EFFECT OF ACRYLAMIDE TREATMENT ON ARGINASE ACTIVITIES AND NITRIC OXIDE LEVELS IN RAT LIVER AND KIDNEY

EDA ÖZTURAN-ÖZER[1], GÜLBERK UÇAR[2], FATMA HELVACıOĞLU[3], DERYA AKAYDIN-ALDEMIR[1], SUNA TÜRKOĞLU[1]
[1]Department of Biochemistry, Faculty of Medicine, Başkent University, 06810 Ankara - [2]Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100 Ankara - [3]Department of Histology and Embryology, Faculty of Medicine, Başkent University, Ankara, Turkey

ABSTRACT

Aim: *To evaluate the effects of acrylamide treatment on the activities of rat liver, kidney arginase activities and nitric oxide levels considering the possible induction of oxidative stress.*

Materials and methods*: Serum aminotransferase activities, blood-urea nitrogen (BUN) and creatinine concentrations and tissue malondialdehyde (MDA), reduced glutathione (GSH), total nitrite concentrations and arginase activities were evaluated in groups. Histopathological analysis was performed.*

Results*: Acrylamide treatment did not modulate liver and kidney serum markers. Hepatic MDA, GSH concentrations did not change whereas they were elevated in kidney tissues of high dose treated group (p<0.05). Arginase activity in gram liver tissue decreased (p<0.0001), but specific activity did not alter. Total nitrite concentrations increased in high dose treated group (p<0.05). In kidney, high dose of acrylamide treatment elevated activity and specific activity of arginase (p<0.05). No alteration was detected in total nitrite levels. Ultrastructural alterations were detected in epithelial cells of proximal tubules in kidney sections of the rats treated with high dose of acrylamide.*

Conclusion: *Liver seems to protect itself against acrylamide toxicity whereas, kidney can be considered as a probable target tissue for acrylamide-induced oxidative stress.*

Key words: *Acrylamide, arginase, nitric oxide , oxidative stress, liver, kidney.*

Received *January 10, 2014;* ***Accepted*** *January 22, 2014*

Introduction

Acrylamide is an important chemical which is extensively used in many industries and also in laboratories[1]. Evidences have indicated that exposure to acrylamide may cause hazardous effects in various tissues and systems due to its strong electrophilic nature in a dose- and tissue-specific manner and also its major metabolite glycidamide is directly involved in mutagenic and carcinogenic effects of acrylamide[2-4]. Besides these, during metabolization via CYP2E1, reactive oxygen species (ROS) are generated[5,6]. The International Agency for Research on Cancer[7] has classified the compound as a “probable human carcinogen (class 2A)”. Hence, the spontaneous formation of acrylamide in carbohydrate rich foods, depending on the preparation conditions, has led to its description also as a food toxicant[8].

Nitric oxide (NO) is a chemical mediator, involved in the maintenance of physiological homeostasis due to its regulatory and protective functions. Besides its known antioxidant property, NO which is produced by inducible nitric oxide synthase (iNOS) can be cytotoxic especially at higher local concentrations. Also, it can react with ROS or oxygen yielding reactive nitrogen species, which cause damage on biological molecules such as enzymes, lipids and DNA by oxidation, nitrosation, and nitration. NOS activity is controlled by a number of molecules including tetrahydrobiopterin, arginine and glutathione[9,10]. Recent studies have indicated that NO is also produced by NOS-independent sources, reduction of nitrite by alternative ways, which is regulated by pH, partial pressure of O_2, nitrite and reducing substrate concentrations[11].

Arginase (E.C.3.5.3.1) is a hydrolytic metalloenzyme that catalyzes the conversion of arginine to ornithine and urea. Hepatic arginase (Type I) is a cytosolic enzyme which plays an essential role in ammonia detoxification as a member of urea cycle. On the other hand, extrahepatic arginase (Type II) is localized in mitochondria. The physiological role of arginase II is associated with the production of ornithine for the synthesis of polyamines, glutamate and proline. In the case of incomplete urea cycle, both isozymes of arginase may also modulate the levels of L-arginine. There is currently increasing interest in the role of arginase in the regulation of NO production since, L-arginine is a substrate of both enzymes NOS and arginase[12,13]. Besides its possible regulatory function on NO production arginase may control also polyamine metabolism. Therefore, the alteration of arginase activity may play a crucial role by contributing to the metabolisms of NO and polyamines.

Experiments have revealed that arginase activity can be modulated in various physiological and pathological situations such as aging, ischemia-reperfusion injuries, inflammation-mediated disorders and certain cancers in which oxidative stress is one of the proposed mechanisms accompanying these pathophysiological conditions[14-19].

The present study was conducted to evaluate the effects of acrylamide treatment on the activities of arginase and NO levels in rat liver and kidney considering the possible existence of oxidative stress. Our presented data will be the first dealing with this concept.

Materials and methods

Chemicals

All chemicals and biochemicals were obtained from either Sigma (MO, USA) or Merck (Darmstadt, Germany). Nitric oxide colorimetric assay kit was purchased from Roche Diagnostic (Mannheim, Germany). Ultrafilter, Centrisart Cut-Off 10000 was from Sartorius (Goettingen, Germany).

Animals and treatments

Eighteen-month-old male Wistar albino rats weighing 220-290 g were obtained and housed in Başkent University Medical and Surgical Experimental Research Center (temperature 20 ± 2oC, humidity 50 ± 10% and 12h light:12h dark cycle). Rats were supplied with standard laboratory diet and tab water ad libitum. All experimental procedures involving animals were approved by Başkent University Institutional Review Board and Ethics Committee, Ankara, Turkey.

Rats were assigned to three groups, six rats in each group, as saline treated group (CONT), low-dose 5mg/kg acrylamide treated group (GRP I) and high dose 50 mg/kg acrylamide treated group (GRP II). LD50 value of acrylamide was reported as 100-150 mg/kg for rats[20].

All treatments were done intraperitoneally for five days. After 48 h of last intraperitoneal (i.p) treatment, rats were anesthetized (i.p.; ketamine 50 mg/kg and xylazine 10 mg/kg). Blood was immediately collected by intracardiac puncture and all the animals were sacrificed. After excision of liver and kidneys, wet weights of the organs were recorded. Liver left lobe and left kidney sections were aliquoted for histopathological examination and remaining tissues and serum samples were kept at -86°C for biochemical analysis. All biochemical studies were performed in duplicate.

Analysis of serum biochemical parameters

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and blood-urea nitrogen (BUN), creatinine concentrations were determined by Roche Hitachi modular system (Mannheim, Germany) using Roche Diagnostic reagents.

Determination of tissue malondialdehyde and reduced glutathione concentrations

For the determination of liver and kidney malondialdehyde (MDA) and glutathione (GSH) concentrations, tissue homogenates were prepared in ice-cold 0.15 M KCl (10%, w/v) using all-glass homogenizer.

MDA, as a marker of lipid peroxidation, was determined according to the thiobarbituric acid reaction described by Buege Aust[21]. One volume of sample was combined with two volumes of the stock reagent containing 15% trichloroacetic acid, 0.375% thiobarbituric acid and 0.25 M HCl. The mixture was kept in a boiling water bath for 15 min and after cooling, centrifuged at 1000 g for 10 min. The absorbances were measured at 535 nm against a reagent blank (Shimadzu-1601, Japan). Quantitations were obtained by using molar extinction coefficient of 1.56 × 105 M-1cm-1. The results were expressed as nmoleMDA/g tissue.

GSH levels were assayed in tissue homogenates according to the method of Ellman[22]. After depro-

teinization of the samples, Ellman color reagent was added onto the supernatant and the absorbance of the generated color complex was detected immediately at 412 nm against a reagent blank. Concentrations were calculated by using GSH calibration curve and results were expressed as μmole GSH/g tissue.

Determination of tissue arginase activity

Tissue samples were homogenized in ice cold 20 mM Tris-HCl buffer, pH 7.68, containing 1% Triton X-100, 0.1 mM PMSF, 0.2 mM leupeptin using all-glass homogenizer. After centrifugation at 15 000 g for 10 min, supernatants were used as enzyme sources. Activity was determined by the method of Geyer and Dabich[23] which is based on the colorimetric estimation of urea formed. Assays were carried out at 37°C in 70 mM Tris HCl/0.5 mM MnCl2, pH 8.5 containing 25 mM arginine with 0.1 ml and 0.2 ml of Mn2+ enriched enzyme source of liver and kidney respectively. After color development, absorbance was measured at 520 nm against sample blank. Quantitation was performed using urea calibration curve. Activity was expressed as μmole urea/min/g tissue (U/g). Protein concentrations were determined according to the method of Bradford(24) and specific activity was described as U/mg protein.

Determination of tissue NO levels

Liver and kidney NO levels were evaluated indirectly by determination of NO3-/NO2-, concentrations, metabolic products of NO. Tissues were homogenized in 20 mM Tris-HCl, pH 7.5, containing 2 mM EDTA using all-glass homogenizer. After centrifugation of homogenates at 2000 x g for 10 min, concentrations of NO3-/NO2- were determined using Nitric Oxide Colorimetric Assay Kit (Roche Diagnostic, Mannheim, Germany) according to the protocol supplied by the manufacturer. The absorbance was measured at 550 nm using microplate reader (Bio-Tek , Winooski-USA). Quantitation was carried out by standard curve and total NO2- concentrations were expressed as nmole/g tissue.

Histopathological Examination

All tissues were fixed in 0.1 M phosphate buffer, pH 7.4, containing 2.5% gluteraldehyde for 2 h. Samples were then post-fixed in 1% osmium tetraoxide for 1h and dehydrated in a series of graded alcohols. Propylene oxide treated specimens were embedded in Araldyte CY 212, according to the protocol supplied by the manufacturer. Semihtin sections, stained with toluidin blue, analyzed under light microscope. Uranyl acetate-lead citrate stained ultrathin sections were prepared and transmission electron microscopy (EM) was performed on a LEO 906E electron microscope (Leo Company, Germany).

Statistical analysis

Data were analyzed with SPSS, Version 11.5 Software. Univariate analysis of variance coupled with Tukey's post-hoc test was performed for parametric data analysis. Non-parametric data was evaluated with Kruskal-Wallis and Bonferroni-Dunn test. Data were expressed as mean ± SEM and/or median (minimum-maximum) for parametric and non-parametric data respectively. p-values less than 0.05 was considered as statistically significant.

Results

Effect of acrylamide treatment on body weight gain, tissue wet weights and the ratios of tissue to body weight

Acrylamide treatment caused a decrease in body weight gain in a dose-related manner. As seen in Table 1, in high dose treated group, i.e. 50 mg/kg, body weight gain reduced significantly comparing to control and group I ($p<0.05$). Tissue wet weights and the ratio of liver wet weight to body weight did not alter by acrylamide treatment. On the other hand, the ratio of kidney wet weight to body weight was affected by acrylamide treatment again in a dose-related manner. High dose acrylamide treatment, 50 mg/kg, significantly increased the ratio compared to control and group I ($p<0.05$).

	CONT	GRP I	GRP II
Body weight gain (g)	4.5; (3-6)	6.0;((-8),(14))	-22.0*;((-34),(-16))
Liver weight (LW, g)	7.7 ± 0.57	7.8 ± 0.29	7.8 ± 0.24
Kidney weight (KW, g)	1.9 ± 0.70	1.8 ± 0.54	1.9 ± 0.56
LW/Body weight	30.3 ± 1.11	30.6 ± 1.96	31.4 ± 2.38
KW/Body weight	7.3 ± 0.48	7.1 ± 0.19	8.2 ± 0.29*

Table 1: Effect of acrylamide treatment on body weight gain, tissue wet weight and the ratio of tissue to body weight. Intraperitoneally five doses of saline (CONT), 5 mg/kg acrylamide (GRP I) and 50 mg/kg acrylamide (GRP II) injected groups. Treatment details are as under Materials and methods section. The sample size of each group is 6. Values represent median; (min-max) or mean ± SEM.

**p< 0.05, GRP II vs GRP I and CONT*

Serum biochemical parameters

The effects of acrylamide treatment on serum biochemical parameters were given in Table 2. Intraperitoneal administrations of acrylamide did not significantly alter the serum AST, ALT activities and BUN and creatinine concentrations.

	CONT	GRP I	GRP II
AST(U/L)	71.8 ± 6.06	77.0 ±7.43	105.0 ±13.47
ALT (U/L)	44.7 ± 4.39	40.3 ± 3.37	42.8 ± 4.22
BUN (mg/dL)	21.0 ± 0.71	17.2 ± 1.22	17.8 ± 1.07
Creatinine (mg/dL)	0.3 ± 0.02	0.2 ± 0.02	0.2 ± 0.03

Table 2: Effect of acrylamide treatment on serum biochemical parameters. Treatments are described in Table 1. Values are mean± SEM. The sample size of each group is 6.

Tissue malondialdehyde and reduced glutathione levels

Tissue MDA concentrations were determined in all groups as a lipid peroxidation index. Acrylamide administration did not significantly alter liver MDA concentrations whereas, kidney MDA concentrations increased in a dose-related manner. As shown in Figure 1, kidney MDA concentration significantly increased when compared to both control and low dose treated groups, GRP II, ($p<0.05$).

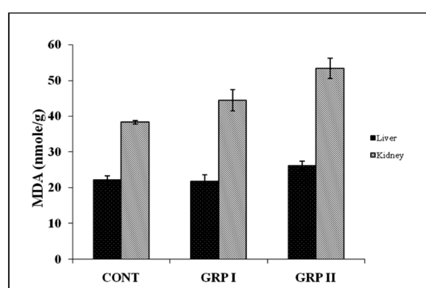

Fig. 1: Tissue MDA concentrations. Treatments are described in Table 1. Values are mean ± SEM. The sample size of each group is 6.
**$p<0.05$; GRP II vs GRP I and CONT*

Tissue GSH concentrations were investigated as one of the parameter of redox status. Administration of applied doses of acrylamide did not alter hepatic GSH concentrations whereas; renal GSH concentrations were increased in a dose-related manner. Comparing to control and 5mg/kg acrylamide treated groups, high dose of acrylamide treated ones showed a significant increase in renal GSH level ($p<0.0001$, Figure 2).

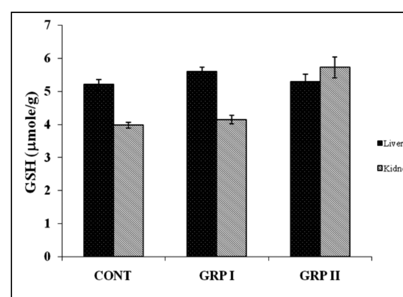

Fig. 2: Tissue GSH concentrations. Treatments are described in Table 1. Values are mean ± SEM. The sample size of each group is 6.
****$p<0.0001$; GRP II vs GRP I and CONT*

Tissue arginase activity and NO concentration

As shown in Table 3, acrylamide treatment caused a decrease in liver arginase activity in both applied doses comparing to control ($p<0.0001$). However, no significant alterations were detected between all groups in term of the specific activity.

In kidney tissues, the effect of acrylamide on arginase activities was dose-related. Arginase activity and specific activity elevated significantly in high dose acrylamide treated group comparing to control and GRP I ($p<0.05$, Table 3).

	CONT	GRP I	GRP II
LIVER			
Arginase			
Activity (U/g)	830.8 ± 10.01	720.5*** ± 19.10	693.4*** ± 12.47
Specific activity (U/mg)	3.6 ± 0.21	3.7 ± 0.16	4.1 ± 0.17
Total nitrite (nmole/g)	3.81 ± 0.219	3.68 ± 0.368	5.35* ± 0.586
KIDNEY			
Arginase			
Activity (U/g)	24.5 ± 1.61	26.9 ± 1.31	35.1* ± 2.47
Specific activity (U/mg)	0.14 ± 0.01	0.17 ± 0.02	0.26* ± 0.02
Total nitrite (nmole/g)	8.56 ± 0.503	10.25 ± 0.397	9.18 ± 0.466

Table 3: Tissue arginase activities and total nitrite concentrations. Treatments are described in Table 1. Values are mean ± SEM. The sample size of each group is 6.
**$p<0.05$, GRP II vs GRP I and CONT; ***$p<0.0001$, GRP II and GRP I vs CONT*

The effects of acrylamide on tissue NO levels (in terms of total nitrite concentrations) were given in Table 3. Treatment caused an increase in hepatic total nitrite concentration in a dose-related manner. In high dose treated group, hepatic total nitrite level was elevated significantly ($p<0.05$). Whereas, no significant alteration was observed in kidney concentrations.

Histopathological observations

Comparative histopathological analysis of both tissue samples from each group was performed, by EM studies, to evaluate the effect of acrylamide treatment. Data indicated that the ultrastructural alterations were predominantly generated in GRP II, high dose of acrylamide treated group. No ultrastructural changes were detected in samples of the 5 mg/kg treated group.

In liver sections, no significant changes were detected in both acrylamide treated groups comparing to control. On the other hand, ultrastructural alterations were detected in epithelial cells of proximal tubules in kidney sections of the rats treated with 50 mg/kg acrylamide. Enhanced vacuolization and widely distributed peroxisomes were detected in the cytoplasm of epithelial cells (Figure 3A). Degenerating cells with electron dense cytoplasm and swollen mitochondria were also present (Figure 3B).

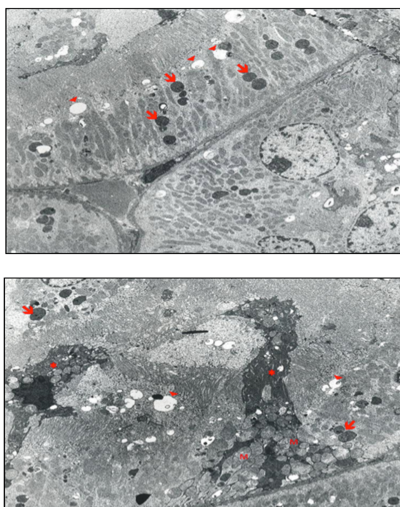

Fig. 3: Transmission electron microscopic examination of kidney tissue of rats treated with sublethal high dose of acrylamide. (A) Section of kidney tissue (x2156). In epithelial cells of proximal tubules, vacuolization (arrow heads) and widely distributed peroxisomes (arrows) were detected. (B) Degenerated cells with electron dense cytoplasm (*) and swollen mitochondria (M) were also observed in these tubule epithelial cells (x2156). Treatment and details of microscopic analysis are described in Materials and methods section.

Discussion

In our experimental model, acrylamide treatment caused an oxidative stress in a tissue- and dose-related manner. Serum hepatic and renal markers were not significantly modulated by applied doses of acrylamide. On the other hand, some ultrastructural changes developed in a tissue and dose specific manner. Tissue related alterations were predominantly detected in rats treated with high dose of acrylamide, i.e. 50 mg/kg.

In present study, body weight gains were decreased in a dose-related manner. Body weight gain of rats treated with high dose of acrylamide, i.e.50mg/kg, was significantly reduced comparing to control and low dose, i.e.5mg/kg, acrylamide treated groups. Experiments have revealed that adverse effect of acrylamide treatment on body weight gain may raise due to the pathological changes of central and peripheral nerves including neurotransmitter metabolism and NO signaling pathway[2,5]. Our data revealed the acceptance of the validity of acrylamide toxicity in our experimental model.

In our model, acrylamide treatment did not modulate serum hepatic markers and liver MDA levels. Molecular mechanism of liver regeneration has been investigated by several laboratories. Experiments have indicated that liver regeneration can be induced by any acute treatment, either surgical or chemical and the regeneration time of hepatocytes is reported as 24-48 h[25-27]. In our experimental model, sacrification was carried on 48 hours after the last acrylamide treatment and data indicated that this period was enough for liver to regenerate itself. Also in the liver, GSH concentrations were not modulated by the treatment indicating the efficiency of hepatic defense system against oxidative stress. Also, no ultrastructural alterations were present in the liver.

Contrarily, our data revealed that kidney was more sensitive to acrylamide induced possible toxicity. MDA levels and GSH concentrations were increased in high dose (50mg/kg) treated group presenting the existence of oxidative stress. Although increase in GSH level may be accepted as a cellular response against oxidative stress, significant elevation of kidney GSH level did not overcome the lipid peroxidation in this group. Therefore, it seems that GSH is not directly capable of protecting tissue against acrylamide-induced oxidative stress in our model. Also, high dose of acrylamide-induced ultrastructural modulations were especially detected in epithelial cells of proximal tubules in kidney. Widely distributed vacuolization and peroxisomes in the cytoplasm of epithelial cells of tubules were detected as a response of cells to the treatment. Degenerating cells with electron dense cytoplasm and swollen mitochondria were also present. In sight of these findings, we may propose that proximal tubules are

on the way of development of an early tissue damage without affecting serum renal markers in the means of organ functions.

The importance of NO levels and arginase activities in hepatic and renal failure/damage has been investigated by several laboratories in different pathological conditions. Several reports have suggested that contribution of NO to hepatic injury is very critical at the initiation step and also it can be contributed to the progression and determination of severity of hepatic damage(9,28, 29). However, NO seems to play a protective role in the pathogenesis of renal failure(30,31). On the other hand, in the case of presence of oxidative stress, overproduction of NO, by iNOS and maybe eNOS, may contribute to the pathogenesis of renal damage(32). This controversy results may arise due to different stages of the pathological conditions and various factors affecting cellular activity of NO(9,28,33,34).

In present study, we have found that the NO levels, in terms of total NO_2 - concentration, altered in a dose-related manner in the liver tissue. High dose of acrylamide treatment enhanced NO generation, in terms of total NO_2- , significantly comparing to low dose treated and control groups. The significant elevation of hepatic total NO_2 - concentration may be accepted as a response against treatment in the liver. While in kidney tissue, our results have revealed that NO levels, in terms of total NO_2 - concentration, did not alter in both applied doses of acrylamide. Hence, NO may show as either cytoprotective or cytotoxic behaviors, acrylamide-induced NO production in the liver should further be evaluated to clarify its exact role. As reported previously, elevated NO level has the ability to induce ɣ-glutamyl cycle(35), apparent GSH concentrations in liver may include the product of this cycle. In our experimental model, - the levels of total NO_2 - did not reduce in both tissues, this situation may be considered as a clue for the availability of arginine.

Experiments have revealed that under pathological conditions the isozyme of arginase other than the predominant one is induced(36,37). Induction of arginase, either I or II plays a crucial role in possible downregulation of NO production by consuming arginine besides providing ornithine for the biosynthesis of polyamines, glutamate and proline.

In the present study, acrylamide treatment altered arginase activity and specific activity in a tissue-dependent manner. Our data have revealed that liver arginase activity decreased in both applied doses of acrylamide.

The reduction of liver arginase activity in gram tissue may indicate decreased concentration of enzyme and/or possible acrylamide-induced reversible/irreversible inhibition. No alterations in specific activity may reveal either acrylamide-induced modulation of turnover rate of the enzyme or the possible induction of expression of arginase II. On the other hand, acrylamide treatment altered renal arginase activity and specific activity in a dose-related manner. In rats treated with high dose of acrylamide, elevations of kidney arginase activity and specific activity may reflect the induction of Type II arginase activity/expression and/or expression of Type I arginase.

Similar situations were reported in several liver and kidney pathologies in which modulation of arginase activity contributed to tissue damage(36-38).

Arginase and NOS may behave in reciprocal manner in terms of induction of synthesis and the regulation of activities. In our experimental model, there was no significant difference between two applied doses of acrylamide on arginase activities in gram hepatic tissue. However, total nitrite level increased only in the high dose of acrylamide treated group comparing to other groups. Therefore, we may conclude about the fact that there is no clear evidence regarding the reciprocal regulation of arginase and NOS activities in the liver. In kidney, renal arginase activity and specific activity increased significantly in high dose acrylamide applied group whereas, in the same group NOS activity, in terms of total NO^2 -, did not change. So, there was again no apparent reciprocal regulation between these two renal enzymes in our experimental model. Since our data indicate that there is no substrate restriction in liver and kidney tissues, acrylamide-induced elevation of arginase activity in kidney tissue may supply ornithine for polyamine biosynthesis and also for the synthesis of glutamate and proline.

Conclusion

Our data indicated that high dose of acrylamide treatment caused an increase in total nitrite concentrations in the liver. Non-altered specific activity of arginase did not prevent the elevation of NO production in the livers of rats treated with high dose of acrylamide. Dose-independent modulation of hepatic arginase activity revealed that arginase was the primarily target enzyme in acrylamide-induced toxicity which was not directly related with NO levels. On the other hand, oxidative stress, in terms of lipid per-

oxidation, occurred in kidney tissues of rats treated with high dose of acrylamide. Acrylamide-induced oxidative stress resulted elevation of arginase activity and specific activity accompanied with non-altered NO production.

Acrylamide-induced toxicity may reveal cumulative or subcumulative effects in target tissues[4,39]. This effect will depend on the dose of acrylamide exposure and the response of tissue against acrylamide-induced toxicity which includes tissue compensation and/or repairing capacity and also existence of earlier damage of target tissue. Since, the modulation of interested enzyme activities either directly or indirectly affects the NO and polyamine metabolisms, also glutamate and proline availability, prolonged exposure by diet may create a potential risk for a series of crucial damage especially in kidneys whereas liver may overcome the possible acrylamide-induced damage. Besides, further evaluation including time course exposure studies should be performed to clarify this organ specific situation.

References

1) Mottram DS, Wedzicha BL, Dodson AT. *Acrylamide is formed in the Maillard reaction*. Nature 2002; 419: *448-449*.

2) Kim K. *Effect of subchronic acrylamide exposure on the expression of neuronal and inducible nitric oxide synthase in rat brain*. J Biochem Mol Toxicol 2002; 19: *162-168*.

3) Takahashi M, Inoue K, Koyama N, et al. *Life-stage differences in susceptibility to acrylamide-induced neural and testicular toxicity*. Arch Toxicol 2011; 85: *1109-1120*.

4) Tareke E, Lyn-Cook B, Duhart H, Newport G, Ali S. *Acrylamide decreased dopamine levels and increased 3-nitrotyrosine (3-NT) levels in PC 12 cells*. Neuroscience Letters 2009; 458: *89-92*.

5) LoPachin RM, Barber DS, Gavin T. *Molecular mechanisms of the conjugated α,β-unsaturated carbonyl derivatives: Relevance to neurotoxicity and neurodegenerative diseases*. Toxicol Sci 2008; 104: *235-249*.

6) Ghanayem BI, Witt KL, Kissling GE, Tice RR, Recio L. Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: Evidence consistent with a glycidamide-mediated effect. Mut Res 2005; 578: 284-297.

7) IARC,. M*onographs on the evaluation of carcinogenesis*. Some industrial chemicals, vol. 60. International Agency for Research on Cancer, Lyon, France, 1994; *389-433*.

8) *EFSA/WHO*. *EFSA/WHO international conference with support of ILSI Europe on risk assessment of compounds that are both genotoxic and carcinogenic*, 2005; *16-8* November 2005. Brussels, Belgium.

9) Diesen DL, Kuo PC. *Nitric oxide and redox regulation in the liver: Part I. General considerations and redox biology in hepatitis*. J Surg Res 2010; 162: *95-109*.

10) Wink DA, Mitchell JB. T*he chemical biology of nitric oxide: insight into regulatory, cytotoxic and cytoprotective mechanisms of nitric oxide*. Free Rad Biol Med 1998; 25: *434-456*.

11) Lundberg JO, Weitzberg E and Gladwin MT. *The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics*. Nature 2002; 7: *156-167*.

12) Mori M, Gotoh T. *Regulation of nitric oxide production by arginine metabolic enzymes*. Biochem Biophys Res Comm 2000; 275: *715-719*.

13) Morris SM. *Recent advances in arginine metabolism: roles and regulation of the arginases*. Brit J Pharmacol 2009; 157: *922-930*.

14) Ikemato M, Tsunekawa S, Toda Y and Totani M. *Liver type arginase is a highly sensitive marker for hepatocellular damage in rats*. Clin Chem 2001; 47(5): *946-948*.

15) Gökmen SS, Aygıt AC, Ayhan MC, Yorulmaz F and Gülen Ş. *Significance of arginase and ornithine in malignant tumors of the human skin*. J Lab Clin Med 2001; 137(5): *340-344*.

16) Porembska Z, Luboinski G, Chrzanowska A, Mielczarek M, Magnuska J and Baranczyk-Kuzma A. *Arginase in patients with breast cancer*. Clin Chim Acta 2003; 328: *105-111*.

17) Ckless K, Lampert A, Reiss J, Kasahara D, Poynter ME, Irvin CG, Lundblad LKA, Norton R, van der Vliet A and M.W. Janssen-Heininger Y. *Inhibition of Arginase Activity Enhances Inflammation in Mice with Allergic Airway Disease, in Association with Increases in Protein S-Nitrosylation and Tyrosine Nitration*. J Immun 2008; 181: *4255-4264*.

18) Mumenthaler SM, Yu H, Tze S, Cederbaum SD, Pegg AE, Seligson DB and Grody WW. *Expression of arginase II in prostate cancer*. Int J Oncol 2008; 32: *357-365*.

19) Rangel MA, Gonzalez-Polo RA, Caro A, del Amo E, Polomo L, Hernandez E, Soler G, Fuentes JM. *Diagnostic performance of arginase activity in colorectal cancer* Clin Exp Med 2002; 2: *53-57*.

20) *Overwiew of Acrylamide toxicity and metabolism*. *JIFSAN /NCFST Workshop on Acrylamide in Food and Toxicology and Metabolic Consequences Working Group*. 2002.

21) Buege JA, Aust AD. *Microsomal lipid peroxidation*. Meth Enzymol 1978; *302-310*.

22) Ellmann, GL. *Tissue sulphydryl groups*. Arch Biochem Biophys 1959; 82: *70-77*.

23) Geyer JW, Dabich D. *Rapid method for determination of arginase activity in tissue homogenates*. Anal Biochem 1971; 39: *412-417*.

24) Bradford MM. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*. Anal Biochem 1976; 72: *248-254*.

25) Mead JE, Fausto N. *Transforming growth factor β may be a physiological- regulator of liver regeneration by means of an autocrine mechanism*. Proc Nat Acad Sci 1988; 86: *1558-1562*.

26) Michalopoulos GK. *Liver generation: Molecular mechanisms of growth control*. Faseb Journal 1990; 4: *176-187*.

27) Furchtgott LA, Chow CC, Periwal V. *A model of Liver Regeneration*. Biophys J 2009; 96: *3926-3935*.

28) Diesen DL, Kuo PC. *Nitric oxide and redox regulation in the liver: Part II. Redox biology in pathologic hepatocytes and implications for intervention*. J Surg Res 2011; 167: *96-112*.
29) Lee WY, Koh EJ, Lee SM. *A combination of ischemic preconditioning and allopurinol protects against ischemic injury through a nitric oxide-dependent mechanism*. Nitric Oxide 2012; 26: *1-8*.
30) Goligorsky MS, Brodsky SV, Noiri E. *Nitric oxide in acute renal failure*. Kid Int 2002; 61: *855-861*.
31) Kim PKM, Zamora R, Petrosko P, Billiar TR. *The regulatory role of nitric oxide in apoptosis*. Int Immunopharmacol2001; 1: *1421-1441*.
32) Sarkar A, Das J, Manna P, Sil PC. *Nano-copper induces oxidative stress and apoptosis in kidney via both extrinsic and intrinsic pathways*. Toxicol 2011; 290: *208-217*.
33) Chirino YI, Pedraza-Chaverri J. *Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity*. Exp Toxicol 2011; 61: *223-42*.
34) Sharma SP. *Nitric oxide and the kidney*. Ind J Nephrol 2004; 14: *77-84*.
35) Kuo PC, Abe KY, Schroeder RA. *Interleukin-1-induced nitric oxide production modulates glutathione synthesis in cultured rat hepatocytes*. Am J Physiol 1996; 271: *851-862*.
36) Moradi H, Kwok V, Vaziri ND. *Effect of chronic renal failure on arginase and argininosuccinate synthetase expression*. Am J Nephrol 2006; 26: *310-318*.
37) Wei CL, Hon WM, Lee KH, Mori M, Gotoh T, Khoo HE. Induction of arginase II in livers of bile duct-ligated rats. Biochem Pharmacol 2002; 63:1043–1050.
38) Sabbatini M, Pisani A, Uccello F, Fuiano G, Alfierri R, Cesaro A, Cianciaruso B, Andreucci VE. *Arginase inhibition slows the progression of renal failure in rats and renal ablation*. Am J Physiol-Ren Physiol. 2003; 284: *F680-687*.
39) Crafton KM, Padilla S, Tilson HA, Raymer JH, MacPhail RC. *The impact of dose rate on the neurotoxicity of acrylamide: The interaction of administered dose, target tissue concentrations, tissue damage, and functional effects*. Toxicol App Pharmacol 1996; 139: *163-176*.

.

Acknowledgements

This study was funded by Başkent University after the approval of Başkent University Institutional Review Board and Ethics Committee (Project no: DA06/01 and DA07/30).

Request reprints from:

EDA ÖZTURAN-ÖZER
Başkent University, Faculty of Medicine
Department of Biochemistry
06810 Ankara
(Turkey)