Nicotine mediated microcystic oedema in white matter of cerebellum: possible relationship to postural imbalance

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ABSTRACT

Background: Nicotine is heavily used addictive drug that has unpleasant side- effects, e.g. dizziness, nausea, emphysema. **Purpose:** The current study was designed to find the possible relationship of nicotine mediated microcystic oedema in white matter of cerebellum to postural imbalance. **Methods:** Nicotine was administered for 8 weeks orally via cannula, using dose rate (5 mg/day, 10 mg/day) to male drukrey rats. The results were compared to control adult rats, given vehicle in identical manner. After 8 weeks exposure, the cerebellum was removed and processed for histopathologic study. **Results:** The cellular microcystic change with interstitial oedema was found in white core of cerebellum of rat received 10 mg/kg of nicotine. Cytoplasmic vacuolation was also observed in most areas of cerebellum. **Conclusion:** These findings suggest that the mature adult cerebellum is susceptible to the damaging effects of nicotine in depleting white core of cerebellum.

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Introduction

Cigarette smoking produces nicotine that is readily absorbed into the physiological system of the smokers. Nicotine, a psychoactive substance is responsible for the development of nicotine dependence among smokers. Simultaneously, several other compounds are also produced that are absorbed in the physiological system of the smoker and producing a wide spectrum of damage to the various organs.[1]

Much of the attention of nicotine research is centered on its addiction aspect while little emphasis is placed on its potential to cause neurotoxicity. Nicotine is earlier reported to cause postural imbalance in smokers.[2] Therefore, nicotine might affect cerebellum. Nicotine is a major tobacco specific alkaloid in both main stream tobacco smoke and environmental tobacco smoke.[3] Nicotine, a major tobacco alkaloid has great physiological and pharmacological effects. It plays crucial role in establishing and maintaining tobacco dependence.[4]

More than 20 metabolites of nicotine have been identified, all of which are less active than the parent compound. The primary urinary metabolites are cotinine (15% of the dose) and trans-3-hydroxycotinine (45% of the dose). Nicotine undergoes stereo-specific processing and trans-isomer is produced. The fatal human data has been estimated to be about 50-60 mg.[5] Interestingly, certain diseases that benefit from nicotine include Alzheimer's Disease and Tourette's Syndrome.[6] As far as health problems are concerned, nicotine may be associated with Cancer, Emphysema, Heart disease and Stroke.

The cerebellum is the central part of the major circuitry that links sensory areas to the motor areas of brain and is required for coordination of important movements. In adults, the weight ratio of cerebellum to cerebrum is 1:10, in infant it is 1:20. Cerebellar output is mainly associated to those structures of the brain that control movement. The cerebellum is currently believed to participate in higher brain functions. Many researchers have studied the effect of nicotine on cerebellum and found significant deficits. The rationale for selecting the long-term exposure regimen and the White core was based on the fact that most smokers are exposed to nicotine for a long period and the cerebellum is a vulnerable target for neurotoxins.[7]

Methods

Subjects

Eighteen adult male drukrey rats were used in the study. They were obtained from the animal house of Industrial Toxicological Research Centre Lucknow after Institute animal ethical approval had been sought and had an mean age of about 90 days. The animals were acclimatized for two weeks after being transferred from their colonies to the working laboratory and were housed in aluminum cages under hygienic and sanitary conditions. The light and dark cycle of twelve hours and temperature was maintained. All investigations reported in study were conducted in conformity with the recommendations from the declaration of Helsinki and the International guiding principles for Biomedical research involved animals.

Experimental procedures

The animals were assigned randomly in following 3 groups with respect to nicotine dose: Control, Experimental I and Experimental II. Each group had 6 animals. Nicotine containing cannula was kept covered with aluminum. The animals were fed on standard pellet diet and water was given *ad-libitum*. This oral administration of nicotine is similar to "chewing tobacco" or the "nicotine gum" route of exposure in human. (-) Nicotine hydrogen tartrate (Lacaster Hysel pharmaceutical) was administered to animals orally via cannula. Experimental Group I (E-I) and Experimental Group II (E-II) were given 5 mg/kg and 10 mg/kg of Nicotine respectively in single dose for 8 weeks. Control rats were given equal volume of normal saline at the same time as vehicle. The animals were sacrificed 2 days following last nicotine administration. Their brains were removed and the cerebellar vermis was dissected and processed for histopathology.

Tissue preparation

The cerebellum was isolated from rest of the brain by cutting the three-cerebellar peduncles. The cerebellum was kept in 10% normal saline. After 48-72 hours of fixation, the cerebellum was removed from the fixative and was kept on the plain surface with its dorsal surface facing up. It was divided in sagittal plane into two halves, right and left.

Processing of Tissues for Making Paraffin Blocks

After removal from fixative, parts of the cerebellum were thoroughly washed with distilled water and gently transferred into sufficient quantity of 30 percent ethanol. The parts of cerebellum were then processed through increasing concentration (50, 70, 90 and 95 percent) of ethanol, each for 60 minutes. Dehydration was completed by two changes of absolute ethanol, each of 30 minutes duration. Dehydrated specimens were 'cleared' in xylene and infiltrated with paraffin wax (melting point 58-60°C). Tissue orientation was done immediately after placing the cerebellar part inside the mould. Rapid cooling was done for solidification of wax.

Cutting of Paraffin Sections

The paraffin blocks were trimmed and attached to block holders. Serial sectioning of each slab was set to 10μm in the sagittal plane on a rotary microtome. The ribbons of sections were spread on a sheet of paper kept over a flap surface. They were lifted by a spatula and transferred to a water bath, the temperature of which was always kept at about 56°C (2 degree below the melting point of the wax used). The flattened sections were carefully lifted by floatation methods on already cleaned glass slides smeared with a thin layer of mayors egg albumin glycerine mixture. The slides with sections were kept in incubator (40-45°C) for fixation and dried slides were stained with luxol fast. In this study, the criterion for determining whether a section was considered a vermal section was the visualization of all 10 cerebellar lobules (not necessarily 10 complete cerebellar lobules). Due to sectioning, one cerebellar vermis (from 5 mg/kg nicotine dose experimental group I) did not meet the criterion and not include in the counting.

Stereological equipment

The Nikon Optiphot microscope used in this study had a motor-driven stage. Slides were viewed under 40x, 100x, 400x magnification with objective lens of 1.4 numerical aperture. The image from microscope was transferred to HCl Computer using CCD-IRIS Color Video Camera.

Results

It was observed that the consumption of food by control animals was good while nicotine fed rats (experimental group) had diminished food intake. During the first few days, the movement and activity of test animals was less. Later they showed signs of excitation and hyperactivity marked by repeated jumping. Subsequently after seven weeks, these animals appeared lethargic.

Morphological Observation

The cerebellar hemispheres were soft, friable, laminated and yellowish brown in colour. There was no significant morphologic change in control and experimental groups.

Histological Observation

A compact mass of white matter, which is continuous between cerebellar hemispheres, extends into the folia as a core of white matter. White matter lies underneath the gray matter of the cortex and made up largely of myelinated nerve fibers running to and from the cortex. The deep nuclei of the cerebellum are clusters of gray matter lying within the white matter at the core of the cerebellum.

The loosening of the white core was present in Experimental Group I whereas the degree of oedema was much (++) increased in Experimental Group II. The cellular microcystic change with interstitial oedema was evident in white core of cerebellum of Experimental Group II. The cells varied greatly in size and appeared to be singly placed. In most of areas they appeared hypertrophied with increased cytoplasmic vacuolation. There are foci of degeneration where the cells appeared swollen and empty with highly stained nucleus.

Discussion

The finding of this study showed that long term nicotine treatment results in significant loss of white matter of cerebellum of drukrey rats. These findings suggest that the white matter are sensitive to damaging effects of nicotine indicated by oedema and cytoplasmic vacuolation which is similar to effects of nicotine reported on the developing white core on maternal exposure of nicotine.[2]

Understanding the mechanism of nicotine mediated depletion of white matter was not investigated, nevertheless it is reasonable to speculate that the interaction of nicotine and the α subunits of nicotine Ach receptor in white core may subsequently trigger apoptotic process that leads to loss of white matter. A recent report has indicated that the activation of nicotine receptors by low doses of nicotine result in apoptotic cell death in primary hippocampal progenitor cell apoptosis[1] demonstrating the capacity of nicotine in promoting apoptosis. Taken together, these finding suggest that the nicotine is a neurotoxic agent regardless of its protective property in many other experimental manipulations.[1,2,5-9]

In the current study, significant difference in oedema was found between two doses of nicotine, possibly due to threshold level for nicotine to exert its effect on white matter loss. The blood nicotine level was not measured in this study, which is a major limitation. Adolescent nicotine exposure has deleterious effects on cell development particularly in purkinje cell layer in cerebellum of albino rats characterized by reduced number of purkinje cell number.[10-12] These findings are substantiated by white matter changes in cerebellum in the current study; we noted cellular oedema in white mater. Our results thus support an emerging pattern wherein adolescent nicotine exposure elicits cerebellar damage leading to abnormality of cellular pattern and corresponding behavioral anomalies.

In order to extend these findings further, detailed microscopic assessment alongwith parallel clinical observation, using MRI techniques, for human studies is imperative. However, the gross measurements from MRI techniques remain one of the most powerful and valuable methods to detect pathology in living organisms. Despite the negative effects of long-term nicotine treatment, it needs to be recognized that there are some reports that show the beneficial effects of chronic nicotine exposure. A few studies have reported that the chronic nicotine treatment improved cognitive performance both in human and animal studies (nicotine skin patch for human and osmotic mini pump for Sprague- Dawley rats).[13] Furthermore, it should be noted that enhancing effects in performances dissipated following the removal of nicotine suggesting that the presence of nicotine in physiological system is required to exert such facilitatory effects.

However, what is lacking in the literature is whether the detrimental performance of long term nicotine exposed patients

following the cessation of treatment is a function of long term nicotine treatment, the withdrawal, or both. In conclusion, this study shows that long-term nicotine exposure during adulthood results in loss of white matter of cerebellum in rat model system. At present, the significance and manifestation of such a loss of white matter remains to be determined. Further behavioral research related to cerebellar functions in the area of neurotoxicity of nicotine will shed some light on this issue.

The article complies with International committee of Medical Journal Editor's uniform requirements for the manuscripts.

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