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Original article

Preventive effect of berberine against DMBA-induced breast cancer in female Sprague Dawley rats

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ABSTRACT

Breast cancer is the prime cause for cancer mortality in women worldwide. The importance of diverse natural and dietary agents to reduce the risk of developing breast cancer is well established. Berberine, a natural isoquinoline alkaloid found in many medicinal plants is widely used in traditional Indian and Chinese medicine. Because of its capability to seize the cell cycle and induce apoptosis of numerous malignant cells, berberine has received considerable attention as a potential anticancer agent. In the present study, breast cancer was induced in Sprague Dawley (SD) rats by intragastric administration of 7, 12-dimethylbenz[*a*]anthracene (DMBA) at a dose of 80 mg/kg of body weight. Treatment of berberine (50 mg/kg BW) to breast tumor bearing rats was found to be effective against DMBA induced mammary carcinoma. The increased levels of lipid peroxide (malonaldehyde), pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α), enzymatic antioxidants (SOD and CAT), non-enzymatic antioxidants (GSH and vitamin C) and transcription factor NF-κB were decreased significantly by administration of berberine. Furthermore, RT-PCR and western blot analysis showed the down-regulation of NF-κB and PCNA in breast tumors. Histopathological studies validated that berberine is effective against DMBA induced ductal carcinoma & invasive carcinoma. Altogether, these findings demonstrate the preventive role of berberine against DMBA induced mammary carcinoma in SD rats.

1. Introduction

Breast cancer is the most recurring carcinoma in women, with an estimated 1.67 million new cancer cases. It ranks as the fifth most cause of death from carcinoma overall (522,000 deaths) and is the most frequent cause of cancer death in women in developing countries (14.3% of total) [1]. Among every eight women, one woman is suffering with breast cancer [2]. Although many therapies are available, they have not been successful enough to divulge significant improvement in the morbidity of breast cancer. Moreover, breast cancer is extremely resistant to chemotherapy and limited effective treatments subsists for advanced disease conditions.

Cells become cancerous when they lose their ability to stop dividing, to stay together at their location, and to die at the proper time. Normal cells undergo apoptosis when they are no longer needed; until then they are protected by several proteins from cell suicide clusters and pathways such as NF-κB transcription pathway. In certain circumstances, genes mutate in a way that makes them permanently incapable of committing suicide [3]. The mutation in genes such as putative protein tyrosine phosphatise (PTEN), breast cancer gene1 and breast cancer gene2 (BRCA1 and BRCA2) by epigenetic factors is one of the origin to breast cancer [4].

The site specific carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA) is commonly employed agent to induce mammary carcinoma in Sprague Dawley (SD) rats. DMBA acts either by initiating or promoting mutations in carcinogenesis responsible genes [5]. The oral administration of DMBA activates cellular cytosolic receptor aryl hydrocarbon receptor (AhR). Activated AhR translocates into the nucleus and combines with AhR nuclear translocation protein. The AhR/ARNT complex induces gene

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transcription by binding to specific DNA recognition sites upstream to AhR responsive genes. This tumorigenesis involves AhR-dependent up-regulation of cytochrome P450 (CYP1A1 and CYP1B1) enzymes, which metabolize DMBA into a mutagenic epoxide intermediate that forms DNA adducts. These DNA adducts are associated with mutations and the malignant transformation associated with polycyclic aromatic hydrocarbons (PAH)-mediated carcinogenesis [6].

Oxidative stress participates in all the three stages of carcinogenesis; initiation, promotion and progression. At initiation step, free radicals activate numerous mechanisms contributing to mutations. During the promotion step initiated cells either enhances the proliferation and/or inhibits the cell death. At the progression step, free radicals contribute to uncontrolled growth of tumor cells, resistance to chemotherapy, genomic instability, metastasis and invasion [7]. Antioxidants detoxify free radicals and ROS (reactive oxygen species) directly or indirectly, thus may protect against cancer. Humongous investigation is carrying out on natural compounds such as alkaloids, flavanoids and poly phenolic compounds as antineoplastic compounds. Berberine, an isoquinoline alkaloid has therapeutic effects on the cardiovascular system particularly in congestive heart failure [8] and also considerable for its anti-inflammatory actions [9]. Berberine is known to suppresses the growth of a wide variety of tumor cells, including breast cancer [10], melanoma [11], pancreatic cancer [12], gastric carcinoma [13], prostate cancer [14], colon cancer [15] and nasopharyngeal carcinoma [16]. Berberine is also active against DMBA induced skin carcinoma [5], and B(a)P induced lung carcinoma [17]. Berberine suppressed NF-κB activation induced by various inflammatory agents and carcinogens [18]. Berberine is evidenced to protect against the side effects of radiation therapy in lung cancer [19]. Although, berberine is known to have several beneficial effects on different organ systems, its role in breast cancer needs to be explored. Therefore, in this study we investigated the chemopreventive and therapeutic potential of berberine against DMBA- induced breast cancer in SD rats.

2. Materials and methods

2.1. Chemicals

DMBA, berberine and corn oil were purchased from Sigma (St. Louis, MO, USA). TNF-α, IL-1β and IL-6 kits were procured from Invitrogen (Invitrogen Corporation, Frederick, USA). All other chemicals used in the study were of analytical grade procured from commercial sources.

2.2. Animals and diet

Female SD rats (170–200 g) of 45–58 days were procured from National Institute of Nutrition, Hyderabad. Animals were acclimatized for two weeks after procurement and were fed with normal pellet diet and water *ad libitum* throughout the experimental period. Standard laboratory conditions were maintained under controlled atmosphere (12:12 h light/dark cycles with an ambient temperature of 22 ± 3 °C and humidity at 50 ± 10%). The study was performed following the CPCSEA guidelines and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC No. MC/05/2015/27) of Gauhati Medical College.

2.3. Study design

SD rats were randomly divided into five groups consisting of six rats in each as follows:

- Group I (Vehicle control): Rats were administered corn oil from the 1st to 12th week *via* intragastric route.
- Group II (Drug control): Berberine (50 mg/kg, orally) was administered thrice a week for twelve weeks.
- Group III (DMBA control): Mammary tumors were induced in the 3rd week by a single dose of DMBA (80 mg/kg in 0.5 ml corn oil) and the rats were given only corn oil in the subsequent weeks (4–12 weeks).
- Group IV (Berberine pre-treatment): Animals were subjected to pre-treatment with berberine (50 mg/kg) weekly thrice from 1 to 12 weeks and DMBA was administered in the 3rd week as in group III.
- Group V (Berberine post-treatment): Animals were subjected to post-treatment with berberine (50 mg/kg, orally) from week-8, and DMBA was administered as in the group III.

All the animals were sacrificed at the end of the 12 weeks. The mammary tumors were dissected and part of tumors were fixed in 10% buffered formalin for histopathological analysis and remaining part were processed for molecular and biochemical analysis.

2.4. Tumor induction and measurement of tumor parameters

DMBA 80 mg/kg body weight was administered to SD rats, as this dose is sufficient to cause 100% tumor incidence [20]. Approximately DMBA took 8–10 weeks to induce mammary tumors in female SD rats. Animals were sacrificed when the tumor diameter reached to two inches and used for further analysis. The tumor volume was calculated using, $\Pi/6 \times (a)^2/(b)$, where '*a*' is the shortest and '*b*' is the longest length of the tumor. Tumor incidence is the percentage of tumors present in a group [21].

2.5. Biochemical analysis

MDA was estimated by Ohkawa et al. [22], Catalase by Sinha et al. [23], GSH by Ellman et al. [24] and Vitamin C by Omaye et al. [25] were followed to estimate the biochemical parameters from breast tissue samples. Superoxide dismutase levels were evaluated using SOD assay kit from Sigma Aldrich.

2.6. Enzyme linked immune sorbent assay

For the estimation of pro inflammatory cytokines TNF-α, IL-1β and IL-6 in breast tissues, 10% tissue homogenate was prepared with PBS containing 1% protease inhibitor cocktail. Then, the homogenates were centrifuged at 12,000 g for 15 min and the supernatant obtained were used for the estimation of cytokines using respective rat ELISA kits (Invitrogen Corporation, USA).

2.7. Western blotting

Total protein in the breast tissue homogenate was estimated using Bradford method [26]. Approximately 40 μg protein sample with an equal volume of 2× laemmli buffer was loaded on each track of 10% polyacrylamide gel and separated by SDS-PAGE. The resolved proteins were transferred to polyvinylidine difluoride membranes (0.2 μm) by electrophoresis. Then the membranes were blocked in 3% BSA in Tris-buffered saline and 0.2% Tween-20 for 1 h at room temperature and probed with PCNA, NF-κB and β-actin rabbit monoclonal antibodies overnight at 4 °C. The blots were washed in Tris-buffered saline and 0.2% Tween-20 and then incubated with anti-rabbit HRP-labelled secondary antibody for 1 h. After extensive washes, the bands were visualized by using 1-Step Ultra TMB blotting solution. ImageJ software was used to estimate the band intensities. Densitometric data was represented as percentage fold change compared with control group.