

Alteration of Oxidative Status in Rats Following Administration of Acrylamide

Hanaa H. El-Sayed¹, Shawkia S. Abd El- Halim¹,
Gehan M. Morsy² and Ayman El-Sayed El- Adawi³

¹ National Nutrition Institute. ² Women's college- Ain Shams University.

³ Home Economics college- Minufiya University.

Abstract

Introduction: Acrylamide (ACR) is a known industrial neurotoxic and carcinogenic chemical in rodents. The recent discovery of acrylamide in wide variety of commonly consumed foods has energized research efforts worldwide to define toxic mechanisms.

Objective: The present study is carried out to investigate the effect of acrylamide administration on *in vivo* malondialdehyde (MDA, a product of lipid peroxidation), reduced glutathione (GSH) as well as copper and zinc superoxide dismutase enzyme activity (Cu/Zn SOD) of rats.

Material and Methods: Fourteen adult male Sprague Dawley rats were divided into two groups each containing "7" rats. Group 1 served as negative control fed on basal diet and group 2 (positive control) received basal diet and acrylamide (0.34g/ kg diet) for 11 days. Levels of MDA, GSH and activity of SOD were determined in liver, kidneys, brain, heart, testes, spleen and lungs of rats.

Results: ACR treatment significantly increased MDA in all organs; the highest increase was detected in testis (87.9%) and heart (71.5%) while the lowest one was found in kidneys (28.2%). On the other hand, GSH levels and SOD activities were significantly reduced in ACR treated rats. However, the reduction of GSH level ranged from 10.2% to 36.5 %.The inhibition of SOD activities were higher in testis (57.3%) and lungs (38.5%).

Conclusion: The present study showed that ACR exerts deteriorated effects on oxidative status of rats.

Keywords: Acrylamide –*In vivo*- Rats- Tissues – Lipid peroxidation – Reduced glutathione- Superoxide dismutase.

Introduction

Acrylamide (NH₂-C=O-CH-CH₂), a highly reactive vinyl, water soluble monomer. It does not occur naturally, but is formed from the dehydration of acrylonitrile (Macwilliam, 1978). Scientists in several countries have reported high concentration of ACR in starch-rich foodstuffs. It was also found in various fried, deep-fried and oven- (e.g. chips, crisps and bread, crackers and breakfast cereals) (Tareke *et al.*, 2002), formed by Maillard reaction from reducing sugars (e.g. glucose) and amino acids (e.g. asparagines) (Mottram *et al.*, 2002, Taubert *et al.*, 2004).

Acrylamide is used in the manufacture of polyacrylamide used in municipal and industrial wastewater treatment (Blumenthal *et al.*, 1995), as well as in paper pulp industry (Dearfield *et al.*, 1988). Polyacrylamides are also used in drilling muds, textile and laminating resins,

flocculation of ores, friction reduction, soil stabilizers, oil-in-water deemulsifiers, gel chromatography and electrophoresis, photography, dyeing and ceramics, and food processing (Bergmark *et al.*, 1991).

Human exposure to acrylamide primarily comes from dermal contact with solid monomer and inhalation of dust and vapor in the occupation setting. The public may be exposed to acrylamide through the ingestion of drinking water that is contaminated with acrylamide or the intake of acrylamide from foods (Jerry, 2005). It has been also reported that acrylamide was formed in the heating of rodent feed, suggesting that human exposure to acrylamide could occur during the cooking of rodent food (Tareke *et al.*, 2002).

Acrylamide has been observed to produce neurotoxic effects in industrial workers and several species of experimental

animals (Sobel *et al.*, 1986; Crofton *et al.*, 1996). Other effects of acrylamide exposure include genotoxic, carcinogenic, reproductive and developmental abnormalities (Erdeich and Friedman, 2004, Yang *et al.*, 2005). Acrylamide can also across the placenta and significant concentration reach the conceptus and produce direct developmental and post-natal effects in rodent offspring (Dearfield *et al.*, 1988).

The current study used the *in vivo* model (rat tissues) to investigate the effect of ACR administration on the oxidative status of different organ tissues of rats (MDA, as a product of lipid peroxidation, reduced GSH and Cu/Zn SOD).

Material and Methods

Chemicals:

Acrylamide was purchased from Merk-schuchardt chemical company (Hohenbrunn, Germany), with molecular formula $\text{NH}_2\text{-C=O-CH-CH}_2$ and purity of > 99%. All other chemicals were of analytical grade.

Animals:

Fourteen adult male Sprague-Dewily rats (weighing 160-180) were obtained from Vaccine and Immunity Organization, Hellwan farm, Cairo, Egypt. Rats were housed individually in mesh bottomed metallic cages under healthy environmental conditions. Water and diet were provided *ad-libitum*.

Experimental design:

Rats were divided into two groups (7rats/ group). Group 1 served as negative control fed basal diet (Philip *et al.*, 1993). Group 2 fed basal diet to which acrylamide is added (0.34g / kg diet) for 11days as described by (Lehning *et al.*, 2002). The consumed food and body weights of rats were recorded twice a week to monitor food intake and body weight gain

- Food intake was calculated as g /day/rat.

- % Body weight Change =

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Biochemical analysis:

At the end of experimental period (11 days), all rats were anesthetized with diethyl ether after overnight fasting and sacrificed. Liver, kidneys, brain, heart, testes, spleen and lungs were isolated immediately, plotted free from adhering blood, washed with cooled saline and dried between filter paper. All organs were frozen at -20°C till further analysis. Glutathione (GSH) was determined according to Beutler *et al.* (1963) and Superoxide dismutase (SOD) according to Beauchamp and Fridovich (1971). Malondialdehyde (MDA) was assayed as described by Uchiyama and Mihara (1978).

Statistical analysis: The data were subjected to statistical analysis using computer programme (SPSS (1996). Independent t-test and one-way analysis of variance (ANOVA) were used, the difference was considered significant at p-value < 0.05 (Zar, 1984).

Results

The focus of this work was to investigate the alteration of oxidative status in acrylamide treated rats. However, No animal mortality was found in rats treated with acrylamide. Results presented in table (1) indicated that food intake and the gained body weights of rats received acrylamide decreased significantly as compared with negative control, with percentages of 79.4% and 25.9% for body weight gain and food intake respectively.

Acrylamide treatment caused a significant increase in MDA levels (table, 2) in tissue homogenates of all examined organs as compared with negative control group. However, this increase was more pronounced in testis (-87.9%) and brain (-76.0%). The least effect was detected in kidneys (-28.2%), while, both liver, spleen (-51.1%) and brain & lung (-30.6%) were equally affected.

The effect of ACR administration on GSH levels of different rat organ tissues is illustrated in table (3), GSH levels were significantly depleted in all tissue homogenates as compared with negative control rats. However, the depletion in GSH

levels were high in kidneys (-36.5%) and testis (-33.5%) and low in lung (-10.2%).

Moreover, SOD activity in tissue homogenates of ACM treated rats (table, 4), was significantly reduced in all organs as compared with the untreated rats. SOD

activity in testis and lung were reduced to 57.3%, 38.5% of the normal control respectively, while the depletion of the enzyme activity in the other organs ranged from 23.8 % to 32.4%..

Table (1): Effect of Acrylamide administration on Food intake and body weight gain of rats (mean ±S.D).

| Parameter | Negative control | Positive control | % change |
|--------------------------|------------------|------------------|----------|
| Food intake (g/ day/rat) | 36.7± 0.93 | 27.2± 2.8* | 25.9 |
| % Body weight change | 23.3± 1.7 | 4.8± 0.3* | 79.4 |

*: Significant difference from negative control (p< 0.05)

Table (2): Effect of Acrylamide administration on mean MDA levels (n mol/g tissue) of different organ tissues of rats (Mean± S.D)

| Organs | Negative control | Positive control | % Change |
|---------|------------------|------------------|----------|
| Liver | 95.7 ± 13.8 | 144.6 ± 17.4* | -51.1 |
| Kidneys | 165.3 ± 24.3 | 211.9 ±15.8* | -28.2 |
| Heart | 60.0 ± 3.6 | 102.91 ± 5.7* | -71.5 |
| Brain | 217.0±9.6 | 382± 6.4* | -76.0 |
| Testis | 162.3 ± 12.0 | 305.0 ± 32.9* | -87.9 |
| Spleen | 51.7 ± 5.3 | 80.19 ± 9.6* | -55.1 |
| Lung | 58.2±1.6 | 76.0±2.7* | -30.6 |

*: Significant difference from negative control (p< 0.05)

Table (3): Effect of Acrylamide administration on mean GSH levels (mg/g tissue) of different organ tissues of rats (Mean± S.D)

| Organs | Negative control | Positive control | % Change |
|---------|------------------|------------------|----------|
| Liver | 51.8 ± 7.5 | 35.8 ± 2.5* | 30.9 |
| Kidneys | 34.8 ± 5.3 | 22.1 ± 1.9* | 36.5 |
| Heart | 32.0±1.4 | 23.0±1.4* | 28.1 |
| Brain | 31.4 ±1.2 | 21.2±0.6* | 32.5 |
| Testis | 48.1 ± 5.7 | 32.0 ± 4.9* | 33.5 |
| Spleen | 33.0±1.1 | 25.0±0.9* | 24.2 |
| Lung | 21.6±1.1 | 19.4±1.8* | 10.2 |

*: Significant difference from negative control (p< 0.05)

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Table (4): Effect of Acrylamide administration on mean SOD activity (U/gm tissue) of different organ tissues of rats (Mean± S.D)

| Organs | Negative control | Positive control | % Change |
|---------|------------------|------------------|----------|
| Liver | 735.8± 2.5 | 549.8± 7.1* | 25.8 |
| Kidneys | 450.9±34.1 | 343.6±9.15* | 23.8 |
| Heart | 487.4±45.3 | 329.3±17.9* | 32.5 |
| Brain | 234.4±13.4 | 177.2±4.0* | 24.4 |
| Testis | 506.0±16.4 | 216.0±12.8* | 57.3 |
| Spleen | 391.4±17.0 | 285.4± 22.0* | 27.1 |
| Lung | 339.8±27.3 | 208.9±4.3* | 38.5 |

*: Significant difference from negative control (p< 0.05)

Discussion

The decrease in body weight gain obtained in the present work (table, 1) is in agreement with observation of Yang *et al.* (2005), who found that body weight was significantly lower in acrylamide treated rats than in the normal control animals. This decrease may be related to the significant accompanied decrease in reduced GSH levels in different organ tissues of rats. Martensson *et al.* (1990) reported that GSH is essential for the function and structural integrity of the gut, and those GSH deficient mice showed severe degradation of jejunum and colonic mucosa and were found to have weight loss and diarrhea.

Free radicals are continuously produced *in vivo* and there are number of protective antioxidant (e.g. superoxide dismutase, reduced glutathione) for dealing with these toxic substances. The balance between the production and catabolism of oxidants is critical for maintenance of the biological function (Sridevi *et al.*, 1998)

The observed marked increase of MDA levels (product of lipid peroxidation) in different tissues (table, 2) is in agreement with the finding of Yousef and El-Demerdash (2006). However, Srivastava *et al.* (1983) suggested that enhancement of lipid peroxidation is a consequence of depletion of glutathione to certain critical levels.

A possible role for the participation of oxidative stress in the toxicity of ACR is

supported by several observations. A major pathway of ACR metabolism is GSH conjugation (Miller *et al.*, 1982), where acrylamide is oxidized to glycidamide (a reactive epoxide) and undergoes conjugation with glutathione. By depleting GSH, ACR may decrease the antioxidant levels of the cells leading to an overall increase of intracellular ROS and oxidative damage. ACR is also metabolized by cytochrome P450 to generate cyanide ions (Raucy *et al.*, 1993) Administration of cyanide has been found to cause lipid peroxidation in the brain in mice (Johnson *et al.*, 1987).

Beiswanger *et al.* (1993) reported that reduced GSH levels in brain and liver of rats were significantly decreased following ACR administration. These findings are in agreement with the present study.

This depletion of GSH levels is related to conjugation of ACR with GSH (as mentioned before). Acrylamide is a highly reactive electrophilic compound with very high water solubility; these properties facilitate its rapid absorption and distribution throughout the body (Solomon *et al.*, 1985). It binds readily to protein thiols (like GSH) and DNA (Bergmark *et al.*, 1991). Depletion of GSH as a result of conjugation with ACR and GSH has been observed *in vivo*, Edwards (1975) found that treatment with ACR rapidly depleted GSH in rat liver and identified a GSH conjugate of ACR in bile. Dearfield *et al.*, (1988) stated that after absorption, ACR is

rapidly metabolized primarily by glutathione conjugation and the majority of applied material is excreted within 24 hours. Moreover, Fennell and Friedman (2005) reported that 59% of the metabolites excreted in urine of rats administered single dose of acrylamide was from acrylamide-glutathione conjugation.

Reduction in the activities of SOD in different tissues is in agreement with the finding of Abdel-Wahhab and Ahmed (2004) who found that SOD activity was decreased in brain homogenate of ACR treated rats. On the contrary, Yousef and El-Demerdash (2006) reported that the activity of SOD was increased by ACR-treatment.

The decreased levels of SOD activity may arise from impairment of antioxidant system as a consequence of an abnormality on the antioxidative metabolisms (Mantovani *et al.*, 2002). Iantomasi *et al.*,(1994) concluded that deterioration of antioxidative glutathione metabolism and increased oxidative damage to proteins and DNA is associated with impaired enzymatic activity of Cu/Zn SOD in patients with Crohn's disease.

Conclusion

On the basis of the present results, we can conclude that acrylamide administration caused disturbances in the oxidative status and antioxidants of rats as well as a risk of damage to the investigated organs.

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التغير في حالة التأكسد في الجرذان بعد تعاطي الأكرلاميد

هناء حسين السيد¹ - شوقية سيد عبد الحليم¹ - جيهان محمد مرسى² - أيمن السيد العدوى³

¹ المعهد القومي للتغذية. ² كلية البنات- جامعة عين شمس.
³ كلية الاقتصاد المنزلي- جامعة المنوفية.

مقدمة: يعرف الأكرلاميد بأنه مادة يصيب الجهاز العصبي ويسبب السرطان . وقد أدى الاكتشاف الحديث لوجود الأكرلاميد في كثير من الأغذية شائعة الاستخدام إلى تنشيط الأبحاث في كل أنحاء العالم لتحديد كيفية إحداثه للسمية. **الغرض من الدراسة:** تم إجراء هذه الدراسة لمعرفة تأثير الأكرلاميد على مستوى كلا من ثنائي الملونالدهيد و الجلوتاثيون و كذلك نشاط إنزيم السوبر أو كسيد ديسميوتيز في الجرذان. **التجربة:** تم تقسيم عدد 14 فأرا ذكرا بالغاً من سلالة الاسبراجو داوولي إلى مجموعتين، الأولى هي المجموعة الضابطة السالبة و التي تغذت على الوجبة الأساسية فقط و المجموعة الثانية (المجموعة الضابطة الموجبة) تغذت على الوجبة الأساسية مضافاً إليها الأكرلاميد بجرعة (0.34 جم / كجم من الوجبة) لمدة 11 يوماً . تم قياس مستوى كلا من ثنائي الملونالدهيد و الجلوتاثيون و كذلك نشاط إنزيم السوبر أو كسيد ديسميوتيز في الكبد و الكلى و المخ و القلب و الخصية و الطحال و الرئة للجرذان. **النتائج:** أدى تعاطي الأكرلاميد إلى زيادة ذات دلالة إحصائية في ثنائي الملونالدهيد في كل الأعضاء المختبرة و ظهرت الزيادة الأكثر في الخصية (87.9%) و القلب (71.5%) بينما كانت الزيادة أقل في الكلى (28.2%)، انخفض تركيز كلا من الجلوتاثيون و نشاط إنزيم السوبر أو كسيد ديسميوتيز في كل الجرذان المعاملة حيث تراوح النقص في الجلوتاثيون من 10.2% إلى 36.5%. بينما كان الانخفاض في نشاط إنزيم السوبر أو كسيد ديسميوتيز مرتفعاً في الخصية (57.3%) و الرئة (38.5%). **الخلاصة:** أظهرت الدراسة الحالية أن تعاطي الأكرلاميد أحدث تأثيرات ضارة على حالة التأكسد في الجرذان