Journal of Trace Elements in Medicine and Biology 47 (2018) 21–30

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Aberrant crypt foci are regionally affected by zinc treatment in a 1,2-dimethylhydrazine induced colon carcinogenesis model

Hichem Moulahoum[a,b,*], Belkacem Mohamed Amine Boumaza[a], Meriem Ferrat[a], Andras-Laszlo Nagy[c], Diana Elena Olteanu[d], Abdelkader Bounaama[a], Simona Clichici[d], Bahia Djerdjouri[a]

[a] Laboratory of Cell and Molecular Biology, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene (USTHB), Algiers, Algeria
[b] Biochemistry Department, Faculty of Sciences, Ege University, Izmir, Turkey
[c] Pathology Department, Universitatea de ştiinţe Agricole şi Medicină Veterinară (USAMV), Cluj-Napoca, Romania
[d] Physiology Department, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania

ARTICLE INFO

Keywords:
Zinc sulfate
Colon cancer
Distal proximal
Dimethylhydrazine
ACF
Oxidative stress

ABSTRACT

Zinc is a trace element widely known for its marked antioxidant properties. To gain more insight into the site- and time- specific mechanisms by which it induces chemoprevention, this study was elaborated over a pre-cancerous model of colon carcinogenesis.

Colon cancer was induced by 1,2-dimethylhydrazine (DMH) in mice (20 mg/kg for 2 weeks) and groups of animals were supplemented with or without zinc sulfate ($ZnSO_4$, 200 mg/L) in drinking water for 4, 10 or 14 weeks. Colon tissues were collected for pathological observation, analyzing aberrant crypt (AC) and aberrant crypt foci (ACF) formations, multiplicity and distribution. Similarly, histological assessment and mucin production, as well as oxidative stress markers estimation was performed for the different groups.

Results showed a significant increase in ACF and AC numbers, ACF multiplicity and demonstrated stronger distal occurrence than in the proximal after DHM administration. Histopathological analysis presented marked structural alterations and mucin loss in the distal than the proximal colons. A significant increase in myeloperoxidase (MPO), nitric oxide (NO), L-ornithine and malondialdehyde (MDA) levels was observed followed by a significant decrease in antioxidant markers (superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH)). Oral $ZnSO_4$ supplementation (continuous or partial) induced significant decrease in ACF, AC numbers and multiplicity, restored histological architecture and mucin production, and a significant decrease in proinflammatory markers while it reduced antioxidants to normal levels.

From this study, insight was obtained on the use of $ZnSO_4$ as a chemopreventive agent and shed light on its potential, as a supplement in nutraceutical approaches.

1. Introduction

Colon cancer is one of the world's leading causes of cancer related deaths in both men and women [1]. It is described as a pathological consequence of recurrent oxidative stress and inflammation, causing DNA damage and silencing of tumor suppressor genes [2]. The end consequence of such events is cellular overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Damaged cells undergo apoptosis or acquire mutations that accumulate in and cause cell proliferation leading to ROS-induced carcinogenesis [3].

1,2-Dimethylhydrazine (DMH) and its metabolite azoxymethane (AOM) are largely used for colon cancer induction [4]. Subcutaneously injected DMH is metabolized in the liver and produces various intermediates such as azoxymethane (AOM) and methylazoxymethanol (MAM). MAM can be either transported to the colon epithelial cells through blood circulation, through excretion with bile acids, or it can even be directly metabolized locally in the epithelium cells or by the help lumen bacteria. The resultant metabolites (Methyl carbonium

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; COX, 2, cyclooxygenase-2; Cu/Zn SOD, copper/zinc superoxide dismutase; DMH, 1;2-dimethylhydrazine dihydrochloride; DSS, dextran sodium sulfate; DTNB, 5;5′-dithio-bis-[2-nitrobenzoic acid] (Ellman's reagent); EDTA, ethylenediamine tetraacetic acid; GI, gastrointestinal tract; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; H&E, hematoxylin and eosin; MAM, methylazoxymethanol; MDA, malondialdehyde; MPL, multiple plaque lesions; MPO, myeloperoxidase; NF-κB, nuclear factor-kappa B; NO, nitric oxide; p53, tumor protein p53; PPAR-δ, peroxisome proliferator-activated receptor delta; ROS, reactive oxygen species; SCFA, short chains fatty acid; TBARS, thiobarbituric acid-reactive substances; ZIP4, Zrt-Irt-like protein 4; $ZnSO_4$, zinc sulfate

* Corresponding author at: Ege University, Faculty of Science, Biochemistry Department, 35100 Bornova, İzmir, Turkey.
E-mail addresses: hmoulahoum@usthb.dz, hic_moul@hotmail.com (H. Moulahoum).

https://doi.org/10.1016/j.jtemb.2018.01.009
Received 4 April 2017; Received in revised form 23 January 2018; Accepted 23 January 2018

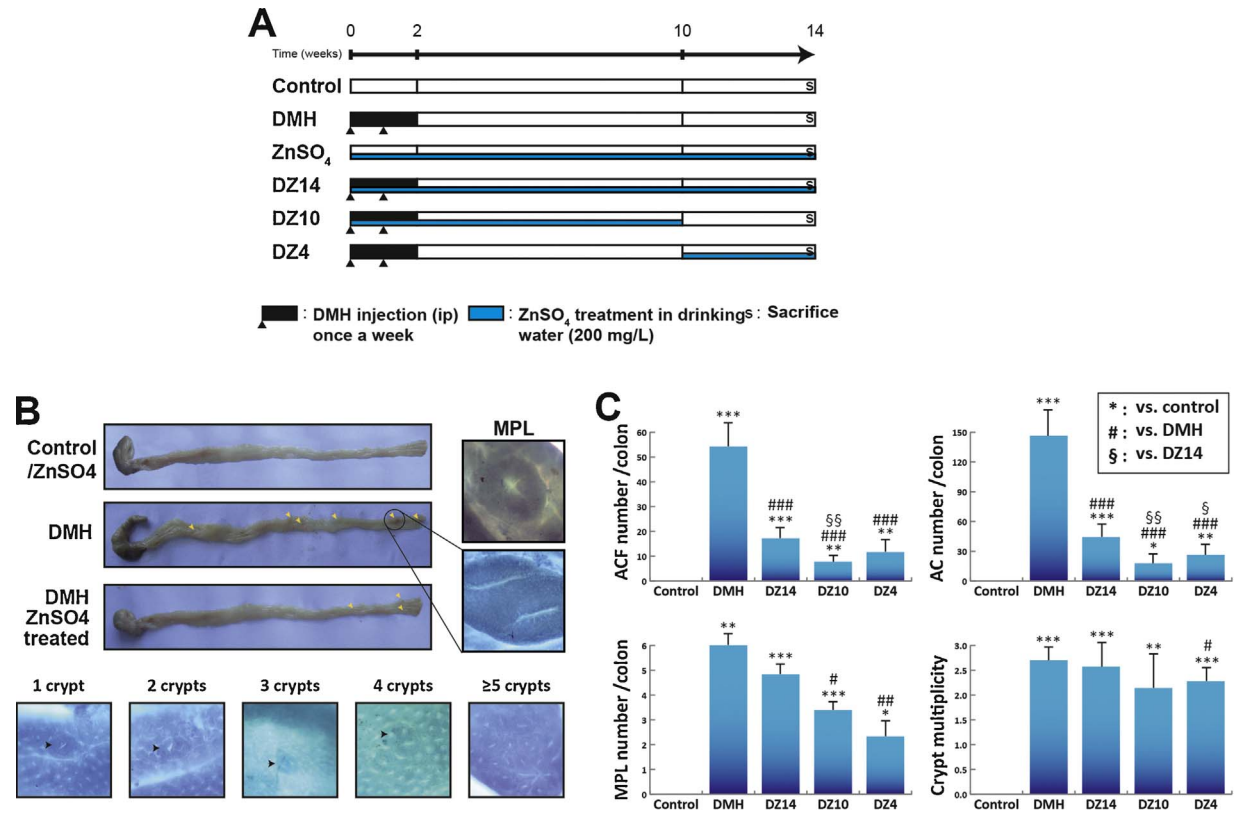

Fig. 1. (A) Experimental design of DMH-induced carcinogenesis. (B) Gross colon appearance and ACF observed under light microscope (magnification × 40). (C) Enumeration of total aberrant crypts (AC), aberrant crypt foci (ACF), multiple plaque lesion (MPL) and ACF multiplicity in colons. *p < .05, **p < .01 and ***p < .001 vs. control; #p < .05, ##p < .01 and ###p < .001 vs. DMH; §p < .05 and §§p < .01 vs. DZ14.

ions) induce DNA methylation and increase mutations in colonic tissue [5].

The public health impacts from colon cancer have led to a growing interest in prevention trials. Several investigations have been conducted on agents with potential chemo-preventive properties and have explained their modes of action. Although a full clarification of causes complexities, development and control of colon cancer is awaiting further investigation, there is abundant scope to explore the use of new agents as interventions at various stages of cancer for the better management of this pathology. Hence, diet products with diverse pharmacological properties are gaining more attention in the prevention and treatment of various diseases including cancer, where others may reduce the risk of it [6].

Zinc is an essential trace element that plays a key role in cellular biochemical and physiological pathways [7–9]. Lack of zinc levels or overdoses are frequently associated to multiple deficiencies [10]. Zinc acts as a cofactor of broad enzymes and may be involved in cell cycle, DNA damage and apoptosis, antioxidant defensive system, DNA synthesis and repair, and metabolism [11,12]. These zinc requiring enzymes can be directly related to a host defense against initiation and progression of cancer, including colon cancer [13–15].

In fact, some studies have demonstrated a protective action of zinc sulfate supplementation against DMH colon carcinogenesis. Tissue zinc levels and antioxidant enzymes have been showed to be reduced during preneoplastic progression and thus, $ZnSO_4$ treatment restored antioxidant enzymes and normal histological architecture [16,17]. In addition, zinc deficiency promoted the development of adenomatous polyps to carcinoma *in situ*, but delayed the progression of carcinoma *in situ* to invasive adenocarcinomas induced by DMH in male mice [18].

The present study was designed to explore the possibility to use zinc salts as a measure of short-term therapy in delaying the compounding events leading to the development of colon tumors. The fundamental concept of regional and preferential effect of both the molecule (i.e. $ZnSO_4$) and the disease in regard to the time of administration were addressed. In our study, we tried to express the differences between the distal and proximal colons regarding the development of preneoplastic lesions (ACF). Moreover, the effect of $ZnSO_4$ supplementation for different periods and starting points through the carcinogenesis development was studied. To our knowledge, this report is the first to evaluate in such details the role of $ZnSO_4$ supplementation in the DMH-induced colon cancer model.

2. Methods

2.1. Drugs and chemicals

Zinc sulfate ($ZnSO_4$), orthodianisidine, Ellman's reagent (DTNB), pyrogallol and other chemicals were purchased from Sigma Chemicals (St. Louis, MO). Acetic acid, ethanol and other reagents were purchased from Panreac (Spain).

2.2. Animals and experimental design

Female Swiss mice of 6–8 weeks age (20 g) were obtained from Institut Pasteur (Algiers, Algeria). They were housed at standard conditions and had access to rodent food and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (ATRSS/PNR 08/N°305). Animal handling was performed in accordance with local and international guidelines for use of laboratory animals.

Mice were randomly divided into six experimental groups (12 per group). Two groups (control and $ZnSO_4$) served as normal control and treatment control, while the rest received 20 mg/kg body weight DMH (in 1 mM EDTA, pH 6.5) subcutaneous injection once a week for a