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Diagnosis of Traumatical and Hypoxial Changes of the CNS – Immunohistochemical Study

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Summary

The immunohistochemical detection of neuron-specific enolase and b-amyloid percursor protein were used in the group of deceased on craniocerebral injury and those who died of prolonged hypoxy without mechanical injury of the brain. Neuron-specific enolase (NSE) is produced by nerve cells and is a suitable marker for both the damage of neurons and axons. While undamaged nerve cells show immunoreactivity with the antibody anti-NSE, a significant decrease of this protein substance was noticed within two hours both in mechanical injury and in cases of prolonged hypoxy. We noticed the presence of NSE in damaged axons already several minutes after the injury whereas the hypoxy of brain without mechanical injury didn't show any or a very slight reaction of axons when examined with anti-NSE without topographic link to axonal lesion. b-amyloid percursor protein (b-APP) is a low molecular protein which the normal values of are not to be found in axons detected by standard immunohistochemistry. We noticed an increased frequency of appearance of this protein substance in axons changed by injury, while a reactive positivity to anti-body b-APP was to be found only rarely at the brain hypoxy without mechanical injury CNS.

Key words: brain injury – immunohistochemistry – neuron specific enolase – b-amyloid percursor protein – diffuse axonal injury – hypoxy

Souhrn
Diagnostika úrazových a hypoxických změn CNS – imunohistochemická studie

Ve skupině zemřelých po kraniocerebrálním poranění a zemřelých s anamnesou prolongované hypoxie bez mechanického poranění CNS byla provedena imunohistochemická detekce neuron-specifické enolázy a b-amyloid prekurzor proteinu. Neuron-specifická enoláza (NSE) je produkována nervovými buňkami a je považována za vhodný ukazatel poranění neuronů i axonů. Zatímco nepoškozené neurony vykazují pozitivitu při vyšetření s protilátkami anti-NSE, v případě mechanického traumatu i prolongované hypoxie byl pozorován signifikantní pokles této bílkovinné substance již během 2 hodin po úrazu. Zaznamenali jsme přítomnost NSE v poškozených axonech již několik minut po poranění, zatímco v případě hypoxie mozku bez mechanického traumatu nebyla pozorována žádná, nebo jen minimální reakce s protilátkou anti-NSE bez topografické vazby na axony.
b-amyloid prekurzor protein (b-APP) je nízkomolekulární bílkovina, jejíž normální hodnoty v axonech nejsou detekovatelné standardními imunohistochemickými metodami. Zaznamenali jsme přítomnost této bílkoviny v úrazově změněných axonech, zatímco v případech hypoxie mozku bez mechanického traumatu byla pozorována přítomnost b-APP jen ojediněle.

Klíčová slova: poranění mozku – imunohistochemie – neuron-specifická enoláza - b-amyloid prekursor protein – difuzní axonální poranění – hypoxie

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In the forensic medicine practice we come very often across the necessity to diagnose very early stages of injury changes of CNS where the common histological methods prove to be insufficient. The use of immunohisto-chemistry (2, 8, 9, 11) in this sphere represents a very important contribution. It should be named especially neuron-specific enolase which is produced by nerve cells and proves to be a suitable marker for damages of both neurons and axons (1, 10, 12, 13). While the undamaged nerve cells show immunoreactivity with antibody anti-NSE, in the damaged neurons a significant decerase of the protein substance could be detected already within 1 to 2 hours. In opposite in injury changed axons it is possible to detect the presence of NSE already within 30 min

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uses following the injury (5, 6). In our oppinion and experience suitable markers of axon damage may be further considered also b-amyloid percursor protein (3, 7). Normal values of axon b-APP are not detectable by standard immunohistochemistry however in injury changed axons a cummulation of this protein appears within 3 hours in a concentration that may be noticed by means of common immunohistochemistry. Some authors noticed the presence of b-APP also in cases of hypoxic damage of the CNS (4). As the literature doesn't mention any influence of hypoxy to combined detection of both neuron-specific enolase and b-amyloid percursor in the cortex and white brain matter, we decided to execute our own research in a group of deceased on craniocerebral injury and group of deceased with prolonged hypoxy without any mechanical injury of the CNS and compare the findings.

Material and methods

We tested 20 autopsy cases without mechanical injury of the CNS, in which the death was preceded by prolonged hypoxy with the period of survival time 6h up to 14d and 20 cases with craniocerebral injury with the period of survival 40 min up to 3d. The age distribution was 1.5 up to 87 years, the post mortal period was at both groups up to 24 h. Formalin-fixed, paraffin-embedded sections of the cortical and subcortical areas, corpus callosum, capsula interna and pons Varoli were stained with hematoxylin-eosin and Palmgren's silver method for axons. Immunohistochemistry was carried from the same sections. In the first layer monoclonal antibodies of mouse origin anti-NSE and anti-b-APP (Zymed comp.) were used. For the visualisation of the gained primer antibody we used biotin-streptavidine system marked by alcaliphophastase that provided us at the place of a positive finding together with Fast red a red colouring.

Results

In the group of 20 deceased on craniocerebral injury 11 cases, that means 55 %, axonal lesion in the sense of axonal swelling up to the creation of retraction balls were proved (tab. 1). In the majority of cases damaged axons showed positivity in both antibodies. The earliest NSE labelled axons could be detected in case with only 50 min of survival. In 16 cases, that means 88 %, we noticed a decrease of NSE in neurons at the place of contusion and these changes were noticed at the earliest stage in the dead body 2 hours after the injury.

Tab. 1. Mechanical trauma

Survival time	No. of cases	Axonal NSE positivity	Axonal β-APP positivity	Decrease of NSE in neurons
40–60 min	4	2	-	-
1 h–6 h	8	5	4	8
6 h–24 h	6	3	3	6
1 d–3 d	2	1	1	2

In the collection of 20 deaths without a mechanical injury of the CNS when the death was preceded by a prolonged hypoxy, axonal lesions in the sense diffuse axonal injury were not noticed and the swelling of the brain tissue was the predominant finding (tab. 2). In 4 cases a positivity of axons in test with the substance anti-NSE was detected. However these changes were noticed especially in the vicinity of damaged neurons showing loss of NSE and were absent diffusionally in the white matter and they were not linked topographicaly to axonal deformities.

Tab. 2. Hypoxia

Survival time	No. of cases	Axonal NSE positivity	Axonal β-APP positivity	Decrease of NSE in neurons
6 h–24 h	3	-	-	2
1 d–3 d	10	2	2	10
3 d-7 d	3	1	1	3
7 d–14 d	4	1	-	3

In 18 cases in this group a decreased reaction with antibody anti-NSE witnessing damage of nerve cells was noticed. At 3 cases a slight positive reaction in tests with antibody anti-b-APP was noted, however these changes were not topographicaly linked to axonal lesions in the same way as they were absent when tested with antibody anti-NSE.

Discussion

Immunochemical methods represent an important contribution in the diagnosis of traumatic lesions of the CNS. In case of early

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stages of diffuse axonal injury the use of immunuchemistry is necessary to improve the diagnosis made by classical histological methods. We consider especially the evidence of neuron-specific enolase and b-amyloid precursor protein regarding our preceding studies a very sensitive method enabling diagnosis of very early stages of diffuse axonal injury as well as early stages of focal injury of the cortex. Bearing in mind that the literature does not mention the influence of hypoxy to combined test of neuron-specific enolase and b-amyloid precursor protein in cortex and white matter of the CNS we tested our own group of violent death cases. At those who died without proved craniocerebral injury and the death was preceded by a prolonged hypoxy in the majority of cases we noticed decrease even diminishing NSE in neurons witnessing the damage of nerve cells. Typical axonal deformations that were found in cases with diffuse axonal injury were not noticed in this group. We proved focal reaction of axons to antibody anti-NSE only in 4 cases. In tests with antibody anti-b-APP a slight positivity of some axons was found only rarely. Therefore we presume that axonal lesions in the sense of diffuse axonal injury may be considered in accordance with literature a primary brain injury that originates in the moment of the injury and the influence of brain hypoxy on its total range, possibly following, only secondary.

Decrease of reaction with antibody anti-NSE in neurons in most cases of prolonged hypoxy proves on the other hand in accordance with well known data a negative influence of hypoxy to nerve cells.

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