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Role of nicotinamide (vitamin B3) in acetaminophen-induced changes in rat liver
Nicotinamide effect in acetaminophen-damged liver

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ABSTRACT

Acetaminophen is a widely used analgesic and antipyretic agent, which is safe at therapeutic doses. However, overdoses of acetaminophen induce severe oxidative stress, which leads to acute liver failure. Nicotinamide has proven effective in ameliorating many pathological conditions that occur due to oxidative stress. This study verifies the prophylactic and therapeutic effects of nicotinamide against the hepatic pathophysiological and ultrastructural alterations induced by acetaminophen. Wistar rats intoxicated with an acute overdose of acetaminophen (5 g/kg b.wt) were given a single dose of nicotinamide (500 mg/kg b.wt) either before or after intoxication. Acetaminophen caused significant elevation in the liver functions and lipid peroxidation marker, and decline in the activities of the hepatic antioxidant enzymes. This oxidative injury was associated with hepatic centrilobular necrosis, hemorrage, vacuolar degeneration, lipid accumulation and mitochondrial alterations. Treating intoxicated rats with nicotinamide (500 mg/kg) significantly ameliorated acetaminophen-induced biochemical changes and pathological injuries. However, administering the same dose of nicotinamide to healthy animals or prior to acetaminophen-intoxication induced hepatotoxicity. Caution should be taken when administering high doses of NAM because of its possible hepatotoxicity. Considering the wide use of nicotinamide, there is an important need for monitoring nicotinamide tolerance, safety and efficacy in healthy and diseased subjects.

1. Introduction

Acetaminophen (*N*-acetyl-*p*-aminophenol (APAP), also known as Paracetamol) is a widely used antipyretic and analgesic drug for relieving fever and mild to moderate pain (Kuffner et al., 2007). APAP is well tolerated and generally safe; thus, it is available as an over-the-counter medication (Schilling et al., 2010). However, deliberate or accidental overdoses of APAP are among the common causes of acute liver failure in the Western world (Lee, 2004).

APAP-induced hepatic toxicity is initiated by a highly reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), generated from the metabolism of APAP by the cytochrome P450 system (Walubo et al., 2004). At therapeutic doses of APAP, NAPQI is detoxified by hepatic glutathione (GSH); but at sufficiently high doses of APAP, GSH becomes depleted, leaving NAPQI free to bind to intracellular proteins. This situation leads to a mitochondrial oxidant stress and initiates protein adduct formation, lipid peroxidation, and DNA fragmentation, which eventually results in necrosis of the liver cells (Jaeschke et al., 2012).

Nicotinamide (NAM; also known as niacinamide) is the amide derivative of nicotinic acid (vitamin B_3). It is a precursor of nicotinamide adenine dinucleotide (NAD^+), a key coenzyme in cellular metabolism and energy production (Belenky et al., 2007). NAM is widely available in foods of both animal and vegetable origin, and is commercially available in many vitamin B complex supplements (Maiese and Chong, 2003). NAM has been used now for over 60 years as a drug. It was originally used to prevent pellagra (Horwitt, 1955), and since then, it has been used in treating a diverse range of dermatological conditions

Abbreviations: 2PY, *N*-methyl-2-pyridone-5-carboxamide; 4PY, *N*-methyl-4-pyridone-5-carboxamide; AIF, apoptosis inducing factor; ALP, alkaline phosphatase; ALT, alanine transaminase; APAP, acetaminophen; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; NAD^+, nicotinamide adenine dinucleotide; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NAM, nicotinamide; NAPQI, *N*-acetyl-*p*-benzoquinone imine; PARP, poly (adenosine diphosphate ribose) polymerase.

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(Namazi, 2003; Khodaeiani et al., 2013; Fabbrocini et al., 2014). Nowadays, NAM is used as a therapeutic agent in abroad spectrum of experimental disease models (Yang et al., 2002; Chen et al., 2008; Abdallah, 2010; Audrito et al., 2011; Kim et al., 2011; John et al., 2012; Ullah et al., 2012; Turunc Bayrakdar et al., 2014). These therapeutic benefits has been attributted to its antioxidant effect (Kundu and Kishore, 2005; Lappas and Permezel, 2011; John et al., 2012; Turunc Bayrakdar et al., 2014), (Yang et al., 2002; Ullah et al., 2012; Turunc Bayrakdar et al., 2014), and anti-inflammatory properties (Lappas and Permezel, 2011; Godin et al., 2011). NAM also plays a significant role in DNA repair and cellular stress tolerance (Surjana et al., 2013). NAM has a good safety profile, having been given safely for 5 years at a dose up to 3 g/per day (Knip et al., 2000).

In view of the fact that APAP overdose induces oxidative stress, apoptosis/necrosis and inflammation, and prompted by the aforementioned therapeutic properties of NAM; the current study examines the potential of this vitamin against the hepatic pathophysiological and ultrastructural alterations induced by an acute overdose of acetaminophen in Wistar rats.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats, weighing 130–150 g, were obtained from the Veterinary Serum and Vaccine Research Institute (Cairo, Egypt). The animals were housed in suitable cages and acclimatised to laboratory conditions for a period of 2 weeks before the commencement of the experiments. A temperature of 26 ± 2 °C and 12 h light/dark cycle were maintained. The animals were given free access to water and standard rodent food pellets. All animals were humanely treated in accordance with the WHO guidelines for animal care, and the experimental protocol was approved by the Ain Shams University Research Ethics Committee.

2.2. Chemicals

Nicotinamide was obtained from Fluka Biochemika (Buchs, Switzerland). Paracetamol (Novadol®) was purchased from Sanovi-aventis Phamaceutical Co. (Cairo, Egypt). Kits for the determination of liver enzymes, bilirubin, creatinine and blood urea nitrogen were purchased from the Egyptian Company for Biotechnology (Spectrum) (Cairo, Egypt). Reduced glutathione, glutathione peroxidase, catalase, and malondialdehyde kits were purchased from Biodiagnostics Co. (Dokki, Egypt). All other chemicals were of analytical grade and obtained from standard commercial suppliers. All solutions were prepared immediately before use.

2.3. Experimental design

Male rats fasted overnight were used herein because unfasted animals show heterogenous responses to APAP (Rofe et al., 1998), and males show greater toxic response to acetaminophen than females (Masubuchi et al., 2011). The animals were randomly assigned to five groups (n = 7) as follows: control group: rats were orally administered 50% propylene glycol; nicontinamide group (NAM): rats were intraperitoneally treated with NAM (500 mg/kg b.wt) prepared in cold saline; Acetaminophen group (APAP): rats were orally intoxicated with a single dose of APAP (5 g/ kg b.wt) prepared in 50% propylene glycol (Ilic et al., 2010); prophylactic group (NAM + APAP): NAM (500 mg/kg b.wt) was administered 1.5 h prior to APAP intoxication; and Therapeutic group (APAP + NAM): NAM (500 mg/kg b.wt) was administered 1.5 h after APAP intoxication.

The dose of nicotinamide was selected according to earlier studies, which indicated the effectiveness of NAM in different experimental models at the dose of 500 mg/kg (Yang et al., 2002; Chen et al., 2008; Turunc Bayrakdar et al., 2014). The 1.5 h time point was selected according to Saito et al. (2010), who reported that most of the administered APAP is metabolized and NAPQI-protein binding is completed at this time point.

2.4. Sample collection

Twenty four hours after APAP intoxication, rats were anesthetized and weighed. Blood samples were collected via heart puncture, centrifuged, and the supernatent serum was divided into 200 μl aliquotes and frozen at −80 °C until being assayed for liver enzymes, bilirubin, creatinine and blood urea nitrogen. After necropsy, the liver was immediately excised, cleaned of the adhering connective tissue, examined for gross lesions, blotted, weighed. Liver index was calculated according to the formula: (liver weight/body weight) × 100. Liver samples were then processed for biochemical, histological and ultrastructural assessments.

2.5. Liver functions

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined as described by Bergmeyer et al. (1978). Serum total bilirubin levels were determined according to the method of Walter and Gerade (1970). Alkaline phosphatase (ALP) was determined as described by Kind and King (1954).

2.6. Oxidative stress markers

Small pieces of liver were weighed and washed using chilled saline solution. Tissues were then minced and homogenized (10% w/v) in ice-cold phosphate buffer (pH 7.2). The homogenate was centrifuged at 10,000*g* for 20 min at 4 °C. The resultant supernatant was used for the determination of oxidative stress and antioxidant biomarkers. Lipid peroxidation was determined by estimating the level of malondialdehyde (MDA) according to the method of Mihara and Uchiyama (1978). Reduced glutathione (GSH) was determined as described by Beutler et al. (1963). Catalase was determined according to Aebi (1984). The protein content was determined according to the method of Bradford (1976).

2.7. Histological procedures

Left lateral lobes of liver were fixed in Bouin's solution at room temperature for 24 h. Samples were then dehydrated in ascending grades of ethyl alcohol, cleared in terpineol and embedded in paraffin wax. Transverse sections, 5 μm thick, were prepared and mounted on slides. Thereafter, slides were dewaxed in xylene, hydrated using graded ethanol, and stained with haematoxylin and eosin for histopathological examination.

2.8. Transmission electron microscopy

Mitochondrial alterations and DNA damage were assessed using electron microscopy. Thin slices of the median lobe of the liver were cut into 1-mm cubes, and then immersed in fresh ice cold 2.5% glutaraldeyde in 0.l M phosphate buffer at pH 7.2 for a few hours. After that, the samples were rinsed three times in the buffer and post-fixed for 2 h in 1% buffered OsO_4, dehydrated through ascending graded series of alcohol, embedded in epoxy resin