Food and Chemical Toxicology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Apricot attenuates oxidative stress and modulates of Bax, Bcl-2, caspases, NFκ-B, AP-1, CREB expression of rats bearing DMBA-induced liver damage and treated with a combination of radiotherapy

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ARTICLE INFO

Article history:
Received 9 January 2014
Accepted 22 April 2014
Available online xxxx

Keywords:
7,12-Dimethylbenz[a]anthracene
Liver damage
Apricot
Radiotherapy
Apoptosis
Oxidative stress

ABSTRACT

We evaluated the ability of apricot to attenuate apoptosis and oxidative stress developed during the process of 7,12-dimethylbenz[a]anthracene (DMBA) and radiotherapy in the liver of rats bearing liver damage. Fifty female Wistar rats were divided into 7 groups; (i) normal control rats; (ii) rats fed with standard diet with apricot (20%), (ii) rats fed with standard diet and administrated 6 gray radiotherapy with Co 60 device applied to a single fraction, (iv) rats fed with standard diet and administered intraperitoneally DMBA (20 mg/kg), (v) rats fed with standard diet and administered DMBA and 6 gray radiotherapy, (vi) rats fed with standard rat diet and administered DMBA and supplemented apricot, (vii) rats fed with standard diet supplemented apricot administered DMBA and radiotherapy (RT) for 6 weeks. Expression of Bax, caspase 3, and glutathione activity decreased in the liver but liver expression of NF-κB, AP-1, CREB, Bcl-2 and ALT, AST, 5′NT, MDA, NO levels increased in DMBA-induced liver damage rats. In conclusion, the results suggest that apricot supplementation and irradiation given in combination, offer maximum protection against DMBA-induced hepatic carcinogenesis.

1. Introduction

Primary liver and bile duct cancers in the United States for 2013 is about 30,640 new cases and 21,670 deaths from these cancers according to the American Cancer Society's estimation (DeSantis et al., 2013; Siegel et al., 2013) Hepatocellular carcinoma (HCC) is a basic malignancy of hepatocytes, is the fourth important reason of cancer related deaths and accounts for 80% majority of liver cancers (Palmer et al., 2013). Polycyclic aromatic hydrocarbons (PAH) are a class of the environmental organic pollutants relased in large quantities. 7,12-dimethylbenz[a]anthracene (DMBA) the most and well known polycyclic aromatic hydrocarbon (PAH) is widespread genotoxic and tumorigenic environmental pollutants (Sharma et al., 2012). The DMBA acts as a potent carcinogen by generating various reactive metabolic intermediates leading to oxidative stress (Beltrami et al., 2013). DMBA is known to induce damage in many enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models (Abedin et al., 2012). Emerging evidences suggest that DMBA induces the production of reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems (Abe, 1986) Change in lipid peroxidation production reactions and antioxidant defense systems were associated with changes in a variety of biochemical pathways (Anbuselvam et al., 2007). Radiation therapy is an effective cancer therapy, which kills cancer and other cells (Agrawal et al., 2011). Application of ionizing radiation can have multiple side effects on cells (Fuchs-Tarlovsky, 2013). There are several general supplementation with antioxidant nutrients were administered prior or/and combined to radiation therapy, these might help protect the tumor cells against the radiation-generated free radicals needed to kill all of the cancer cells (Fuchs-Tarlovsky, 2013).

Apricots (*Prunus armeniaca* L.) are rich in minerals and vitamins most famous fruit in Malatya located in Eastern of Turkey (Ozturk et al., 2009). Apricot has a high content of carotenoids, mainly β-carotene and vitamins C and E and Se besides their antioxidant properties (Parlakpinar et al., 2009). Apricot containing this protective

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http://dx.doi.org/10.1016/j.fct.2014.04.036
0278-6915/

agent for the body increasing the resistance of healthy cells and is useful as a preventive against cancer (Apak et al., 2008). For example, in previous studies, it was reported that MK615, a compound extracted from the Japanese apricot "Prunus mume" has been reported to have anti-tumor activities against several cancer cell lines, including HCC (Sakuraoka et al., 2010; Hoshino et al. 2013). However, the effects of apricot and radiotherapy for DMBA induced liver carcinogenesis in rats have not been evaluated.

Transcription factors play an important role in cancer tumorigenesis and progression. The activator protein 1 (AP-1), NFκ B; Bcl-2, Bax, and caspases 3 as apoptosis-related cysteine peptidase, cell survival genes and cAMP response element-binding protein (CREB) induce cell proliferation, and tumor metastasis are very important apoptosis related parameters. It regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, cancer (Azuma et al., 2007; Okoh et al., 2011).

The objective of the present study was, therefore, to determine whether combination of apricot supplementation and radiotherapy exerts protective effect by stimulating apoptosis during DMBA induced liver carcinogenesis using the expression of proteins, NF-κB; Bcl-2, Bax, and caspases 3, AP-1 and CREB as the apoptosis-associated markers as the apoptosis-associated markers and GSH, MDA and total nitrite levels as an example of oxidative stress marker's.

2. Materials and methods

2.1. Animals

FemaleWistar rats (200 ± 4.5 g) aged 6 weeks were obtained from İnonu University Laboratory of Experimental Animal, Malatya, Turkey and housed in a room maintained at a constant temperature (22 ± 2 °C) and humidity (55 ± 5%) under 12 h of light and 12 h of darkness per day. The rats were acclimated to the environment for 1 week prior to the initiation of the experiment. All procedures involving rats were conducted in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the Institutional Animal Care and Use Committee of the Inonu University.

2.2. Experimental design

The rats were divided according to body weights (BW), which were similar, into five equal groups, 7 each. The groups were as follows: Group (i), normal control rats, did not receive any treatment and fed with standard rat diet. Group (ii), rats fed with standard rat diet supplemented with apricot (20%), Group (iii), rats fed with standard rat diet and administrated 6 gray RT with Co 60 device applied to a single fraction, Group (iv) was fed with standard rat diet and administered intraperitoneally DMBA (20 mg/kg body weight), Group (v), rats fed with standard rat diet and administered DMBA and 6 gray RT; Group (vi), rats fed with standard rat diet and administered DMBA and supplemented apricot; Group (vii) (DMBA + RT + Apricot), rats fed with standard rat diet and administered DMBA and RT and supplemented apricot. Treatment was continued for 6 weeks. The apricot was supplied by Fruit of Research Institute Malatya, Turkey.

Animals were observed daily, and all the necessary data were recorded. All animals were sacrificed by cervical dislocation after an overnight fast. Blood was collected and normal, and suspicious lesions were rapidly removed, measured, and rinsed in physiological saline. Liver tissue samples for histological evaluation were prepared in 10% buffered formalin and later embedded in paraffin. The sections were stained with hematoxylin and eosin (HE). Fresh tissues were used for each experimental process. Blood samples were centrifuged at 3000×*g* for 10 min, and the serum was carefully removed and stored at −80 °C until further analysis.

2.3. Laboratory analyses

For analyses, the frozen liver was thawed and homogenized gently for about 45 s in 1/10 volume of ice-cold 10 mM phosphate-buffered saline (PBS, pH 7.4) containing 1.15% KCl, and centrifuged at 800*g* to remove cell debris and nuclei. Supernatant was further centrifuged at 10,000*g* for 10 min and kept for analysis.

2.4. Biochemically analysis

NO is rapidly oxygenated to NO_2 and further to NO_3. As the direct assessment of NO is almost impossible (in vivo), the combined production of NO_2 and NO_3 can be used to assess NO in vitro and in vivo. NO levels were determined by a modification of the cadmium reduction method. After the liver tissues were homogenized in 1.15% KCl buffer (1:9 w/v) using a manual glass homogenizer (Tempest Virtishear, model 278069; The Virtis Company, Gardiner, NY). Results are expressed as mmol/mg tissue. Lipid peroxidation was measured in terms of MDA formation, which is the major product of membrane lipid peroxidation done by a previously described by the method of modified by Karabulut et al. (2010). The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances of lipid peroxidation and are given as nmol/g tissue. For total glutathione activity, the homogenate of the tissues with trichloroacetic acid solution was used the enzymatic method based on the use of Ellman's reagent. Results were expressed as nmol/g wet tissue. Serum AST, ALT levels were measured with Abbott kits (Abbott Diagnostics, Abbott Park, Ill, United States) on the Abbott clinical autoanalyser (Architect c16000). 5′NT levels was studied by Biocompare rat Elisa kits with the full otomatic ELISA reader.

2.5. Western blot analysis

For western blot analysis, liver was homogenized in PBS with protease inhibitor cocktail (Calbiochem, San Diego, CA, USA) and the protein concentration was quantitated. The sample (20 mcg of protein per lane) was mixed with sample buffer, boiled for 5 min, separated by SDS-polyacrylamide (12%) gel electrophoresis under denaturing conditions, and electroblotted onto nitrocellulose membrane. Nitrocellulose blots were washed in PBS and blocked with 1% bovine serum albumin in PBS for 1 h prior to application of the primary antibody. Primary antibody was diluted (1:1000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4 °C with protein antibody. Antibodies against NF-κB, Bcl-2, Bax, cleaved caspase-3 (19-kDa), and β-actin were purchased from Santa Cruz Biotechnology Inc, CA, USA. The next day, the immunoreaction was continued with the secondary goat antirabbit horseradish-peroxidase-conjugated antibody after washing for 2 h at room temperature. Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. Protein levels were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

2.6. Statistical analysis

Data are described median as minimum and maximum values, Kruskal–Wallis test was used for group comparisons. Kruskal–Wallis test after multiple comparisons were made by the method of Conover. 0.001 and 0.05 level of significance for all tests was considered.

3. Results

The effects of apricot on selected biochemical parameters (ALT, AST and 5′NT) were presented in Table 1. Administration of DMBA resulted in a significant increase in all tested enzyme activities in blood plasma in comparison to the values from control group (by 63% for ALT, 58% for AST, 59% for 5′NT). However, the treatment with apricot and RT significantly decreased the levels of these parameters elevated by DMBA. For example, ALT, when compared with the control group, decreased 47% in rats treated with apricot ($P < 0.05$). The effects of apricot, RT and DMBA on the activities of levels of MDA and NO in rat liver were shown in Table 2. Apricot intake and RT treatment decreased the levels of MDA and NO by 27.7% and 14.4%, respectively, in comparison with the results from the control.

The effects of apricot and RT on the expression of Bcl-2, Bax, caspase 3, and NF-κb, AP-1, CREB in the liver tissue were shown in Fig. 1(A–G). DMBA treated group was lower levels of caspase 3 compared to other groups ($P < 0.005$). Higher levels of caspase 3 were found in apricot group compared to other groups. Administration of DMBA significantly increased Bcl-2, AP-1, CREB, NFκB

Table 1
ALT, AST and 5′NT levels are expressed as mean ± SD in all the groups. Q3

Groups	ALT (IU/l)	AST (IU/l)	5′NT (mIU/ml)
Control	42.43 ± 2.8	60.14 ± 3.5	2.56 ± 0.2
Apricot	45.32 ± 3.21	65.36 ± 3.2	2.89 ± 0.3
RT	52.36 ± 3.98[ab]	68.96 ± 3.78[b]	4.02 ± 0.59[ab]
DMBA	115.23 ± 8.56[a]	145.63 ± 9.45[a]	6.23 ± 0.35[a]
DMBA + RT	95.36 ± 4.35[a]	125.28 ± 7.48[a]	5.26 ± 0.23[ab]
DMBA + Apricot	80.12 ± 2.36[ab]	92.32 ± 5.36[ab]	4.89 ± 0.98[ab]
DMBA + RT + Apricot	68.56 ± 2.25[ab]	75.38 ± 4.51[ab]	3.56 ± 0.48[ab]

Please cite this article in press as: Karabulut, A.B., et al. Apricot attenuates oxidative stress and modulates of Bax, Bcl-2, caspases, NFκ-B, AP-1, CREB expression of rats bearing DMBA-induced liver damage and treated with a combination of radiotherapy. Food Chem. Toxicol. (2014), http://dx.doi.org/10.1016/j.fct.2014.04.036