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Ameliorative effect of nicorandil on high fat diet induced non-alcoholic fatty liver disease in rats

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is an accumulation of excessive amounts of fats in the liver that is not caused by alcohol consumption. It is considered as the most common liver disease in Western societies. The aim of this study is to investigate the possible protective effects of nicorandil and pioglitazone, the benefits of their combination and the possible mechanism underlie these effects in NAFLD. Rats were fed a high-fat diet (HFD) for eight weeks to induce NAFLD. In the next eight weeks, rats were fed the HFD along with pioglitazone (4 mg/kg) or nicorandil in two dose levels (3 or 15 mg/kg), alone or in combination. Chronic HFD administration resulted in significant elevations in serum levels of liver enzymes, total cholesterol, triglycerides, glucose, insulin and HOMA-IR index as compared with the control group. This was coupled with significant increments in liver triglycerides, MDA content and TNF-α as well as a significant reduction in liver GSH content. In comparison with the control group; liver expression of NF-κB was significantly elevated while liver eNOS expression and nitric oxide content were significantly decreased in HFD group. Treatment with pioglitazone or nicorandil either alone or in combination successfully ameliorated the deleterious effects of HFD on the all previous parameters.

In conclusion, this investigation indicates a novel role of nicorandil in rats with NAFLD. This effect is mediated through, nitric oxide donor, antioxidant and anti-inflammatory properties, leading to improvement of insulin resistance. It is worth mentioning that the combinations were more effective than the individual drugs.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is an accumulation of excessive amounts of fats in the liver that is not caused by alcohol consumption (Clark et al., 2002).

It is considered as the most common liver disease in Western societies and affects up to 35% of the population in several countries (Clark, 2006). Notably, 1–5% of patients with simple steatosis can eventually develop actual cirrhosis; and 10–15% of patients with NASH can progress to cirrhosis and even to hepatocellular carcinoma (Hashimoto et al., 2009; Ascha et al., 2010). Considering current obesity epidemic, it is expected that NAFLD prevalence will rise.

NAFLD comprises a spectrum of liver disorders starting with isolated hepatic steatosis, progressing to the more ominous non-alcoholic steatohepatitis (NASH), to the end-stage of cirrhosis, and ultimately which underlie the development of hepatocellular carcinoma (Matteoni et al., 1999).

The pathogenesis of hepatic fat accumulation in NAFLD and progression to NASH is incompletely understood. The most widely supported theory in the pathogenesis of NAFLD is a “two-hit” theory (Day and James, 1998).

According to this theory, the “first hit” involves fat accumulation in the hepatocytes, while insulin resistance (IR) is suggested to be the “second hit” (Sanyal et al., 2001; Pagano et al., 2002). The “first hit” increases the sensitivity of the liver to multiple factors that participate in the “second hit” and both hits leading to hepatic injury, inflammation and fibrosis. A combination of oxidative stress and subsequent lipid peroxidation, inflammatory cytokines, hormones derived from adipose tissue (adipocytokines) and mitochondrial dysfunction are included among these factors (Rolo et al., 2012).

The effective therapy for NAFLD has not been established, but there are many proposed strategies and agents used for liver support. Gradual loss of body weight with change in lifestyle or

Abbreviations: FFA, free fatty acids; GSH, glutathione; HFD, high fat diet; HOMA-IR, homeostasis model assessment index for insulin resistance; iNOS, induced nitric oxide synthase; IR, insulin resistance; MDA, malondialdehyde; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NIC 3, nicorandil (3 mg/kg/day); NIC 15, nicorandil (15 mg/kg/day); NF-κB, nuclear factor kappa-B; PIO, pioglitazone

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bariatric surgery, which may improve liver function tests and liver histology in patients with NASH is among these strategies (Lam and Younossi, 2009). However, this strategy has poor compliance in many patients.

Many researchers used different agents which may target a different step in the pathway of hepatic steatosis or its progression to steatohepatitis (Oh et al., 2008). Hundal et al. (2000) stated that metformin may ameliorate fat-induced hepatic insulin resistance by decreasing gluconeogenesis and enhancing peripheral glucose uptake. The present investigation follows different pathways such as oxidative stress, inflammation or IR by two different drugs to improve the liver state after HFD feeding.

Nicorandil (N-(2-hydroxyethyl) nicotinamide nitrate ester), potent NO donor, is generally accepted as an effective therapy for the treatment of ischemic heart diseases. It also acts as a potassium (k-ATP) channel opener (Sakai et al., 2000). It is effective in the treatment of several diseases such as bronchial asthma, urinary incontinence, erectile dysfunction and neurodegenerative diseases (Hedlund et al., 1994; Zhou et al., 1995). Activation of the ATP-K+ channel leading to decrease in the mitochondrial membrane potential resulting from the enhanced K+ permeability of the inner mitochondrial membrane correlates with an accelerated oxygen consumption by muscle cells or isolated mitochondria treated with nicorandil (Debska et al., 2002). Martineau (2012) stated that inhibition of the respiratory chain leading to reduce fatty acid oxidation and decrease the insulin sensitivity. Therefore, the stimulation of respiration following pharmacological opening of mitochondrial potassium channels could be a remedy for insulin resistance, restoring the proper cellular response to this hormone (Dymkowska et al., 2014).

In addition to its K-ATP channel opening activity, it has free radical scavenging property; reduce NF-κB and increase eNOS expression (Tsuchida et al., 2002). Accordingly, it is expected to exert a beneficial effect on NAFLD via donating NO, increasing eNOS, reducing NF-κB and insulin resistance.

Pioglitazone, PPAR γ agonist, belongs to the thiazolidinedione (TZD) class of antidiabetic drugs (Gerstein et al., 2006). Pioglitazone is able to manage fat-induced hepatic insulin resistance by increasing insulin sensitivity in adipose tissues. It is also used in the treatment of polycystic ovary syndrome (Mayerson et al., 2002; Jia-sheng et al., 2012). This class has anti-inflammatory properties, as demonstrated by a decrease in NF-κB level and an increase in adiponectin levels, which secreted by adipose cells (Lutchman et al., 2006).

Relying on the aforementioned, this study aimed to evaluate the hepatoprotective effect of pioglitazone (insulin sensitizer) and nicorandil (NO donor) in two dose levels in an experimental model of NAFLD and the benefits of the combination of the two drugs. In addition to the possible mechanism underlie these effects.

2. Materials and methods

2.1. Ethics statement

Experimental design and animal handling were performed in accordance with the guidelines of the animal ethics committee of the Faculty of Pharmacy, Zagazig University and were handled following the International Animal Ethics Committee Guidelines, ensuring minimum animal suffering.

2.2. Animals

Adult male Wistar rats weighing 130–150 g were obtained from the animal facility of Veterinary Medicine Faculty, Zagazig University, Zagazig, Egypt. Rats were housed in clean cages and kept under controlled temperature (25 ± 3 °C) and constant light cycle (12 h light/dark). Rats were allowed free access to a standard rodent chow diet and water ad libitum.

2.3. Chemicals and drugs

Pioglitazone powder was kindly provided by Medical Union Pharmaceuticals (MUP, Ismailia, Egypt). Nicorandil was obtained from Adwia Pharmaceutical Company, Egypt. All used chemicals were of analytical grade. Both drugs were dissolved in normal saline. Cholesterol was purchased from GFS chemicals and reagents (Texas, USA) and bile salts were purchased from SAS Chemicals Co. (Mumbai, India).

2.4. Study protocol

Rats were randomly divided into seven groups, 10 rats each. Group 1 served as a normal group and was maintained on normal rat chow diet throughout the experiment (16 weeks). The remaining six groups were maintained on a HFD containing 87.7% standard diet (w/w), 10% pork fat (w/w), 2% cholesterol (w/w) and 0.3% bile salts (w/w) (Pan et al., 2006) for eight weeks. In the next eight weeks, HFD was given in addition to the following treatment regimens; group 2 (HFD group) received distilled water (1 ml/kg/day, p.o.); group 3 (PIO) received pioglitazone (4 mg/kg/day, p.o.) (Zaitone et al., 2011); group 4 and 5 (NIC 3 and NIC 15, respectively) received nicorandil (3 or 15 mg/kg/day, p.o., respectively) (Ahmed et al., 2011; Serizawa et al. 2011), group 6 and 7 (PIO+NIC 3 and PIO+NIC 15, respectively) received a combination of pioglitazone and nicorandil in the same aforementioned doses.

At the end of the experiment, the final body weight of each animal was recorded and fasting blood glucose was determined with an automatic blood glucose meter (Super Glucocard, Japan) using blood samples from the tail tip. Blood was collected from the retro-orbital plexus and centrifuged at 1000g for 15 min using heraeus sepatech centrifuge (Labofuge 200) to separate serum that was divided into aliquots and stored at −80 °C till analyzed for liver enzymes, total cholesterol, triglycerides and fasting insulin levels.

2.5. Tissue sampling

Animals were sacrificed under anesthesia with urethane (1.3 g/kg, I.P). The liver was rapidly dissected and washed free of blood with ice cold 0.9% NaCl solution. The liver was weighed to calculate the liver index (*liver weight*/*body weight* × 100). One part of the liver (0.3 g) was immersed in liquid nitrogen and kept at −80 °C for determination of hepatic triglycerides, MDA, reduced glutathione and TNF-α contents and gene expression of NF-κB and eNOS. The other part was also removed and kept in 10% phosphate buffered formalin for histopathological examination.

2.6. Measurement of serum biochemical parameters

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the method described by Reitman and Frankel (1957).

Serum total cholesterol was determined according to the principle of Allain et al. (1974) and triglycerides were determined according to the methods described by Werner et al. (1981). These parameters were determined colorimetrically using Bio diagnostic kits supplied by the Egyptian Company for Biotechnology, Egypt, following the manufacturer's instructions.

Fasting serum insulin level and liver TNF-α content were assayed by sandwich enzyme-linked immunosorbent assay (ELISA) (Millipore, Cairo, Egypt) which uses microtiter plate coated with mouse