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The protective role of ferulic acid on sepsis-induced oxidative damage in Wistar albino rats

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ABSTRACT

Oxidative stress has an important role in the development of sepsis-induced multiorgan failure. Ferulic acid (FA), a well-established natural antioxidant, has several pharmacological activities including anti-inflammatory, anticancer and hepatoprotective. This study aimed to investigate the effects of FA on sepsis-induced oxidative damage in Wistar albino rats. Sepsis-induced DNA damage in the lymphocytes, liver and kidney cells of rats were evaluated by comet assay with and without formamidopyrimidine DNA glycosylase (Fpg). The oxidative stress parameters such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and total glutathione (GSH) and malondialdehyde (MDA) levels were also measured. It is found that DNA damage in sepsis + FA-treated group was significantly lower than the sepsis group. FA treatment also decreased the MDA levels and increased the GSH levels and SOD and GSH-Px activities in the sepsis-induced rats. It seems that FA might have ameliorative effects against sepsis-induced oxidative damage.

1. Introduction

Sepsis, the systemic response to an infection, is a common cause of morbidity and mortality in intensive care units that can progress to multiple organ failure which is clinically characterized by the liver, pulmonary, cardiovascular, renal and gastrointestinal dysfunction (Zapelini et al., 2008). Reactive oxygen species (ROS) are believed to be involved in the development of sepsis (Cassol-Jr et al., 2010; Kaymak et al., 2008; Zhou et al., 2012). The pro-inflammatory effects of ROS include endothelial damage, formation of chemotactic factors, neutrophil recruitment, cytokines release, mitochondrial impairment, lipid peroxidation, and DNA damage (Andrades et al., 2009, 2011; Barichello et al., 2006; Hotchkiss and Karl, 2003), all contributing to a free radical overload and to oxidant–antioxidant imbalance (Andrades et al., 2011). It has been demonstrated that ROS lead to the breakage in single and double strands, base modifications, fragmentation of deoxyribose, formation of DNA-protein cross-links as well as abasic sites (Andrades et al., 2009; Barzilai and Yamamoto, 2004; Cooke et al., 2003; Evans and Cooke, 2004).

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Natural products are widely used as dietary supplements because of their potential antioxidant properties. Plant polyphenols may act as antioxidants by different mechanisms such as free radical scavenging, metal chelation and protein binding (Maurya and Devasagayam, 2010). Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA), a phenolic compound, arises from the metabolism of phenylalanine and tyrosine by Shikimate pathway in plants (Prasad et al., 2006; Ramar et al., 2012; Sudheer et al., 2008). This phenolic compound is present in fruits, vegetables, rice and wheat and has been suggested to have several properties such as antioxidant, antihyperlipidemic, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic, neuroprotective, and antihypertensive (Balakrishnan et al., 2008; Ramar et al., 2012; Sudheer et al., 2008). It is also shown that FA had protective effects against cardiovascular diseases, Alzheimer's disease, and ultraviolet radiation (Mancuso and Santangelo, 2014). FA has been used as a food additive and antioxidant in Japan, whereas sodium ferulate is used for treatment of cardiovascular and cerebrovascular diseases in China (Itagaki et al., 2009; Zeni et al., 2012; Zhao and Moghadasian, 2008).

Due to its phenolic nucleus and an extended side chain (Fig. 1), FA readily forms a resonance stabilized phenoxy radical which accounts for its free radical-scavenging effects (Graf, 1992; Palacios and Perez, 1990; Zhao and Moghadasian, 2008). This enables FA to protect DNA and lipids against oxidation through ROS (Kanski et al., 2002; Srinivasan et al., 2006; Zhao and Moghadasian, 2008). FA was shown to scavenge both ROS and reactive nitrogen species (RNS) (Trombino et al., 2013). Through its free radical scavenging activity, and enhancing the cell stress response, FA has antioxidant activity (Calabrese et al., 2008; Barone et al., 2009). FA was also shown to be effective as a neuroprotector in several *in vitro* and *ex vivo* models of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and cerebral ischemia/reperfusion injury. A preclinical study with FA has showed a therapeutic activity in preventing noise-induced auditory loss (Fetoni et al., 2010).

The aim of this study was to assess the protective effects of FA on sepsis-induced oxidative damage in the lymphocytes, liver and kidney cells of Wistar albino rats. To determine the oxidative DNA damage, Comet assay was used. The oxidative stress parameters such as superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities and total glutathione (GSH) and malondialdehyde (MDA) levels in the liver and kidney tissues were also measured to investigate the effects of FA on sepsis-induced oxidative stress.

2. Materials and methods

2.1. Chemicals

The chemicals used in the study were purchased from the following suppliers: normal melting agarose (NMA) and low melting point agarose (LMA) from Boehringer Manheim (Mannheim, Germany); sodium chloride (NaCl), sodium hydroxide (NaOH), potassium chloride (KCl) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) from Merck Chemicals (Darmstadt, Germany); formamidopyrimidine DNA glycosylase (Fpg), bovine serum albumin, dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), Triton-X-100, phosphate-buffered saline (PBS) tablets, trichloroacetic acid, thiobarbituric acid, n-butanol and FA from Sigma–Aldrich Chemicals (St Louis, MO, USA); ethylendiaminetetraacetic acid disodium salt dihydrate (EDTA-Na_2), natriumlauroylsarcosinate, and Tris from ICN Biomedicals Inc. (Aurora, OH, USA), SOD assay kit, GSH-Px assay kit and GSH assay kit from Cayman Chemicals Co. (Ann Arbor, MI, USA).

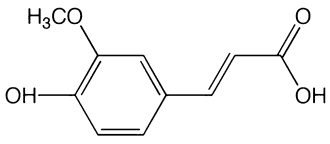

Fig. 1 – Structure of FA.

2.2. Animals

Wistar albino rats (3 months old, male, weight range 200–300 g) were used in all experiments. Animals were obtained from Refik Saydam National Public Health Agency, Ankara, Turkey. They were housed in plastic cages with stainless steel grid tops. Rats were maintained on a 12 h light–dark cycle, at controlled temperature (23 ± 2 °C) and humidity (%50). Animals were fed with standard laboratory chow and allowed to access feed and drinking water *ad libitum* before and after operation. The animals were treated humanely and with regard for alleviation of suffering and the study were approved by Hacettepe University Animal Ethical Committee.

2.3. Cecal ligation puncture (CLP) model

The animals were subjected to sepsis by cecal ligation puncture (CLP) as previously described (Comim et al., 2009; Ritter et al., 2003; Wichterman et al., 1980). In this model, the rats become bacteremic with Gram-negative enteric organisms (Cassol-Jr et al., 2010), in which caecum is ligated distal to the ileocecal valve and perforated using two needle punctures (Parker and Watkins, 2001). Under aseptic conditions, rats were anesthetized with intraperitoneal (i.p.) injection of 90 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı Warner-Lambert, Istanbul, Turkey). A midline laparotomy was performed using minimal dissection under the anesthesia and the cecum was ligated just below the ileocecal valve with 3-O silk ligatures so that intestinal continuity was maintained. The cecum was perforated on the antimesentric surface of the cecum at two locations 1 cm apart and was gently squeezed to extrude a small amount of feces. Finally, all rats were resuscitated with saline (5 mL/100 g b.w.) subcutaneously (s.c.). The rats were deprived of food but had free access to water after the operation. The sham operated group underwent laparotomy; the cecum was manipulated but was not ligated or perforated. All animals were maintained under the same conditions after the surgery.

2.4. Experimental design

The rats were divided into four groups:

Group 1: Sham group ($n = 8$). This group consisted of animals treated with 0.5 ml i.p. saline alone following laparotomy.