Original Article

NON-CONVENTIONAL LIGHT MICROSCOPIC VIEW OF THE BRAIN MICROANATOMY

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Summary

In the search of an easy and more contrasted light microscopic visualization of the degenerative changes in hamster brain we found that a prolonged treatment with 3,-3' diaminobenzidin (DAB) of brain tissue sections effects more optimal view of the microanatomy of the central nervous system (CNS). The longer exposure to DAB takes place after a non-specific classic avidin-biotin complex incubation of the objects.

In this study we show various morphological signatures of the brain cellular microanatomy using this unconventional method for visualization of the objects in CNS. The final result is a real big contrast of the light microscopic image of the brain microanatomy. Thus we propose a new modification of the histochemical colouration permitting more clear registration of the process of spongiosis in the brain during the development of an experimentally provoked transmissible spongiform encephalopathy in hamster. The quality of the obtained images of the brain microanatomy and especially of the degenerative changes is higher than in other conventional histological procedures. This modification could be useful in the study and diagnostics of the formation of degenerative spongiosis in CNS.

Key words: 3,-3'diaminobenzidin, spongiosis, microanatomy, brain degeneration

Introduction

The detection of the features of the fine brain microanatomy by light microscopy is limited from various factors - generally from the type of the staining (tissue colouration) and the quality of the tissue visualization by the each of the used histochemical procedures. A new optimised result is demonstrated here with a prolonged incubation of the brain tissue sections in 3',3 diaminobenzidine. The DAB application in histology is originally included as component of the different immunohistochemical methods for cell and tissue visualization. By the way, the earliest procedures of immunohistochemistry have been developed as qualitative methods for antigen recognition with a growing use as quantitative assay [1]. Immunocytochemical and enzyme histochemical analyses of the cells and tissues detect the expression of various molecules [2]. In our study we try to obtain a more efficient and contrasted image of the CNS tissue by light microscopy during a routine and longitudinal observation of the brain tissue in the search of degenerative changes. Serial tissue sections from hamster brain were subjected finally to a prolonged exposition to DAB. It's well known that the most of the immunohistochemical methods are invented as approaches to the specific needs of the histology and the microanatomy. As example could be

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mentioned the wide use of the bromodeoxyuridine (BrdU) immunohistochemistry intended and applied for a specific aim - the detection of the newly generated cells [3]. In our case as method of choice we searched a more contrast visualization of the brain tissue for light microscopy facilitating the demonstration of the finest changes in the brain cell structure during the development of degenerative changes. Having in mind that human and animal prion diseases (or so called transmissible spongiform encephalopathies) are manifested with multiple spongiform destructions in CNS we have trying to make easier the registration of the spongiosis. In the incurable (for now!) prion disorders the spongiosis can be classified as the most common histopathological sign during their progression [4]. In this paper we assume the results of applying of a modified histochemical procedure for brain tissue visualization for light microscopy and its use for registration of spongiosis during the development of experimentally provoked by scrapie 263K strain prion neurodegeneration.

Materials and Methods

Animals: Adult five week-old female outbred golden Syrian hamsters (18 animals) were used as source of healthy and degenerative brain tissue.

Infection of 12 animals is made by intracerebral injection into the right hemisphere of 50 µl of 1% w/v brain stock homogenate from 263K scrapie strain ($2.2x10^{11}$ 50% lethal dose/g). Anaesthetized hamsters (injected and controls) were sacrificed at 70^{th} and 87^{th} days after agent inoculation (florid and terminal stages of the experimental prion encephalopathy).

Experimental prion disease scrapie 263K was diagnosed by its progressive clinical signs, and confirmed pathomorphologically for each animal.

Histopathological material

Diseased brains (final number 9) and healthy control brains were fixed overnight in Carnoy's solution at room temperature. After their embedding in paraffin were obtained serial transversal sections 5-7 µm thick from selected levels using *Leica* paraffin microtome.

A basic *non-specific incubation* of each group sections was performed for 30 min using avidin-biotin complex (R.T.U. Vectastain, ABC Reagent, VECTOR Labs, cat.No: PK-7100) containing both DH avidin and biotinilated horseradish peroxidase H.

The visualization of the brain microanatomy and especially spongiosis was made histochemically by a longer exposure (10-15 min) of the tissue sections to DAB substrate kit for peroxidase (cat. N. SK-4100, VECTOR, CA USA).

***Studied CNS regions*:** cortex cerebri, thalamus, hippocampus, cerebellum.

Results

A really contrasted and detailed light microscopic image of the brain microanatomy is the result of the application of described above histochemical staining modification. Digitised images were taken from all studied CNS regions (thalamus, hippocampus, cortex and cerebellum) where we searched earliest signs of the brain degeneration and especially spongiosis. Due to the maximally contrasted staining of the brain tissue the spongiform vesicles become easy to register in neurodegenerative model (Fig.1). One essential contribution of the proposed here procedure is its capacity for demonstration of any initial spongiform changes in the brain without the help and using of electron microscopy or other more complicated methods. This staining procedure applied on brain tissue from control healthy animals shows very detailed and contrasted brain microanatomy (Fig. 2). In terminal stage of the experimental spongiforme encephalopaty (in this model the 80^{th} and more days after agent inoculation) the same procedure detects the appearance and increases in size of oval spongiform destructions of the brain structure. During initial clinical period (70^{th} day of experimental disease) could be seen rarely dispersed very small perforations of the tissue integrity situated generally in the neuronal cytoplasm (Fig. 3). It is interesting that the process is initialised firstly always in the cytoplasm of the biggest neurons of the cerebrum (cortex and thalamus). Later begins the formation of most large spongiforme vesicles usually in the places of disappeared apoptotic neurones. As illustrated in Fig. 4, the brain sections taken from degenerative cortex and prepared following proposed staining modification display very dramatic picture of seriously injured tissue. A drastically lacerated regions in CNS could be observed light microscopically in the places of gradually disappeared big neurons.

Compared to the healthy hamster brains as control spongiform vesicles in the terminal stage of the experimental scrapie 263K reveal specific histologic view that is not easy to miss during a serial microscopic observation of the sections (Fig.5). Another important priority of the proposed staining procedure is the possibility for registration by light microscopy the initial appearance of the spongiform objects and their origin (Fig. 6).

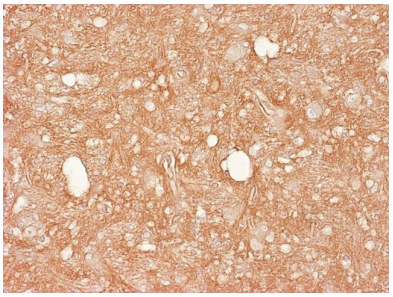

Figure 1. Modification of the brain tissue histochemical staining. Spongiform vesicles in hamster CNS. Thalamic brain region during terminal stage of neurodegeneration. Scrapie 263K strain model in hamster. Light microscopy. X 100.

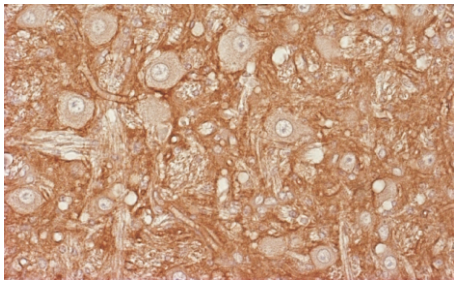

Figure 2. The same staining procedure applied in control healthy animals shows detailed and contrasted light microscopic view of the local brain microanatomy. Cortex. Light microscopy. X200.

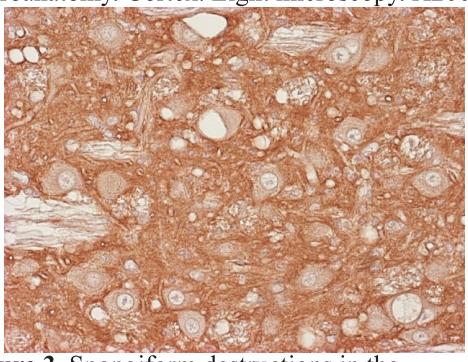

Figure 3. Spongiform destructions in the degenerative brain. Initial small perforations in the neuronal cytoplasm. 70th day after agent inoculation. Hippocampal area. Light microscopy. X200.

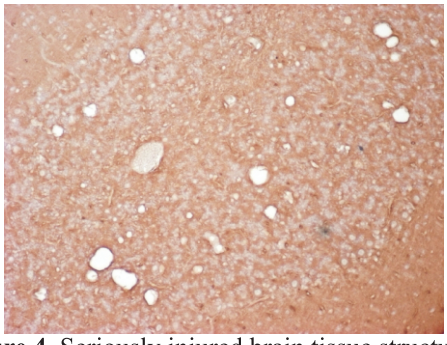

Figure 4. Seriously injured brain tissue structure during the terminal stage of experimental scrapie 263K. Cerebellum. Light microscopy. X63.

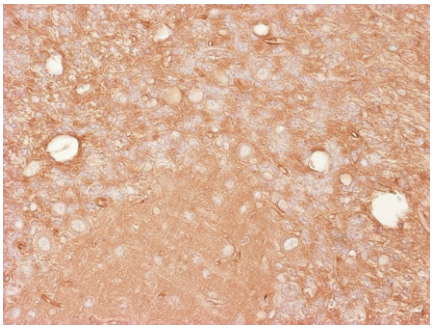

Figure 5. Histochemical visualization of the spongiosis in the terminal stage of the experimental scrapie 263K. A specific view that is not easy to miss during a serial light microscopic observation of the brain regions. Cerebellum. Light microscopy. X100.

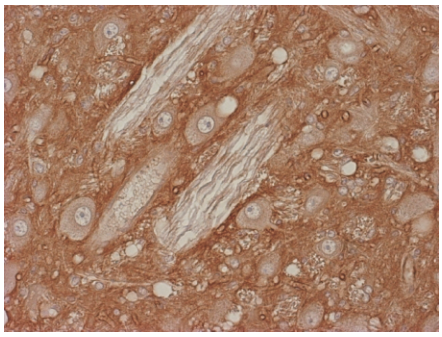

Figure 6. Diagnostics of the initial appearance of spongiform vacuoles during the early clinical stage of the experimental scrapie 263K. Cortex. Light microscopy. X 200.

Discussion

The growing need for new and easy applicable histochemical procedures for serial and routine light microscopic diagnostics of the brain degenerative changes is the reason for development of proposed staining modification. The efforts for increasing the effectiveness of the studies on brain degeneration at light microscopic level lead to proposition of staining procedures with more contrast tissue colouration of CNS applicable on cryostat and microtome tissue sections. Here, based on non-specific but selective binding of biotinilated horseradish peroxidase H to the brain tissue components the visualization of CNS microanatomy is obtained by a prolonged incubation of the brain sections with 3,-3' diaminobenzidine derivatives from DAB substrate kit for peroxidase. Having in mind that brain spongiosis is a general anatomo-pathologic characteristic of the transmissible spongiform encephalopathies (prion diseases) the rapid diagnostics of these fatal disorders in very great extent count on histochemical study of the degenerative tissue [5]. Generally, in a classic screening of the neurodegeneration the serially taken brain slices are assessed morphologically and scored for spongiosis, prion protein deposition and neuronal lost [6]. Spongiosis is the hallmark lesion in the degenerative prion diseases [7] and this justifies our attempts for developing a rapid checking of this indicative for degeneration. The field of application of the proposed procedure is wider - there are many other types of spongiform formation in CNS. Another degenerative status in CNS is registered independently from the prion infection. This is so called cerebellar spongiform degeneration described earlier [8] and presenting a type of non-prion degenerative changes provoked in brain by heavy metal intoxication. Other places in human pathology where proposed staining could be applied are the approaches in the studies of the spongiphorm-like vacuolations during the development of several metabolic and toxic human encephalopaties [9, 10]. The question is: why is necessary to improve the simple light microscopic procedure like our when there are many other most efficient methods for brain

tissue visualization? The answer is that for the needs of each routine histological screening of the degenerative changes an easy-accomplishable and rapid procedure has a preference for other specific methods. Modifications of light microscopic researches in neurobiology are applicable for many other aims: studies of the neuronal shape, analysis of the cellular networks in the brain or dynamic changes of the cell microenvironment during neuronal degeneration [11, 12, 13]. In all these cases there is place for more simple light microscopic methods together with the specific Golgi impregnation, electron microscopy, immunohistochemistry and genetic reporters used in a complex study of the brain microanatomy.

Conclusions

In this paper we propose a new modification for routine histochemical tissue colouration permitting more clear and contrasted study of the brain microanatomy on the light microscopic level. This modification is suitable for serial histochemistry and especially for large series of brain tissue where rapid and not so expensive histochemical methods are indispensable. The quality of the obtained light microscopic images is higher than in the conventional histological procedures and could be useful for diagnostics of the formation of spongiform objects in CNS.

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