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RESEARCH ARTICLE

Effects of Acrylamide Treatment on Oxidant and Antioxidant Levels in Rats

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Summary

The aim of this study was to investigate serum total antioxidant status (TAS), total oxidant status (TOS) and ischemia-modified albumin (IMA) levels in long term acrylamide (ACR) given rats, compared to control rats. In total, 25 male and 25 female Wistar rats were involved in this experiment. Animals in each sex were segregated into three groups. Two of them were treatment groups and one of them was control group. Each treatment group consisted of ten animals and each control group consisted of five animals. ACR was administered to the treatment groups at 2 and 5 mg/kg/day via drinking water for 90 days. In the end of the experiment, serum samples were analyzed for IMA, TAS, TOS and albumin levels with the spectrophotometric method. Serum IMA and adjusted IMA levels were significantly higher at concentrations of 2 mg/kg and 5 mg/kg in the male rats when compared with those of the control male rats. Serum TAS levels significantly decreased at concentrations of 5 mg/kg in the male rats when compared with those of the control rats. We also observed a significant increase in the levels of serum TOS at concentrations of 5 mg/kg in the male rats. There were no significant differences between serum IMA, TAS, TOS and albumin levels at concentrations of 2 mg/kg and 5 mg/kg in the female rats. Our findings show that long term treatment with 2 mg/kg and 5 mg/kg doses of ACR led to a significant depletion of serum TAS levels and overproduction of serum TOS and IMA levels, consequently, to an increase in oxidative stress.

Keywords: *Acrylamide, Total antioxidant status, Total oxidant status, Ischemia-modified albumin, Oxidative stress*

Ratlarda Akrilamid Kullanımının Antioksidan ve Oksidan Değerleri Üzerine Etkisi

Özet

Bu çalışmada, uzun süre akrilamid verilen sıçanlar üzerinde total antioksidan durum (TAS), total oksidan durum (TOS) ve iskemi modifiye albumin'in (IMA) serum düzeylerinin nasıl değiştiğinin araştırılması amaçlanmıştır. Çalışmada 65-75 g ağırlığında ve yaşları 3-4 haftalık 25 erkek ve 25 dişi Wistar cinsi sıçanlar kullanılmıştır. Hayvanlar 90 gün boyunca standart sıçan yemi ile beslenmişlerdir. Bununla beraber, günlük tüketecekleri içme suyuna 2 mg/kg/gün ve 5 mg/kg/gün dozunda akrilamid ilave edilmiştir. Akrilamid uygulaması sonrası hayvanlar anestezi altında servikal dislokasyonla öldürülmüş ve serumlarında IMA, TAS, TOS ve albumin düzeyleri spektrofotometrik yöntem ile ölçülmüştür. 2 mg/kg ve 5mg/kg akrilamid verilen erkek sıçanlara ait serum IMA düzeyleri kontrol grubuna göre önemli derecede yüksek bulunmuştur. Ayrıca, 5mg/kg akrilamid verilen erkek sıçanlara ait serum TAS düzeyleri kontrol grubuna göre önemli derecede düşük ve serum TOS değerleri önemli derecede yüksek bulunmuştur. 2 mg/kg ve 5mg/kg akrilamid verilen dişi sıçanlara ve kontrol grubuna ait serum IMA, TAS, TOS ve albumin düzeyleri arasında istatistiki açıdan önemli bir fark bulunamamıştır. Bu sonuçlara bağlı olarak bulgularımız, akrilamidin oksidatif stresi artırdığını göstermektedir.

Anahtar sözcükler: *Akrilamid, Total antioksidan durum, Total oksidan durum, İskemi modifiye albumin, Oksidatif stres*

INTRODUCTION

Acrylamide (C_3H_5NO) (ACR) is a small hydrophilic molecule that polymerizes readily in the presence of an initiator because of the double bond between the first and second C-atoms, which makes it a versatile industrial chemical [1]. Polymers of ACR (polyacrylamide) are extensively used in modern chemical technology for a variety of purposes [2]. In the last years, relevant amounts of ACR have been identified in heat-treated carbohydrate-rich foods

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such as fried potatoes, cookies, bread, cold breakfast cereal, biscuits and crackers [3,4]. It was shown that ACR might be formed through the Maillard reaction from amino acids (e.g. asparagine) and reducing sugars (e.g. glucose) [3]. Maillard reaction is proposed the major pathway for the formation of ACR in food [5,6]. Several different reaction routes have been proposed for the further reaction to ACR such as decarboxylation of the Schiff base [6-8], decarboxylated Amadori product [6,7], formation of a 3-Aminopropionamide [8,9], Strecker degradation of asparagine [5].

The biological consequences of ACR exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound. Subsequently, experimental exposure of rodents to ACR has also revealed a carcinogenic mode of action for this chemical [10]. It has been reported that most investigations of ACR toxicity have mainly focused on their genotoxic and carcinogenic properties; however, accumulating evidences seem to indicate that ACR also possesses cytotoxic properties by affecting the redox status of the cells [11].

Oxidative stress results from an imbalance between the production of free radical and antioxidant activity, leads to damage of biological macromolecules and disruption of normal metabolism and physiology [12]. Oxidative stres in the cells or tissues refers to enhanced generation of reactive oxygen species (ROS) and/or depletion of antioxidant defense system. ROS can attack the polyunsaturated fatty acid in the biological membranes and induce free radical chain reactions, leading to the enhancement of lipid peroxidation [13]. The methods used for the determination of oxidative stress are generally of board range. An often used and easily detectable parameter for the serum anti-oxidative and oxidant properties are the total antioxidant status (TAS) and total oxidant status (TOS) [14,15]. It has been showed that the high concentrations of ACR exert deteriorating effects on antioxidant enzyme activities, lipid peroxidation process and haemolysis in human erythrocytes *in vitro* [16].

Ischemia-modified albumin (IMA) is a novel marker of tissue ischemia [17]. IMA is serum albumin in which the N-terminus has been chemically modified [18]. The diagnostic albumin Co^{2+} binding test is based for IMA on the observation that the affinity of serum albumin for Co^{2+} is reduced after N-terminus modifications [18]. Proposed mechanisms for the conversion of serum albumin to IMA include hypoxia, acidosis, superoxide-radical injury, energy-dependent membrane distruption and exposure to free iron and copper [19]. Nowadays, IMA is accepted as a marker of oxidative stress. It has been proposed that ROS such as superoxide (O_2^-) and hydroxyl ($^{\cdot}OH$) radicals generated during ischaemia modify the N-terminus of serum albumin resulting in IMA formation [18].

Studies describing the role of oxidative stress in ACR toxicity are limited, although the main pathways for metabolism of ACR occur either through conjugation with reduced glutathione (GSH) or via epoxidation to glycidamide [16]. Also, to our knowledge, the effects of intermediate doses of ACR on serum IMA, TAS and TOS levels in rats have not been investigated yet. Therefore, the present experiment was carried out to investigate the effects of 2 and 5 mg/kg/day doses of ACR on serum IMA, TAS and TOS levels in rats.

MATERIAL and METHODS

Chemicals

ACR (Cas 79-06-1) was received from Sigma Aldrich Chemical Company. The material was a white odourless crystalline solid, with a chemical purity of>99% (for electrophoresis). The test material was prepared weakly and stored at room temperature. All other chemicals used were of analytical grade.

Animals and Husbandry

Male and female weaned Wistar rats, weighing 65-75 g and aged 3-4 weeks old, were obtained from Selcuk University Experimental Medicine Research and Application Centre (Konya, Turkey). The rats were housed in wire-topped opaque polycarbonate cages and maintained under constant environmental conditions with a 12 h light/dark schedule. The environmental temperature was 20°C and humidity was 50%. Commercial food pellets and drinking water were provided *ad libitum*. The protocols of the animal experiments were approved by the internal ethical commitee of the Selcuk University (number: 2010-98).

Dosing

We choosed the doses of ACR applied as 2 and 5 mg/kg/day in our study since not to occure a characteristic sign of ACR-induced neurotoxicity such as hindlimb foot splaying [20,21].

Experimental Design

In total, 25 male and 25 female Wistar rats were involved in this experiment. Animals in each sex were segregated into three groups. Two of them were treatment groups and one of them was control group. Each treatment group consisted of ten animals and each control group consisted of five animals. ACR was administered to the treatment groups at 2 and 5 mg/kg/day via drinking water for 90 days. Tap water was administered to five animals of control group in the same manner as in the treatment groups. After 24 h from the last application all animals were anaesthesized with ketamine in combination with a sedative. Blood samples were collected from the cardiac puncture of all rats under anaesthesia. After collecting blood samples rats were sacrificed by cervical dislocation.

Blood samples were obtained after suitable centrifugation and samples were stored frozen at -80°C until the day of analysis.

Measurement of Total Antioxidant Status

TAS of serum were determined using an automated measurement method which was based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants [14]. The results were expressed in mmol Trolox equivalents/L.

Measurement of Total Oxidant Status

TOS of serum were determined using a novel automated measurement method.[15] Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar H_2O_2 equivalent per liter (μmol H_2O_2 equiv./L).

Measurement of Ischemia-Modified Albumin Levels

IMA level was measured by a colorimetric assay developed by Bar-Or et al.[17] based on measurement of unbound cobalt after incubation with patient serum. Increased amounts of IMA results in less cobalt binding and more residual unbound cobalt available for complex with a chromogen [dithiothreitol (DDT)], which can be measured photometrically. The procedure was as follows:

50 μL of 0.1% cobalt chloride was added to 200 μL of serum, gently mixed, and waited 10 min for adequate cobalt-albumin binding. Fifty microliters of DTT, at a concentration of 1.5 mg/ml, was added as a colorizing agent and the reaction was stopped 2 min later by adding 1.0 mL of 0.9% NaCl. The colored product was measured at 470 nm and compared to a serum-cobalt blank without DTT and reported in absorbance units (ABSU).

Adjusted IMA was calculated as (individual serum albumin concentration/median serum albumin concentration of the population)×IMA ABSU value. This formula was applied to correct IMA values for serum albumin (Median serum albumin concentration of each group of the subjects were used separately) [22].

Measurement of Albumin Levels

Serum albumin levels were measured by commertially available kits based on routine methods on the Synchron LX System (Beckman Coulter, Fullerton CA).

Statistical Analysis

All data are expressed as mean±standart deviations (SD). Statistical analyses were done using SPSS v. 16.0 (SPSS Inc.,IL, USA). Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Differences were considered significant at a probability level of $P<0.05$.

RESULTS

Serum IMA, adjusted IMA, TAS, TOS and albumin levels of the groups are presented in *Table 1*. As seen from the table, serum IMA and adjusted IMA levels were significantly higher at concentrations of 5 mg/kg ($P<0.001$ for IMA and $P<0.01$ for adjusted IMA) and 2 mg/kg ($P<0.01$) in the male rats when compared with those of the control male rats. However, there were no significant differences between the same parameters at concentrations of 5 mg/kg and 2 mg/kg in the female rats and the control female rats *(Fig. 1)*. Serum TAS levels significantly decreased at concentrations

Table 1. *Serum IMA, adjusted IMA, TAS, TOS and albumin levels of the groups (All values are mean±standart deviations)*
Tablo 1. *Grupların serum IMA, ayarlanmış IMA, TAS, TOS ve albumin düzeyleri (Bütün değerler ortalama±standart sapma)*

Parameter	Group	Control	ACR 2 mg/kg	ACR 5 mg/kg
IMA (ABSU)	Male	0.53±0.09	0.71±0.07[b]	0.72±0.07[a]
	Female	0.64±0.09	0.69±0.06	0.73±0.03
Adjusted IMA (ABSU)	Male	0.54±0.09	0.71±0.07[b]	0.73±0.07[b]
	Female	0.63±0.1	0.70±0.08	0.73 ± 0.02
TAS (mmol Trolox equivalents/L)	Male	3.65±1.2	3.34 ± 1.2	1.77±1.1[c]
	Female	3.69±1.5	3.67±1.1	3.31±1.0
TOS (μmol H_2O_2 Eq/L)	Male	8.88±2.3	12.67±2.0	14.72±5.0[c]
	Female	9.95±1.9	11.18±2.5	14.28±4.1
Albumin (g/dL)	Male	2.72±0.1	2.66±0.2	2.63±0.1
	Female	3.17±0.1	3.06±0.4	2.88±0.3

IMA- *ischemia-modified albumin;* ***TAS-*** *total antioxidant status;* ***TOS-*** *total oxidant status;* [a] *P<0.001,* [b] *P<0.01,* [c] *P<0.05 compared with control group*

of 5 mg/kg (P<0.01) in the male rats when compared with those of the control rats. But, this difference was not found at concentrations of 5 mg/kg in the female rats. On the other hands, there were no significant differences between serum TAS levels at concentrations of 2 mg/kg in the male and female rats and the control rats *(Fig. 2)*. We also observed a significant increase in the levels of serum TOS at concentrations of 5 mg/kg (P<0.05) in the male rats. No differences were found in serum TOS levels between at concentrations of 5 mg/kg in the female rats and control rats. Also, no differences were found in serum TOS levels between at concentrations of 2 mg/kg in the male and female rats and control rats *(Fig. 3)*. There were no significant differences between serum albumin levels of the groups. In addition, no sign of sickness and decreased activity of the rats or mortality was observed during the study.

DISCUSSION

ROS are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms, which remove them via enzymatic and non-enzymatic antioxidative mechanisms. Under some conditions, the increase in oxidants and decrease in anti-oxidants cannot be prevented, and the oxidative/anti-oxidative balance shifts toward the oxidative status [23]. Several reports have reported that ACR promotes the generation of ROS and the depletion of anti-oxidants [13,24].

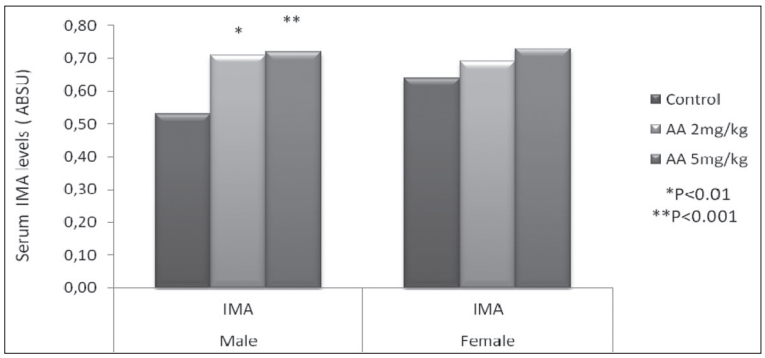
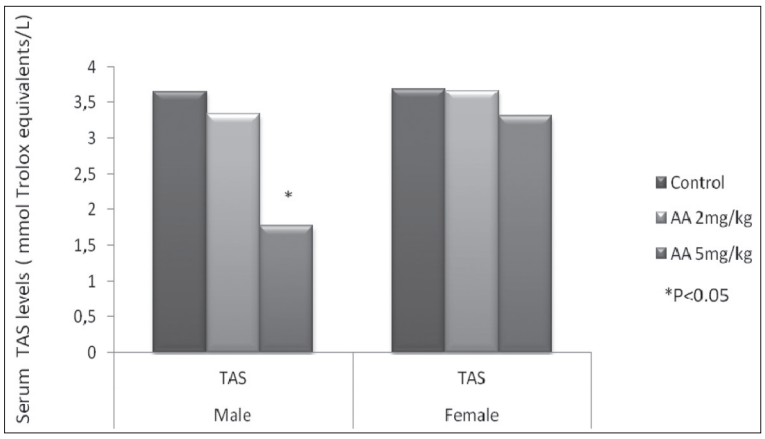


Fig 1. Serum ischemia-modified albumin (IMA) levels in all groups

Şekil 1. Bütün gruplarda serum iskemi-modifiye albumin (IMA) düzeyleri

Fig 2. Serum total antioxidant status (TAS) levels in all groups

Şekil 2. Bütün gruplarda serum total andioksidan durum (TAS) düzeyleri



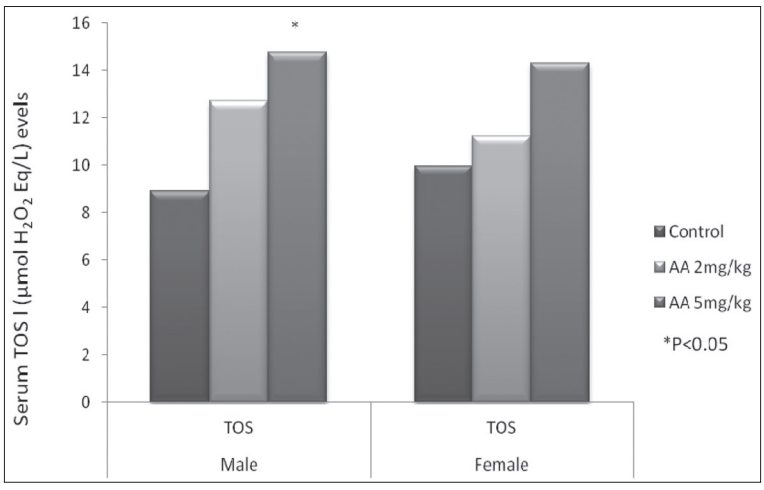


Fig 3. Serum total oxidant status (TOS) levels in all groups

Şekil 3. Bütün gruplarda total antioksidan (TOS) durum düzeyleri

The present study shows that ACR given via drinking water to male rats induced to increase in TOS and IMA levels and decrease in TAS levels.

Conjugation with GSH catalyzed by glutathione-S-transferase is the main pathway of ACR metabolism and detoxification [25]. GSH is one of the essential compounds for the maintenance of the cell integrity because of its reducing properties and participation in the cell metabolism [26]. ACR is capable of interacting with vital cellular nucelophiles possessing –SH, $–NH_2$ or –OH. Therefore, it reacts with glutathione in a similar manner and forms glutathione *S*-conjugates, which is the initial step in the biotransformation of electrophiles in to mercapturic acids [27]. Decreasing GSH content in the tissues with the increase of ACR concentration could be due to increased formation of *S*-conjugates between ACR and GSH [28]. ACR can be reactive unlike the above mentioned way. Firstly, ACR can undergo radical-mediated polymerization. Secondly, it can be metabolized to an epoxide derivate, glycidamide, presumably by cytochrome P4502E1, being readily reactive toward DNA and other macromolecules [16].

In the literature, our finding supported in studies which made with different oxidative stress marker after ACR -treatment. For example, Allam et al.[29] have reported that ACR administered either prenatally or perinatally has been shown to induce significant increase of thiobarbituric acid reactive substances (TBARS) and oxidative stres (significant reductions in total thiols, superoxide dismutase (SOD) and peroxidase activities) in the developing cerebellum of rats [29]. Alturfan et al.[30] have found that 8-OHdG levels as an indicator of oxidative DNA damage, significantly increased in ACR group. In addition, they demonstrated that in ACR-treated rats, GSH levels decreased significantly while the malondialdehyde (MDA) levels, myeloperoxidase activity and collagen content increased in the tissues suggesting oxidative organ damage. Yousef et al.[28] have found that the concentration of TBARS, and the activities of glutathione *S*-transferase and SOD in plasma, liver, testis, brain, and kidney were increased in ACR -treated male rats [28]. Catalgol et al.[16] have demonstrated that *in vitro* treatment of ACR results in haemolysis and induction of lipid peroxidation and changes the activities of antioxidant enzymes at high doses in human erythrocytes.

The results of the present study indicate that serum IMA levels increase in ACR-treated rats, compared to control rats. Albumin is the most abundant serum protein, and is a powerful extracellular antioxidant. The biochemical mechanism modifying the N-terminal region of albumin during ischemia is unclear, but reperfusion after an ischemic event may damage serum albumin as much as, if not more than, ischemia itself [31]. These modifications to albumin may involve hypoxia, acidosis, or free radical damage, most of which occur within minutes [23]. According to our hypothesis, IMA is increased in conditions that produce free radicals, such as ACR -treatment. Naruszewicz et al.[32] have reported that the first time that chronic ingestion of dietary ACR might induce oxidative stress in humans through leukocyte activation and increased production of ROS [32]. In addition, they have reported that a significant increase in the oxidized low-density lipoprotein, interleukin-6, and high sensitivity C-reactive protein concentrations occurred, with all these factors recognized as capable of enhancing atherosclerosis progression [32]. According to their hypothesis, long-term ingestion of high ACR doses with food may cause chronic inflammation and contribute to the development of early atherosclerosis as well as increase the risk of coronary artery disease. IMA was considered to be related as a potential cardiovascular risk factor [18]. Previous studies have found that IMA levels increased in acute coronary syndromes, during percutaneous coronary intervention, and myocardial ischemia [17,33,34]. Our finding supports Naruszewicz et al.'s [32] hypothesis. Because, long term intermediate doses ACR -treatment, which increased serum IMA and TOS levels, may be risk for cardiovascular diseases.

On the other hand, a significant increase serum IMA and TOS levels and decrease TAS levels did not observed in female rats. We think that estrogen may be cause this situation. The hormone estrogen, (estradiol) provides natural anti-oxidant properties because it increases the expression of antioxidant enzymes and decreases troublesome NADPH oxidase enzyme activity and superoxide production. Estrogens have a phenolic hydroxyl group at position 3 and a methyl group at position 13. The presence of this phenol group gives estrogen its antioxidant property by neutralizing oxygen free radicals. The estrogens estriol and estradiol confer significant antioxidant activity [35].

In our study, ACR administered rats grew normally and gained body weight over a 12-week period, and there were no significant differences in the average body weight compared with control rats. This finding is in accordance with that of Park et al.[36]. However, previous studies indicated that rats exposed to ACR showed a decrease in body weight [37,38]. According to our hypothesis, there were no changes in body weight in intermediate doses.

As a result, our findings show that long-term treatment with intermediate-ACR doses led to a significant depletion of serum TAS levels and overproduction of serum TOS and IMA levels especially in male rats, and cause oxidative stress.

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