

ORIGINAL ARTICLE

Medicine Science 2022;11(4):1478-81

Does dietary intake of acrylamide affect hydroxyproline levels? an animal study

¹Tugba Agbektas¹, ²Ozhan Pazarcı², ³Ayca Tas³, ⁴Nese Keklikcioglu Cakmak⁴,
⁵Seyran Kilinc⁵, ⁶Yavuz Silig¹

¹Sivas Cumhuriyet University Faculty of Medicine, Department of Biochemistry, Sivas, Türkiye

²Adana City Training and Research Hospital, Department of Orthopedics and Traumatology, Adana, Türkiye

³Sivas Cumhuriyet University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Sivas, Türkiye

⁴Sivas Cumhuriyet University, Faculty of Engineering, Department of Chemical Engineering, Sivas, Türkiye

⁵Sivas Cumhuriyet University Faculty of Medicine, Department of Orthopedics and Traumatology, Sivas, Türkiye

Received 05 August 2022; Accepted 23 August 2022

Available online 03.10.2022 with doi: 10.5455/medscience.2022.04.082

Copyright@Author(s) - Available online at www.medicinescience.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International.



Abstract

Acrylamide is a chemical that occurs due to high temperatures during cooking. It consists of an amino acid found in foods and sugars. Studies have shown that cancer formation occurs within the scope of oxidant reagents and DNA damage due to exposure to acrylamide. Our study aims to examine the effects of dietary acrylamide intake on plasma hydroxyproline levels in rats. In this study, 4 groups were formed with 8 rats in each group (total number=32). Blood samples were collected on days 14 and 28. Acrylamide solution was applied to each rat in the treatment group by gastric gavage process at 5 mg/kg three times a week. Hydroxyproline levels in rats' plasma samples were measured. The median (IQR) hydroxyproline levels were 7.40(2.45) µg/L in group 1 (14. days control group) and 7.98(3.34) µg/L in group 2 (14. days acrylamide applied) who received acrylamide. The mean hydroxyproline levels were 7.25(1.96) µg/L in group 3 (28. days control group) and 9.76(2.64) µg/L in group 4 (28. days acrylamide applied) who received acrylamide. No difference was observed between the groups. Dietary acrylamide intake did not have a significant effect on hydroxyproline levels at the application dose and duration in our study.

Keywords: Hydroxyproline, acrylamide, rat, serum level

Introduction

Hydroxyproline is found in the body's collagen. Which is one of the basic structural proteins of the body, such as bone cartilage. Collagen production and destruction in the body can change with food intake. Nutrition with collagen-boosting foods is especially important for reducing the effects of aging and bone metabolism. Collagen has a great place in the healing of post-traumatic bone and soft tissue injuries [1]. In recent years, studies on hydroxyproline metabolism and nutrition have increased [2,3]. Hydroxyproline is one of the biomarkers of bone resorption. Hydroxyproline is an amino acid derived from the post-translational hydroxylation of proline. Hydroxyproline provides approximately 12-14% of the total amino acid content of mature collagen. During the

breakdown of bone collagen, about 90% of hydroxyproline is released and subsequently metabolized in the liver [4]. In a study, it was shown that the increase in urinary hydroxyproline increased the degradation of type I collagen from the bone matrix in osteoporotic women [5]. Although hydroxyproline is primarily used as a resorption biomarker, about 10% of hydroxyproline is derived from newly synthesized procollagen peptides during bone formation. In addition, hydroxyproline can be found in other tissues such as skin and cartilage [6]. As a result, hydroxyproline has been accepted as a non-specific bone resorption biomarker of the collagen cycle [7].

Acrylamide; is a chemical formed during cooking above 120°C. It is formed after exposure to high heat from asparagine. This substance, first discovered in 2002, has been shown to have a carcinogenic effect in rat models. Large amounts of acrylamide are taken daily through diet. Acrylamide is present in many everyday foods such as fries, coffee, and chips. In addition, it has neurotoxic and apoptosis-increasing effects on the body. What are the interactions of these two substances, whose metabolism in the body is directly affected by the foods we take? The effect of dietary

*Corresponding Author: Ozhan Pazarcı, Adana City Training and Research Hospital, Department of Orthopedics and Traumatology, Adana, Türkiye
E-mail: dr.pazarci@gmail.com

acrylamide intake on hydroxyproline has not been investigated in previous studies [7,8]. Our study aimed to examine the effects on plasma hydroxyproline levels in rats that received and did not receive acrylamide.

Material and Methods

Animal grouping

Local animal ethics committee approval was obtained for 32 adult Wistar albino male rats weighing 215-260g included in this study (date:24.02.2020, no:65202830-050.04.04-365).

The application of acrylamide and taking the samples from the experimental animal model was carried out at the Sivas Cumhuriyet University Experimental Animal Unit. The rats were cared for and fed by providing water and feed ad libitum in metal cages with a base, at room temperature of 22–24°C and humidity of 55%, in a 12-hour light/12-hour dark environment.

In the study, 8 rats were formed from 4 groups, each treatment, and a control group on the 14th and 28th days. (a total of 32 rats) (group 1:14. days control group, group 2:14. days acrylamide treatment group, group 3:28 days control group, group 4:28 days acrylamide treatment group). Acrylamide (Sigma-Aldrich Chemical Co. St. Louis, USA) solution was applied to each rat in the treatment group by gastric gavage process at 5mg/kg three times a week. Acrylamide was given three times a week to prevent esophageal irritation. Then, without animal sacrifice, blood samples were taken from groups 1 and 2 on the 14th day, and from groups 3 and 4 on the 28th day.

Collection of Blood Samples

For the measurement of hydroxyproline in treatment and control groups of rats, 2mL blood samples were collected into sterile citrate tubes. Blood sample collection through a cardiac puncture in the rat. Plasma was obtained by centrifuging the collected blood samples at 1000xg for 15 minutes. The obtained samples were placed in Eppendorf tubes and stored at -80°C until analysis.

Hydroxyproline Levels Assay

Hydroxyproline levels in rats’ plasma samples were measured using the ELISA kit (Catalog no: SG-20734 Sinogeneclon Co., Ltd., CHINA) according to the manufacturer's protocol instructions. The range of maximum and minimum detectable values of the hydroxyproline ELISA kit was 1–20µg/L and the sensitivity was 0.5µg/L. The plate was washed with the Bio-Tek ELX50 automatic washer (BioTek Instruments, USA), and absorbance readings were measured with the Microplate Reader device (BioTek, Epoch, USA). Test results of hydroxyproline were expressed in µg/L.

Statistical analysis

The conformity of the data in the study to the normal distribution was evaluated with the Shapiro-Wilk test. As a result of the evaluation, the Mann-Whitney U test was used to compare the distributions of two independent groups. Descriptive statistics were expressed as the Median (IQR). Statistical significance was accepted as p<0.05. To evaluate the data, the IBM SPSS Statistics version 22 (IBM SPSS for Windows version 22, IBM Corporation,

Armonk, New York, United States) package program was used.

Results

Figure 1 shows the flowchart of the study. None of the rats in all groups died during the study period. All rats well tolerated the administration and no significant weight loss was observed until the end of the experiment. Table 1 shows the hydroxyproline plasma levels. When the distribution of group 1 and group 2 was examined, no statistically significant difference was found, although the concentration increased in group 2 (p>0.05). Similarly, when the distributions of Group 3 and Group 4 were compared, no statistically significant difference was found, although the concentration of group 4 increased compared to group 3 (p>0.05). When the distributions of group 2 (control on the 14th day) and group 4 (acrylamide on the 28th day) were examined, the prolongation of the time increased the amount of concentration, but no statistically significant difference was found in this increase (p>0.05) (Table 1).

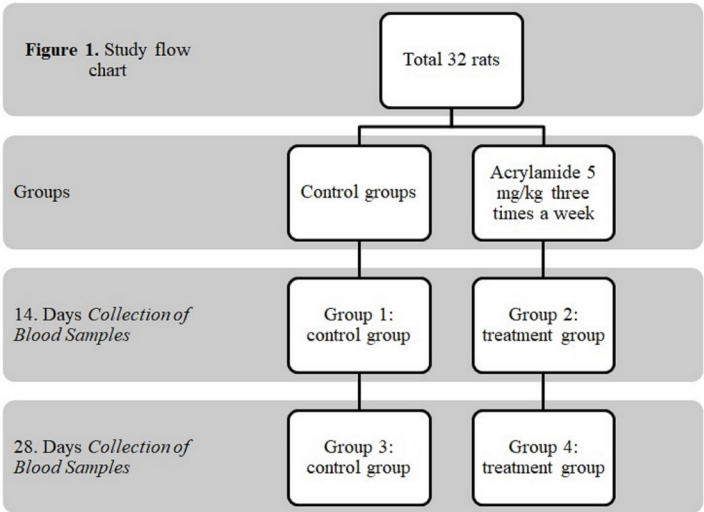


Table 1. All groups hydroxyproline plasma levels (µg/L)

Hydroxyproline levels	n	Median a<	p-Value
Grup 1 Control (14. day)	8	7.40(2.45)	0.270
Grup 2 Acrylamide (14. day)	8	7.98(3.34)	
Grup 3 Control (28. day)	8	7.25(1.96)	0.066
Grup 4 Acrylamide (28. day)	8	9.76(2.64)	
Grup 2 Acrylamide (14. day)	8	7.98(3.34)	0.248
Grup 4 Acrylamide (28. day)	8	9.76(2.64)	
Mann whitney u testi, p<0.05*			

Discussion

This study was designed to investigate the effect of 5 mg/kg three times a week dose administration of acrylamide on rat hydroxyproline plasma levels. The present study showed that the controls and the rats administered with acrylamide did not show any significant difference in terms of hydroxyproline plasma levels. Several studies examine the effects of acrylamide on body health [9]. This is the first study examining the effect of acrylamide administration on plasma hydroxyproline levels.

Our study stands out in terms of showing the interaction of

acrylamide, which is often taken with diet, and hydroxyproline, an important structural protein of the body. The daily dietary intake of acrylamide is gradually increasing. There is acrylamide contamination between 25-38% of the foods in the daily diet in the USA [10]. Therefore, this dose range suitable for daily dietary exposure was chosen in our study. Some studies; argues that the amounts of acrylamide consumed in the normal diet are insufficient to show that it is likely to cause adverse effects on human health, especially cancer [10,11]. On the other hand, acrylamide cytotoxicity was seriously emphasized in some rat studies [12,13]. According to the studies, it has been reported that oxidative DNA damage occurs in the liver tissues of rats due to the administration of acrylamide [14,15]. In another study, it was observed that acrylamide increased oxidative DNA damage and reactive oxygen species (ROS) levels in the BRL-3A rat liver cell line [16]. Acrylamide causes oxidative DNA damage; It has been determined that ROS is produced by apoptosis induction and disruption of mitochondrial membrane potential [17].

Hydroxyproline has an important role in the body. Therefore, their levels may be affected by diet. It is also important to know the factors that will affect it [18]. An appropriate nutritional regimen is very important for wound healing, especially after surgical treatment. Hydroxyproline is a good marker for determining the level of wound healing [19]. Hydroxyproline is required for glycine synthesis and glutathione (GSH) is synthesized from glycine [1,20]. GSH provides redox balance by playing a role in the scavenging of radical oxygen species [21]. Hydroxyproline increases upon degradation of collagen in cells due to oxidative stress response and hypoxia. Accordingly, hydroxyproline plays a role as an antioxidant [22,23]. It has been proven that hydroxyproline scavenges reactive oxidants [2,24] and affects the expression of antioxidant enzymes to maintain intracellular balance [1].

Our study did not show a significant difference in hydroxyproline blood levels in 28 days of dietary intake. Long-term and human studies are needed on this subject. Similar to our study, the difference in the long- and short-term effects of acrylamide was emphasized in the literature [25]. Our study was important in terms of the effects of acrylamide in the acute phase. In addition, the effects of acrylamide in the chronic period should be examined in future studies.

How does acrylamide affect bone-collagen metabolism as well as hydroxyproline metabolism? This is not clear in the literature. But we know that acrylamide inhibits autophagy via blocking autophagic flux and induces cell apoptosis [26]. Besides exposure to acrylamide dysregulated the expression of skeletal development-related genes. Exposure to acrylamide suppressed the maturation of osteoblasts and cartilage matrix and promoted the formation of osteoclasts. The toxicity of acrylamide might translate to the next generation [27]. Our study emphasized the effect of short-term acrylamide when exposed to the diet. Long-term dietary acrylamide exposure also should be investigated in future research.

The authors are aware that the study has some limitations. The first of these; is the fact that the study was conducted in a single dose and was a short-term study. Second, the results of experimental animal studies are difficult to directly apply to daily clinical practice.

Conclusion

The present pilot study aimed to investigate the effects of acrylamide on hydroxyproline in an experimental model in rats. According to our results, no significant effect of dietary acrylamide intake on short-term hydroxyproline levels was demonstrated at the application dose. Long-term experimental and clinical trials are needed to confirm our findings.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

All authors declare no financial support.

Ethical approval

This study was approved by the Ethical Committee of Sivas Cumhuriyet University, Faculty of Medicine (Date: 24.02.2020, Decision no: 65202830-050.04.04-365).

References

1. Wu G, Bazer FW, Burghardt RC, et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids*. 2011;40:1053-63.
2. Phang JM, Donald SP, Pandhare J, Liu Y. The metabolism of proline, a stress substrate, modulates carcinogenic pathways. *Amino Acids*. 2008;35:681-90.
3. Srivastava D, Zhu W, Johnson WH, et al. The structure of the proline utilization a proline dehydrogenase domain inactivated by N-propargylglycine provides insight into conformational changes induced by substrate binding and flavin reduction. *Biochemistry*. 2010;49:560-9.
4. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomarker research*. 2017;5:1-9.
5. Lowry M, Hall DE, Brosnan JT. Hydroxyproline metabolism by the rat kidney: distribution of renal enzymes of hydroxyproline catabolism and renal conversion of hydroxyproline to glycine and serine. *Metabolism*. 1985;34: 955-61.
6. Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. The biosynthesis of collagen and its disorders. *New England J Med*. 1979;301:77-85.
7. Virk-Baker MK, Nagy TR, Barnes S, Groopman J. Dietary acrylamide and human cancer: a systematic review of literature. *Nut Cancer*. 2014;66:774-90.
8. Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *Eur J Cancer Prev*. 2012;21:375-86.
9. Pedreschi F, Mariotti MS, Granby K. Current issues in dietary acrylamide: formation, mitigation and risk assessment. *J Sci Food Agriculture*. 2014;94:9.
10. Lineback DR, Coughlin JR, Stadler RH. Acrylamide in foods: a review of the science and future considerations. *Annu Rev Food Sci Technol*. 2012;3:15-35.
11. Riboldi BP, Vinhas ÁM, Moreira JD. Risks of dietary acrylamide exposure: A systematic review. *Food Chemistry*. 2014;157:310-22.
12. Raju J, Roberts J, Taylor M, et al. Toxicological effects of short-term dietary acrylamide exposure in male F344 rats. *Environ Toxicol Pharmacol*. 2014;39:85-92.
13. Yener Y. Effects of long term low dose acrylamide exposure on rat bone marrow polychromatic erythrocytes. *Biotech Histochem*. 2013;88:356-60.
14. Ansar S, Siddiqi NJ, Zargar S, et al. Hepatoprotective effect of Quercetin supplementation against Acrylamide-induced DNA damage in Wistar rats. *BMC Complement Alter Med*. 2016;16:1-5.

15. Alturfan AA, Tozan-Beceren A, Şehirli AÖ, et al. Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative stress in rats. *Mol Biol Rep.* 2012;39:4589-96.
16. .Hong Y, Nan B, Wu X, et al. Allicin alleviates acrylamide-induced oxidative stress in BRL-3A cells. *Life Sci.* 2019;231:116550.
17. Nowak A, Zakłos-Szyda M, Żyżelewicz D, et al. Acrylamide decreases cell viability, and provides oxidative stress, DNA damage, and apoptosis in human colon adenocarcinoma cell line Caco-2. *Molecules.* 2020;25:368.
18. Yerlikaya FH, Toker A, Yener Y. Effects of acrylamide treatment on oxidant and antioxidant levels in rats. *Kafkas Universitesi Veteriner Fakultesi Dergisi.* 2013;19:607–12.
19. Hemmati AA, Larki-Harchegani A, Habib S, et al. Wound healing property of milk in full thickness wound model of rabbit. *Int J Sur.* 2018;54:133-40.
20. Wu Z, Hou Y, Dai Z, et al. Metabolism, nutrition, and redox signaling of hydroxyproline. *Antioxid Redox Signal.* 2019;30:674-82.
21. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med.* 1996;20:933-56.
22. Chandel NS. Mitochondrial regulation of oxygen sensing. *Membrane Receptors, Channels and Transporters in Pulmonary Circulation.* 2010:339-54.
23. Wenger RH, Hoogewijs D. Regulated oxygen sensing by protein hydroxylation in renal erythropoietin-producing cells. *Am J Physiol-Renal Physiol.* 2010;298:F1287-F96.
24. Phang JM, Liu W, Zabinryk O. Proline metabolism and microenvironmental stress. *Annual Rev Nutrition.* 2010;30:441.
25. Yang G, Luo C, Yan X, et al. Extracorporeal Shock Wave Treatment Improves Incisional Wound Healing in Diabetic Rats. *Tohoku J Experimental Med.* 2011;225:285–92.
26. Song D, Xu C, Holck AL, et al. Acrylamide inhibits autophagy, induces apoptosis and alters cellular metabolic profiles. *Ecotoxicol Environ Saf.* 2021;208:111543.
27. Zhu F, Wang J, Jiao J, Zhang Y. Exposure to acrylamide induces skeletal developmental toxicity in zebrafish and rat embryos. *Environ Pollut.* 2021;271:116395.