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Effect of Acute Acrylamide Administration in PTZ Induced Convulsions in Mice

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ABSTRACT Epilepsy is a neurological disorder which causes seizures. Epilepsy treatment is conducted with administration of antiepileptic drugs. Acrylamide is a chemical substance which is used for manufacturing, drainage treatment, cosmetics and laboratory studies. It can also be found in potato chips or French fries, infant formulas, beer, biscuits and cookies due to heating of carbohydrates at certain degrees. This study was conducted to assess effect of acute acrylamide administration on epileptic seizures in mice. 36 female Swiss-albino mice were separated into 6 groups. Acrylamide was administered via i.p. route. Groups A5 and A10 were administered with only 5 and 10mg/kg acrylamide respectively. PA5 and PA10 groups received five days of both 5 and 10mg/kg acrylamide respectively and also administered 80mg/kg(i.p.) pentylenetetrazol(PTZ) at the end of the 5th day. Control group was administered with saline. PTZ group was administered solely with 80mg/kg(i.p.) PTZ. Results show no convulsions or any tremor due to lone acrylamide administration in myoclonic convulsions. Results reveal a significant reduction in time required for myoclonic convulsions in PA5(15.5s) and in PA10(15.7s) groups compared to PTZ(62.3s) groups. Time required for tonic-clonic convulsion period was also significantly lowered in PA5(19.3s) and PA10(21s) groups compared to PTZ(267s). Present study suggests a proconvulsive effect of acrylamide on PTZ induced convulsions. Studies concerning diets of epilepsy patients are required to evaluate its relevance in human subjects.

Keywords: *Epilepsy, Convulsion, Anticonvulsive, Acrylamide, Seizure, Pentylenetetrazol*

ÖZ

Farelerde PTZ Kaynaklı Konvülsiyonlarda Akut Akrilamit Uygulamasının Etkisi

Epilepsi nöbetlere neden olan nörolojik bir hastalıktır. Epilepsi tedavisi antiepileptik ilaçlarla yapılmaktadır. Akrilamit, üretim sanayinde, atık su sistemlerinde, kozmetikte ve laboratuvar çalışmalarında kullanılan kimyasal bir maddedir. Akrilamit aynı zamanda patates cipsi ve kızartmasında, bebek ürünlerinde, birada, bisküvilerde ve kurabiyelerde karbonhidratların belli derecelerin üzerinde ısıtılmasıyla ortaya çıkabilmektedir. Bu çalışma akrilamitin farelerde epileptik nöbetlere etkisini değerlendirmek için gerçekleştirilmiştir. 36 adet dişi Swiss-albino fare 6 gruba ayrıldı. Akrilamit i.p. yolla verildi. Grup A5'e 5 ve grup A10'a 10mg/kg akrilamit 5 gün boyunca verildi. PA5 ve PA10 gruplarına hem 5 gün boyunca 5 ve 10mg/kg akrilamit hem de 5.günün sonunda 80mg/kg i.p. pentilentetrazol(PTZ) verildi. Kontrol grubuna serum fizyolojik verildi. PTZ grubuna sadece 80mg/kg i.p. PTZ verildi. Sonuçlar tek başına uygulanan akrilamitin herhangi bir nöbet ya da titremeye neden olmadığını gösterdi. PA5(15.5s) ve PA10(15.7s) gruplarında PTZ(62.3s) gruplarına göre miyoklonik nöbete girmek için gereken sürede anlamlı azalma gözlendi. Tonik-klonik nöbete girme süresinde de PA5(19.3s) ve PA10(21s) gruplarında PTZ(267s) grubuna göre anlamlı kısalma gerçekleşti. Bu çalışma akrilamitin PTZ ile indüklenen nöbetler üzerindeki prokonvülsif etkisini göstermektedir. Bu sonuçların insanlardaki karşılığının değerlendirilebilmesi için epilepsi hastalarının diyetleri ile ilgili çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Epilepsi, Konvulsiyon, Antikonvulsif, Akrilamid, Nöbet, Pentilentetrazol*

INTRODUCTION

Epilepsy is a common neurological disease which occurs in brain with uncontrolled electrical activity. It causes seizures which is decreasing life quality of patients and even may be lethal in serious cases. It is encountered in different forms including generalized, absence, focal, myoclonic types. Medical treatment of epilepsy is via administration of antiepileptic drugs which avoids or alleviates convulsions. Epilepsy can be triggered by

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exposure to rapid flash lights, hyperventilation, hunger, fatigue or some chemicals such as organophosphates (Pearson and Patel 2016).

Some plant based compounds may also aggravate epileptic symptoms and act as a proconvulsive substance. *Salvia officinalis* L. is known to increase synaptic transmission via inhibiting acetylcholine esterase (Kennedy et al. 2006) and acting as proconvulsive in epilepsy patients. Etiology of epilepsy includes trauma, infectious diseases and genetic predisposition. Current findings reveal that oxidative stress is also a counterpart of this disease (Waldbaum and Patel 2010). Antioxidant level of epileptic patient blood is lower than healthy people suggesting the imbalance in oxidants and antioxidants in such patients (Sudhaa et al. 2001). In addition, continuation of an antiepileptic therapy decreases oxidant status (Morimoto et al. 2017). These findings are supported with animal studies. Hassanzadeha et al (Hassanzadeha et al. 2017) found that administration of Ferulic acid causes antiepileptogenic effect and avoids oxidative stress in kindled rats. Other plant originated substances also revealed similar results which reveal a decrease in oxidative stress parameters is correlated with attenuated convulsive activity (Katare and Ganachari 2001; Gupta at al. 2003).

Acrylamide is a chemical substance which is used in different applications in laboratory science and also manufacturing processes. Among many uses its polymer namely polyacrylamide has many applications such as drainage treatment, cosmetic and fabric production. Its polymer, polyacrylamide is an important component in laboratory studies (Friedman 2003). However foods can be another unintended source of acrylamide apart from industrial production. It is formed due to heating of carbohydrates at certain degrees which is known as Maillard browning (Demirok Soncu and Kolsarici 2017). Potato chips or French fries, infant formulas, beer, biscuits and cookies are rich sources of acrylamide (Stadler et al. 2002).

Acrylamide was blamed for different untoward effects in organisms. It binds to DNA but since this binding is on the sugar side it is classified as "potential" carcinogen. It binds to hemoglobin as an adduct (Sumner et al. 2003) and decreases erythrocyte deformability which is an important parameter for proper functioning of erythrocytes (Arihan et al. 2011). Its effects on neurological system are also investigated. Both acrylamide and its epoxide metabolite glycidamide are reported as neurotoxic.

Acrylamide neurotoxicity was shown in different animal models from *Drosophila melanogaster* (Muralidhara 2012) to cats (Kuperman 1958). Acrylamide affects potassium channels thereby changing channel function and cause alterations in neuronal conductivity (Bentzena et al. 2006). Acrylamide is thought to interact with neuronal proteins and affects nerve terminals mainly (LoPachin 2002; LoPachin 2004). Acrylamide causes oxidative stress in nervous system (Zhu et al. 2008) and also in liver, kidney, brain and testes by increasing lipid peroxidation and altering activities of superoxide dismutase and Glutathione-S-Transferase (Yousef and El-Demerdash 2006) and in intestinal cells by attenuating reduced gluthathione (Zödl et al. 2007).

This study aims to assess effect of acute acrylamide administration on PTZ induced epileptic convulsions in mice.

MATERIALS and METHODS

Animals

Prior to experiments an ethical permission was obtained from Van Yuzuncu Yil University Animal Experiments Ethical Committee. All administrations were conducted according to Helsinki Declaration. Swiss albino female mice weighing 25-40 grams were used. Humidity (55±15%) and temperature (24±2°C) were kept constant in a 12h dark and light cycle. All experiments were conducted between 9:00 to 12:00 a.m.

Drugs

The following drugs were used; Pentylenetetrazol (PTZ) (Sigma) 80mg/kg and acrylamide (Sigma) at 5 and 10 mg/kg. Drugs were dissolved in saline and were administered intraperitoneal (i.p.) in a volume of 10 ml/kg of body weight. Control group was administered with saline.

Acrylamide dosage

There exist different dosage and administration schemes in literature from micrograms to 40 mg/kg (Alturfan et al. 2012), oral or i.p. and from 5 days (Husain et al., 1987) to longer chronic administrations. We have administered it in 5 days and at 5 and 10 mg/kg doses.

Experimental procedure

36 female mice were separated into 6 groups. Number of animals were decided according to result of previous studies and with the permission of ethical committee. Acrylamide was administered via i.p. route.

Groups A5 and A10 were administered with only 5 and 10 mg/kg acrylamide respectively. PA5 and PA10 groups received five days of both 5 and 10 mg/kg acrylamide respectively and also administered 80mg/kg i.p. PTZ at the end of the 5th day. Control group was administered with saline. PTZ group was administered solely with 80 mg/kg i.p. PTZ. PTZ is a commonly used chemical for inducing convulsions in animal models (Loscher, 2011). In this study PTZ test is used to assess any pro/anticonvulsive effect of acrylamide.

Time required for first myoclonic and tonic-clonic convulsion and duration of the latter were recorded. Increase in latency of start of both convulsion types are accepted as anticonvulsive effect and vice versa. In addition number of ex animals due to tonic-clonic convulsions was also recorded. An attenuation of number of ex animals shows anticonvulsive activity or vice versa.

Statistical analysis

All data are expressed as the mean±SEM. Data were analyzed with Kruskal-Wallis and Tukey tests. Statistical significance is set to $P<0.05$.

RESULTS

Following i.p. PTZ injection, all animals showed myoclonic convulsions in PA5, PA10 and PTZ groups. In myoclonic convulsions, results revealed a significant reduction in time required for myoclonic convulsions in PA5 (15.5s) and in PA10 (15.7s) groups compared to PTZ (62.3s) group (Table 1).

Time required for tonic-clonic convulsion period was also significantly lowered in PA5 (19.3s) and PA10 (21s) groups compared to PTZ (267s) (Table 1). No significant difference between those groups was observed and this finding shows no dose dependent response between 5 and 10 mg/kg of acrylamide.

Table 1. Effect of different administrations on the PTZ-induced convulsions in Mice

Treatment	MCC(s)	TCC(s)	C (s)	N of TCC	N of Ex
Control	-	-	-	-	-
PTZ	62.3±5.5 [a]	248.2±48.0[a]	11.0±1.4	6/6	6/6
A5	-	-	-	-	-
A10	-	-	-	-	-
PA5	15.5±2.2[b]	19.5±1.2[b]	12.5±1.8	6/6	6/6
PA10	15.7±0.3[b]	21±0.9[b]	12.7±0.5	6/6	6/6

Results are presented as mean±S.E.M. MCC: Latency for myoclonic convulsions, TCC: Latency for tonic-clonic convulsions, PTZ: Pentylentetrazol, A5: 5 days of i.p. 5mg/kg acrylamide group, A10: 5 days of i.p. 10mg/kg acrylamide group, PA5: 5 days of i.p. acrylamide 5mg/kg+last day 80 mg/kg PTZ group, PA10: 5 days of i.p. acrylamide 10 mg/kg+last day 80 mg/kg PTZ group. C: Convulsion period in seconds, N of TCC: Number of animals having tonic clonic convulsions, N of Ex: Number of Ex animals due to tonic-clonic convulsions. Significance: $P<0.05$. Statistically homogenous subsets are given in same letters as a, b.

DISCUSSION

Epilepsy is a common neurological disease which is induced by various reasons. Certain diet ingredients or toxins may also cause convulsions or exert proconvulsive effect. On the other hand some of the toxins inhibit seizures such are Botulinum toxin (Lee et al., 2017). Acrylamide is well known for its neurotoxic properties and this study was conducted to test its pro/anticonvulsive nature. No deaths or morbidity were occurred due to acrylamide administration during study period. Results also show no convulsions or any tremor in lone acrylamide administration this period. In literature convulsions due to acrylamide were observed by Kuperman (Kuperman, 1958) when administered in doses close to lethal dose. Therefore in accordance with the initial expectation no convulsion was observed in our study at 5 and 10 mg/kg dosage (A5 and A10 groups). Present study may suggest a possible proconvulsive effect of acrylamide on PTZ induced convulsions due to acrylamide administration. It may act as a proconvulsive diet ingredient also in human epileptic patients.

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