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Effect of pentoxifylline and/or alpha lipoic acid on experimentally induced acute pancreatitis

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ABSTRACT

Acute pancreatitis is a sudden inflammation of the pancreas that may be life threatening disease with high mortality rates; particularly in presence of systemic inflammatory response and multiple organ failure despite of the conventional antibiotic and symptomatic treatment. Oxidative stress has been shown to be involved in the pathophysiology of acute pancreatitis. This study was designed to investigate the possible effect of pentoxifylline and alpha lipoic acid respectively and in combination on rats with L-arginine induced acute pancreatitis. Rats were divided as follow; Group 1: served as control, Group 2 and Group 3: sacrificed after 24 h and 7 days; respectively, from induction of acute pancreatitis by L-arginine 250 mg/100 g, Group 4 and Group 5: rats treated by pentoxifylline (12 mg/kg) and sacrificed after 24 h and7 days; respectively, from induction of acute pancreatitis, Group 6 and Group 7: treated by alpha lipoic acid (1 mg/kg) and sacrificed after 24 h and 7 days; respectively, from induction of acute pancreatitis, Group 8 and Group 9: treated by pentoxifylline and alpha lipoic acid and sacrificed after 24 h and 7 days; respectively, from induction of acute pancreatitis. Serum samples were collected to assay levels of amylase enzyme, C-reactive protein, IL-6, catalase enzyme activity, malondialdehyde and pancreases were excised for histopathological examination and assay of pancreatic myeloperoxidase. L-arginine induced-acute pancreatitis was evident by increased in serum marker enzymes and by histopathological findings compared to control group. Pentoxifylline and alpha lipoic acid respectively provided protection against L-arginine induced acute pancreatitis possibly by their antioxidant and anti-inflammatory effect. Treatment with alpha lipoic acid exhibited pronounced improvement in the course of pancreatitis when compared to treatment with pentoxifylline. Moreover, the combination of pentoxifylline and alpha lipoic acid offered the most evident protection when compared to groups that received monotherapy; pointing to the effectiveness of such combination therapy.

1. Introduction

Acute pancreatitis is a sudden inflammation of the pancreas that may be life threatening with high mortality rates; particularly in presence of systemic inflammatory response and multiple organ failure despite of the treatment. In addition it could be misdiagnosed with other causes of acute abdomen (Lankisch and Lerch, 2006; Whitcomb, 2006). Acute pancreatitis has numerous etiologies; alcohol beverages and biliary tract disease are most common causes. Moreover, many other etiologies are present as; viral infection as mumps and hepatitis type A&B, drugs as tetracycline, valproic acid, estrogens and furosemide as well as hypercalcemia, hypertriglyceridemia and developmental abnormalities of the pancreas. In 10% of cases, the cause is unknown, although recent studies have suggested that up to 70% of cases of idiopathic pancreatitis are secondary to biliary microlithiasis (Ramsingh, 1997; Martinez et al., 2006).

It is believed that acute pancreatitis involves intracellular activation of digestive enzymes and autodigestion of the pancreas as a central pathophysiologic cause that quickly induces non infectious inflammatory reaction at the site of injury with digestion of surrounding organs (Beckingham and Bornman, 2001). Oxidative stress has been shown to be involved in the pathophysiology of acute pancreatitis, where oxygen free radicals and lipid peroxidation play an important role in the development of local and systemic inflammatory reactions and complication during acute pancreatitis (Szabolcs et al., 2006).

L-arginine is an amino acid present in the proteins of all life forms. In small dose, L-arginine supplemented in diet may have anti-atherogenic, antioxidant and immunomodulatory actions. However previous reports stated that receiving excessive doses of L-arginine supplementation without medical advice induce acute pancreatitis (Saka et al., 2004). As a model of acute pancreatitis, it has been demonstrated that L-arginine induces it through oxygen free radicals

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generation and inflammatory mediators which have a key role in the development of the disease.

So, the use of drugs with antioxidant and/or anti-inflammatory properties could be proposed as a therapeutic intervention in acute pancreatitis to improve the outcome of the disease (Hardman et al., 2005). Pentoxifylline is a methyl-xanthine derivative that has hematologic and an immunomodulating properties which lead to vasodilatation, improves erythrocyte flexibility, enhances blood flow, inhibits platelet aggregation, reduction in blood viscosity and inhibits production of tumor necrosis factor-α (TNF-α). It is recently demonstrated to exhibit strong anti-inflammatory and antioxidant effects (Schandene et al., 1992; Meiners et al., 2004). Alpha Lipoic acid is a thiol antioxidant compound which demonstrated direct free radical scavenging properties. Alpha lipoic acid and its reduced form dihydrolipoic acid, have been referred to as "universal antioxidant" that functioning in both membrane and aqueous phases (Atmaca, 2004).

The present study was designed to investigate the possible protective effect of pentoxifylline and alpha lipoic acid each alone and in combination against l-arginine induced pancreatitis.

2. Material and methods

2.1. Drugs and chemicals

- L-arginine, powder, Sigma; prepared as a solution by distilled water to a final concentration of 500 mg/ml.
- Pentoxifylline, powder, Aventis; prepared as a solution by distilled water to a final concentration of 2.4 mg/ml.
- Alpha lipoic acid, ampoule, Eva Pharm; prepared as a solution by distilled water to a final concentration of 1 mg/ml.
- Other chemicals and reagents used in this work are of analytical gradient; obtained from Sigma-Aldrich Chemical Co.

2.2. Animals and treatment

This experiment was carried out on 90 male albino rats weighted 150–200 g, the rats obtained from Tanta Faculty of Medicine and handled in accordance with the guideline principles in the use of animals. They were kept under the same condition and had free access to food and water all over the period of the work. They were divided into 9 groups (each of 10 rats) as follow:

- Group 1: Normal rats served as control group.
- Group 2: Acute pancreatitis was induced by a single intraperitoneal injection of L-arginine in a dose 250 mg/100 g body weight. Rats were sacrificed 24 h after induction of acute pancreatitis (Mervi et al., 2006).
- Group 3: Acute pancreatitis was induced by a single intraperitoneal injection of L-arginine in a dose 250 mg/100 g body weight (Mervi et al., 2006). Rats were sacrificed 7 days after induction of acute pancreatitis.
- Group 4: Rats were received pentoxifylline as a single intraperitoneal injection in a dose of 12 mg/kg body weight (Gomez-Cambronero et al., 2000) then rats was sacrificed 24 h after induction of acute pancreatitis.
- Group 5: Rats were received pentoxifylline in a dose of 12 mg/kg body weight/day for 7 days by intraperitoneal injection then rats were sacrificed 7 days after induction of acute pancreatitis.
- Group 6: Rats were received alpha lipoic acid as single intraperitoneal injection in a dose of 1 mg/kg body weight (Park et al., 2005) then rats were sacrificed 24 h after induction of acute pancreatitis.
- Group 7: Rats were received alpha lipoic acid in a dose of 1 mg/kg body weight/day for 7 days by intraperitoneal injection then rats were sacrificed 7 days after induction of acute pancreatitis.
- Group 8: Rats were received pentoxifylline as a single intraperitoneal injection in a dose of 12 mg/kg body weight and alpha lipoic acid as a single intraperitoneal injection in a dose of 1 mg/kg body weight then rats were sacrificed 24 h after induction of acute pancreatitis.
- Group 9: Rats were received pentoxifylline in a dose of 12 mg/kg body weight/day and alpha lipoic acid in a dose of 1 mg/kg body weight/day for 7 days by intraperitoneal injection then rats were sacrificed 7 days after induction of acute pancreatitis.

In the treated groups; the drug administration started simultaneously with induction of pancreatitis. At the end of the experiment the rats were sacrificed after 24 h for acute study (groups 2, 4, 6, 8) and after 7 days for follow up (groups 3, 5, 7, 9), blood samples were collected immediately, centrifuged at 3000 × *g* and the serum was separated and stored at −20 °C for further assay of serum levels of amylase enzyme, C-reactive protein (CRP), IL-6, catalase enzyme activity and malondialdehyde (MDA). The abdomen of each rat was dissected and the pancreas was removed immediately and part of it fixed in 10% formalin. Paraffin sections of 5 μm were cut and stained with heamatoxyllin and eosin then examined by light microscope for histopathological changes. Assessment of pancreatic damage was scored by grading acinar cell degeneration, interstitial inflammation, edema and hemorrhage as described by Schmidt's standards (Schmidt et al., 1992) with modification as follows: Grading for edema was scaled as 0: absent or rare; 1: edema in the interlobular space; 2: edema in the intralobular space; 3: isolated-island shape of pancreatic acinus. Inflammation was scored as 0: absent; 1: mild; 2: moderate; 3: severe. Acinar cell degeneration was scaled as 0: absent; 1: focal (<5%); 2: and/or sublobular (<20%); 3: and/or lobular (>20%). Parenchyma hemorrhage was scored as 0: absent; 1: mild; 2: moderate; 3: severe. The maximum score for inflammatory infiltration was 12.

The other part of pancreas was blotted to remove excess blood, weighed and immediately put in 5 ml of PBS (phosphate buffered saline) at 4 °C. Tissue samples were homogenized with 0.5% hexadecyltrimethylammonium bromide in 50 mmol /L PBS (pH 6.0). The suspension was subjected to four cycles of freezing and thawing, and further disrupted by sonication (40 seconds). The sample was then centrifuged (10,000 g, 5 min, 4 °C), and the supernatant was used for myeloperoxidase activity (MPO) assay (Li et al., 2009).

Serum amylase enzyme level (U/L) was measured using kits obtained from Eli tech diagnostic Co. according to the method of Winn-deen et al. (1988).

Serum C-reactive protein (CRP) level (mg/L) was measured using kits obtained from Biosystems Co. according to the method of Otsuji et al. (1982).

Serum IL-6 (pg/ml) was measured using a sandwich ELISA kits for rats (Quantikine), following the manufacturer's protocol instructions.

Serum catalase activity was assayed according to method by Aebi (1984). Briefly, 0.1 ml of 30% H_2O_2 was added to 50 ml of 0.05 M phosphate buffer pH 7. The A_{240} of the solution was observed for reproducible results. A_{240} must be between 0.550 and 0.526. If higher than this range add buffer to decrease A_{240} If too low adds H_2O_2 to increase the absorbance. One unit will decompose 1.0 μmole of H_2O_2 per minute at pH 7.0. The rate of disappearance of H_2O_2 is followed by observing the rate of decrease in the absorbance at 240 nm.

Serum malondialdehyde (MDA) level (nmol/ml) was assayed using the principle and method described by Yagi (1984). It depends on the fact that MDA react with TBA producing thiobarbituric acid reactive substance (allegedly a $[TBA]_2$-Malondialdehyde adduct) a pink chromogen, which can be measured spectrophotometrically at 532 nm. Malondialdehyde-bis-Diethyl acetate (1,1,3,3-tetramethoxypropane) was used as a reference standard. Briefly, 0.5 ml of serum was shaken with 2.5 ml TCA in 10 ml centrifuge tube, then 1 ml of 0.67% TBA was added and mixed. The tube was shaken and heated in a boiling water bath 100 °C for 30 minutes followed by rapid cooling in ice bath. N-