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Original Article

The effects of alpha-lipoic acid on breast of female albino rats exposed to malathion: Histopathological and immunohistochemical study

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ABSTRACT

Background: The wide use of the organophosphate insecticide malathion is accompanied by the risk of human exposure and may be involved in the etiology of breast cancers, especially in developing countries. Alpha (α)-lipoic acid, a natural molecule, present in our diet has antioxidant and protective effects in cases such as aging, diabetes mellitus, and vascular and neurodegenerative diseases all in which free radicals are involved. However, there is only scarce data regarding the efficacy and biological activity of α-lipoic acid on malathion-induced breast histopathological changes.

Aims: To investigate whether malathion can induce mammary histopathological changes, to immunohistochemically analyze the modulations in proliferation-apoptosis balance associated with these changes, to assess the associated metabolic parameters, antioxidant stress and hormonal profile changes and to elucidate the possible protective effect of α-lipoic acid on malathion induced alterations in rats.

Materials and methods: Forty Wistar female rats weighing 150–170 g were divided into four groups. Group 1: control group were injected intraperitoneally (ip) with saline solution. Group2: animals were injected (ip) with malathion twice a day for five days. Group 3: animals were orally given α-lipoic acid, after three hours of treatment with malathion at the same dose given to group 2. Group 4: animals were treated with α-lipoic acid at the same dose given to group 3. Rats were sacrificed on the 90th day, and breast tissues were analyzed for histopathological and immunohistochemical alterations. Blood samples were collected for biochemical tests.

Results: α-Lipoic acid exhibited a striking reduction of malathion-induced mammary tumor incidence, and reversed intra-tumor histopathological alterations. Alpha lipoic acid suppressed proliferating cell nuclear antigen (PCNA) and p53 expression, induced apoptosis, upregulated proapoptotic protein Bax.

Conclusions: Our results provide the experimental evidence that α-lipoic acid exerts chemopreventive effect in the breast hyperplastic and malignant changes by suppressing abnormal cell proliferation and inducing apoptosis with an oncostatic effects during an early-stage breast cancer.

Introduction

Breast cancer is considered major and common health problem in both developing and developed countries. The insecticide malathion which is widely used in agriculture and public health can generate certain harmful effects on humans. Hence, there is need for more research to be focused in this area. The etiology of breast cancers is dictated by both internal factors (inherited mutations, hormones, and immune conditions) and environmental/acquired factors (such as smoking, diet, drugs, radiation, and infectious organisms) [1,2]. Behavioral and environmental factors are considered to be among the major influencing components causing increase in the incidence of breast cancer risk [3]. *In vivo* and *in vitro* studies have shown that environmental substances (e.g., DDT, polychlorinated biphenyls, 4-nonylphenol, 4-octylphenol) can promote mammary cancer [4,5].

Organophosphorus compounds are organic ester of phosphoric or thiophosphoric acid and are increasingly used in agriculture, medicine, and industry. Because of growing concerns about health

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and environmental problems associated with pesticides, several governments are seeking to employ safe, convenient and economically feasible biological methods for combating pests.

The organophosphorus compound malathion (O,O-dimethyl-S-1,2-bis ethoxy carbonyl ethyl phosphorodithionate), is extensively used throughout the world to control a variety of outdoor insects in both agricultural and residential settings [6]. Malathion insecticide is widely used in Saudi Arabia to control motile stages of mites and some other insects on fruits and vegetables and has limited plant systemic activity [7,8].

Malathion is converted into malaoxon that inhibits acetylcholinesterase (AChE) [9]. Malathion has been shown to be mutagenic and there is suggestive evidence of carcinogenicity for malathion in animals [10]. The association between cancer risks in humans and agricultural pesticides has been studied in both occupational and nonoccupational exposures [11]. *In vitro* studies showed that malathion induced malignant transformation of breast epithelial cell lines [12]. A previous study showed synergistic effect of malathion and estrogen on mammary gland carcinogenesis [4,13]. Currently used pesticides such as malathion affect the function of sex hormone receptors thus possessing endocrine-disrupting potential [14].

There is a consensus that malathion induces toxicity and carcinogenicity through the inhibition of acetylcholinesterase (AchE) [2,12]. Moreover, several organophosphorus pesticides including malathion have been reported to cause oxidative stress [15]. Oxidative stress as defined by Dare et al. is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates [16]. Oxidative stress with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses is associated with production of reactive oxygen species (ROS). The reactive oxygen species released by the mitochondrial respiratory chain can damage a wide range of essential biomolecules resulting in lipid peroxidation, DNA damage and enzyme inactivation [17,18]. Antioxidant enzymes, mainly Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) are the first line of defense against free radical induced oxidative stress. SOD is known to convert superoxide radicals to H_2O_2 and molecular oxygen [19]. Catalase as an important regulator of oxidative stress, is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water [20,21]. GPx, responsible for enzymatic defense against hydrogen peroxide, it catalyses the reaction between glutathione and hydrogen peroxide to form glutathione disulphide (GSSG) and the reduction product of H_2O_2 [22].

Resistance to chemotherapy is a major obstacle to successful treatment of breast cancer [23,24]. Earlier and current literature shows that over 60% of breast cancer patients use some form of complementary and alternative medicine [25,26]. Studies have shown that phytochemicals that display potent anticancer effects in both *in vitro* and *in vivo* rodent models can be potential candidates for chemoprevention in humans [27,28]. These phytochemicals have different chemical properties and can block tumorigenesis by multiple mechanisms that include prevention of pro-carcinogen activation, inhibition of cell proliferation, invasion, angiogenesis, and stimulation of apoptosis [29].

Alpha (α)-lipoic acid (thioctic acid) is a naturally occurring dithiol compound. It was discovered in 1951. Alpha-lipoic acid catalyzes oxidative decarboxylation process converting pyruvate to acetyl CoA. Hence, α-lipoic acid is essential for energy production in cells. Lipoic acid is found mainly in animal foods such as meat and liver and at low or undetectable levels in plant foods such as potato [30,31]. There is some evidence for the protective effect of Alpha lipoic acid in cases such as rheumatoid arthritis, lyme disease, diabetes mellitus, and vascular and neurodegenerative diseases and age-related conditions in which free radicals are involved [32–36]. Alpha lipoic acid has also been suggested for cataracts, glaucoma, multiple sclerosis, and Alzheimer's disease. Studies are generally dealing with the biological consequences of α lipoic acid administration in cases associated with oxidative stress or the differences between the antioxidant activities of α-lipoic acid and its derivatives [32–38].

The mammary gland is a suitable model for examining its susceptibility to different carcinogenic agents because of its high cell proliferation and differentiation. Cell proliferation in the mammary gland is related to both topography of the mammary parenchyma and specific stages of the gland development that are modulated by age, hormonal variations, and parity history. The intralobular terminal duct is equivalent to the terminal ductal lobular unit in the human breast, considered the site of origin of human breast carcinomas [39–42].

Expression of proliferating cell nuclear antigen (PCNA) is elevated in the nucleus during late G1 phase immediately before the onset of DNA synthesis, becoming maximal during S-phase and declining during G2 and M phases. Its level correlates directly with rates of cellular proliferation and DNA synthesis. Aberrant cell cycle protein expression plays important pathways involved in the etiopathogenesis of breast carcinoma. Tumors with overexpression of PCNA have significantly worse outcomes [43].

Data on the efficacy and biological activity of α-lipoic acid on malathion mammary alterations are meager. The present study was carried out to elucidate the possible protective effect of α-lipoic acid treatment on malathion induced breast histopathological and biochemical alterations in rats. Moreover, the immunohistochemical changes in proliferation-apoptosis balance induced by malathion were analyzed.

Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Qassim University, College of Medicine, Buridah, Qassim Region, KSA.

Experimental design

Forty Wistar female rats aged 39 days with average weights of 150–170 g were obtained from Qassim University Animal Facility, Faculty of Medicine, Qassim University, Buridah, KSA. They were housed in Animal facility, with room temperature maintained at 27 °C, relative humidity of 50–70% and an airflow rate of 15 exchange/h. Also, a time controlled system provided 07.00–21.00 h light and 21.00–07.00 h dark cycles. All rats were given ad libitum access to Teklad rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes. Acclimatization was for 1 week before the experiment. The female rats were divided into four groups, ten animals each: Group 1: control group was injected intraperitoneally (ip) with saline solution. Group2: experimental animals were injected (ip) with 170 mg/kg body weight of malathion twice a day for five days (malathion (CAS 121-75-5) sc-211768, Santa Cruz Biotechnology, Inc., USA) Purity: ≥95%. Group 3: experimental animals were orally given α-lipoic acid solution at a dose of 20 mg/Kg body weight, after three hours of treatment with malathion at the same dose given to group 2 (α-lipoic acid solution (CAS 1077-28-7) sc-202032, Santa Cruz Biotechnology, Inc., USA) Purity: ≥95%. Group 4: animals were treated with α-lipoic acid at the same dose given to group 3. Twenty four hours and 90 days after the last treatment of animals, ten animals from each group were anaesthetized and opened by a midline incision from the pubis to the submaxillary area to remove the mammary glands. The skin was dissected to expose the six pairs of mammary glands (thoracic, abdominal and inguinal).

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