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Experimental Study

Effect of long term-administration of aspartame on the ultrastructure of sciatic nerve

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ABSTRACT

Aspartame is the most widely used artificial sweetener worldwide. There is much controversy about the effect of aspartame on different cells in the body. The aim of this study was to evaluate the effect of aspartame on the structure of the sciatic nerve. Thirty adult male albino rats were divided into three groups. Group I served as control; Group II received aspartame orally in a dose of 250 mg/kg/d for 3 months; Group III received aspartame at the same dose and for the same period, and was allowed to recover for 1 month. Specimens of sciatic nerve were processed, and semithin and ultrathin sections were examined by light and electron microscopy. A morphometric study was done to evaluate the g-ratio, which is the ratio between the axon diameter and total fiber diameter. Long-term aspartame administration resulted in many degenerative changes affecting mainly the myelin sheath, in the form of focal and extensive demyelination; disruption and splitting of myelin lamellae with loss of compact lamellar structure; and excessive enfolding with irregular thickening of myelin sheaths. Less frequent than those observed in the myelin sheath, some axonal changes were detected, such as compression and distortion. Dilated rough endoplasmic reticulum and vacuolation of the cytoplasm of Schwann cells were also detected. Partial improvement was observed in the recovery group. It was concluded that long-term administration of aspartame had a harmful effect on the structure of sciatic nerve and 1 month stoppage of aspartame was not enough to achieve complete recovery.

1. Introduction

Aspartame is an artificial sweetener consumed by > 200 million people worldwide and it is used as non-nutritive sweetener in ~6000 food products, including dry beverage mixes, chewable multi-vitamins, breakfast cereals, chewing gum, puddings and fillings, carbonated beverages, refrigerated and nonrefrigerated ready to drink beverages, yoghurt products, and pharmaceuticals [1].

It has been found that aspartame induces toxicity at various levels. Recently, many experimental studies have confirmed that aspartame is a multipotential carcinogenic agent and increases the risk of lymphoma, leukemia, urinary tract tumors, and neurological tumors, even at a daily dose (20 mg/kg) that is much less than the acceptable daily dose (40 mg/kg) [2]. In addition, several studies have suggested an association between aspartame consumption and the risk of type 2 diabetes [3], preterm delivery [4], nephrotoxicity [5], hepatotoxicity [6], and induction of histopathological changes in the parotid salivary glands [7].

With regard to neurotoxicity of aspartame, most of the previous studies were concerned about central nervous system toxicity. Aspartame affects the cerebral cortex [8] and cerebellar cortex [9,10], affecting memory, learning, and behavior [11]. The aim of the present study was to

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evaluate the effect of aspartame on the structure of the sciatic nerve in adult male albino rats.

2. Materials and methods

2.1. Preparation of animals and study design

The present study was carried out on 30 adult male albino rats, weighing 150–200 g. The animals were kept in adequate ventilation and temperature and were fed standard laboratory food and water. The rats were divided into three equal groups. Group I (Control Group) received ordinary diet and water. Group II (Aspartame Group) received aspartame (250 mg/kg/d) [12] dissolved in distilled water and administered by gastric tube for 3 months. This dose corresponded to the acceptable daily intake in humans (defined by the World Health Organization) of 40–50 mg/kg/d. Species correction required a five to six times higher dose in rats than humans, as rats metabolize aspartame faster than humans [13]. Group III (Recovery Group) received aspartame as in Group II, but rats were left for 1 month after stopping aspartame to recover before they were killed.

2.2. Preparation for microscopy

Samples from the sciatic nerves were obtained at the end of the experiment. Rats were killed by decapitation and the sciatic nerves were carefully dissected out, sectioned transversely into small pieces of 1 mm^2 and fixed in 2.5% glutaraldehyde for 24 hours. Specimens were washed in 0.1 M phosphate buffer at 4 °C, then postfixed in 1% osmium tetroxide at room temperature. Specimens were dehydrated in ascending grades of ethanol, and embedded in Epon resin (Embed; pure Epon/Araldite mixture) for 24 hours at 35 °C, 48 hours at 45 °C. and 24 hours at 60 °C (gradual method) (Mammalian Soft Tissue Protocol - In-situ and Immersion fixation) then place labels into BEEM (Better Equipment for Electron Microscopy) capsules, add degassed resin, and then add tissue blocks. Tissue blocks descended to the tip of the BEEM capsules before polymerization of resin. Semithin sections (1 μm) were stained with toluidine blue and examined by light microscopy. Ultrathin sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate [14]. Specimens were examined and photographed with a JEM transmission electron microscope.

2.3. Morphometric and statistical study

Using a Leica LAS software program (Leica Geosystems, St. Gallen, Switzerland) connected to a microscope (Leica Microsystems, Wetzlar, Germany), the diameter of the regular myelinated fibers of the sciatic nerve and their axons in five nonoverlapping fields was measured manually, and then the axon/fiber ratio was estimated, which is known as the g-ratio [15]. Morphometric data were analyzed, and differences were considered significant at $p \leq 0.05$. Morphometric study was carried out at the Image Analysis Unit, Faculty of Medicine, Tanta University, Tanta, Egypt.

3. Results

3.1. Light microscopy

Toluidine-blue-stained sections of the sciatic nerve of the Control Group revealed normal histological features for nerve axons, myelin coat, Schwann cells, and endoneurium (Figures 1A and 1B). Examination of the Aspartame Group revealed increased spaces between the nerve fibers, and the endoneurium contained dilated blood vessels with multiple cells with irregular nuclei (Figures 1C and 1D), in addition to focal lysis of some myelin sheaths and formation of myelin loops (Figure 1E). Marked demyelination, degeneration (Figure 1F), and irregular thickening (Figure 1G) of myelin sheaths were also detected. In the Recovery Group, toluidine-blue-stained sections revealed partial improvement. Some fibers appeared similar to those of the Control Group, while others showed irregularity of the myelin sheath with focal lysis (Figure 1H).

3.2. Electron microscopy

Ultrathin sections of the Control Group revealed Schwann cells surrounded by myelinated and unmyelinated axons. Myelinated axons were surrounded with regular myelin sheaths with preserved compact lamellar structure and uniform thickness, and the axoplasm contained multiple microtubules and mitochondria (Figures 2A and 2B). Ultrathin sections of the sciatic nerve of the Aspartame Group showed many monocytes with their distinctive nuclei, which were indented, kidney-shaped, or C-shaped, within dilated blood vessels (Figures 2C and 2D), in addition to marked degenerative changes affecting mainly the myelin coat. Most samples showed focal lysis of myelin sheaths (demyelination; Figure 2E) with redundant myelin (Figures 2F and 2G) and formation of myelin loops (invagination of myelin sheaths toward the axoplasm); Figure 2H), incisures (wide separations) of myelin lamellae and splitting (loss of compact lamellar structure; Figures 3A and 3B). Some nerve fibers with marked disruption (discontinuous and disorganization), demyelination, and degeneration of the myelin sheath were also detected (Figures 3C and 3D). However, excessive enfolding with irregular thickening of the myelin sheaths was detected in some areas (Figures 3E and 3F). Changes in axons were less frequent than those observed in the myelin sheath; some axons appeared shrunken and compressed with distorted axoplasm (Figures 3E and 3F). Regarding the Schwann cell coat, the most prominent findings were destroyed swollen mitochondria, dilated rough endoplasmic reticulum (RER), in addition to vacuolation of the cytoplasm (Figures 3G and 3H). Examination of ultrathin sections obtained from the Recovery Group revealed partial improvement, with some fibers appearing similar to those in the Control Group (Figures 4A and 4B). Some fibers showed focal lysis, focal appearance of redundant myelin, focal separation of the myelin sheath from the axon, and the axoplasm of some nerve fibers contained few vacuoles (Figures 4C–4H).

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