Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 45 (2007) 1634–1640
www.elsevier.com/locate/foodchemtox

Garlic oil ameliorates ferric nitrilotriacetate (Fe-NTA)-induced damage and tumor promotion: Implications for cancer prevention

Mukesh Kumar Agarwal [a], Mohammad Iqbal [b,*], Mohammad Athar [a]

[a] *Department of Medical Elementology and Toxicology, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi-110062, India*

[b] *Biotechnology Research Institute, University Malaysia Sabah, Locked Bag No. 2073, 88999 Kota kinabalu, Sabah, Malaysia*

Received 2 April 2006; accepted 23 February 2007

Abstract

Intraperitoneal injection of ferric nitrilotriacetate (Fe-NTA) to rats and mice results in iron-induced free radical injury and cancer in kidneys. This study was designed to investigate the effects of garlic oil on Fe-NTA-induced damage and tumor promotion. Pretreatment of rats with garlic oil at a dose regimen of 50–100 mg/kg body weight for a week significantly and dose dependently protected against Fe-NTA induced damage as well as tumor promotion. Garlic oil afforded protection against hepatic lipid peroxidation, generation of hydrogen peroxide, preserved glutathione levels and activities of antioxidant enzymes. A protection against Fe-NTA induced hepatic tumor promotion was also apparent as inhibition in the modulation of hepatic tumor markers viz., ornithine decarboxylase activity and DNA synthesis. These results clearly demonstrate the role of oxidative stress and its relation to tumor promotion and suggest protective effects of garlic oil against Fe-NTA induced hepatic toxicity and it can serve as potent chemopreventive agent to suppress oxidant-induced tissue injury and carcinogenesis.

Keywords: Iron nitrilotriacetate; Garlic oil; Chemoprevention; Oxidative stress; Antioxidant enzymes; Tumor promotion

1. Introduction

Ferric nitrilotriacetate (Fe-NTA), a free radical generating compound, which is formed by the interaction of iron and nitrilotriacetic acid, a known substitute for pyrophosphate used in various kinds of detergents (Anderson et al., 1985). It is a potent nephrototoxic agent (Awai et al., 1979) and induces apoptosis in mouse renal proximal tubules (Kawabata et al., 1997). An enhanced formation of 4-hydroxy-2-nonenal modified proteins in renal proximal tubules of rats treated with Fe-NTA has been shown (Toyokuni et al., 1994). Fe-NTA administration is subsequently associated with high incidence of renal damage and renal carcinoma in male mice and rats (Li et al., 1987; Okada and Midorikawa, 1982). It is assumed that Fe-NTA-mediated generation of free radicals plays an important role in renal tumorogenesis (Li et al., 1987; Okada, 1996). Renal DNA damage leading to the single strand and double strand brakes (Toyokuni and Sagripanti, 1993), DNA protein cross-links (Toyokuni et al., 1995) and enhanced formation of 8-hydroxy deoxyguanosine (8-OH-dG) has been observed following exposure of animals to Fe-NTA (Umemura et al., 1990). Our laboratory have previously reported that treatment with Fe-NTA induced a variety of changes *in vivo*, i.e. enhanced lipid peroxidation, depletion of enzymatic and non-enzymatic antioxidant molecules, induction of protein carbonyl content, increased prostaglandin $F_2\alpha$, increased expression

Abbreviations: Fe-NTA, ferric nitrilotriacetate; GSH, reduced glutathione; CDNB, 1-chloro-2, 4-dinitrobenzene; ODC, ornithine decarboxylase; ROS, reactive oxygen species; TBA, thiobarbituric acid; TCA, trichloroacetic acid; H_2O_2, hydrogen peroxide; PMS, post-mitochondrial supernatant; PMSF, phenylmethyl sulfonyl fluoride.

* Corresponding author.
E-mail address: m_iqbal2k@hotmail.com (M. Iqbal).

0278-6915/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.fct.2007.02.029

of HNE-modified proteins, increased ornithine decarboxylase activity and DNA synthesis in kidney (Athar and Iqbal, 1998; Iqbal et al., 1997, 1999a,b). In addition, Fe-NTA has been shown to induce hepatic oxidative damage in HepG2 cells (Sakurai and Cederbaum, 1998) and a potent hepatic tumor promoter and it depletes GSH levels and causes a decrease in the activities of GSH-metabolizing and antioxidant enzymes in liver (Iqbal et al., 1995, 1996). The prevention of Fe-NTA induced hepatotoxicity by vitamin E has also been reported (Agarwal et al., 2005).

Diet has been suggested to have a significant impact on the process of the cancer development (Armstrong and Doll, 1975). Recent investigations suggest that several dietary constituents may be important factors capable of increasing or decreasing cancer incidence (Wattenberg, 1983). Thus, specific and predetermined manipulations of the diet may represent a promising non-invasive approach to minimize cancer. These studies promoted us to search for a novel and effective agent that may suppresses toxicity and carcinogenicity of Fe-NTA.

Allium sativum commonly known as garlic, a member of lily family is a common constituent of Indian diet. It is used with spices to give the food a special flavor and fragrance. It is also used as a medicinal herb in many countries. A very extensive literature exists concerning medicinal uses and chemical composition of garlic. Amongst the biological effects found is chemoprevention of cancer. A number of pharmacological effects of garlic constituents have been reported. These include bactericidal (Stoll and Seeback, 1951; Weisberger and Pensky, 1957), antibiotic (Lewis, 1977), antitumor (Weisberger and Pensky, 1957), hypolipidemic (Lewis, 1977), hypoglycemic and antiatherosclereosis activities (Nandkarni, 1954). Garlic oil also inhibits 7, 12-dimethyl benz(*a*)anthracene (DMBA) and benzo(*a*)pyrene (BP) induced skin carcinogenesis in mice (Patnaik et al., 1980; Sadhana et al., 1988). In other organs, too, garlic oil reduces the tumor induction response (Brady et al., 1988; Wargovich et al., 1988; Wargovich and Goldberg, 1995). Additionally, dietary garlic powder inhibits diethylnitrosamine-induced rat hepatocarcinogenesis (Kweon et al., 2003).

Recent epidemiological studies revealed that gastric cancer mortality was 10 times lower in areas of china where garlic consumption is higher as compared to regions where the intake of garlic is low (Mei et al., 1985). Similarly, a lower risk of stomach cancer has been reported in residents of Italy who consumes greater quantities of garlic (Buiatti et al., 1989). Several studies have shown that garlic powder and/or garlic constituents can reduce the incidence of chemically induced tumors in experimental animals (Wattenberg, 1983). We also observed that pretreatment of animals with garlic oil reduces the toxicity of Fe-NTA in rat kidney (Iqbal and Athar, 1998). Therefore, the consumption of garlic may provide some kind of protection from cancer development. As garlic and its purified constituents have been shown to protect against various kinds of injuries and neoplasm involving oxidative stress, we therefore, hypothesize that pretreatment of animals with garlic may suppress Fe-NTA induced hepatic injury. In the view of the above observations, we decided to investigate whether dietary component garlic could inhibit Fe-NTA induced damage in rats and to assess the possible preventive mechanism(s) expressed by garlic. We report herein the *in vivo* protective effects of garlic oil toward Fe-NTA induced damage and tumor promotion. The significance of these results can be implicated in relation to the cancer chemopreventive effects of garlic against the induction of tumors in various target organs.

2. Materials and methods

2.1. Chemicals

Chemicals and biochemicals used in this study were either of analytical grade or highest purity grade available commercially. Bovine serum albumin, nicotinamide adenine dinucleotide phosphate reduced, tris HCl, 2-mercaptoethanol, γ-glutamyl-*p*-nitroanilide, phenylmethylsulphonyl fluoride, pyridoxal-5-phosphate, dithiothreitol, glutathione reductase, thiobarbituric acid, 1-chloro-2,4-dinitrobenzene, hydrogen peroxide, 5,5′-dithio-bis-2-nitrobenzoic acid, glucose-6-phosphate, nitrilotriacetic acid disodium salt (NTA), oxidized and reduced glutathione were purchased from either Sigma Chemical Company, St Louis, MO, USA or Aldrich, USA. [^{14}C]Ornithine (specific activity 56 mCi/mmol) and [^{3}H]thymidine (specific activity 82 Ci/mmol) were obtained from Amersham Corporation, UK. Garlic oil was obtained from Ranbaxy Research Lab Ltd., New Delhi, India and was used as such without any further processing.

2.2. Preparation and injection of Fe-NTA solution

A solution of Fe-NTA was prepared by the method of Awai et al. (1979). Briefly, ferric nitrate ($FeNO_3$) and NTA was dissolved in double distilled water. The respective solutions were mixed to achieve a molar ratio of 1:3 of Fe-NTA. The pH was adjusted to 7.4 with sodium bicarbonate with constant stirring. All solutions were prepared fresh immediately before its use. Fe-NTA was intraperitoneally injected into animals.

2.3. Animals and treatments

Male Wistar rats (4–6 weeks old, weighing 125–150 g) pathogen free were procured from Central Animal House Facility of Jamia Hamdard University, New Delhi, India and used for all biological experimental work. All animals were housed in an air-conditioned room in polypropylene cages usually in groups of six rats in each unless mentioned otherwise and were allowed to acclimatize for one week before study. They had free access to pellet diet (Hindustan Lever Ltd., Bombay, India) and water ad-libitum. The animals were kept at room temperature of 25 °C (±2 °C) and were exposed to a 12-h light/dark cycle.

For ODC activity and various biochemical studies different groups of animals were used. We took thirty rats to study the effect of garlic oil on Fe-NTA-mediated generation of hepatic oxidative stress and ODC induction studies. These rats were divided into five groups and each group has six rats. Group-I received saline and served as negative control. Group-II received an oral treatment of garlic oil (100 mg/kg body weight) and also served as control. Group-III animals received only corn oil (vehicle of garlic oil) daily for 7 days through the gavage. Group-IV and V animals received 50 mg and 100 mg garlic oil/kg body weight, respectively for 7 days through gavage. Twenty four hours after the last treatment of garlic oil or corn oil, the animals of group-III, IV and V received an i.p. injection of Fe-NTA (9 mg Fe/kg body weight). All these animals were sacrificed 12 h after saline or Fe-NTA-treatment within a period of 1-h by cervical dislocation.

For [^{3}H]thymidine incorporation studies, animal treatment and dose regimen were the same as described above. However, at 18 h after