European Review for Medical and Pharmacological Sciences 2013; 17: 467-471

Protective effect of omega-3 fatty acids on diethylnitrosamine toxicity in rats

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Abstract. – OBJECTIVES: In this study, it was aimed to investigate the effect of n-3 fatty acids (n-3 FA) on diethylnitrosamine (DEN) toxicity with respect to alterations including nitric oxide (NO) formation, uric acid level as well as some liver related enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in rats.

MATERIALS AND METHODS: Forty male Wistar albino rats were used as animal materials. Animals were divided into 4 groups and treated as follows: Rats in group 1 (control) were intraperitoneally (i.p) injected with single dose of saline; rats in group 2 were i.p. injected with DEN at a dose of 150 mg/kg/body weight; rats in group 3 were treated with DEN (via single i.p. injection at 150 mg/kg/body weight) plus n-3 FA (at a dose of 0.4 g /kg/day via subcutaneous route in fish oil) for 7 days, and group 4 received n-3 FA via s.c. route at a dose of 0.4 g/kg/day in fish oil for 7 days. The plasma samples were analyzed for NO, uric acid levels as well as for activities of AST, ALT and ALP.

RESULTS: Uric acid level was lower in DEN group than in control. In addition, NO level and AST, ALT, ALP activities in DEN group were significantly higher than in control. Nitric oxide concentration, ALT and ALP activities in DEN + n-3 FA treated rats were lower than in DEN alone. Uric acid level in DEN + n-3 FA group was higher than in DEN group.

CONCLUSIONS: These results suggest that n-3 fatty acids could ameliorate the toxic effects of DEN in part by means of its free radical scavenging activity and may be of therapeutic value in the protection of liver against toxic effects of DEN.

Key Words:
Diethylnitrosamine, Omega-3 fatty acids, Nitric oxide, Rat.

Introduction

N-nitroso compounds are environmental toxicants, which are widely used in industrial solvents. They are also known as carcinogens and could be found in different types of foodstuff including milk, meat, salted fish, alcoholic beverages and several vegetables[1]. Both environmental and food-born N-nitrosamines pose a health risk for human and animals. Diethylnitrosamine is experimentally used to induce liver carcinoma and study the mechanisms of cytotoxic injury. The cytochrome P-450-dependent enzymes of monooxygenase system also catalyze diethylnitrosamine. Some of the by-products produced during these reactions react covalently with cellular components leading to cellular necrosis, mutation and cancer[2]. DEN is reported to induce generation of free radicals leading to oxidative stress and cell injury through its metabolized end product (ethyl radical). The action of active metabolite ethyl radical on DNA is considered to play a role in carcinogenesis[3]. DEN causes alterations in some enzymes found in the serum and tissues. When the liver cells are injured, several types of liver-specific enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phospatase (ALP) and γ-glutamyltransferase (GGT), are elevated[4]. Studies indicated that DEN elevates the activities of these enzymes[4,5]. AST, ALT and ALP are the group of enzymes, which are used to evaluate the status of liver damage and are considered more sensitive parameters to assess liver injury in rodent species[4,6].

It is known that one of the major mediators in chronic inflammatory processes is the nitrite oxide (NO). It is produced by the liver parenchymal and non-parenchymal cells from L-arginine via nitric oxide synthase (NOS)[7,8]. In normal physiological conditions, NO could be either protective or cytotoxic. Uncontrolled, prolonged and/or massive production of NO by inducible NOS (iNOS) may cause liver damage, inflammation and even tumour development[9].

Uric acid constitutes 33 to 58% of the plasma antioxidant capacity, and it is considered the second important antioxidant molecule following the thiol

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groups within the antioxidant system in the plasma[10].

Biologically important long chain omega-3 polyunsaturated fatty acids, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linoleic acid (ALA), exist in large amounts in fish and fish oils[11]. Recent studies indicate that n-3 fatty acids could have beneficial effects on cancer[12,13], cardio-vascular diseases[14], immune system[15], cirrhosis[16] and nervous system disorders[17]. In addition, omega-3 essential fatty acids are reported to have antioxidant properties by reducing oxidative stress and preventing generation of reactive oxygen species (ROS)[18,20-23].

This study aimed to investigate the effect of n-3 fatty acids (n-3 FA) supplementation on the NO formation, uric acid level and AST, ALT and ALP activities in experimentally induced DEN toxicity in rats.

Materials and Methods

Animal and Treatments

Forty male Wistar albino rats, weighing 185-215 g with the average age of 16 week old, were used as animal materials in this study. The research work was carried out with the approval of the Institutional Ethics committee of Kafkas University, Faculty of Veterinary Medicine. Rats were housed in stainless steel cages in a quite room maintained with 12:12 hr light-dark cycle, at 20-23°C and relative humidity 50-55%. Rats were fed with standard pelleted chow and water *ad libitum*. Animals were divided into 4 groups. Group 1 (control) received a single dose of saline via intraperitoneal (i.p.) injection. Group 2 (DEN alone) was given a single dose of 150 mg/kg/body weight DEN via i.p. injection. Rats in group 3 (DEN + n-3 FA) were treated with single dose of DEN (150 mg/kg body weight) via i.p. injection plus n-3 fatty acid in fish oil (Marincap, Kocak Pharma, Turkey; the composition of the Marincap capsule was EPA 18% and DHA 12%) at a dose of 0,4 g/kg/day via subcutaneous (s.c.) injection for 7 days. Group 4 (n-3 FA alone) received 0,4 g/kg/day n-3 fatty acid in fish oil via s.c. injection for 7 days. At the end of experiment (at day 8), blood samples were collected into the heparinized tubes via cardiac puncture under light ether anaesthesia. Following blood collection, blood was centrifuged at 3000 rpm for 15 min.

Biochemical Analysis

Plasma NO level was measured by the method of Miranda et al[7]. Nitric oxide is known to have very short half-life, and it is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-). Level of NO can be determined indirectly by measuring the concentration of nitrite (NO_2^-) and nitrate (NO_3^-). Initially, plasma samples were deproteinized with 10% zinc sulphate. Total NO concentrations (nitrate and nitrite) were determined colorimetrically by the acidic Griess reaction (via reaction involving reduction of nitrate to nitrite by vanadium (III) chloride). Levels of plasma uric acid and activities of AST, ALT and ALP were analyzed spectrophotometrically using commercially available kits (BioMérieux, Marcy l'Etoile, France).

Statistical Analysis

Statistical analysis were performed by the statistical package SPSS, version 10.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) followed by Duncan test. Results were expressed as mean ± standard error (mean ± SE). p values less than 0.05 were considered significant.

Results

The data obtained from the biochemical assays are presented in Table I. NO levels were found to be higher ($p < 0.01$) in group 2 (DEN alone) than in group 1 (control). Similarly, NO concentration was lower ($p < 0.01$) in group 3 (DEN + n-3 FA) than in group 2 (DEN alone). No difference was found in NO level between control and group 4 (n-3 FA).

Uric acid concentration was found to be lower ($p < 0.01$) in group 2 than in control. Uric acid concentration was higher ($p < 0.01$) in group 3 than in group 2, whereas there was no difference in uric acid levels among control, group 3 and group 4.

Activities of AST, ALP and ALT enzymes in group 2 were elevated compared with that of control. ALT and ALP activities of group 3 were lower ($p < 0.01$) than that of group 2.

Discussion

Increased plasma level of NO was observed in DEN group. It may be due to the enzymatic or chemical break down of N-NO bound found in the chemical structure of DEN, which leads to the release of NO. A significant increase in NO concentration observed in group 2 is in accordance with the previous reports[24,25] where it was speculated that DEN could potentially serve as NO/NO^+

Table I. Effects of omega-3 fatty acids on plasma NO, uric acid levels and activities of AST, ALT and ALP.

Parameters	Control	DEN	DEN + n-3 FA	n-3 FA
NO (μM)	60.81 ± 2.78[b]	74.56 ± 4.56[a]	62.62 ± 3.76[b]	51.20 ± 4.24[b]
Uric acid (mg/L)	29.15 ± 2.77[a]	17.01 ± 2.07[b]	23.19 ± 1.14[a]	27.09 ± 2.07[a]
ALT (U/L)	54.29 ± 1.41[b]	66.50 ± 3.90[a]	58.67 ± 2.22[b]	53.22 ± 1.70[b]
AST (U/L)	99.71 ± 4.30[c,b]	129 ± 9.13[a]	113.78 ± 4.01[a,b]	91.56 ± 3.08[c]
ALP (U/L)	2.26 ± 0.01[c]	2.34 ± 0.01[a]	2.29 ± 0.01[b]	2.26 ± 0.01[c]

Values were represented as mean ± SE (n=10). Means with different superscripts (a, b, c) in the same row indicate significant difference. Control: A single dose of saline given via intraperitoneal (i.p.) injection, DEN: A single dose of 150 mg/kg/body weight DEN given via i.p. injection, DEN + n-3 FA: A single dose of 150 mg/kg/body weight DEN given via i.p. plus 0,4 g/kg/day subcutaneously (s.c.) n-3 FA for 7 days, n-3 FA: 400 mg/kg/day s.c. n-3 FA for 7 days.

donor. The carcinogenesis induced by N-nitrosamines is reported to be due to α-hydroxylation by the catalytic action of cytochrome p-450-dependent oxidases and oxygenases. The break down of α-hydroxy-N-nitroso compounds could indirectly result in DNA damage via the generation of alkylating agents that are produced from N-nitrosamines[25]. Nitric oxide can interact with superoxide ($O_2^{\cdot}$) to form peroxynitrite ($ONOO^-$) which is also a powerful oxidant capable of oxidizing potentially dangerous reactions including thiol groups of proteins that lead to cellular damage. Other than its own oxidizing effect, breakdown of peroxynitrite could give rise to the production of hydroxyl radical ($^{\cdot}OH$) which is a potent oxidant leading to cell injury[18]. It has been shovn that exogenous reduced glutathione (GSH), containing thiol group, can altered levels of NO in rabbits[19].

Studies indicate that n-3 FA treatment in rats brought about a reduction of NO levels in erhytrocytes[18], plasma[26] and tissues[22]. In the present study, plasma NO concentration in rats treated with DEN alone was found to be higher than in control and DEN + n-3 FA group. These findings suggest that concurrent administration of n-3 FA could reduce NO level in DEN group. The results obtained in this study suggest that n-3 FA can react with free radicals acting as a free radical scavenger, which may lead to reduce NO concentration[18,22].

Uric acid constitutes 33 to 58% of the plasma antioxidant capacity. It is considered the second important antioxidant molecule following the thiol groups within the antioxidant system in the plasma[10]. In addition, it was reported that uric acid serves as a peroxynitrite scavenger[27]. In this investigation, we have observed that plasma uric acid level in DEN group was lower than that in the control group. We speculate that this reduction could be due to the fact that DEN-induced elevation in NO level results in generation of peroxynitrite, which can interact with uric acid leading to its depletion in the plasma. The level of uric acid concentration in DEN + n-3 FA group was similar to the values observed in control. The normalization of uric acid levels in DEN + n-3 FA group could be explained by the antioxidant effect of n-3 FA. However, Schacky et al[28] showed that n-3 FA administration in humans did not change the uric acid concentrations. Uric acid concentration in group 4 (n-3 FA alone) was found to be similar to that in control. These findings also suggest that n-3 FA could show antioxidant effect during a toxic insult.

DEN-induced free radical generation may be due to the action of mixed-function oxidase system through the cytochrome p-450 dependent enzymatic process. Free radicals generated via this route are thought to play an important role in many toxicities and cell injury[3]. AST, ALT and ALP enzymes are frequently used to evaluate the status of liver damage and considered more sensitive parameters to measure liver injury in rodent species[3,4,6]. Sahin et al[5] and Bansal et al[3] reported that DEN administered at doses of 100 and 200 mg/kg resulted in increased AST, ALT and ALP activities in the plasma of rats. Similar to these reports, we observed significantly increased AST, ALT and ALP activities in the plasma of DEN-treated rats compared with that of control indicating that DEN treatment could induce a liver damage in the rats. On the other hand, Hatzitolios et al[29] reported that the administration of n-3 FA to the patients with hiperlipidemia reduced the levels of AST and ALT activities and prevented the occurrence of fatty liver in 35% of the affected patients. Alwayn et al[30] reported that in a nonalcoholic fatty-liver model, n-3 FA administration in murines, receiving high carbohydrate intake, led to reduced AST, ALT and ALP activities compared with the group without n-3 FA treatment. Similarly,

the concurrent administration of n-3 FA in our study gave rise to a decreased level of ALT and ALP in DEN + n-3 FA group. These results suggest that n-3 FA could protect liver from DEN-induced liver damage in rats.

The similarity observed between control and n-3 FA groups with respect to assayed parameters indicates that n-3 FA alone has no effect on the liver. ALT is primarily found in the liver whereas AST could also be found in liver, heart, kidneys, brain and muscle tissues. For this reason, ALT is known to be a more liver-specific enzyme compared with AST[6]. The discrepancy in ALT and AST activities could be explained by the specificity of these two enzymes to different tissues.

In conclusion, administration of n-3 fatty acids reduced NO released and increased uric acid level in DEN-treated rats. In addition, n-3 fatty acid normalized activities of AST, ALT and ALP enzymes. The results suggest that n-3 fatty acids could ameliorate the toxic effects of DEN in part by its free radical scavenging activity and may be of therapeutic value in the protection of liver against toxic effects of DEN.

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