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Promising role of ferulic acid, atorvastatin and their combination in ameliorating high fat diet-induced stress in mice

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ABSTRACT

Aims: The present study evaluated a comparative and combined hepatoprotective effect of atorvastatin (AS) and ferulic acid (F) against high fat diet (HFD) induced oxidative stress in terms of hyperlipidemia, anti-oxidative status, lipid peroxidation and inflammation.
Main methods: Male Swiss albino mice were given a diet containing high fat (H) (23.9% wt/wt), supplemented with AS (10 mg/kg) or F (100 mg/kg) and both (10 and 100 mg/kg) for 8 weeks. The control mice (C) were fed with normal diet.
Key findings: The H mice exhibited increased body weight; hyperlipidemia; serum level of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6); hepatic lipid profile; lipid accumulation; reactive oxygen species (ROS) of hepatocytes, lipid peroxidation and liver antioxidant capacity was decreased. Immunofluorescent and Western blot assay revealed activation of nuclear factor kappa B (NF-κB) signaling pathway. The addition of F or AS and both in the diet significantly counteracted HFD induced body weight gain; hyperlipidemia; TNF-α, IL-6; hepatic lipid profile; fatty infiltration; NF-κB signaling pathway; ROS; lipid peroxidation and moreover elevated levels of hepatic antioxidant enzymes activity were observed.
Significance: Simultaneous treatment with AS, F and their combination protected against HFD induced weight gain and oxidative stress. The protection may be attributed to the hypolipidemic and free radical scavenging activity of AS or F and their combination. This study illustrates that AS and F have relatively similar hypolipidemic, antioxidative, anti-inflammatory actions and the AS + F combination along with HFD has shown outstanding effects as compared to other treated groups.

Introduction

High dietary fat intake promotes the development of obesity in humans and rodents as a result of an imbalance between energy intake and energy expenditure (Bray et al., 2004). High fat induced weight gain promotes hepatic oxidative stress, triggers the redox-sensitive transcription factor NF-κB, some inflammatory processes, prevalence of hepatic steatosis and other liver lesions called steatohepatitis (Milagro et al., 2006; Hotamisligil et al., 1993; Pessayre et al., 2002; Jaskiewicz et al., 2008; Leclercq, 2007; Zou et al., 2006). Indeed, chronic fat ingestion (inflammation) plays a role in the development of insulin resistance and other obesity-related features commonly recognized as metabolic syndrome in both developed and developing countries (Xu et al., 2003; Dandona et al., 2004; Roberts and Sindhu, 2009). Proinflammatory molecules, including cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), are able to activate NF-κB, initiating a signaling cascade of activation. The molecules, which regulate the activation of the NF-κB heterodimer, RelA (p65) and p50, controls inflammation has been identified. Signaling systems induced by a variety of stimuli activate two serine kinases, termed IκB kinases (inhibitor of nuclear factor kappa B kinase alpha subunit also called IKK α or IKK1 and inhibitor of nuclear factor kappa B kinase beta subunit also called IKK β or IKK2), which target the inhibitors of κB (IκB). The subsequent phosphorylation by these kinases leads to eventual ubiquitination and proteasome-dependent degradation of IκB, releasing the latent dimeric transcription factor to the nucleus, where it binds to promoter sites for gene transcription (DiDonato et al., 1997; Nakano et al., 2006; Carlsen et al., 2004; Blackwell and Christman, 1997).

Atorvastatin, a hydrophobic statin is indicated for the treatment of dyslipidemia, specifically hypercholesterolemia, combined hyperlipidemia and non-alcoholic fatty liver disease with high cardiovascular risk factors (Lupattelli et al., 2012; Malhotra and Goa, 2001; Lea and McTavish, 1997; Foster et al., 2011; Chalasani, 2005). The antioxidant and anti-inflammatory effects of this compound have well studied (Stoll et al., 2004). There is a therapeutic dilemma about the use of statins in the treatment of dyslipidemia and associated conditions particularly in the presence of elevated liver aminotransferase levels (Calderon et al., 2010). One of the major drawbacks of statin therapy

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is inhibition of coenzyme Q 10 (CoQ 10) synthesis. In humans, CoQ 10, a potent antioxidant and free radical scavenger, preserves cellular integrity. These functions are relevant to human health, leading to the logical conclusion that patients on long term statin therapy should receive supplemental CoQ10 (Bliznakov, 2002). Many reports are also coming about adverse effects, drug interactions and controversy about statin therapy in this field (Ozdemir et al., 2000; Gajski et al., 2008; Graham et al., 2004; Thompson et al., 2003).

Therefore, a number of recent studies have focused on the prevention and treatment of obesity and its associated health risks using naturally-occurring phytochemicals. Ferulic acid is a major phenolic acid abundantly present in various fruits and vegetables, such as banana, citrus fruits, bamboo shoots, eggplant, cabbage, and broccoli and is reported to have strong antioxidant activities (Rukkumani et al., 2004; Srinivasan et al., 2007). Moreover ferulic acid is a phenolic acid of low toxicity and it can be absorbed, easily metabolized in the human body (Zhao et al., 2004). It possess various physiological properties, such as inhibition of tumor promotion, reduction of serum and hepatic lipid profile mainly total cholesterol, TG level, and also protective action against liver injury (Wilson et al., 2007; Yasukawa et al., 1998; Jin Son et al., 2010; Srinivasan et al., 2005). The liver is a metabolically active organ; thus it should reflect any systemic derangement on high fat diet feeding. Therefore, we searched for a natural protective agent that can provide protection against HFD induced hepatic stress from a mechanistic viewpoint.

Regarding health aspects of atorvastatin and ferulic acid that have been studied in the past, there have been few reports on the pathophysiological roles of ferulic acid and atorvastatin in relation to high fat diet and hepatic inflammation. Moreover, the comparative and combined effects of these two compounds on high fat diet induced obesity, antioxidative status, lipid peroxidation (LPO) and hepatic inflammation remain unclear. Thus, this study was conducted to evaluate a comparative and combined effect of dietary supplementation of atorvastatin and ferulic acid on the high fat diet induced obesity, antioxidant status, LPO and liver inflammation in the mice. In addition, a sustainable health care system must foster the development of dietary initiatives that are effective in preventing the incidence of high fat diet induced hepatic stress and following disorders.

Material and methods

Chemicals and animals

Atorvastatin, ferulic acid, GSH, protease inhibitor cocktail, and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were procured from Merck (Darmstadt, Germany). All reagents were of highest analytical grade. Male Swiss albino mice were purchased from Bengal chemical and pharmaceuticals Ltd. (Kolkata, India).

Animal treatment & diet

The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Animal room was maintained at a temperature of 25 ± 1 °C, a humidity of 50 ± 10% and a 12-h light/dark cycle and the mice were allowed to access food and water ad libitum. Male Swiss albino mice, weighing 14–18 g, were divided into eight (8) different groups containing six (6) animals, viz., (1) control (C) (2) ferulic acid (F) (3) atorvastatin (AS) (4) ferulic acid + atorvastatin (AS + F) (5) high fat diet (H) (6) high fat diet fed with ferulic acid (H + F) (7) high fat diet fed with atorvastatin (H + AS) and (8) high fat diet with atorvastatin + ferulic acid (H + AS + F). Control group was supplied with standard laboratory diet that contains (for 100 g) 13.9 g protein (14.2% of total energy), 61.8 g carbohydrate (63.4%), 3.9 g fat (9%) and the rests were vitamins, minerals etc. The positive control groups of animals were treated with F (100 mg/kg body weight), AS (10 mg/kg body weight) (Zadelaar et al., 2007), and AS + F (100 + 10 mg/kg body weight) along with standard laboratory diet. The high fat diet contains (per 100 g) 11.1 g protein (11.4% of total energy), 32.8 g carbohydrate (33.6%) and fat 23.9 g (54.9%) as published elsewhere (Das et al., 2012; Anai et al., 1999). We tested three different doses of ferulic acid (F), atorvastatin (AS), and ferulic acid + atorvastatin (F + AS) — (i) 50 mg (F), 5 mg (AS), 50 + 5 mg (F + AS); (ii)100 mg (F), 10 mg (AS), 100 + 10 mg (F + AS); and (iii) 150 mg (F), 15 mg (AS), 150 + 15 (AS + F) mg/kg body weight to evaluate their protective effects (data not shown). The dose of 100 mg (F), 10 mg (AS), 100 + 10 (AS + F) mg/kg body weight was found to be more effective than the first set of dose and similarly effective with the third set of dose. The animals were treated with F (100 mg/kg) and AS (10 mg/kg) (Zadelaar et al., 2007) and AS + F (100 + 10 mg/kg) body weight on the basis of most advantageous level of efficacy. After the end of 8 weeks of treatment, the animals were kept in overnight fasting condition before collection of blood from retro-orbital plexus and then sacrificed by cervical dislocation followed by liver collection, and stored at −80 °C for further analysis.

Body weight

Body weight was monitored by a calibrated weighing scale prior to feeding the mice with the experimental diets every week.

Measurement of plasma and hepatic lipids

The hepatic lipid, cholesterol (TC) and triglyceride (TG) were extracted using the procedure developed by Folch et al. (1957). Total serum cholesterol was measured by enzymatic method using kit from Randox Laboratories Ltd. (Antrim, United Kingdom) according to the manufacturer's protocol. Triglyceride in serum was measured using kit from Merck (Darmstadt, Germany).

Cytokine ELISA

The levels of murine serum tumor necrosis factor-α and interleukin-6 were measured using a sandwich ELISA Kit purchased from Endogen Inc. (Rockford, IL, USA) according to the manufacturer's protocol.

Determination of hepatic antioxidant enzyme activities

The livers were homogenized using Tris buffer (pH 7.4) in the presence of protease inhibitor cocktail for biochemical estimation.

Catalase activity was measured by monitoring the decrease in absorbance resulting from the elimination of H_2O_2 by the action of catalase according to Yumoto et al. (2000). *Superoxide dismutase* activity was determined by utilizing the involvement of superoxide anion radical in the auto oxidation of pyrogallol according to the modified protocol of Marklund and Marklund (1974).

Determination of lipid peroxidation

The lipid peroxidation was evaluated by the thiobarbituric acid reactive substance (TBARS) level in the homogenate using a standard protocol (Buege and Aust, 1978). The amount of lipid peroxidation was determined using $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as amount of TBARS produced in nanomoles/g of tissue.

Estimation of reduced glutathione (GSH) content

GSH was determined according to the method described by Moron et al. (1979). The GSH present in liver homogenate reacts with 5, 5-dithio-bis (2-nitrobenzoic acid) to form a yellow complex. The absorbance was read at 412 nm.