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Modulation of carbon tetrachloride-induced hepatic oxidative stress, injury and fibrosis by olmesartan and omega-3

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ABSTRACT

This study was designed to investigate the potential effects of omega-3, olmesartan and their combination on established hepatic fibrosis in the carbon tetrachloride (CCl_4) rat model. Male Wistar rats received subcutaneous injections of CCl_4 twice weekly for 12 weeks, as well as daily oral treatments of olmesartan (1 and 3 mg/kg), omega-3 (75 and 150 mg/kg) and their combination during the last 4 weeks of intoxication. Our results indicated that omega-3 and, to a lesser extent, olmesartan dose-dependently blunted CCl_4-induced necroinflammation scoring and elevation of liver injury parameters in serum. Besides, omega-3 and, to a lesser extent, olmesartan treatments in a dose dependent manner attenuated CCl_4-induced liver fibrosis, as demonstrated by hepatic histopathology scoring and 4-hydroxyproline content. The mechanisms behind these beneficial effects of both omega-3 and olmesartan were also elucidated. These include (1) counteracting hepatic oxidative stress and augmenting hepatic antioxidants; (2) preventing the activation of hepatic stellate cells (HSCs), as denoted by reducing α-smooth muscle actin (α-SMA) expression in the liver; (3) inhibiting the proliferation and chemotaxis of HSCs, as evidenced by downregulating platelet-derived growth factor receptors-β (PDGFR-β) expression in the liver; and (4) inhibiting the fibrogenesis response of HSCs, as indicated by inhibiting the secretion of transforming growth factor-β1 (TGF-β1). Unexpectedly, when olmesartan was co-administered with omega-3, it interfered with the hepatoprotective and anti-fibrotic activities of omega-3. In conclusion, this study introduces the first evidence regarding the pronounced anti-fibrotic activity of omega-3 and suggests that it may be beneficial in the treatment of hepatic fibrosis in humans.

1. Introduction

Despite the scientific progress in the field of liver disorders, hepatic fibrosis still remains a major medical problem with significant morbidity and mortality. Fibrosis represents the wound healing response of the liver to repeated injury that engages a range of cell types and mediators [1]. After a chronic liver injury of any etiology, the damaged hepatocytes, their membrane components, metabolites of toxic agents, and infiltrating inflammatory cells activate Kupffer cells, which release a number of soluble agents, including cytokines, such as platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), insulin-like growth factor-1 (IGF-1) and endothelin-1 (ET-1), as well as reactive oxygen species (ROS) [2]. These factors act on the hepatic stellate cells (HSCs), which undergo a response known as activation and start expressing new receptors, such as PDGF and TGF-β1 receptors, and new proteins, such as α-smooth muscle actin (α-SMA) [3]. As a result, activated HSCs secrete large amounts of extracellular matrix (ECM) proteins, resulting in accumulation of ECM proteins and fibrogenesis [1].

Inhibition of renin-angiotensin system (RAS), either by angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists, is used clinically to treat hypertension, congestive heart failure, myocardial infarction and renal insufficiency. Apart from this, a large amount of evidence supports that RAS plays an axial role in hepatic fibrosis [4] and its complications, such as portal hypertension and hepatocellular carcinoma [5,6]. Among the various inhibitors for RAS, olmesartan is considered the most important blocker for angiotensin II type 1 receptors. This latest agent has been shown to have longer half-life/duration of action, fewer side effects and greater potency in comparison to the old and new agents of this pharmacological group [7]. With respect to the efficacy and safety of olmesartan in several cardiovascular disorders, its effect against established liver fibrosis is not tested yet.

Omega-3 fatty acids are a type of polyunsaturated fat and are derived from either fish or plant sources. Regardless of their origin, omega-3 polyunsaturated fatty acids, mainly eicosapentaenoic acid and docosahexaenoic acid, have been shown to display pleiotropic benefits against inflammatory, malignant and cardiovascular

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diseases [8,9]. Omega-3 fatty acids supplements have been recently shown to decrease hepatic injury and steatosis [10,11]. Nevertheless, data about their efficacy in established liver fibrosis are still lacking.

Therefore, this study was designed to investigate the potential effects of omega-3, olmesartan and their combination on established hepatic fibrosis in the carbon tetrachloride (CCl_4) rat model. Our results indicated that omega-3 fish oil and, to a lesser extent, olmesartan dose-dependently possessed hepatoprotective and anti-fibrotic activities in established liver fibrosis. The mechanisms behind these beneficial effects of both omega-3 and olmesartan were also elucidated. These include (1) counteracting hepatic oxidative stress and injury; (2) preventing the activation of HSCs as denoted by reducing α-SMA expression in the liver; (3) inhibiting the proliferation and chemotaxis of HSCs as evidenced by down-regulating PDGF receptors-β (PDGFR-β) expression in the liver; and (4) inhibiting the fibrogenesis response of HSCs as indicated by inhibiting the secretion of TGF-β1. Unexpectedly, when olmesartan was co-administered with omega-3, it interfered with the hepatoprotective and anti-fibrotic activities of omega-3.

2. Materials and methods

2.1. Drugs and chemicals

Omega-3 fish oil (containing eicosapentaenoic acid and docosahexaenoic acid in a 2:1 ratio) was purchased from Jarrow Formulas (Los Angeles, CA, USA). Olmesartan medoxomil was purchased from Dalian Richon Chemical (Beijing, China). Silymarin (milk thistle powder containing 80% silymarin) was purchased from Bulk Nutrition (Northborough, MA, USA). 1-Methyl-2-phenylindole, 1,1,3,3-tetramethoxypropane, 5,5′-dithiobis(2-nitrobenzoic acid), vanadium III chloride (VCl_3) and *trans*-4-hydroxy-L-proline were purchased from Sigma–Aldrich (St. Louis, Mo., USA). Methanesulfonic acid was purchased from Merck (Darmstadt, Germany). 3,3′,5,5′-Tetramethylbenzidine and hexadecyltrimethylammonium bromide were purchased from MP Biomedicals (Irvine, CA, USA). L-glutathione reduced and pyrogallol were purchased from Fluka (Buchs SG, Switzerland).

2.2. Animals

Adult male Wistar rats (250–275 g) were allowed access to food and tap water *ad libitum* throughout the acclimatization and experimental periods. All animals received humane care in compliance with the National Institutes of Health and the Research Ethics Committe Criteria for Care of Laboratory Animals at Mansoura University.

2.2.1. Induction of hepatic fibrosis

Hepatic fibrosis was induced by subcutaneous (s.c.) injections of 50% (v/v) of CCl_4 in olive oil (1 ml/kg) twice a week for 12 weeks as previously described [12,13] with a slight modification. The initiation of treatments (i.e. prophylaxis) before the appearance of fibrosis does not mimic the clinical application of anti-fibrotic drugs. Therefore, all treatments under investigation were administered during the last 4 weeks of CCl_4-intoxication via the oral route to mimic the clinical application of these drugs. Silymarin treatment was also included in the study as a standard hepatoprotective and antioxidant for comparing its efficacy with those of omega-3 and olmesartan.

2.3. Experimental design

The rats were divided into eight groups as follows: (1) Control: received biweekly olive oil (1 ml/kg, s.c.) in addition to saline (1 ml/kg/day, oral); (2) CCl_4: received biweekly CCl_4 as described in addition to saline (1 ml/kg/day, oral); (3) CCl_4 + OLM 1: received CCl_4 and olmesartan (1 mg/kg/day, 0.1% w/v in saline; 1 ml/kg, oral); (4) CCl_4 + OLM 3: received CCl_4 and olmesartan (3 mg/kg/

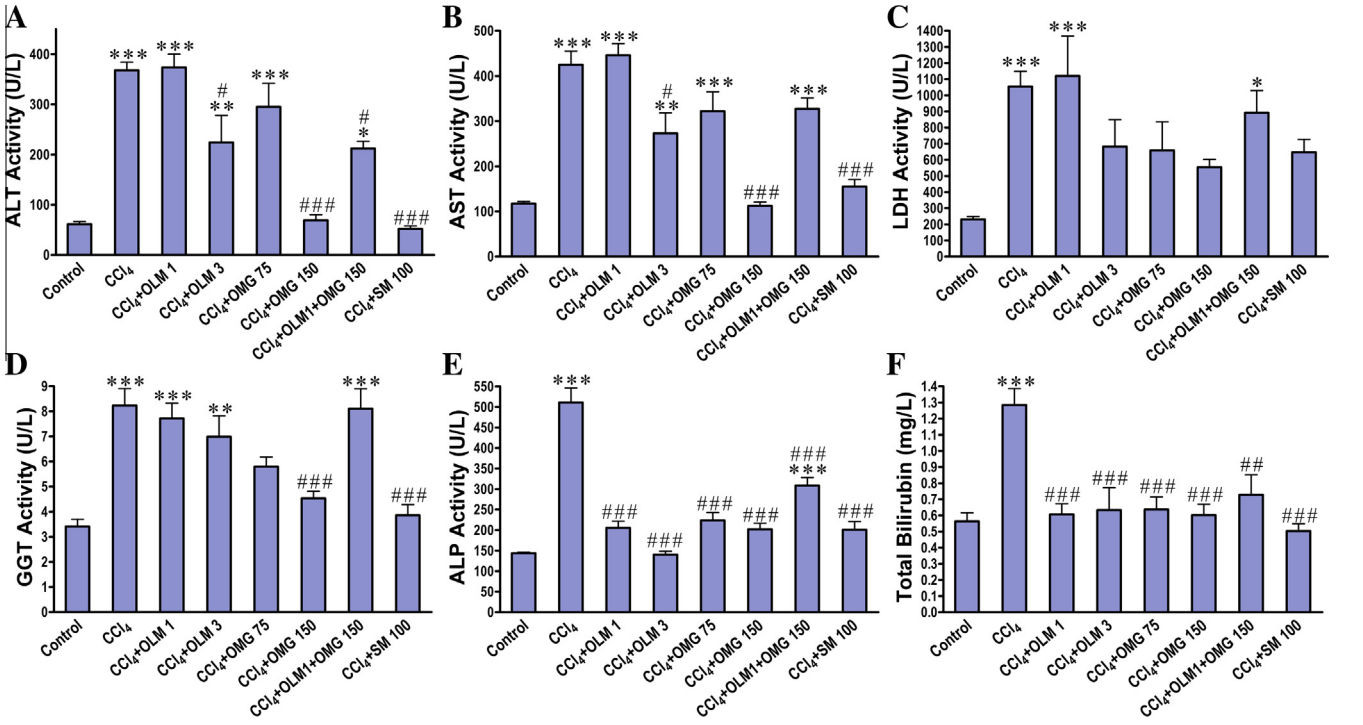

Fig. 1. Effects of olmesartan (1 and 3 mg/kg), omega-3 (75 and 150 mg/kg) and their combination on serum activities of alanine aminotransferase (ALT) (A), aspartate aminotransferase (AST) (B), lactate dehydrogenase (LDH) (C), γ-glutamyl transpeptidase (GGT) (D) and alkaline phosphatase (ALP) (E), as well as level of total bilirubin (F) in carbon tetrachloride (CCl_4)-induced fibrosis. Bars are means ± SE (n = 6–8). Statistically significant differences are indicated as: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. CCl_4. OLM, olmesartan; OMG, omega-3; SM, silymarin.