Experimental and Toxicologic Pathology 65 (2013) 409–418

Contents lists available at SciVerse ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

The effect of rosmarinic acid on 1,2-dimethylhydrazine induced colon carcinogenesis

Karthikkumar Venkatachalam, Sivagami Gunasekaran, Victor Antony Santiago Jesudoss, Nalini Namasivayam*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608002, Tamilnadu, India

ARTICLE INFO

Article history:
Received 7 October 2011
Accepted 18 December 2011

Keywords:
Rosmarinic acid
1,2-Dimethylhydrazine
Chemoprevention

ABSTRACT

This study was carried out to investigate the chemopreventive potential of rosmarinic acid (RA) against 1,2-dimethylhydrazine (DMH) induced rat colon carcinogenesis by evaluating the effect of RA on tumour formation, antioxidant enzymes, cytochrome P450 content, p-nitrophenol hydroxylase and GST activities. Rats were divided into six groups and fed modified pellet diet for the entire experimental period. Group 1 served as control, group 2 received RA (10 mg/kg b.w.). Groups 3–6 were induced colon cancer by injecting DMH (20 mg/kg b.w.) subcutaneously once a week for the first four weeks (groups 3–6). In addition, RA was administered at the doses of 2.5, 5 and 10 mg/kg b.w. to groups 4–6 respectively. DMH treated rats showed large number of colonic tumours; decreased lipid peroxidation; decreased antioxidant status; elevated CYP450 content and PNPH activities; and decreased GST activity in the liver and colon. Supplementation with RA (5 mg kg/b.w.) to DMH treated rats significantly decreased the number of polyps (50%); reversed the markers of oxidative stress (21.0%); antioxidant status (38.55%); CYP450 content (29.41%); and PNPH activities (21.9%). RA at the dose of 5 mg/kg b.w. showed a most pronounced effect and could be used as a possible chemopreventive agent against colon cancer.

1. Introduction

Colorectal cancer is the second most common cause of cancer related deaths around the world. As of 2008, it is ranked as the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the Western world (American Cancer Society, 2010). It is one of the fastest emerging gastrointestinal cancers in the Asia Pacific region (Goh et al., 2005). In India, there are about 3.5 million cases of cancer of which about 35,000 are found to suffer from colorectal cancer (Shrikhande et al., 2007). Though the estimated incidence of colorectal cancer is low in India its prevalence is increasing at an alarming rate with changing life style.

Worldwide epidemiologic studies reveal an interplay between diet and the prevalence of gastrointestinal tract tumours, especially colorectal cancer, which can be promoted by a diet rich in fat and meat (Aggarwal, 2008). Red meat, including beef, pork, lamb, goat (Singh and Fraser, 1998), processed meat (smoked, cured, salted, or preserved) (Chao et al., 2005) and alcohol (Murata et al., 1996) have been linked with an increase in the risk of colon cancer (World Cancer Research Fund, 2007). It may be related to increased concentrations of secondary bile acids within the colon, which may increase cell proliferation in the colonic mucosa, and have been found to be carcinogenic in animal models. Thus, cancer prevention using diet-based intervention is considered safe and without any side effects.

In this study we have used 1,2-dimethylhydrazine (DMH), a cycasin derivative to induce colon cancer in rats. This rat model has a similar pathology with that of the human disease, during the development of colon cancer. DMH is converted to azoxymethane (AOM), which is further metabolized to methylazoxymethanol (MAM) and then to methyldiazonium ion. Once methyldiazonium ion is formed it generates a carbonium ion that is responsible for the methylation of nucleic acids (Taketo, 1998). The activated carcinogen reaches the colon either via the blood or bile. The primary effect of the carcinogen in the colonic epithelial cells is methylation of DNA. As like other alkylating agents, metabolite of DMH, AOM is also hydroxylated to MAM by CYP2E1, which undergoes oxidation, catalysed by CYP2E1 or alcohol dehydrogenase (ADH) in the liver. The oxidized product, methylazoxyformaldehyde, yields methyldiazonium ion and formic acid by the addition of water. Methyldiazonium ion methylates DNA bases in the target organs. So the production of free radicals by DMH might be due to P-450-dependent enzymes which augment oxidative stress by the formation of H_2O_2 and O_2^* (Farber and Gerson, 1984).

Moreover, DMH itself can generate H_2O_2 in the presence of copper ions (Yagi, 1987). In the presence of metallic ions such as Fe^{2+}/Cu^{2+}, H_2O_2 can react with O_2 and can convert it into a

* Corresponding author. Tel.: +91 4144 239141; fax: +91 4144 238343.
E-mail address: nalininam@yahoo.com (N. Namasivayam).

0940-2993/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.etp.2011.12.005

more reactive OH radical. If there are not enough catalase (CAT) or glutathione peroxidase (GPx) to decompose H_2O_2 (Cheeseman and Slater, 1993), the generated OH radical is capable of attacking DNA. It is also believed that copper ions present at G-C sites of DNA molecules (Pezzano and Podo, 1980) can induce point mutation in the p53 tumour suppressor gene, and other cellular genes that in turn can be converted into oncogenes responsible for the development of cancer (Lane, 1994; Bose, 1988).

Oxidative stress is known to be associated with the pathogenesis of inflammation related colorectal cancer (Seril et al., 2003). One of the key characteristics of tumour cells is their increased ability to survive as compared to normal cells. Repeated exposure to reactive oxygen species (ROS) are considered to be tumourigenic, by virtue of their ability to increase proliferation, survival and cellular migration. ROS can induce DNA damage, leading to genetic lesions that initiate tumourigenicity and subsequent tumour progression. Although antioxidants can clearly protect cells from mutagenesis at the initiation stage of carcinogenesis, their role in the destruction of transformed cells is less clear.

Superoxide dismutase (SOD) is the first characterized antioxidant enzyme (McCord and Fridovich, 1969), which is able to dismutate two O_2^- anions to H_2O_2 and molecular oxygen. CAT is subsequently responsible for the detoxification of H_2O_2 to water. GPx are another group of enzymes capable of reducing hydroperoxides, including lipid hydroperoxides, using glutathione (GSH) as substrate. The oxidized form of GSH, the glutathione disulphide (GSSG) is again reduced by a specific enzyme glutathione reductase (GR). The cytochrome P450 and its isoenzyme P4502E1 is constitutively expressed in human liver and catalyzes the oxidation of many known or suspected carcinogens of low molecular weight. Activity of this isoform in mammals is often measured as a rate of hydroxylation of p-nitrophenol (PNP) to 4-nitrocatechol (Koop et al., 1989; Zamaratskaia et al., 2009) in the presence of p-nitrophenol hydroxylase (PNPH), which is used as a marker of CYP2E1.

Apoptosis is a strictly regulated pathway responsible for the ordered removal of superfluous, aged, and damaged cells. It not only plays an important role in the development and maintenance of tissue homeostasis, but it also represents an effective mechanism by which harmful cells can be eliminated (Thompson, 1995; Kroemer et al., 1995). The role of ROS as intermediates for apoptosis signaling and cancer development is well supported by numerous investigations (Alexandre et al., 2006; Wiseman and Halliwell, 1996). Polyphenols can act as antioxidants as well as prooxidants depending on the tumour environment. Oxidation of polyphenols produces O_2, H_2O_2 and a complex mixture of semiquinones and quinones, all of which are potentially cytotoxic (Jornot et al., 1998; Hockenbery et al., 1993; Tamarit et al., 1998; Cai and Jones, 1998). Mammalian cells have developed biochemical defence systems in order to protect the cellular micro environment against deleterious effects of endogenous and exogenous stress. These early events are likely to determine whether a cell will necrose, senesce, apoptose or survive and proliferate (Limoli et al., 1998).

Chemopreventive agents are typically natural products or their synthetic analogues that inhibit the transformation of normal cells to premalignant cells or the progression of premalignant cells to malignant cells by modulating processes associated with xenobiotic biotransformation, along with the protection of cellular elements from oxidative damage (Sun et al., 2008).

Polyphenols are major plant compounds with antioxidant activity and various beneficial biological effects. Rosmarinic acid (RA) is a naturally occurring polyphenol widely distributed in Labitae plants. It is one of the main components responsible for the high antioxidant activity of commercial rosemary extracts (the only spice commercially available for use as an antioxidant in food processing, in Europe and US) (Yanishlieva-Maslarova and Heinonen, 2001). RA is also found in sage, basil, peppermint and lavender (Petersen and Simmonds, 2003). The biological activities and potential applications of RA have been studied widely. It exhibits antioxidant (Erkan et al., 2008), anti-inflammatory (Kuhlmann and R€ohl, 2006), hepatoprotective (Lima et al., 2006) and neuroprotective (Iuvone et al., 2006) effects, also inhibits the enzyme thrombin activity (Melzig and Henke, 2005) and induces apoptosis (Hur et al., 2007).

The potent beneficial role of RA in DMH induced experimental rat colon carcinogenesis has not been studied so far. Thus, our aim was to examine the effect of RA on colon tumours formation and also an attempt to explain the mechanism of its anti-tumourigenic activity.

2. Materials and methods

2.1. Animals

Male albino Wistar rats weighing 130–150 g were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University, Tamilnadu, India. The animals were maintained as per the principles and guidelines of the 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). The rats were housed at room temperature ($23 \pm 1\,^{\circ}C$) and humidity ($55 \pm 5\%$) with a 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 20% total fat in the feed (Table 1). The modified pellet diet and water were fed ad libitum.

2.2. Experimental design

Male Wistar rats were divided into six groups of six rats each and fed modified pellet diet for the entire experimental period. Group 1 served as control, group 2 received RA (10 mg/kg b.w.). Groups 3–6 were induced colon cancer by injecting DMH (20 mg/kg b.w.) subcutaneously once a week for the first four weeks (groups 3–6). In addition, RA was administered at the doses of 2.5, 5 and 10 mg/kg b.w. to groups 4–6 respectively. The detailed experimental design is shown in Fig. 1.

2.3. Chemicals

1,2 Dimethylhydrazine (DMH), rosmarinic acid, Nitroblue tetrazolium (NBT), reduced glutathione (GSH), 5,5′dithiobis-2-nitrobenzoic acid (DTNB), 1′-chloro-2,4-dinitrobenzene (CDNB), nicotinamide adenine dinucleotide phosphate (NADP) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals and solvents used were of analytical grade and obtained from Hi-Media Laboratories, Mumbai, India.

2.4. Body weight changes

The body weight of control, DMH and RA treated rats was measured throughout the study period. The rats were weighed at the

Table 1
Composition of modified pellet diet.

	Commercial diet (84.2%)	Peanut oil (15.8%)	Total (100%)
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