Pol. J. Environ. Stud. Vol. 21, No. 5 (2012), 1243-1247

Original Research

Investigation of Possible Ecotoxic Effects of Acrylamide on Liver with the Azaserine-Rat Model

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Received: 13 January 2012
Accepted: 25 April 2012

Abstract

In this study, when acrylamide is taken into the body by nutrition, it was aimed to investigate its ecotoxic and/or carcinogenic effects on the liver. The Azaserine-Rat model developed by Longnecker and Curphey was used in this research, and in total 60 Wistar Albino race male rats were used, including 10 rats in each group. The rats were fed for 16 weeks by adding acrylamide at rates of 5 mg/kg/day and 10 mg/kg/day in drinking water. Moreover, neoplastic structures were formed by azaserine application and its effect on the development of these neoplastic structures was also investigated. As a result of this study, it was determined that ecotoxic and histopathological alterations, together with atypical cells, focuses formed in the livers of the rats in the group to which azaserine was applied and in the livers of the rats in the groups that included 5 and 10 mg/kg/day acrylamide in their drinking water. Moreover, it was found that the development (average focus diameter and focus volume) of neoplastic structures formed with azaserine was increased by 5 and 10 mg/kg/day acrylamide. These results make it possible for the probability of acrylamide to be a cancer initiator in the livers of rats.

Keywords: acrylamide, ACF, carcinogenic effects, quantitative analysis, liver

Introduction

Acrylamide is a monomer having the molecular formula C_3H_5NO (CH_2=CH-$CONH_2$) and 71.08 molecular weight, which is colorless and odorless, and in crystalline form can be dissolved in water and in solvents such as acetone, ethanol, and methanol, and is transformed into acrylic acid when hydrolyzed [1]. Acrylamide, which is used in the production of polyacrylamide, also is extremely useful in the treatment of drinking water and wastewater, in paper production, in petroleum industry, in mine production, asphalt, and in the treatment of soil and sand. Moreover, it's also commonly used as an additive in the cosmetic industry, in electrophoresis (used in molecular biology applications), in the manufacture of photographic film, in the production of adhesives, polishes and dyes and in the preparation of some alloys used in dentistry [2, 3]. It's necessary to carry out studies in order to reveal the potential detrimental effects of acrylamide on human beings since it was revealed that acrylamide forms by itself during cooking at high temperatures, roasting, and frying of carbohydrate-rich foods [4, 5], and this is taken into the body together with foods at high ratios [6]. Because it is indicated that "80% of cancers commonly observed in human beings are defined as a case including lifestyle dimensions together with nutrition, social, and cultural habits and are sourced from environ-

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Table 1. Average body weights together with weights of liver of rats in each experimental group (Average±Standard Deviation) P<0.05.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	Control	5 mg/kg/day Acrylamide	10 mg/kg/day Acrylamide	Azaserin Control	Azaserin 5 mg/kg/day Acrylamide	Azaserin 10 mg/kg/day Acrylamide
Body weights (g)	254.9± 37.5	267.6± 23.2	257.8±15.9	261.1± 31.2	346.3[a]±37.5	358.4[a]±25.9
Liver weights (g)	9.77± 1.3	10.75± 1.5	10.99± 0.9	10.59±1.7	13.05[a]±1.5	13.23[a]±0.4

[a]Statistically different group from both control group and azaserine control group

mental factors." In the Western world, liver cancer is the third highest cause of death [7]. Nowadays, increases in the observation frequency of liver cancer depending on environmental factors necessitates more careful research on this cancer's epidemiology. Researchers have investigated experimentally neoplastic variations and toxic effects in cells of rats [8-12]. In this study, possible ecotoxic and/or carcinogenic effects of the acrylamide were investigated in the livers of rats, where it was taken into the body as a result of environmental factors or cooking of foods at high temperatures.

Experimental Procedures

Experimental Animals

In our research, 14-day *Wistar albino* race male rats were used, the weights of which varied between 22 and 30 g (n=60). The rats were grouped into six, with 10 rats in each group. Before starting the experiments, the research ethics committee approved.

Application Dosages and Azaserine-Rat Method Applied to Experimental Animals

The rats in control group (Group 1) were fed by normal drinking water and standard rat feed. The rats in Group 2 were fed by adding 5 mg/kg/day acrylamide to their drinking water and the rats in Group 3 were fed by adding 10 mg/kg/day acrylamide. The Azaserine-Rat model developed by Longnecker and Curphey [13] was used in order to investigate experimentally occurring neoplastic variations in liver cells. Recently, this model was used successfully in many studies [8, 14, 15]. 0.3 ml injectable water-soluble azaserine (30 mg/kg body weight) was injected to two-week-old rats in Group 4 (Azaserine control), Group 5 (Azaserine + 5 mg/kg/day acrylamide), and Group 6 (Azaserine + 10 mg/kg/day acrylamide) experimental groups for the formation of carcinogenesis in their liver cells. Injection was applied intraperitoneal once a week throughout the successive three weeks. During one month subsequent of injection, the formation of atypical cell focuses (ACF) was possible in the liver and the effect of acrylamide on these occurred neoplastic variations was investigated after adding acrylamide to their drinking water. After one week following the last injection, the rats were fed with normal standard diet (Purina) and water, including acrylamide *ad libitum* for each group as mentioned before. The rats were always fed with feed and water that were suitable for their groups during 16 weeks.

Tissue Sample Collection and Evaluation

Before starting the dissection on rats, their body weights were recorded. After the application of anaesthetic agents (ketamine (60 mg/kg) and ether, they were sacrificed by cervical dislocation. The livers were preserved in 10% formalin solution for 24 hours. First, general tissue follow-up was applied in order to quantitatively evaluate the amount and microscopic determination of atypical cell foci (ACF) in the liver of rats. After general tissue follow-up, tissues were embedded in hard paraffin and hematoxylin and eosin staining was performed on 5 μm thick sections cut on a rotary microtome (Thermo Scientific, Shandon Finesse 325).

Statistical Analysis

In our study, Student-Newman-Keuls Multiple Comparison statistical analysis method (ANOVA) was used (ProStat version 5.04 for Windows). The results of the experiments were given as arithmetic means and ±standard deviations and P<0.05 values were accepted as statistically important. A mathematical formula was applied in order to determine the properties (ACF per mm^2, ACF per mm^3, average focus diameter, average focus volume, percent rate of ACF size to the size of whole liver) of atypical cell focuses in the liver (PTSDC; Planar-to-Spatial Data Converter by Anthony Flaks).

Results

Results Related to Weights of Body and Liver

The body weights and the weights of liver belonging to rats in the experimental groups are given in Table 1. It was determined that there was no significant difference in terms of statistics when body weights and liver weights of azaserine+5 mg/kg/day acrylamide group (Group 5) and azaserine+10 mg/kg/day acrylamide group (Group 6) were compared between each other. However, when body and liver weights of control group (Group 1), azaserine control group (Group 4), 5 mg/kg/day acrylamide group (Group 2), and 10 mg/kg/day acrylamide group (Group 3) were com-

pared, a significant difference was found in terms of statistics (P<0.05). When liver weights of 5 mg/kg/day acrylamide group (Group 2) and 10 mg/kg/day acrylamide group (Group 3) were compared between each other and with those of other groups, it was determined that there was no significant difference in terms of statistics (P>0.05).

Results of Histological and Quantitative Analysis

Atypical cell focuses (ACF), adenoma, and carcinoma were not observed in the livers of rats in control group (Group 1) whose drinking water was not applied with acrylamide. In the groups of 5 mg/kg/day (Group 2) (Fig. 1) and 10 mg/kg/day (Group 3) (Fig. 2) to which acrylamide was applied, it was observed that ACFs were formed. It was also observed that average focus diameters (mm) in azaserine+5 mg/kg/day acrylamide group (Group 5) increased being statistically significant when compared with azaserine control group (Group 4) (Table 2). In the livers of rats in this group, it was determined that atypical cell focuses occurred and atypical cell adenoma or adenocarcinoma were not encountered anyway.

Moreover, it was indicated that the percentage rate of ACF size to the size of whole liver, average focus diameters, and average focus volumes in azaserine+10 mg/kg/day acrylamide group (Group 6) increased statistically significantly when compared with azaserine control group (Group 4) (Table 2). ACF load increased significantly in all quantitative parameters of the group to which 10 mg/kg/day acrylamide was applied when compared with the group to which 5 mg/kg/day acrylamide was applied. In the group to which azaserine+10 mg/kg/day acrylamide was applied, it was observed that ACF load in all quantitative parameters increased significantly when compared with the group to which azaserine+5 mg/kg/day acrylamide was applied (Table 2).

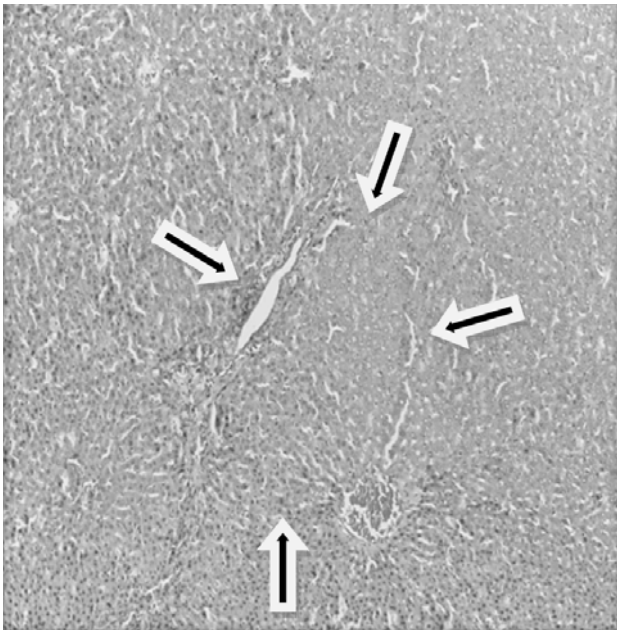

Fig. 1. Light microscopy view of atypical cell foci in Rat liver after 5 mg/kg/day acrylamide application for 16 weeks Mag: (10×20).

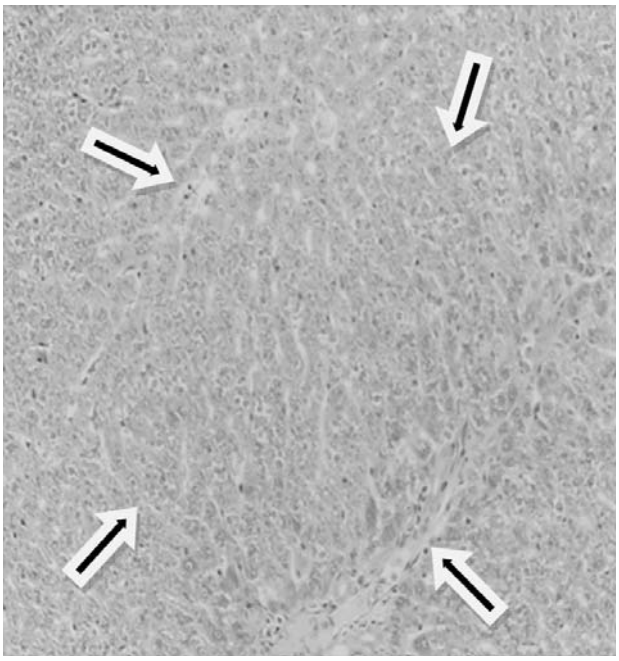

Fig. 2. Light microscopy view of atypical cell foci in Rat liver after 10 mg/kg/day acrylamide application for 16 weeks Mag: (10×20).

Discussion of Results

As a result of this study, in consideration of the effect of acrylamide on the body and liver weights of rats, it was determined that there was no significant difference when body weights of the azaserine+5 mg/kg/day acrylamide group (Group 5) and the azaserine+10 mg/kg/day acrylamide group (Group 6) were compared between each other. However, a statistically significant difference was found when compared with the body weights of the control group (Group 1), the azaserine control group (Group 4), the 5 mg/kg/day acrylamide group (Group 2), and the 10 mg/kg/day acrylamide group (Group 3) (P<0.05). In the research of Tyl et al. [16], it was indicated that there was a decrease in the body weights of babies of pregnant rats to which 5 mg/kg/day acrylamide was applied. On the other hand, Garey et al. [17] determined that there was a decrease in the body weights of babies of pregnant rats to which 1.0 mg/kg/day acrylamide was applied. These results were in accordance with our results obtained from the groups to which azaserine and acrylamide were applied. Wise et al. [18] show that the decrease observed in the body weights of babies was the most sensitive indicator of developmental toxicity. When body weights of 5 mg/kg/day acrylamide group (Group 2) and 10 mg/kg/day acrylamide group (Group 3) were compared between each other and with those of other groups, it was determined that there was no significant difference in terms of statistics (P>0.05).

When liver weights of azaserine+5 mg/kg/day acrylamide group (Group 5) and azaserine+10 mg/kg/day acry-

Table 2. Quantitative values of ACF occurring in rat liver (average±standard deviation) P<0.05.

Groups	Area of ACF per mm^2	Volume of ACF per mm^3	% rate of ACF size over the whole liver size	Average focus diameter (mm)	Average focus volume (mm^3)
Group 1 Control	0	0	0	0	0
Group 2 5 mg/kg/day Acrylamide	0.082±0.02	0.347[a]±0.157	0.226±0.003	0.240±0.053	0.007[e]±0.003
Group 3 10 mg/kg/day Acrylamide	0.130±0.06	0.443[a]±0.273	0.545[b]±0.063	0.308±0.041	0.019[e]±0.015
Group 4 Azaserin Control	0.250±0.045	1.520±0.128	0.210±0.019	0.236±0.008	0.034[e]±0.004
Group 5 Azaserin 5 mg/kg/day Acrylamide	0.073±0.017	0.195[a]±0.085	0.650[b]±0.168	0.409±0.065	0.028[e]±0.011
Group 6 Azaserin 10 mg/kg/day Acrylamide	0.15±0.11	0.233[a]±0.259	5.160[abcd]±0.197	0.975±0.557	1.135±0.12

[a] Statistically different group from azaserine control group
[b] Statistically different group from 5 mg acrylamide group
[c] Statistically different group from 10 mg acrylamide group
[d] Statistically different group from azaserine 5 mg acrylamide group
[e] Statistically different group from azaserine 10 mg acrylamide group

lamide group (Group 6) were compared between each other, it was determined that there was no significant difference in terms of statistics. However, when compared with liver weights of the control group (Group 1), the azaserine control group (Group 4), the 5 mg/kg/day acrylamide group (Group 2), and the 10 mg/kg/day acrylamide group (Group 3) were compared, a significant difference was found in terms of statistics (P<0.05). When liver weights of 5 mg/kg/day acrylamide group (Group 2) and 10 mg/kg/day acrylamide group (Group 3) were compared between each other and with those of other groups, it was determined that there was no significant difference in terms of statistics (P>0.05).

In research that has been carried out for two years with rats and mice in order to determine the carcinogenic effects of acrylamide, it was determined that acrylamide given in various applications caused the formation of tumors [19]. In a study performed by mixing acrylamide in the drinking water of rats, it was indicated that acrylamide had carcinogenic properties and, moreover, testicle and thyroid tumors in male rats and fibroadenoma of breasts together with thyroid tumor in female rats were observed [20]. As a result of subacute treatment of acrylamide, it was observed that synthesis of DNA increased, in which acrylamide has a role for cancer in target tissues such as thyroid, testicle mesothelium, and adrenal medulla [21]. In laboratory tests performed as *in vivo* and *in vitro*, it was also determined that acrylamide and glycideamide (its principle metabolite) indicated genotoxic effects at high dosages.

In order to minimize the negative effects of acrylamide taken with foods into the body, it is necessary to decrease the formation of acrylamide in food. This is only possible by decreasing either carbohydrate content or asparagine content. Moreover, since acrylamide is formed while cooking carbohydrate-rich foods at high temperatures, damage to food-borne acrylamide can be minimized by adjusting the heating time and heating temperatures of foods to decrease the formation of acrylamide. Some researchers have reported that acrylamide concentration of potato chips is decreased at a rate of approximately 60% as a result of decreasing its sugar content by boiling or bleaching [22, 23]. In addition, heating with a microwave oven establishes a suitable medium for the formation of acrylamide [24, 25]. Moreover, levels of acrylamide that occur as a result of interaction between asparagines-glucose, asparagines-fructose, and asparagines-sucrose were measured and it was determined that the amount of acrylamide increased when high temperature short-time or low temperature long-time heating was used for asparagines-glucose and asparagines-fructose, whereas the amount of acrylamide increased when both temperature and period of heating for asparagines-sucrose were increased [26]. From this point of view, to minimize the use of microwave ovens, which are used commonly in our daily lives, will provide benefits in order to minimize the amount of acrylamide formed in foods.

Conclusions

As a conclusion of our study, it is possible for acrylamide to be a potential cancer initiator in the livers of rats, since cell groups with neoplastic variations were observed and the development of neoplastic structures was increased by the application of 5 mg/kg/day acrylamide and 10 mg/kg/day acrylamide in rats.

Acknowledgements

The authors gratefully acknowledge the Selcuk University Research Fund (BAP) for financially supporting this work (Project No. 09401045).

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