ARRIVE guidelines and were carried out in accordance with both Spanish and European laws regarding the handling and care of experimental animals (EU Directive 2010/63/EU).

Thirty male SAMP8 mice and thirty male SAMR1 mice of 2 (young) and 10 (old) months of age were used in this study (total $n = 60$).

Animals were divided into eight experimental groups (7–8 animals per group), four of each strain: two non-treated young mice groups, two non-treated old mice groups and four groups of old mice treated either with 1 mg/kg/day or 5 mg/kg/day XN. Young and old SAMR1 were used as controls.

They were all housed in cages in a room under controlled environmental conditions (22 °C; 70% humidity), with automatic light cycles (12 h light/dark) and standard diet *ad libitum*. Food and water were available at all times with the quantity and frequency of consumption being the free choice of the animal. All the animals received humane care according to the Guidelines for Ethical Care of Experimental Animals of the European Union.

2.2. Treatment

Xanthohumol powder (≥98% purity, NIC Nookandeh Institute for Chemistry of Natural Substances, Homburg, Germany) was dissolved in absolute ethanol and added to the animals' drinking water at two different dosages (1 or 5 mg/kg/day). After previous monitoring, we estimated the water consumption of individual animals to be 0.8 mL/10 g body weight/day. In our study mice weighed 30 g. This implied a water intake of about 2.5 mL/day for each mouse. The dose of XN administered was 1 mg/kg and 5 mg/kg, i.e. 0.03 and 0.15 mg/mouse (30 g) respectively. Solutions of 1.2 mg/100 mL and 6 mg/100 mL were prepared, which were 0.03 and 0.15 mg in the estimated 2.5 mL of water/day respectively. Non-treated animals were supplied only with ethanol 1%. XN solution was prepared daily and was available to animals during the 24 h of the day. Water bottles were covered with aluminum foil to be protected from light.

After 30 days of treatment, animals were sacrificed by cervical dislocation followed by decapitation and hearts were collected. Sample tissues were placed into cryotubes immediately after extraction, frozen in liquid nitrogen and stored at -80 °C until the biochemical determinations were made.

2.3. RNA isolation and cDNA synthesis

The hearts of the mice were used for RNA isolation using the method described by Chomczynski (Chomczynski & Sacchi, 2006) and the TRI Reagent kit (Molecular Research Center, Inc., Cincinnati, OH, USA) following the manufacturer's protocol. RNA concentration was determined by spectrophotometry (260 nm) by BioDrop™, and the purity of the RNA was estimated by electrophoresis using 1.3% agarose gels.

Reverse transcription of 2 µg RNA for cDNA synthesis was performed using the Reverse Transcription System (Promega, Madison, WI, USA) and a pd(N)6 random hexamers.

2.4. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to determine the mRNA expression of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), heme oxygenase (HO) isoforms 1 and 2, B-cell lymphoma-2-associated X protein (BAX), apoptosis-inducing factor (AIF), and sirtuin 1 (SIRT1). It was performed using an Applied Biosystems 7500 Fast apparatus with the SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and 300 nM concentrations of specific primers. The sequences are listed in Table 1.

Cycling conditions were adjusted according to the respective thermal cycler and primer/template combinations (Kim, Yang, Bae, & Park, 2008): 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, 60 °C for 30 s and finally 95 °C for 15 s. For the normalization of cDNA loading in the PCR, the amplification of 18S ribosomal RNA for every sample was used. Relative changes in mRNA expression were calculated using the $2^{-\Delta\Delta CT}$