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Effect of intermittent fasting on sciatic neurofilaments in acrylamide administered rats

Elif Kaval Oğuz

Department of Science Education, Van Yüzüncü Yıl University, 65080, Van, Turkey

ABSTRACT

Acrylamide is a widely used chemical in industrial manufacturing and laboratory studies. Long term use of this chemical causes axonal degeneration. In this study, 20 mg/kg of acrylamide was administered to Wistar-albino rats daily and effects on sciatic nerve and any potential protective activity of intermittent fasting were investigated. Effect of acrylamide and intermittent fasting was assessed by immunefluorescent method. As a result paralysis in hind legs and coordination problems during walking was observed in acrylamide administered group. These problems were less frequent in intermittent fasting group. In addition, a decrease in neurofilament number was observed in acrylamide group whereas neurofilament level was similar with control group in intermittent fasting group. Concomitant administration of acrylamide and intermittent fasting caused ameliorating effect of intermittent fasting. A potential protective effect of intermittent fasting against acrylamide can be considered according to such results.

Key Words: Acrylamide, Neuropathy, Axon, intermittent fasting

Introduction

Acrylamide is a water soluble vinyl monomer (CH_2=CHCONH$_2$) which has wide industrial applicability. In addition it can also be found in various food sources in daily consumed diet (1). Some of those food sources which are prepared in high temperatures are potato chips, biscuits, breads and bakery products and coffee. It is also a monomer of protein gel electrophoresis which is a widely used technique in molecular biology studies.

Knowledge about neuropathic effects of acrylamide in peripheral nervous system start to accumulate since 1950s from human cases and experimental animal studies. Dense filamentous accumulation, debris in axolemma and mitochondrial degenerations were observed in animals exposed to acrylamide. In addition acrylamide is also accused of disruption in neurotransmission (2). Following such events a decrease in axoplasmic flow occurs. Wallerian type of degeneration was reported in peripheral nerves of acrylamide exposed mammals (3, 4). Apart from peripheral nerves an increase in neurofilament brain gene expression also occurs (5). In addition in pheochromocytoma cells an increase in neurofilament expression was also observed in a dose dependent fashion (6).

Neurofilament proteins are investigated in three categories according to their molecular weights. Those are NF-L (light), NF-M (medium) and NF-H (heavy). Neurofilament disorders are observed features of Alzheimer, Parkinson and other neurodegenerative diseases (7,8).

Intermittent fasting is a type of diet which includes food intake restricted into determined time periods. Intermittent fasting or other types of food restrictions were reported to have protective effect against diabetes, heart disease (ischemic injury), liver damages and obesity (9,10,11). Intermittent fasting was also known for its protective effects against different kinds of damages occurring in nerve tissue (12,13).

Aim is of this study is to investigate effect of acrylamide (which is a carcinogenic chemical with endocrine disrupting and neurotoxic effects) on rat sciatic nerve and also to evaluate a potential protective effect of intermittent fasting against acrylamide.

Material and Methods

Experimental Design: Study material was obtained from an experimental protocol which is performed under approval of Van Yuzuncu Yil University Ethical Commission (with approval date 24.08.2017 approval number 08 and with further decision 2018/03).

*Corresponding Author: Elif Kaval Oğuz, Van Yuzuncu Yil University, Department of Science Education, TR-65080, VAN Turkey, E-mail: ekoguz@yyu.edu.tr, Tel: +0 (432) 444 50 65; fax: +0 (432) 225 13 68
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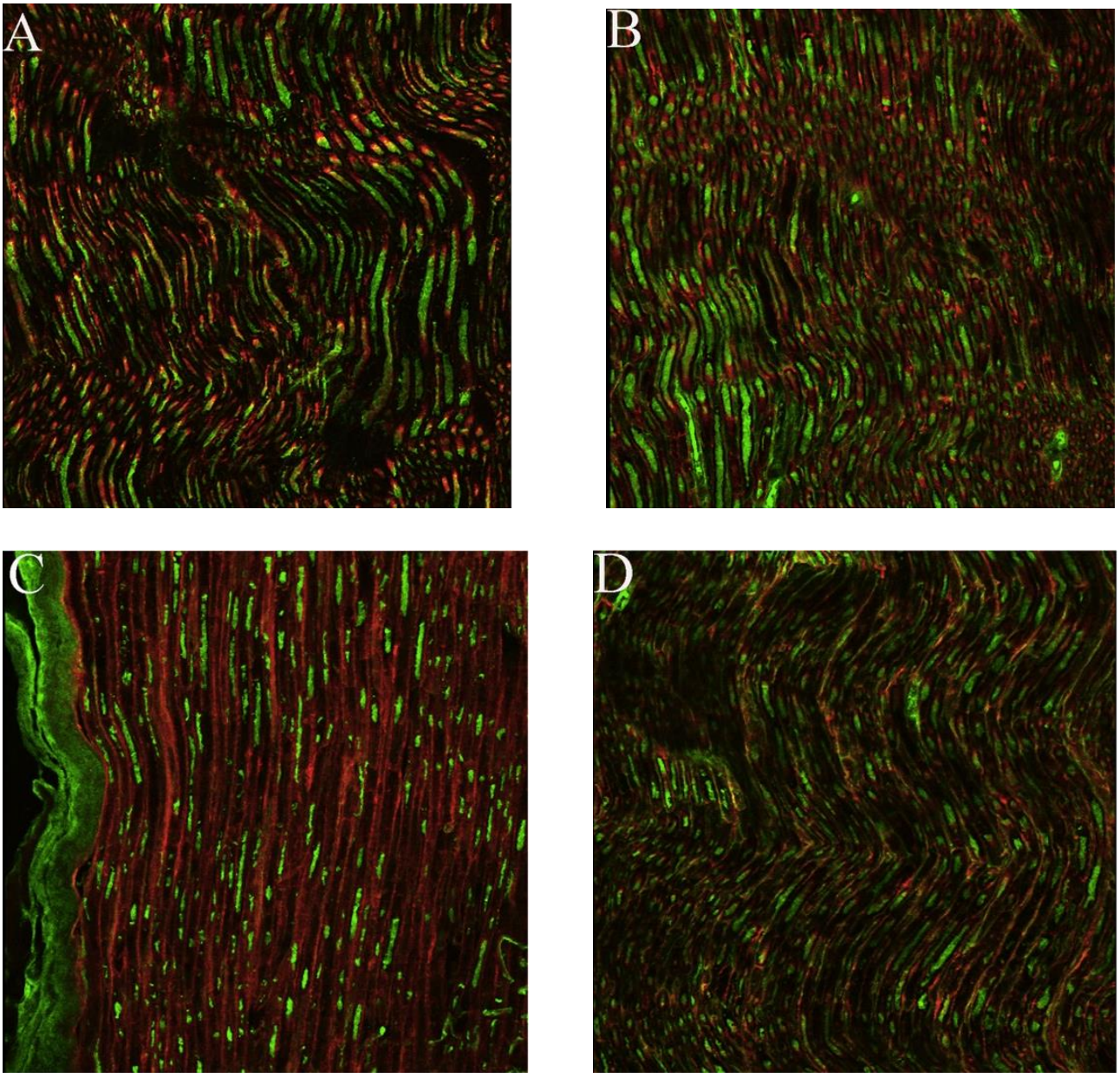

Fig. 1. Immunefluorescent staining of neurofilaments and myelin on sciatic nerves of experimental groups (20x). A) Control group, B) Intermittent fasting group, C) Acrylamide group, D) Acrylamide+intermittent fasting group. (Green color represents neurofilaments, red color represents myelin).

Experimental protocol is defined below;

Fourty female Wistar-Albino rats were divided into 4 groups including 10 animals in each group. Control group; no administration was conducted. Acrylamide group; 20 mg/kg acrylamide was administered daily with gastric gauge. Intermittent fasting group; rats were fasted for 12 hours each other day (14). Acrylamide+intermittent fasting group: 20 mg/kg acrylamide was administered daily with gastric gauge and rats in this group were fasted for 12 hours each other day. Animals were kept in a temperature, humidity and light controlled environment and in separate cages. Following 30 days of this experimental protocol rats were anesthetized and sciatic nerves of rats from all groups were transferred into 4% paraformaldehyde. Obtained tissues were than kept in 30% sucrose solution overnight in refrigerator.

Immunohistochemical detection of neurofilaments in axon: To evaluate axoskeletal changes and the effects of acrylamide on the sciatic nerves, we performed double immunofluorescent staining. Immunohistochemistry technique was applied with slides of rat sciatic nerves. There were four groups of experiments for slides. The first group was control, the second was acrylamide group, the

third was intermittent fasting and the fourth group was acrylamide+intermittent fasting. The sciatic nerves were removed and fixed in ice-cold 4% paraformaldehyde and immersed in 30% sucrose solution in PBS for at least 24 hours for cryoprotection. The sciatic nerves were then cut with a cryostat (Leica, Germany) at 10µm thickness, mounted on poly-lysin coated slides. Slides were permeabilized and blocked with phosphate buffer saline salt solution (PBS) containing 0.1% triton-x, 3% bovine serum albumin and 5% goat serum for 30 mins at 4ºC then immunostained with primary antibodies overnight; these were mouse monoclonal anti-neurofilament heavy IgG (NE14, Sigma-Aldrich, St. Louis, MO, USA), and polyclonal Anti S100 (Z00311, Dako, Carpinteria, CA, USA). The slides were washed with PBS for three times, incubated with Alexa 488 goat anti mouse IgG, Alexa 568 goat anti rabbit IgG secondary antibodies (Invitrogen, Carlsbad, CA, USA) overnight. All primary and secondary antibodies were used at 1/100 dilution. Then preparations were washed with PBS three times and mounted. Negative control sections were incubated under the same conditions without primary antibody. The slides were visualized with a Zeiss LSM 510 META Axioplain 2 imaging (Fluorescence Microscope) laser scanning confocal microscope, images were photographed with a Zeiss Axiocam MRC camera, using the program Axiovision, version 4.5 (Zeiss) for image acquisition.

Results

Administration of acrylamide cause locomotor abnormalities in lone acrylamide administered group in the third week of acrylamide administration. Animals were having difficulty in walking and coordinated movements in their cages. No such abnormal behavior was observed in control or intermittent fasting groups. Animals in acrylamide+intermittent fasting group showed less abnormal locomotor movements compared to animals in lone acrylamide administered group.

Staining of neurofilament showed a similar neurofilament level in axon of peripheral nerves of control and intermittent fasting. However neurofilament staining was decreased in acrylamide administered group (Figure 1C). In acrylamide+intermittent fasting group this staining level was found similar with control group. No difference between groups was observed in myelin staining (Figure 1). Negative controls (without primary antibodies) were not stained (data not shown).

Discussion

Acrylamide is reported to cause general symptoms such as paralysis in rear extremities and abnormalities in walking in experimental animals (15). In our study observed symptoms (paralysis and abnormailities in walking) are in parallel with previous studies on the subject (2, 16).

Increase in neurofilament level was reported in many studies focusing on acrylamide exposure to experimental animals (5, 6). Yu et al., (2006) found a decrease in neurofilament types (NF-H, NF-M and NF-L) in high doses of acrylamide (40 mg/kg). However they reported no difference between control group in a lower level of acrylamide dosage (20 mg/kg) (15). They also found an increase in neurofilament level in central nervous system. In this study an attenuation of neurofilament level was observed in sciatic nerve due to acrylamide administration. Dissimilarities observed in different studies dealing with acrylamide exposure may arise from differences in age and strain of experimental animals, acrylamide concentration, route of exposure and exposure period.

Neurofilament accumulation in nervous system is an observed finding in acrylamide toxicity. It was reported to cause damage via decreasing axoplasmic movement in nervous system (2, 3). Stone et al., (2001) showed no contribution of neurofilament accumulation in nerve damage caused by acrylamide. It must be kept in mind that alterations in different axonal proteins apart from neurofilament alterations can be also affecting nerve damage (17).

Although humans do not expose to acrylamide dosage administered to experimental animals (20 mg/kg/day) it is clear that they are exposed to much longer time periods of detoriating effects of acrylamide. Nevertheless intermittent fasting which is an important scientifically proven tool for longevity and a healthier life is ameliorating alterations observed in sciatic nerve tissue due to acrylamide exposure. Further studies are needed to set up experimental models to test effects of acrylamide exposure and potential methods to ameliorate its untoward effects.

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Conflict of interest: The authors declare that they have no conflict of interest.

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