Saudi Journal of Biological Sciences (2011) **18**, 29–36

King Saud University

Saudi Journal of Biological Sciences

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CASE STUDY

Protective effect of L-carnitine against acrylamide-induced DNA damage in somatic and germ cells of mice

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Received 14 June 2010; revised 18 July 2010; accepted 23 July 2010
Available online 29 July 2010

KEYWORDS

Acrylamide;
L-carnitine;
Antimutagenicity;
Micronuclei;
Chromosomal aberrations;
Sperm abnormalities;
Mice

Abstract Recent findings of acrylamide (AA) in many common foods have sparked renewed interest in assessing human health hazards. AA was evaluated by the International Agency for Research on Cancer as probably carcinogenic to humans. For this reason, the aim of this study is to evaluate the potential genotoxic effect of AA using chromosomal aberration analysis and micronucleus (MN) test in mouse bone-marrow cells and morphological sperm abnormalities. The result of the present work indicated that treatment with a single dose of 10, 20, or 30 mg/kg b.wt. of AA for 24 h and the repeated dose of 10 mg/kg b.wt. for 1and 2 weeks induced a statistically significant increase in the percentage of chromosomal aberrations and micronuclei in bone- marrow cells. These percentages reduced significantly in all groups treated with AA and the protective agent L-carnitine. Also the results indicated that the dose 10, 20 and 30 mg/kg b.wt. of AA induced a statistically significant percentage of morphological sperm abnormalities compared with the control group. Such effect reached its maximum (7.24 ± 0.61) with the highest tested dose which reduced to (4.02 ± 0.58) in the group treated with the same dose of AA and L-carnitine. In conclusion, the results confirm the protective role of LC against the mutagenicity of AA.

1. Introduction

Acrylamide (AA) is a high-production vinyl compound whose polymeric form is used in the construction and oil industry, in the manufacture of paper, plastics and textiles, as a flocculant in the treatment of waste water, and in cosmetics (Friedman, 2003). A worldwide public concern has been caused by the finding that the same compound is generated in many common foods during cooking at high temperatures (Svensson et al., 2003). For example, AA reaches parts per million concentrations in French fries, potato and tortilla chips, bread crust, various baked foods, breakfast cereals and coffee. Further studies showed that AA is formed during the Maillard browning reaction from a heat-induced reaction between the amino acid asparagine and the carbonyl group of glucose (Stadler et al., 2002).

With respect to the AA genotoxicity data, AA has been shown to be clastogenic and mutagenic in rodents *in vivo*

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doi:10.1016/j.sjbs.2010.07.004

Production and hosting by Elsevier

(Dearfield et al., 1995). AA could induce DNA damage in the PC Cl3 and FRTL5 rat thyroid cell lines, as well as in human lymphoblastoid TK6 cells in the Comet assay (Koyama et al., 2006). Additionally, the results presented recently by Park et al. (2002) suggested that AA itself appears to be involved in AA-induced cellular transformation and that cellular thiol status is involved in AA morphological transformation. AA decreased glutathione (GSH) levels in Syrian hamster embryo (SHE) cells (Park et al., 2002). It has also been found to be conjugated with GSH (Tong et al., 2004). It is possible that AA itself can be cytotoxic and genotoxic by decreasing the oxidative defense system in the cells (Zamorano-Ponce et al., 2006), as well as, releasing the reactive oxygen species (ROS) (Liping et al., 2007). However, AA itself reacts quite slowly with DNA (Solomon et al., 1985). Within the cells AA can be oxidized to the more reactive glycidamide (GA) by cytochrome P450-depended monooxygenase (CYP2E1) (Sumner et al., 1999). GA has been reported to be 100–1000 times more reactive with DNA than AA (Segerback et al., 1995). GA is known to be clastogenic and mutagenic *in vitro* and *in vivo* (Paulsson et al., 2003; da Costa et al., 2003).

Much attention of preventive medicine research is focused on natural antioxidants. This interest refers not only to isolation and identification of new biologically active molecules by the pharmaceutical industry, but also because of the emergent public interest in using crude plant extracts (Dragland et al., 2003).

L-carnitine (LC) is a vitamin-like substance that is structurally similar to amino acids. Most carnitine is obtained from diet such as mushroom, carrot, bread, rice and tomato. It can also be synthesized endogenously by skeletal muscle, heart, liver, kidney and brain from the essential amino acids lysine and methionine (Rebouche and Seim, 1998). It is known that LC and its derivatives prevent the formation of reactive oxygen species (ROS) and protect cells from per oxidative stress (Dokmeci et al., 2006).It is generally accepted that exogenous carnitine, either at normal dietary intake levels or in pharmacological amounts can be beneficial in a number of physiopathological conditions. The dependence on carnitine uptake is evident from patients suffering from primary systemic carnitine deficiency (CDSP), an autosomal recessive disorder of fatty acid oxidation, caused by mutations in the *OCTN*2 gene encoding, an organic cation/carnitine transporter (Wang et al., 1999). It has been reported that carnitine protects the myocardium against ischemia (Reznick et al., 1992), myocardial infarction (Singh et al., 1996) and skeletal muscle myopathy in heart failure (Vescovo et al., 2002). Recent reports have also suggested that carnitine has additional pleiotropic functions in tissues that are not considered lipogenic, such as brain (Llansola and Felipo, 2002). Some studies indicate a carnitine-dependent reduction of either DNA single-strand breaks in isolated human lymphocytes after *in vitro* treatment with an oxygen radical-generating system (Boerrigter et al., 1993) or DNA cleavage induced by H_2O_2 UV-photolysis (Vanella et al., 2000). Also, LC has DNA-repair capability and decreased induction of aberrations in *Ataxia telangiectasia* (A-T) patients (Berni et al., 2008).

Due to common industrial application of AA and its presence in food to find an agent to decrease the cytotoxicity and genotoxicity of AA is a good choice. The present study is therefore aimed to investigating the possibility that LC may inhibit AA cytotoxicity and genotoxicity in somatic and germ cells of the mice.

2. Materials and methods

2.1. Animals

Male white Swiss mice aged 9–12 weeks were used in all experiments. The animals were obtained from a closed random-bred colony at the College of Pharmacy, University of King Saud in Rhiyad. The mice used for any one experiment were selected from mice of similar age (±1 week) and weight (±2 g). Animals were housed in polycarbonate boxes with steel-wire tops (not more than five animals per cage) and bedded with wood shavings. Ambient temperature was controlled at 22 ± 3 °C with a relative humidity of 50 ± 15% and a 12-h light/dark photoperiod. Food and water were provided *ad libitum*. Animals were sacrificed after treatment by cervical dislocation.

2.2. Chemicals

L-carnitine is produced by Sigma-Tau Pharmaceuticals, Pomezia, Roma, Italy. Acrylamide was purchased from Sigma (St. Louis, MO). All other chemicals used were of the highest analytical grade.

2.3. Treatment and cytological preparations

2.3.1. Micronucleus and chromosome aberrations

For the analysis of micronuclei and chromosome aberrations in bone marrow cells, mice were orally treated (using a stomach tube) with a single dose of AA at doses of 10, 20 and 30 mg/kg b.wt. Samples were taken 24 h after treatment. For the repeated dose experiment, mice received daily oral doses of 10 mg/kg b.wt. AA for 1 and 2 weeks (7 and 14 days). In the repeated dose treatments, other groups of mice were given 100 and 200 mg LC, simultaneously with the AA. A negative – non-treated – control group of mice was examined. In addition, other groups of mice were given the oral doses of LC (100 and 200 mg/kg b.wt.) for 1 and 2 weeks.

2.4. Slide preparation and scoring

2.4.1. Micronucleus test

The micronucleus assay from mouse bone-marrow cells was performed following the standard procedure described by Schmid (1973). 5000 polychromatic erythrocytes (PCE) were counted, five mice per treatment (1000 PCE per mouse) for scoring MN. The significance of the experimental data from control data was calculated using differences between 2 proportions (Daniel, 1974), in the case of PCE and the tables of Kastenbaum and Bowman (1970) for micronuclei in polychromatic erythrocytes (MPCE).

2.4.2. Chromosome aberrations

Mice were injected i.p. with colchicine 2–3 h before sacrifice. Bone marrow preparations were made according to the technique described by Yosida and Amano (1965). A group of five mice was used for each treatment and 100 well-spread metaphases were analyzed per animal scoring for different kinds of abnormalities including gaps, breaks, fragments, deletions, Robertsonian translocations and polyploid metaphases.