Journal of Genetic Engineering and Biotechnology (2011) 9, 65–72

Academy of Scientific Research & Technology and National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

Benzo-a-pyrene induced genotoxicity and cytotoxicity in germ cells of mice: Intervention of radish and cress

A.M. Hassan [a,*], S.S. Alam [a], S.H. Abdel-Aziem [a], K.A. Ahmed [b]

[a] Cell Biology Department, National Research Center, 12622 Dokki, Giza, Egypt
[b] Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt

Received 10 May 2011; accepted 10 May 2011
Available online 21 June 2011

KEYWORDS
Chemoprotection;
Cress;
Radish;
Benzo-a-pyrene;
Mice sperm;
DNA damage

Abstract Exposure to chemicals like benzo(a)pyrene (BaP) can lead to structural changes in DNA and as a consequence to increased incidence of diseases with a genetic basis, as well as oxidative stress in the testis. However its ability to induce oxidative DNA damage in germ cells is not fully investigated. In the present study, BaP was used to induce 8-hydroxydeoxyguanosine (8-OHdG), a specific DNA adducts for oxidative DNA damage, in testis and epididymal sperm and the possible protection role of radish and/or cress was investigated. The results revealed that BaP induced a significant increase in DNA damage in both tissues, as indicated by increased DNA strand breaks in a fluorimetric analysis of DNA unwinding (FADU). Furthermore, it increased the oxidative damage in epididymal sperm, as indicated by the increase in sperm abnormalities, lipid peroxidation (LPO), accompanied with a decrease in glutathione content (GSH), sperm count and sperm motility as well as induction of filtration in the histology of the testis. Treatment with radish and/or cress oil prior to BaP injection succeeded in reducing the germ cell genotoxicity as indicated by the decrease in DNA damage, 8-OHdG levels, sperm abnormalities, LPO level and increased sperm counts, motility and GSH content. Moreover, cress was found to be effective than radish and the combined treatment was more effective than the single treatment. It could be concluded that, pretreatment with radish and/or cress improved the epididymal sperm quality and reduced the genotoxicity and DNA damage induced by BaP, thereby declaring the protective role of radish and cress.

* Corresponding author.
E-mail addresses: azizayahassan@yahoo.com, Dr_azizahassan@yahoo.com (A.M. Hassan).

1687-157X © 2011 Academy of Scientific Research and Technology. Production and hosting by Elsevier B.V. All rights reserved.

Peer review under National Research Center, Egypt.
doi:10.1016/j.jgeb.2011.05.006

Production and hosting by Elsevier

1. Introduction

Benzo(a)pyrene (BaP) is an important polycyclic aromatic hydrocarbon (PAH) carcinogen that undergoes metabolic activation through CYP1A1, CYP1A2, and CYP1B1. BaP is a potent systemic and local carcinogen which induces skin, lung, and stomach tumors in animal models [42]. During the metabolic process, BaP produces reactive oxygen species (ROS) via cytochrome P4501A1 (CYP1A1) [9]. These ROS and their metabolites (benzo(a)pyrene diol epoxide) can

cause oxidative DNA damage and form adducts with DNA. The reactive intermediates of BaP metabolism also have the ability to alkylate nucleophilic sites of DNA [18,9], which can lead to the formation of basic sites and DNA strand breaks. Exposure to BaP can lead to structural changes in DNA of somatic cells and as a consequence to increase incidence of somatic diseases with a genetic basis [14,45] and [44]. The mutagenic potential of BaP in male germ cells, however, has still not been fully established. BaP-related DNA damage was observed at all stages of spermatogenesis and in testis [44,30], but it is largely unknown how germ cells deal with DNA damage to protect their genetic material, and to prevent the accumulation of mutations in the germ line [30]. However, there are dietary agents which have been found to be beneficial in promoting good health and reducing the carcinogenic risks associated with the exposure to some of these dietary agents [28,25].

Increased vegetable intake, particularly of cruciferous vegetables such as cabbage, cauliflower, radish, watercress and mustard greens, is associated with a decreased risk of several cancers in human population studies [40,52] and [6]. Cruciferous vegetables have been shown to display several anticarcinogenic properties *in vivo*, as reviewed by Steinkellner et al. [41] with various underlying mechanisms. Some of these mechanisms include alterations in the activities of metabolic enzymes [29] and [21], through reduction of oxidative DNA damage levels in humans after supplementation with Brussels, Cuciferous and Leguminous sprouts [17].

In terms of active chemical species, cruciferous vegetables are rich sources of glucosinolates, a class of sulfur- and nitrogen-containing glycosides that are hydrolyzed (by plant myrosinase or intestinal microflora) to form isothiocyanates. Isothiocyanates protect laboratory animals against chemically-induced cancer through inhibition of phase I enzymes and/or induction of glutathione-*S*-transferase [50,23]. 4-(Methylthio)-3-butenyl isothiocyanate (MTBITC) is one of the most extensively studied compounds in this class, and recent epidemiological and experimental studies showed that MTBITC and other isothiocyanates may possess promising cancer chemo-preventive agent towards different classes of environmental carcinogens [19]; Ben [2]. Cruciferous vegetables such as cress *(Lepidium sativum*) and radish (*Raphanus sativus*) contain glucotropaeolin, the precursor compound from which butenyl isothiocyanate is formed. Therefore, we used garden cress and radish as a model cruciferous plant in the present study. Although the chemo-preventive effects of isothiocyanates were found to be due to protection against DNA damage caused by the different carcinogens, only few reports are available on the antimutagenic effects of cruciferous vegetables [21] and none of the available reports indicate potential protective effects of radish and cress in germ cells. The aim of the current study was to investigate the chemo-preventive role of radish and cress oil-extracted from Egyptian *R. sativus* and *L. sativum*-against the genotoxic and cytotoxic effect of benzo-a-pyrene in sperm and testicular tissue of mice.

2. Materials and methods

2.1. Chemicals

Benzo(a)pyrene (BaP) (CAS No. 50-32-8. Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml. Radish and Cress seed oils were purchased from El-Captain Company (CAP PHARMA), 6th October City, Egypt. The major fatty acid in Cress seed oil was α-linolenic acid (34%) followed by oleic (22%), linoleic (11.8%), eicosanoic (12%), palmitic (10.1%) erucic (4.4%), arachidic (3.4%) and stearic acids (2.9%). The total tocopherol and carotenoid content of Cress seed oil was 327.42 and 1.0 μmol/100 g oil, respectively. The oil was stable up to 4 months at 4 °C. While Radishes seed oil are rich in ascorbic acid, folic acid, and potassium. They are a good source of vitamin B6, riboflavin, magnesium, copper, and calcium. All other chemicals were of analytical grade.

2.2. Animals

Adult Swiss male mice (10–12 week-old, 25 gm) were randomly drawn from the stock colony of "National Research Center". Mice were housed in polypropylene cages in a controlled atmosphere with a temperature range of 25 ± 5 °C and mean relative humidity of 50 ± 5%. The animals were maintained on commercial mouse pellets ad libitum and had free access to water during a week of acclimatization and throughout the experiment.

2.3. Experimental protocol

The mice were randomly divided into nine groups (10 mouse/group). Group I, served as control and received normal diet throughout the experimental period. Group II, intraperitoneally injected with BaP (175 mg/kg b.w.) dissolved in DMSO at a concentration of 1.0 mg/ml for 2 consecutive days. Group III, served as negative control and intraperitoneally injected with DMSO (4 ml/kg body weight) for 2 consecutive days. Groups IV, V, VI, VII, VIII and IX orally administered radish and/or cress (0.1 ml/25 gm b.w/day) alone or 2 weeks prior to BaP injection for 2 days. At the end of the experimental period, all animals were killed by decapitation. The epididymis and testes were excised immediately and processed for the following analysis.

2.4. Cytogenetic techniques

2.4.1. Quantification of DNA damage in testis and epididymal sperm

The fluorimetric analysis of sperm and testis DNA unwinding (FADU) was performed according to the procedure described by Birnboim [5] with minor modifications. In brief, final cell pellet was suspended in known volume of Krebs Ringer bicarbonate solution. An aliquot of testicular cell suspension (1×10^6 cells) or epididymal sperms (2×10^6) were transferred into test tubes and cells were lysed for 10 min. The assay was performed in triplicates. The pH was increased by adding, successively and carefully, the alkaline solutions in order to allow DNA unwinding. Following neutralization, the percentage of double-stranded DNA (ds DNA) formed was detected by measuring the fluorescence of samples after addition of ethidium bromide. Measurements were performed in a Shimadzu F-2000 fluorescence spectrophotometer with 520 and 575 nm as excitation and emission wavelengths, respectively. The percentage of ds DNA remaining after the unwinding process was calculated according to the method of Birnboim [5] by the ratio (unwound DNA fluorescence–denatured DNA