Food and Chemical Toxicology 49 (2011) 887–892

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effect of gallic acid on xenobiotic metabolizing enzymes in 1,2-dimethyl hydrazine induced colon carcinogenesis in Wistar rats – A chemopreventive approach

J. Giftson Senapathy [a,*], S. Jayanthi [b], P. Viswanathan [c], P. Umadevi [a], N. Nalini [b]

[a] Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Karunya Nagar 641 114, Tamil Nadu, India
[b] Department of Biochemistry & Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
[c] Division of Pathology, Faculty of Medicine, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 1 October 2010
Accepted 13 December 2010
Available online 21 December 2010

Keywords:
Chemoprevention
1,2-dimethyl hydrazine
Xenobiotic-metabolizing enzymes
Gallic acid

ABSTRACT

Colon cancer risk may be influenced by phase I and II xenobiotic–metabolizing enzyme systems. The chemopreventive agent gallic acid (GA), a plant polyphenol, is found in various natural products. Our aim was to evaluate the potential role of GA on drug-metabolizing enzymes in 1,2-dimethyl hydrazine (DMH) induced rat colon carcinogenesis. The total experimental duration was 30 weeks. The effect of GA (50 mg/kg b.w.) on the activities of phase I enzymes (cytochrome P450 and cytochrome b5) and phase II enzymes (glutathione S-transferase, DT-diaphorase and gamma glutamyl transpeptidase) were assessed in the liver and colonic mucosa and the colons were also examined visually. In DMH induced rats, there was a decrease in the activities of phase II enzymes and an increase in the activities of phase I enzymes. On GA supplementation, there was a significant increase in the activities of phase II enzymes and a significant decrease in the activities of phase I enzymes, in addition to the decreased tumor incidence. Histopathological changes also confirm this. Thus, the marked potential of GA in modulating the phase I and II xenobiotic-metabolizing enzymes suggests that GA may have a major impact on colon cancer chemoprevention.

1. Introduction

Colon cancer is a multifactorial disease that results from the interaction of multiple genetic and environmental factors. Colon cancer is one of the most common forms of cancer in the western world, where it has been found to be intimately linked to diet (Lipkin et al., 1999).

The colon specific carcinogen 1,2-dimethyl hydrazine (DMH) has been shown to induce the formation of methyl adducts with DNA bases, point mutations, micronuclei and sister chromatid exchanges (Choudhary and Hansen, 1998). This adduct formation interferes with normal cell growth by causing genetic mutations and altered normal gene transcription.

DMH is not a direct carcinogen and carcinogens that are not chemically reactive are metabolically activated by the phase I and phase II xenobiotic-metabolizing enzymes in order to exert their mutagenic and carcinogenic effects. The activation of carcinogen is primarily catalyzed by phase I enzymes. In phase I reactions, the procarcinogens undergo hydroxylation catalyzed by microsomal cytochrome P450 dependant monooxygenases to produce strong electrophiles capable of interacting with cellular nucleophiles such as DNA to form adducts eventually culminating in mutagenesis and neoplastic transformation. These electrophilic intermediates are detoxified by phase II biotransformation enzymes such as glutathione S-transferase (GST) and DT-diaphorase (DTD) (Hodgson, 2001; Sheweita and Tilmisany, 2003).

Many chemopreventive agents have been shown to modulate gene expression including induction of phase II detoxifying enzymes, such as GST. Induction of phase II enzymes in general leads to protection of cells/tissues against exogenous and/or endogenous carcinogenic intermediates. The antioxidant or electrophile response element (ARE/EpRE) found at the 5′-flanking region of these phase II genes may play an important role in mediating their induction by xenobiotics including chemopreventive agents (Kong et al., 2001).

Gallic acid (3,4,5-trihydroxybenzoic acid, GA) (Fig. 1), a naturally occurring plant phenol, is found in various other natural products like gallnuts, sumac, tea leaves, oak bark, apple peels, grapes, strawberries, pine apples, bananas and lemons. GA

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DMH, 1,2-dimethyl hydrazine; DTD, DT-diaphorase; GA, Gallic acid; GGT, γ-glutamyl transpeptidase; GST, glutathione S-transferase.

* Corresponding author. Tel.: +91 9865151763; fax: +91 422 2615615.
E-mail addresses: meetgiftson@gmail.com, meetgiftson_j@yahoo.co.in (J. Giftson Senapathy).

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.12.012

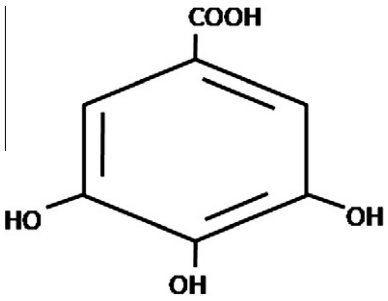

Fig. 1. Chemical structure of gallic acid (3,4,5-trihydroxybenzoic acid).

possesses cytotoxicity against cancer cells, and has anti-inflammatory and antimutagenic properties. GA was described as an excellent free radical scavenger and as an inducer of differentiation and programmed cell death in a number of tumor cell lines (Jagan et al., 2008).

Even though many reports revealed that GA plays an important role in the prevention of malignant transformation and cancer development, its effect on colon carcinogenesis in vivo, is not yet documented. In this investigation, we delineated the role of GA on DMH induced colon carcinogenesis in rats with special focus on phase I [cytochrome P450 and cytochrome b5] and phase II [glutathione S-transferase (GST), DT-diaphorase (DTD) and γ-glutamyl transpeptidase (GGT)] xenobiotic metabolizing enzymes and histopathological changes in the colon.

2. Materials and methods

2.1. Chemicals and their sources

Gallic acid (GA) and DMH were procured from Sigma Chemicals Co., (St. Louis, MO, USA). All other chemicals used were of high grade unless otherwise specified.

2.2. Tumor induction

DMH, was dissolved in 1 mM EDTA, adjusted to pH 6.5 with 1 mM NaOH and administered subcutaneously in the right thigh at a dose of 20 mg/kg body weight once a week for the first 15 weeks.

2.3. Preparation of gallic acid

Gallic acid (GA) was solubilized in water just before treatment and was administered everyday orally at a dose of 50 mg/kg body weight (Hsu and Yen, 2007) for 30 weeks.

2.4. Animals and diet

Male albino Wistar rats were procured and maintained at the Central Animal House, Raja Muthiah Medical College & Hospital, Annamalai University. The animals were kept in polypropylene cages (four per cage) and fed standard pellet diet for one week. Thereafter, the animals were randomly divided into six groups each containing eight rats and maintained under controlled conditions of temperature (24 ± 2 °C), humidity (50 ± 10%), and 12-h light/dark cycle. Commercial pellet diet containing 4.2% fat (Hindustan Lever Ltd., Mumbai, India) was powdered and mixed with 15.8% peanut oil making a total of 20% fat in the diet (Table 1). This modified powdered diet was fed to rats throughout the experimental period of 30 weeks (including 1 week for acclimatization), and tap water was provided ad libitum.

Table 1
Composition of the modified diet.

	Commercial pellet diet 84.2%	Peanut oil 15.8%	Total %
Protein	17.7	–	17.7
Fat	4.2	15.8	20.0
Carbohydrate	50.5	–	50.5
Fibre	3.4	–	3.4
Minerals	6.7	–	6.7
Vitamins	1.7	–	1.7

The animals were cared for incompliance with the principles and guidelines of Ethical Committee for Animal Care of Annamalai University in accordance with the Indian National Law on animal care and use (Reg. No.160/1999/CPCSEA/482).

2.5. Treatment schedule

Group 1 served as control rats, group 2 rats were given GA, 50 mg/kg body weight by intragastric intubation everyday; groups 3–6 were injected DMH (s.c. injections 20 mg/kg body weight) once a week for the first 15 weeks. Group 4 rats received GA as in group 2 starting 1 week before DMH injections and continued till the final exposure [DMH + GA (initiation-I)]. Group 5 rats received GA as in group 2 after the cessation of DMH injections and continued till the end [DMH + GA (post-initiation)] of the experimental period. The rats in group 6 were supplemented with GA as in group 2 from the day of carcinogen treatment and continued till the end of the entire experimental period of 30 weeks [DMH + GA (Entire period)]. For more clarity experimental protocol is shown in Fig. 2.

2.6. Preparation of cytosol and microsome fractions

At the end of the experimental period all the animals were sacrificed under anesthesia (i.p. administration of ketamine hydrochloride 30 mg/kg body weight) by cervical dislocation after an overnight fast. The liver and the intestinal mucosal scrapings were homogenized in ice-cold 0.15 M Tris–KCl buffer (pH 7.4) and centrifuged at 10,000 rpm for 20 min. The supernatant was transferred into pre-cooled ultracentrifugation tubes and centrifuged at 1,05,000*g* for 60 min. The resulting supernatant (cytosolic fraction) was used for assaying total cytosolic glutathione S-transferase (GST, EC 2.5.1.18), DT-diaphorase (DTD, EC 1.6.99.2) and γ-glutamyl transpeptidase (GGT, EC 2.3.2.2). The pellet representing microsomes was used for assaying cytochrome b5 and cytochrome P450.

2.7. Biochemical estimations

2.7.1. Estimation of cytochrome b5 and cytochrome P450

Cytochrome P450 and cytochrome b5 content were assayed as described by Omura and Sato (Omura and Sato, 1964). To the mixture containing 1.0 ml of buffer, 0.1 ml of tissue homogenate, a few milligrams of solid sodium dithionate were added. The absorbance was scanned between 400 and 500 nm. CO was gently bubbled for approximately 1 min and the samples were read from 400 to 500 nm. The difference in absorption spectrum was used to calculate cytochrome P450 content using the extinction coefficient 91 mM^{-1} cm^{-1}. Cytochrome b5 was measured from the difference spectrum between reduced and oxidized cytochrome b5. The reaction mixture containing 1.0 ml of buffer and 0.5 ml of tissue homogenate was read from 400 to 500 nm. The difference in absorption spectrum was used to calculate cytochrome b5 content using the extinction coefficient 185 mM^{-1} cm^{-1}.

2.7.2. Determination of GST, DTD and GGT

The activity of GST was determined as described by Habig et al. (1974). The reaction was started by the addition of 0.1 ml of 30 mM GSH to the reaction mixture containing 2.0 ml of phosphate buffer, 0.1 ml of 30 mM 1-chloro-2,4-dinitrobenzene (CDNB), and 0.1 ml of tissue homogenate and 1.7 ml of distilled water. The reaction mixture was preincubated at 37 °C for 5 min. The increase in absorbance at 340 nm was read using CDNB as substrate.

The activity of DT-diaphorase was assayed as described by Ernster (1967). The reduction with NADPH as the electron donor and 2,6-dichlorophenolindophenol (DCPIP) as the electron acceptor and measured at 550 nm. The reaction was started by the addition of 0.5 ml of sample to the reaction mixture containing 2.4 ml of Tris-buffer, 0.05 ml of 0.3 mM NADH, 0.02 ml of 0.4 mM DCPIP, 0.1 ml of 33 mM cytochrome C and 0.02 ml of 0.07% bovine serum albumin. The enzyme activity was calculated using the extinction coefficient 21 mM^{-1} cm^{-1}.

The activity of GGT was assayed using the method of Fiala et al. (1972). The reaction mixture containing 22 mM glycylglycine, 5 mM of gamma glutamyl *p*-nitroanilide and 3 mM of 0.1 mM Tris–HCl buffer in a total volume of 4.0 ml was incubated for 10 min at 37 °C. The reaction was arrested by immersing the tubes in a boiling water bath and the absorbance of the liberated *p*-nitroaniline was read at 410 nm.

2.7.3. Estimation of protein

The protein content was determined following the method of Lowry et al. (1951) using BSA as standard, at 660 nm.

2.8. Statistical analysis

Data were analyzed using one-way analysis of variance and a significant difference among treatment groups were evaluated by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant at $P < 0.05$. All statistical analyses were made using SPSS 12.0 software package (SPSS, Tokyo, Japan).