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Nicotinic acid prevents experimental liver fibrosis by attenuating the prooxidant process

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ABSTRACT

Liver fibrosis is the excessive accumulation of extracellular matrix proteins that occurs in most chronic liver diseases. Nicotinamide treatment has been shown to prevent collagen accumulation and fibrogenesis in a bleomycin model of lung fibrosis. In this study, we evaluated the effects of nicotinic acid (NA) on experimental liver fibrosis and investigated its underlying mechanism.

Methods: Fibrosis was induced by chronic TAA administration and the effects of co-administration with NA for 8 weeks were evaluated, including control groups.

Results: TAA administration induced liver fibrosis, which was prevented by nicotinic acid. NA prevented the elevation of liver enzymes and prevented hepatic glycogen depletion. Liver histopathology and hydroxyproline levels were significantly lower in the rats treated with TAA plus NA compared with TAA only. NA demonstrated antioxidant properties by restoring the redox equilibrium (lipid peroxidation and GPx levels). Western blot assays showed decreased expression levels of TGF-β and its downstream inductor CTGF. Additionally, NA prevented hepatic stellate cell activation due by blocking the expression of α-SMA. Zymography assays showed that NA decreased the activity of matrix metalloproteinases 2 and 9.

Conclusions: NA prevents experimental fibrosis; the mechanisms of action are associated with its antioxidant properties and the reduction in TGF-β expression. The decrease in TGF-β levels may be associated with the attenuation of the oxidative processes, thus resulting in a reduction in HSC activation and ECM deposition. The findings suggest a possible role for NA as an antifibrotic agent for liver injury.

1. Introduction

Cirrhosis is the end-stage consequence of hepatic parenchyma fibrosis and results in nodule formation and altered hepatic function. Fibrosis and cirrhosis result from a sustained wound-healing response to chronic liver injury from a variety of causes including viral, autoimmune, drug-induced, cholestatic and metabolic diseases [1]. Liver fibrosis is initiated by mechanisms that lead to inflammation, which activates a wound-healing response due to the production of the fibrogenic cytokine transforming growth factor-β (TGF-β) [2]. TGF-β appears to be a key cytokine/growth factor mediator in human fibrogenesis because it activates hepatic stellate cells (HSCs) to increase the production and accumulation of extracellular matrix (ECM) [3]. One protein that has shown potential as a downstream mediator of TGF-β signalling in fibroblastic cells is the cysteine-rich peptide connective tissue growth factor (CTGF) [3,4]. CTGF has been suggested to be an important downstream modulator of TGF-β activity and is capable of amplifying the TGF-β profibrogenic action in the liver and in other tissues. TGF-β is not only mitogenic and chemotactic in fibroblasts, but it also stimulates the synthesis of at least two extracellular matrix components: (1) type I collagen and (2) fibronectin [5,6].

Nicotinamide is the amide form of vitamin B3 (niacin) and is obtained via synthesis in the body or as a dietary source and supplement [7]. Nicotinic acid (NA) is the other form of the water-soluble vitamin B3 (Fig. 1). Over the years, NA has been used to treat various diseases such as schizophrenia and type I diabetes [8]. NA has beneficial effects on plasma lipoproteins and has demonstrated clinical benefits in reducing cardiovascular events and atherosclerosis progression. Moreover, NA also exerts anti-inflammatory actions that may be beneficial to patients with inflammatory skin diseases [9]. The side effects of NA have limited its use in general clinical practice. Serious hepatic toxicity has been reported at doses above 3 g/day [7]; these side effects are rare when NA is prescribed at lower doses. Therefore, it is generally considered safe as a food additive and as a component in cosmetics and

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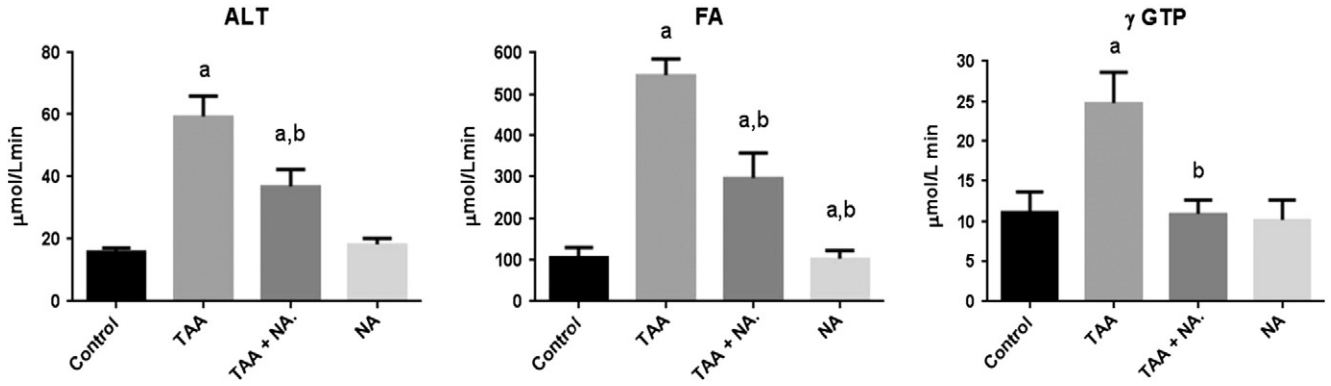

Fig. 1. ALT, AP and γ-GTP activities were determined in serum from control rats following chronic TAA administration for 8 weeks. Control (CONTROL), TAA-treated (TAA), TAA plus nicotinic acid (TAA + NA), and nicotinic acid (NA) rats. Data represent the mean values of the experiments performed in triplicate ± S.E. (n = 6). An "a" denotes a significant difference from the control ($P < 0.05$) and a "b" denotes a significant difference from the TAA-treated rats ($P < 0.05$).

medicines [10]. Evidence from several studies suggests that NA is a potential antifibrotic agent. Treatment with NA was found to attenuate collagen accumulation and lung fibrosis in a bleomycin hamster model [11–13]. In vitro, NA can induce apoptosis in hepatic stellate cells and reduce the expression of collagen I and proinflammatory cytokines [8]. Hepatotoxins, like TAA, initially damage the centrilobular regions of the liver, where there are high levels of cytochrome P450 oxidase that mediate their conversion to toxic intermediates, followed by the production of reactive oxygen species (ROS), lipid peroxidation, and the release of pro-inflammatory cytokines [14]. P450 2E1 enzymes located in the microsomes of liver cells, which convert TAA to a highly reactive toxic intermediates known as thioacetamide sulphur dioxide ($TASO_2$) through oxidation [15,16], inducing hepatotoxicity in experimental animals and different grades of liver damage, including nodular cirrhosis, production of pseudolobules, proliferation of hepatic cells, and necrosis of parenchyma cells [17].

It has been reported that NA inhibits liver fibrosis in rats intoxicated with TAA by suppressing DNA synthesis and enhancing apoptosis of hepatic stellate cells [18]. In CCl_4-induced liver injury, NA has shown protective effects since 1967 [19], it prevents liver necrosis by restoring mitochondrial ability for Ca^{2+} uptake [20], also NA prevents CCl_4-induced liver toxicity in sheep [21]. The molecular mechanisms by which NA exerts these effects have not been identified. In this study, we aimed to evaluate the potential antifibrotic properties of NA on liver injury induced by repeated thioacetamide (TAA) administration to rats and to explore if the action mechanism is associated with attenuation of oxidative stress and downregulation of TGF-β and CTGF.

2. Materials and methods

2.1. Chemicals

Nicotinic acid, sodium thiosulfate, anthrone, thiobarbituric acid, chloramine-T, p-dimethylaminobenzaldehyde, γ-glutamyl-p-nitroanilide, L-γ-glutamyl-p-nitroaniline, p-nitrophenyl phosphate, bovine serum albumin and thioacetamide were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Sodium hydroxide, glacial acetic acid, hydrochloric acid, sulphuric acid, ethanol, methanol, toluene, and formaldehyde were obtained from J.T. Baker (Xalostoc, Mexico City, Mexico). All of the reagents were of analytical quality.

2.2. Study design

Wistar male rats initially weighing 100–110 g and fed a Purina chow rat diet ad libitum were used in the study. Four or five animals were housed per polycarbonate cage under controlled conditions (22 ± 2 °C, 50–60% relative humidity and 12 h light–dark cycles). Cirrhosis was induced by i.p. administration of TAA (Sigma Chemical Company St. Louis, MO, USA) (200 mg/kg of body weight) dissolved in saline three times a week for 8 weeks. In order to determine the capacity of NA (Sigma Chemical Company St. Louis, MO, USA) to prevent liver fibrosis, four groups were formed and treated for 8 weeks. Group 1 (n = 8) consisted of the control animals receiving the vehicle only (saline, i.p.); group 2 (n = 15) was administered TAA; group 3 (n = 15) received TAA plus NA 50 mg/kg dissolved in a saline solution, p.o., daily; and group 4 (n = 8) received NA only. All of the animals were killed

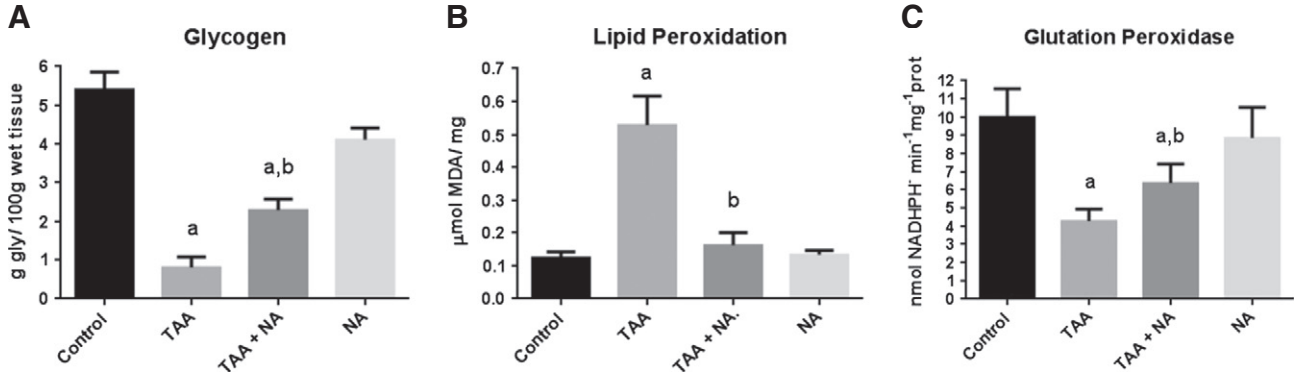

Fig. 2. (A) Liver glycogen content, (B) glutathione peroxidase and (C) lipid peroxidation determined in the livers from control rats following chronic TAA administration for 8 weeks. Control (CONTROL), TAA-treated (TAA), TAA plus nicotinic acid (TAA + NA), and nicotinic acid (NA) rats. An "a" denotes a significant difference from the control ($P < 0.05$) and a "b" denotes a significant difference from the TAA-treated rats ($P < 0.05$).