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Cancer Detection and Prevention 29 (2005) 54–58

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Comparison of chemopreventive effects of Vitamin E plus selenium versus melatonin in 7,12-dimethylbenz(*a*) anthracene-induced mouse brain damage

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Accepted 23 June 2004

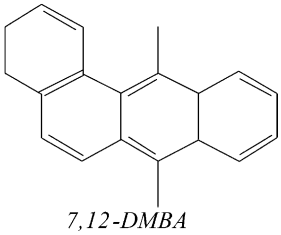
Abstract

In this work, the protective effect of Vitamin E plus selenium (Vit E + Se) and melatonin against 7,12-dimethylbenz(*a*)anthracene (7,12-DMBA)-induced changes in superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase (CAT) and carbonic anhydrase (CA) activities and malonedialdehyde (MDA) levels of mouse brain were compared. 12-month old mice were divided into four groups each including 10 animals. The first group served as control group. The second group was treated with 7,12-DMBA (20 mg/(kg day)). The third group was treated with 7,12-DMBA and Vitamin E (90 μg/(individual day)) and selenium (1.8 μg/(individual day)) simultaneously. The fourth group was treated with 7,12-DMBA and melatonin (4.2 mg/(kg day)) simultaneously. Treatment continued for 21 days after which the mice were sacrificed and brain homogenates were prepared. 7,12-DMBA treated group exhibited significantly decreased levels of brain SOD, GSHPx, CAT and CA activities and increased MDA levels as compared to control. Vitamin E + Se fully or partially restored enzyme inhibition except for SOD. Lipid peroxidation was also reduced in Vitamin E + Se treated group. Melatonin provided a better protection for SOD, GSHPx and CAT, and a plausible protection for CA activity. Protection against lipid peroxidation measured as MDA in melatonin treated group was appreciable although slightly lesser than the protection provided by Vitamin E + Se. The results imply that Vitamin E + Se and melatonin both provide chemoprevention against 7,12-DMBA-induced oxidative stress in mouse brain.

Keywords: Chemical carcinogenesis; 7,12-DMBA; Free radicals; Oxidative damage; Melatonin; Vitamin E; Selenium; Brain

1. Introduction

Polycylic aromatic hydrocarbons (PAH) such as 7,12-DMBA have been shown to form free radicals and these compounds play a critical role in carcinogenesis [1]. This role is accompanied by the generation of reactive oxygen species, such as peroxides, hydroxyl and superoxide anion radicals, which induce cellular oxidative damage through DNA strand breaks and lipid peroxidation [2–4].



7,12-DMBA

In the organism, there are some antioxidant defense systems responsible for detoxifying free radicals whose concentration

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0361-090X/$30.00
doi:10.1016/j.cdp.2004.06.006

increases depending on various reasons. Those systems are composed of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), and non-enzymatic compounds such as glutathione (GSH), melatonin, albumin, uric acid, Vitamins A, E and C, flavonoids, and selenium. If the sensitive balance between the levels of free radicals and the members of the antioxidant defense system is disturbed, this may cause for many pathological changes leading to the cell and tissue damage [5]. Brain cells are prone to free radical damage because of their high content of iron and polyunsaturated fatty acids, the latter being a substrate for lipid peroxidation, and because of their relatively deficient antioxidative defense mechanism [6,7].

There is great clinical interest in oxidative stress and lipid peroxidation, due to the suggestion that many significant disease states are associated with oxidative injury. When various primary and secondary products of lipid peroxidation in biological systems are decomposed, aldehydes are formed. One intensely studied aldehyde is malondialdehyde (MDA), and it is commonly used as a marker for the lipid peroxidation process [8].

It has been demonstrated that Se plays an important role in the active center of the Se-dependent GSHPx. The enzyme bears four subunits and each subunit contains one Se atom [9]. Epidemiological studies have pointed out inverse associations between nutritional Se status and cancer risk, suggesting that a relatively low Se status may be among the determinants of cancer risk. Animal experiments have shown supranutritional levels of Se to reduce tumorigenesis. Most recently trials have indicated that supplemental Se can reduce cancer risk [10].

CA is found in several tissues such as lung, liver, kidney and brain. This enzyme not only regulates the pH of several media in organisms, it also indirectly involves in the antioxidant enzyme system. The important role that carbon dioxide (CO_2)–bicarbonate (HCO_3^-) equilibrium plays in the chemical reactivities and biological actions of peroxynitrite anion ($ONOO^-$) receives special attention, since the reactivity of peroxynitrite anion is significantly influenced by the formation of the reactive nitrosoperoxocarbonate intermediate ($ONOOCO_2^-$). It has been proposed that CO_2 might serve as a catalytic scavenger of $ONOO^-$ by accelerating its decomposition [11]. It is also reported that CA functions as an oxyradical scavenger and thus protects cells from oxidative damage and CA over expressing cells exhibit lower free radical levels [12].

This study is planned to search the changes in the antioxidant defense system after 7,12-DMBA administration and to observe and compare the possible protective effects of melatonin and Vitamin E plus selenium against 7,12-DMBA-induced mouse brain damage.

2. Materials and methods

Chemicals: xanthine, xanthine oxidase, cytochrome c, oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione reductase, bovine serum albumine (BSA), SOD, Vitamin E, sodium selenite, 7,12-DMBA, n-butanol, thiobarbutiric acid, 1,1,3,3-tetramethoxypropane and all other reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Animals: experiments were carried out with 40 female mice (Mus musculus) aged 12 months. They were housed five per cage, and fed standard pellet diet (Aytekinler Feed Co.) and water ad libitum. The animals were maintained in a controlled environment under standardized conditions of temperature and humidity with an alternating 12 h light:12 h dark cycle. Animals were acclimated for 2 weeks.

Treatment of animals: mice were divided into four groups of 10. The first group served as the control and was subjected to i.p. injections of 25 μL corn oil daily. The second group had daily i.p. injections of 7,12-DMBA (20 mg/(kg body weight day) in corn oil, for 21 days. The doses selected in the present experiments are similar to those used in carcinogenesis and in biochemical studies [13] except that the administration of total dose was achieved in 21 days. The third group had the same procedure of 7,12-DMBA injections as the second group and received Vitamin E + selenium [(90 μg Vitamin E + 1.8 μg Se)/day], simultaneously. The fourth group had 7,12-DMBA injections and melatonin (4.2 mg/kg body weight), simultaneously.

At the end of 3 weeks, the mice were killed by cervical dislocation. Brains were promptly dissected and perfused with 50 mM (pH 7.4) cold phosphate buffer saline solution (PBS). Brains were homogenized in 1/5 (w/v) PBS using PCV Kinematica Status homogenizer. Homogenates were divided into two portions and one part was directly used for MDA measurements immediately. The second was sonicated four times for 30 s with 20 s intervals using VWR Bronson Scientific sonicator. Then, homogenates were centrifuged at 20,000 × *g* for 15 min in a Beckman L8–70 M ultracentrifuge. Supernatants were separated and kept −80 °C until enzyme activity measurements were performed. Care was taken to keep the temperature at +4 °C throughout the preparation of homogenates and supernatants.

Measurement of MDA levels: the level of MDA in brain tissue homogenate was determined using the method of Uchiyama and Mihara [14]. Half a milliliter of homogenate was mixed with 3 ml H_3PO_4 solution (1%, v/v) followed by addition of 1 ml thiobarbituric acid solution (0.67% w/v). Then the mixture was heated in water bath for 45 min. The colored komplex was extracted into *n*-butanol, and the absorption at 532 nm was measured using tetramethoxypropane as standard. MDA levels were expressed as nanomole per milligram of protein.

Determination of protein concentration: protein determinations in supernatants were done according to Lowry and Rosebrough [15] using BSA as standard. A Shimadzu 1601 UV–vis spectrophotometer with a connected PC and a Grand LTD 6G thermostability unit adjusted to 37 ± 0.1 °C was employed for all spectrophotometric assays.