WHITE CORE OF CEREBELLUM IN NICOTINE TREATED RATS - A HISTOLOGICAL STUDY

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ABSTRACT

Although numerous studies explored the consequences of nicotine exposure on cerebellum and on its various layers, little research is focused on nicotine exposure on White core of cerebellum. In the present study, we assessed the effects of long-term nicotine exposure on White matter of cerebellum. Nicotine was administered for 60 days orally via cannula, using dose rate (5mg / day , 10 mg / day) to Male Drukrey rats. The results were compared to control adult rats, given vehicle in identical manner. After 60 days exposure, the cerebellum was removed and processed for histopathologic study. The results showed that long term nicotine treatment regime did result in significant loss of White core of cerebellum. These findings indicated that mature adult cerebellum is susceptible to the damaging effects of nicotine in depleting White core of cerebellum.

KEY WORDS: Cerebellum ; Histopathology; Neurotoxicity; Smoking ;Tobacco

INTRODUCTON

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

Much of the attention of nicotine research is centered on its addiction issue and less focus is placed on its potential to cause neurotoxicity. Nicotine is reported to cause postural imbalance in smokers[2]. Therefore, nicotine might be the circuitry involving the cerebellum. Nicotine is a major tobacco specific alkaloid in both main stream tobacco smoke and environmental tobacco smoke[3]. Nicotine, major tobacco alkaloid has great physiological and pharmacological effects. It plays crucial role in establishing and maintaining tobacco dependence[4]. The fatal human data has been estimated to be about 50-60 mg[5]. Some diseases that nicotine might improve include Alzheimer's Disease and Tourette's Syndrome[6]. As far as health problems are concerned nicotine may improve Cancer, Emphysema, Heart disease and Stroke.

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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 fine movements. In health, it provides connections during movement which are the basis for precision and accuracy and it is initially involved in motor learning and reflex modification. Cerebellar output is mainly to those structures of the brain that control movement. The cerebellum is currently believed to participate in higher brain functions. Many workers studied the effect of nicotine on cerebellum and found significant depleting changes. 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 very little basic research is focused on its effect on white core of cerebellum.

MATERIAL & METHOD

Eighteen adult male Drukrey rats were used in the study. They were obtained from the animal house of Industrial Toxicological Research Centre Lucknow around 90 days of age. The animals were assigned randomly to three-treatment groups with respect to nicotine dose, in following 3 groups: Control, Experimental I and Experimental II. Each group had 6 animals. Nicotine containing cannula was covered with aluminum foil due to light sensitive nature of nicotine. The animals were fed on standard pallet 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 per 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 removed and the cerebellum was dissected and processed for paraffin embedding. Sections of 5 microns were cut and stained with Enrlich Haematoxyline and Eosine

Stereological equipment :-

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

OBSERVATION

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

Morphological Observation

The cerebellar hemispheres were soft, friable, laminated and yellowish brown in color. 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 (Fig 1 A). White matter lies underneath the gray matter of the cortex and made up largely of myelinated nerve fibers running to and from the cortex (Fig 1A). 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 (Fig 1B) whereas the degree of oedema was much (++) increased in Experimental Group II (Fig.1C). The cellular microcystic change with interstitial oedema was evident in white core of cerebellum of Experimental Group II. (Fig.1C).

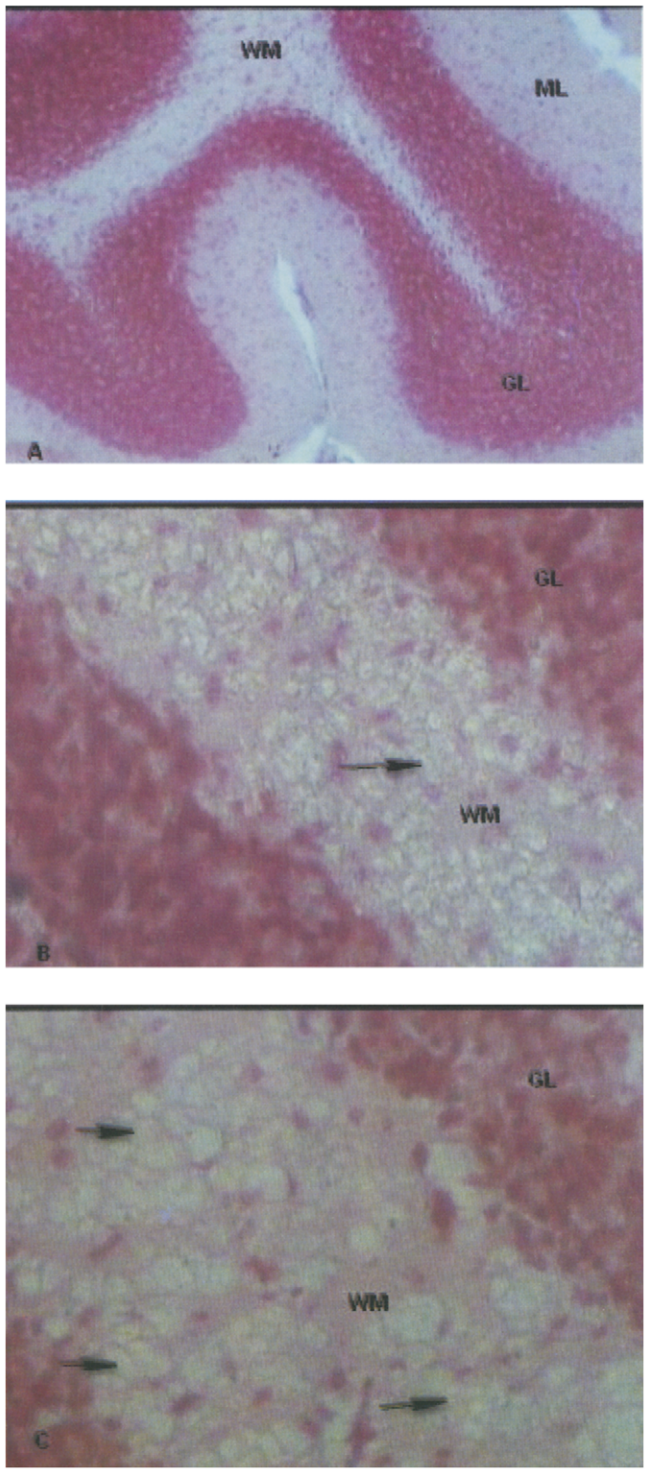

Fig. 1:

The photomicrographs of White core of cerebellar hemisphere, A: control, B:5mg/kg nicotine treatment. C:10mg/kg nicotine treatment. All photomicrographs were takenfrom lobule & near the midsagittal plane. Arrow head on the photomicrographs indicates microcystic odema in the white core of cerebellum.

The cells varied greatly in size and appeared to be singly placed. In most of areas they appeared hypertrophied with increased cytoplasmic vauolation (Fig 1C). There are foci of degeneration where the cells appeared swollen & 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 albino rats. These findings suggest that the white matter are sensitive to damaging effects of nicotine indicated by odema 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 how nicotine depletes white matter is beyond the scope of this research, nevertheless it is reasonable to speculate that the interaction of nicotine and the subunits of nicotine Ach receptor in white core may subsequently trigger apoptotic process that leads to loss of white matter. A recent report indicated that the activation of nicotine receptors by low doses of nicotine results in apoptotic cell death in demonstrating the ability of nicotine in mediating apoptosis. Taken together, these finding suggest that the nicotine is a neurotoxic agent regardless of its protective property in many other experimental manipulations[6,7,8].

In the current study significant difference in edema 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 in determining the blood nicotine level that is needed to mediate the white core loss following nicotine exposure. 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[9]. These findings are paralleled by white matter changes in cerebellum in current study; we found eventual cellular edema in white mater. Our results thus support an emerging pattern where in adolescent nicotine exposure elicits cerebellar damage leading to abnormality of cellular pattern and corresponding behavioral anomalies.

However, the current results and finding from[10] validate the need to use more sensitive microscopic assessment in animal studies to confirm the clinical observation using MRI techniques. On the other hand, the gross measurements from MRI techniques remain one of the most powerful and valuable methods to detect morphological pathology in living organisms.

Despite the positive effects of long-term nicotine treatment, it needs to be recognized hat there are some reported beneficial effects of chronic nicotine exposure[11,12,8] reported that 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). Furthermore, it should be noted that enhancing effects in performances dissipated following the removal of nicotine suggesting that 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.

CONCLUSION

This study showed that long-term nicotine exposure during adulthood resulted 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.

REFERENCES

1. Denissenko M.F., Pao A,, Tang M., Pfifer G.P. Preferential formation of Benzo(a)pyrene adducts at lung cancer mutational hot spots in p53.Science 1996;274: 430-432,.
2. Pereira M., Strupp T., Holzleitner T., Brandt.Smoking correlation of nicotine induced nystagmus & postural disbalances. Neuroreport 8: 1223- 1226, 2001.
3. Xu Xu, Michael M. Iba & Clifford P. Weisel.Simultaneous and sensitive measurement of Anabasine, nicotine and nicotine metabolites in human urine by liquid chromatography - Tandem Mass Spectrometry. Clinical Chemistry2004; 50(12): 2323-2330.
4. Zhang X., Ameno K., Ameno S., Kinoshita H., Kubota T., Kumihashi M., Mostofa J., Twahashi K. and Igiri I. Effect of whole depletion of CYP2A6 on nicotine metabolism in humans. Drug & Chemical

Toxicology 2002; 25(2): 203-213.
5. Lazutka F.A., Vasilyauskene A.D., Gefen S.G.Toxicological evaluation of the insecticide nicotine sulfate. Gig Sanit.1969; 34(5): 3033 (translated).
6. Costa G., Abin-Carriquiry J.A., Dajas F. Nicotine prevents striatal dopamine loss produced by 6-hydroxydopamine lesion in the substantia nigra. Brain Res. 2001;888: 336-342.
7. Prendergast M.A., HarrisB.R., Mayer S.,Holley R.C.,Littleton J.M.Nicotine exposure reduces N-methyl-D-aspartratetoxicity in the hippocampus relation to distribution of the alpha 7 nicotinic acetylcholine receptor subunit. Med.Sci. Monit. 2001;7.1153-1160.
8. Wright S.C., Zhong J., Zheng H.Nicotine inhibition of apoptosis suggest a role in tumor production . Faseb J. 1993;7: 1045-1051.
9. Chen A., Russell B. Edwards, Ronald D. Romero, Scott E., Parnell Rebecea J. Monk. Longterm nicotine exposure reduces Purkinje cell number in the adult rat cerebellar vermis. Neurotoxicology & Teratology 2003;25: 325-334.
10. West J.R., Parnell S.F.,chen W.A.J.,Cudd T.A.Alcohol mediate Purkinje cell loss in absence of hypoxemia during third trimester in ovine model system. Alcohol Clin.Exp.Res.2001; 25.1051-1057.
11. Levin E.D., White H.K.Four weeks nicotine ski patch treatment effects on cognitive perfommance in Alzheimer's disease. Psychopharmacology 1999; 143: 158-165.
12. Rezwani A.H., Levin F.D.Cognitive effects of nicotine. Biological Psychiatry 2001; 258-267.