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Changes of Cytoskeletal System in Neurodegenerative Disease : Immunohistochemical Characterization of Neurofibrillary Changes Induced by Mitotic Spindle Inhibitors*

Heasoo Koo

Department of Pathology, College of Medicine, Ewha Womans University

= Abstract =

In experimental neurogenerative diseases, aluminum(Al) intoxication and axotomized sensory neurons showed an abnormal accumulation of neurofilaments(NF) in the neuronal perikarya. These NF contain phosphorylated(ph) epitopes that are not detectable in normal perikaryal NF. Antimitotic drugs also cause accumulations of NF probably by depolymerization of microtubules(MT). The present study was designed to examine immunohistochemical changes of NFs following administration of antimitotic drugs, which result in accumulation of NFs in the cell body through a different pathogenetic mechanism than aluminum. Adult rabbits were injected intracisternally with 25-50 μg of maytansine and maytanprine, two antimitotic agents. Tissues were obtained from experimental and control animals and processed for histological and immunocytochemical examinations. Large bundles of NF in the perikarya and proximal processes of large neurons from experimental animals reacted intensely with monoclonal antibodies(mAb) to ph epitopes of 200 KDa NF subunit as well as with mAb recognizing nonphosphorylated(non-ph) NF epitopes. Neuronal perikarya from control animals immunoreacted only with mAb to non-ph NF. Immunoreaction of Ab to the microtubule associated protein 2(MAP-2) and to tubulin was similar in neurons from experimental and control animals. No immunoreaction was detected with antibodies to tau proteins. The abnormal presence of ph epitopes in accumulating NF under different conditions indicate that neurons affected in different diseases show aberrant phosphorylation of NF proteins associated with functional impairment.

KEY WORDS : Cytoskeletal system · Neurodegenerative disease · Maytansine · Maytanprine.

Introduction

Alterations of cytoskeletal system such as accumulation and disorganization of neuronal cytoskeletal components have been recognized in major degenerative disease of human central nervous system(CNS)[1-3], as well as in normal aging[4] and in experimental conditions[5-7]. These abnormal fin-

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Table 1. Antibodies

Monoclonal Ab against ph epitopes of 200 kDa subunit of MF : I : I : I(1) and SM_{34}(2)	
Monoclonal Ab against non-ph epitope of doublet 200 and 145 kDa subunits of NFs : SM_{33}(2)	
Anti-tubulin : Tyrosinated and detyrosinated α-tubulin(3)	
Anti-MAP-2 : Kosik MAP-2 mouse $5F_9$-$H_{10\text{-}13}$(4)	
Anti-tau : Kirshner(5) and Connolly(6)	
(1) Banbury Report(In press)	(4) Proc Natl Acad Sci USA 83 : 4044-4048
(2) Sternberger-Meyer Inc	(5) J Cell Biol 98 : 1090-1097
(3) Cell 38 : 779-789	(6) Exp Cell Res 127 : 341-350

dings had been intensively studied by light and elecetron microscope. With the development of immunohistochemistry, monoclonal antibodies specifically recognizing the ph epitopes of each subclasses of NF have been available for investigation of the distribution of the ph NF in normal and diseased conditions[8-10].

Aluminum salts intoxication and axotomy have been used as model to produce and understand the abnormal accumulation and phosphorylation of NFs[5)6)11]. The antimitotic drugs, Maytansine (MYT) and Maytanprine(MYTPR), known to induce specific injuries of tubulin-microtubule system by binding to tubulin, are also known to induce neurofibrillary changes in vivo and in neuronal cultures[7)12]. I have tried to evaluate immunohistochemically the distribution of epitopes of NFs as well as other components of cytoskeleltal system in antimitotic drug injected animals.

Material and Method

Adult New Zealand albino rabbits, weighing 3.0~3.5kg, were used. MYT 50μg or 25μg in 100ml of Ringer solution were administered to 4 and 3 rabbits respectively by injection into the cisterna magna. Three control animals were injected with 50ml of Ringer's solution. The animals were sacrificed at various times between 66 and 96 hours after injection by intracardiac perfusion with 1.5% formalin with 0.25% glutaraldehyde in 0.1M sodium cacodylate buffer(pH 7.35). Brain and spinal cord were dissected and postfixed in the fixative used for perfusion. The tissue blocks were processed for paraffin embedding.

Sections cut 5~7μm thick were stained with Hematoxylin and Eosin and Bodian's silver stain or processed for immunostaining with peroxidase-anti-peroxidase method described by Sternberger et al[8]. The following antibodies were used : 1) two mAbs directed against ph epitope of 200 KDa subunit of the NF(1 : 1 : 1 and SM34), 2) mAb to non-ph epitope of doublet 200 and 145 KDa subunits of NFs(SM33). Selected sections were stained with various antibodies : anti-tau, anti-MAP-2, and anti-tubulin(Table 1).

Results

Compare to the controls, animals injected with MYT and MYTPR showed accumulation of NF bundles in both neuronal cell bodies and proximal segments of neuronal processes in brain stem and upper spinal cord(Fig. 1). MYT and MYTPR induced NF appeared as tangled masses distending and distorting the perikarya and proximal portion of neuronal processes. Antibodies to ph NF did not stain neuronal perikarya nor their proximal portion of neuronal processes in control animals but reacted with distal portion of neuronal processes(Fig. 2A). In MYT and MYTPR treated animals, the antibodies to ph NF stained perikarya of neurons containing accumulated NF bundles(Fig. 2B). Neurons without NF accumulation showed staining of proximal segments of their processes and weak or no perikaryal staining with the antibodies to ph NFs.

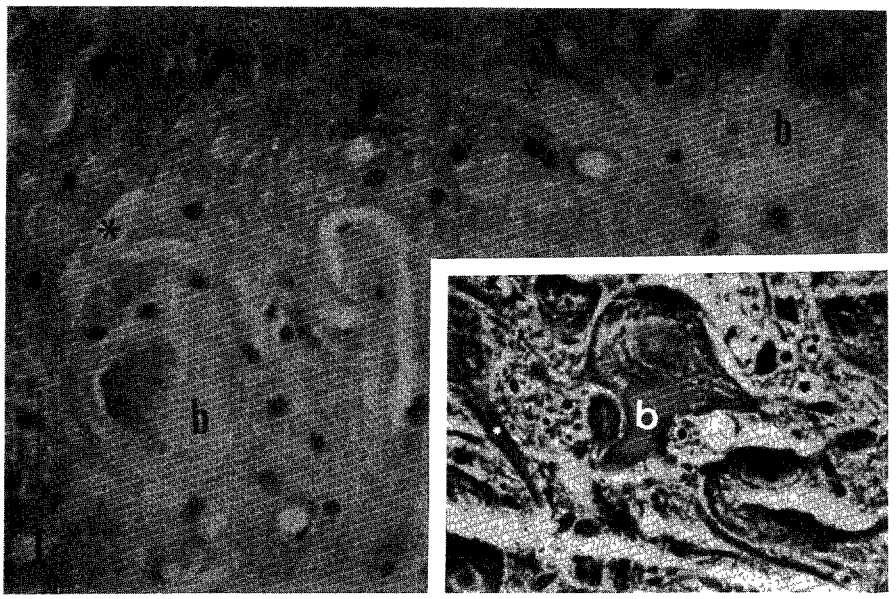

Fig. 1. Accumulation of neurofilament bundles in neuronal perikarya(b) and proximal segment of neuronal processes(*) (H-E, ×100 original magnification) ;
Inset : Tangled neurofilament bundles(b) distending and distorting the perikarya and proximal portion of neuronal process (Bodian's silver stain, x100 original magnification)

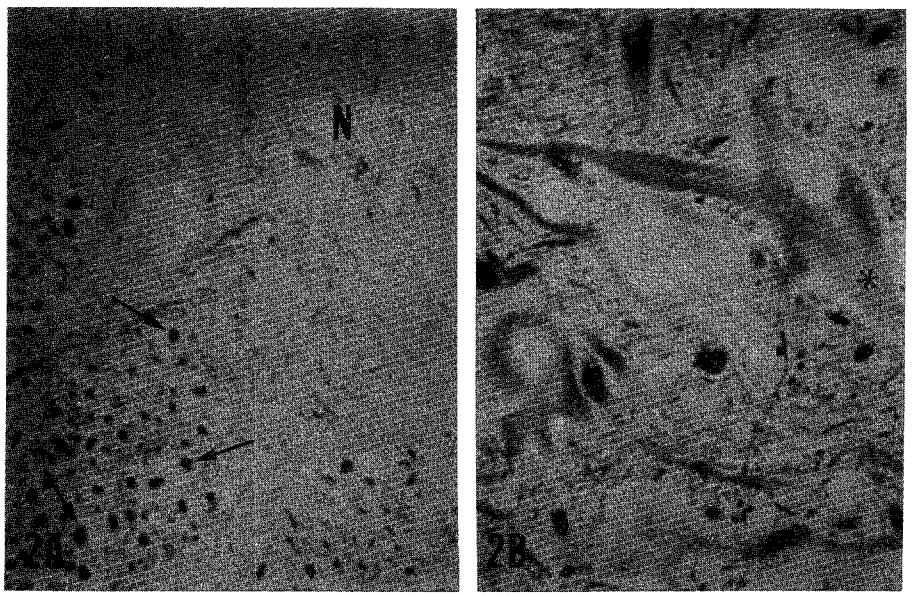

Fig. 2. Immunostain with anti-ph NF antibody (PAP, ×100 original magnification)
A(Control) : Negative reaction in neuronal cell body(N) and positive reaction in distal portion of neuronal processes (Arrows)
B(MYT-treated) : Positive reaction in neurofilament bundles in neuronal perikarya and proximal portion of neuronal processes(*)

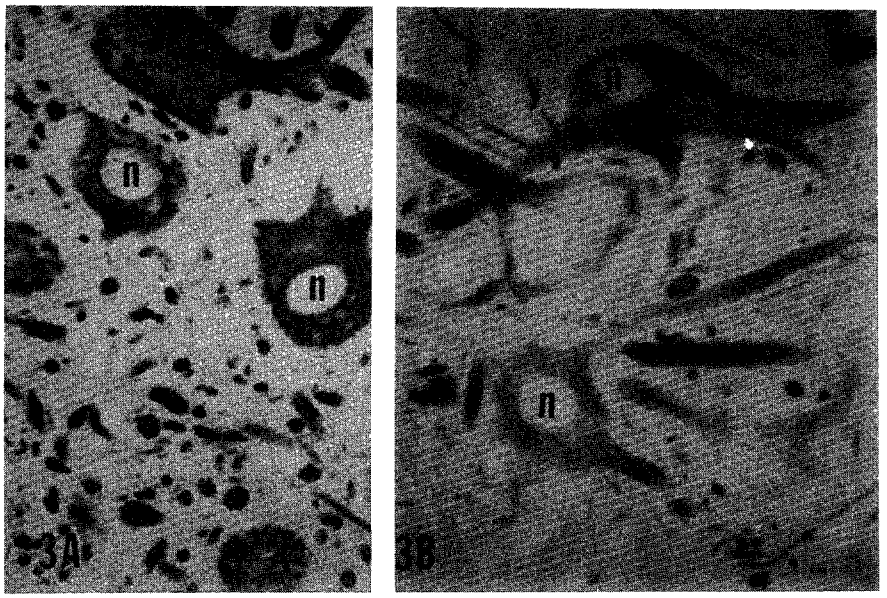

Fig. 3. Immunostain with anti-non-ph NF antibody (PAP, ×100 original magnification)
A(Control) : Positive reaction of neuronal perikarya and processes
B(MYT-treated) : Positive reaction in neurofilament bundles(b) in neuronal perikarya and processes
n : nucleus

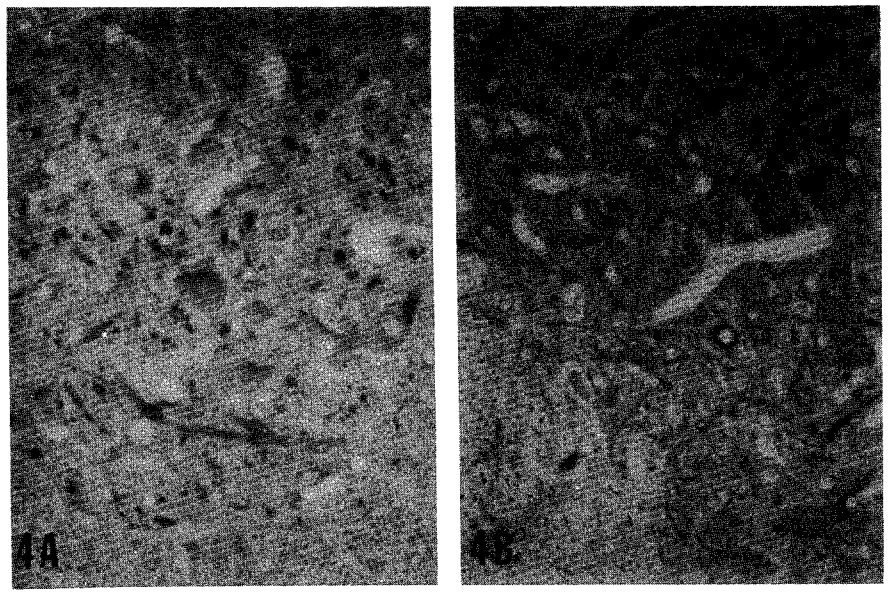

Fig. 4. Immunostain with anti-tubulin antibody (PAP, ×40 original magnification)
: Similar reaction in control(A) and MYT-treated cases(B)

The antibody to non-ph NF stained all neurons in controls and MYT and MYTPR treated animals regardless of presence of acumulated NF bundles (Fig. 3A and 3B). Antibodies to tubulin and MAP-2 showed similar reaction in controls and MYT and MYTPR treated animals(Fig. 4). No immunoreaction was noted with antibodies to tau proteins.

Discussion

Neurofilaments are a major component of neuronal cytoskeleton and are composed of three distinct polypeptides with approximate molecular mass of 68, 145, and 200 KDa subunit (NF-H200, NF-M150, and NF-L68, respectively)[13)14)]. The central core of the NFs is enriched in the NF-L, whereas periodic side arms contain the NF-H. Each of the three subunits is coded by separate genes on murine chromosome 8. In humans, NF-L and NF-M genes are located on chromosome 8, whereas NF-H is found on chromosome 22[15)]. Difference in the regulation of genes coding for each subunit could influence NF structure that could affect NF phosphorylation, transport, and ultimate function[16)17)].

NFs are synthesized and assembled in neuronal perikarya, assembled into a filamentous form, and transported distally through the axons with slow component a(SCa) of axonal transport[18)]. During the axonal transportation phosphorylation of NFs occurs at multiple sites[19)]. Consequently, in normal neurons the ph epitopes of NF proteins are present exclusively in axons and non-ph epitopes are widely distributed in perikarya, dendrites and axons. Most of the phosphorylation is of the two high molecular weight subunits(145 and 200 KDa). These carboxy-terminal domains have been shown to form the side arm projections that connect NFs with each other and NFs and MT to produce a cross-linked matrix of cytoskeleton[14)]. NFs comigrate with MT in a NF-MT superstructure in which side arms made of NFs and other proteins connect NFs and MT in a tridimensional meshwork[20)]. It has been shown that the phosphorylation of NFs results in their conformational and antigenic changes[14)]. Lewis and Nixon[21)] showed that the phosphorylation of NF protein in optic axons advanced in association with the transition of NF from moving to stationary forms. Based on this data, they suggested a hypothesis that the progressive phosphorylation of the tail domain facilitates the radial projection of side arms to favor interactions with axonal elements.

The present study, showing that ph NF proteins accumulate in the perikarya, demonstrates that antimitotic drugs are associated with changes in the processing of NFs within perikarya. Maytansinoids have a specific affinity to tubulin and induce disruption of cytoplasmic microtubules, block axoplasmic transport, and induce an accumulation of 10 nm filaments[7)]. MYT appears to share a binding site on the tubulin molecule with vincristine, however, it has been also hypothesized that an additional binding site specific for MYT exits[22)]. Pathogenetic mechanisms for accumulation of NF after administration of Maytansinoids could be hypothesized as an increased synthesis of NF proteins, and enhanced assembly of NFs from preexisting protein, or a reduction in NF breakdown. Increased synthesis of NF proteins can be examined using labeled cDNA probes to quantitate NF mRNAs on Nothern blots and in situ hybridization preparations[23)]. The presence of ph epitopes of NFs in perikarya could result from several mechanisms. NF proteins retained in perikarya could be aberrantly phosphorylated. On the other hand, NF proteins may become phosphorylated in perikarya and have greater difficulty being transported with slow axonal transport system. Therefore, the perikaryal ph NFs may be retained there for a longer period of time and thus increase the cytoplasmic volume of the neurons affected by various disease. This finding suggests that the abnormal posttranslational modification of the NFs may play an important role in the perikaryal and axonal swellings.

The abnormal occurrence of ph NFs in neuronal perikarya or in proximal axonal segments and dendrites has been documented in other experimental disorders. Rabbits intoxicated with Al salt developed reversible accumulation of ph NFs in perikarya and proximal axons[6)11)12)24)]. Several pathogenic mechanism have been suggested to explain this observation : increased synthesis of NFs, decreased degradation of NFs or alternatively a primary effect on other elements of neuronal cytoskeleton with secondary involvement of NFs. Impairment of axonal transport had been considered as the most like pathogenic mechanism[25)]. NF transport appears to be selectively blocked in the peripheral axons 5~10 mm from the medulla. Selective impairment of NFs transport indicated to a direct action of Al on NF proteins or on other proteins involved in NF transport rather than to a general toxic effect. Recently, Muma et al[26)] showed normal rate of axonal NF transport in Al toxicity. In addition, chronically intoxicated animal with acrylamide showed ph NF protein in perikarya of dorsal root ganglia[27)]. In their model, the alterations in axonal transport may reflect a regenerative response of nerve cells whose distal axons are undergoing degeneration. Ph NFs have been also noted in motor neurons and dorsal root ganglia sonsory neurons following sciatic nerve injury[28)] as well as in nerve transsection[29)]. Similar patterns of NF immunoractivity was also described in perikarya of retinal ganglion cells following transection of optic nerve[30)].

Abnormal distribution of NF immunoreactivity have been also noticed in neurons in major human CNS degenerative disease. In AD, ph epitopes of NF are described in neurofibrillary tangles in a variety of cell populations including hippocampal pyramidal neurons[31)]. In Parkinson's disease, perikarya of dopaminergic neurons in the substantia nigra showed Lewy bodies containing ph NFs[32)]. Several reports demonstrated that ph NFs are abnormally increased in the perikarya and axons of anterior horn motor neurons of sporadic and familial ALS[33)]. In addition, similar findings were described in infantile neurodegenerative diseases[34)], some neurodegenerative disease[35)], and spontaneously occurring animal disorders[36)], as well as in swollen cortical neurons in Creutzfeldt-Jakob disease[37)]. It is not yet known whether these abnormalities in human neurodegenerative disease represent primary cytoskeletal pathology or abnormal response to axonal degeneration.

NFT of AD and Lewy body of Parkinson's disease showed MAP-2 and ubiquitin positivity. MAP-1 and tubulin were positive only in Lewy body and tau reported to be positive in NFT[38-40)]. The meaning of these positive reactions are not yet known. The present study showed similar reactions in experimental and control animals with anti-tubulin, MAP-2, and tau antibodies. Further studies with better defined antibodies may give the different results and may help to understand biochemistry of neuronal degeneration.

Conclusion

The present study showing the presence of ph NF proteins in perikarya and proximal cell processes demonstrates that similar abnormalities occuring in different pathogenetic conditions. In human disease, it is not clear whether theses abnormalities represent primary cytoskeletal pathology or abnormal response to degenerative changes of axon. But, the abnormal presence of ph epitopes in NF accumulating in several different experimental conditions suggest that abnormal phosphorylation of NF is undoubtedly associated with alterations in NF biology and altered functions of affected neurons.

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퇴행성 신경질환에서 보이는 세포골격군의 변화 : 감수분열억제제 투여시의 신경섬유소의 변화에 대한 면역화학적 검색

구 혜 수

이화여자대학교 의과대학 병리학교실

= 국문초록 =

신경세포내의 신경섬유소의 축적과 변화는 사람에서 발생하는 여러질환들과 실험동물에서 일으키는 퇴행성 신경질환에서 흔히 보이는 소견이다. 그중에서 신경섬유의 인화(phosphorylation)의 변화는 여러 퇴행성 질환에서 보고되는 잘 알려진 변화로서 그 정확한 기전은 아직도 모른다. 본연구에서는 감수분열 억제제인 maytansine과 maytanprine을 토끼의 뇌간에 투여하여 신경세포내에 신경섬유소의 축적을 유발하였고 여러가지 항체를 사용하여 신경섬유소의 변화에 대해 면역화학적으로 검색하였다. 본실험에서 감수분열 억제제를 투여한 다음 축적된 신경섬유소는 정상 신경세포에서와 달리 인화된 형태로 세포질내에 존재하는 것이 관찰되었다.

비슷한 결과가 알루미늄중독과 같은 신경세포내의 섬유소 축적의 기전이 다른 경우에서도 관찰되고 있는 것으로 보아 이러한 변화는 신경섬유 축적의 원인으로서 보다는 오히려 결과로서 나타나는 것으로 여겨진다.