ZEBRAFISH EXPERIMENTAL MODEL FOR AUTOIMMUNE ENCEPHALOMYELITIS

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Abstract: *Background: Demyelinating diseases place a significant burden on both patients and health systems. Experimental autoimmune encephalomyelitis is used in demyelinating disease research, usually on mammalian species. Zebrafish are increasingly used in biomedical research due to their low cost and relative similarity with humans. Objective: Generation of a zebrafish model of autoimmune encephalitis. Materials and methods: Adult zebrafish lots received an intramuscular injection of salmon central nervous system emulsified in complete Freund's adjuvant in volumes of 1 and 2 μl, respectively. A control lot was kept in similar conditions. After 4 weeks, demyelination was evaluated with Luxol Fast Blue staining and image analysis in FIJI, and histological examination for inflammatory changes. Results: Over 60% of the lots exposed to both 1 and 2 μl of central nervous extract showed microglial hyperplasia and significantly higher demyelination compared with controls. Conclusions: The results suggest autoimmune demyelination. However, additional investigations are required to validate the model.*

INTRODUCTION

A demyelinating disease is any disease of the nervous system in which the myelin sheath of neurons is damaged.(1) The reduction in conduction ability causes deficiency in sensation, movement, cognition, or other functions depending on which tracts are involved.

Demyelinating diseases are severely debilitating pathologies, with a significant impact on the quality of life of the patient and on public health systems.

The etiologic agents of demyelinating diseases include genetic factors, autoimmune reactions, infectious agents, and unknown factors.(2)

Experimental autoimmune encephalomyelitis (EAE), or experimental allergic encephalomyelitis, is an animal model of inflammatory demyelinating diseases of the central nervous system (CNS, including multiple sclerosis and acute disseminated encephalomyelitis.(3)

EAE can be induced in a number of species, including mice, rats, guinea pigs, rabbits and primates.(4) In rodents, the most commonly used antigens are spinal cord homogenate, purified myelin, myelin protein such as myelin basic protein MBP), myelin proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), or peptides of these proteins, all resulting in distinct models with different disease characteristics regarding both immunology and pathology.(5,6)

Zebrafish (Danio rerio) is used as an experimental model due to its small size, high fecundity, lower maintenance costs than rodents, and relative similarities with man.

Thus, a zebrafish model of EAE is a potentially useful addition to demyelinating disease research strategies.

MATERIALS AND METHODS

Animals

Wild type zebrafish with ages between 4-6 months, with a weight of 562±115 mg, were obtained from a store (Hornbach) and divided into 3 lots: control (n=6), low-dose CNS extract (n=8) and high-dose CNS extract (n=9).

Preparation and administration of CNS extract

Commercially obtained salmon (Salmo salar) encephalus was frozen and extracted whole, homogenized with a pestle, and emulsified in complete Freund's adjuvant (Sigma-Aldrich, Germany) by aspiration and ejection with a 2 ml syringe for 10 minutes. The emulsion was prepared fresh on the morning of administration, in concentration of 1 g encephalus/1 ml complete Freund's adjuvant.

The fish were anesthetized by immersion for 5 minutes in a solution of lidocaine (0.3 mg/ml). The emulsion was administered by intramuscular injection with a Hamilton syringe, 2 mm ventral to the lateral line and 3 mm caudal to the genitourinary orifice, in doses of 1 µl (low dose lot) and 2 µl (high dose lot), respectively.

Euthanasia and histopathological processing

Four weeks after the injection, the zebra fish were euthanized by immersion in ice water, as recommended by the RSPCA zebrafish guide, followed by fixation in 10% formaline. The brains were removed, paraffin embeded and serially sectioned.

Six to eight microtome sections were made for each individual. The slides were stained with Hematoxylin-Eosin (HE) for morphologic evaluation, Luxol Fast Blue (LFB), to determine myelin content, and CD3 immunohistochemistry kit, to determine T lymphocyte presence.

Histological analysis

HE stained slides were examined for lymphocyte and histiocyte infiltrates, oligodendroglial depletion, microglial hyperplasia and axonal degeneration. LFB stained slides were examined for the presence of demyelination areas, determined by reduced staining intensity (hypochrome areas). In order to avoid including staining artifacts or anatomical structures, only clearly demarcated hypochrome areas, present on at least two consecutive sections, without bilateral symmetry were considered relevant.

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Image acquisition and computerized analysis

Each section was examined with a Nikon Eclipse E200 microscope and photographs were taken under constant light and exposure conditions.

To determine demyelination extent, the percentage of hypochrome areas out of the total brain section area was measured in the FIJI distribution of ImageJ.

Parameter values per individual were obtained by averaging the values obtained for each section.

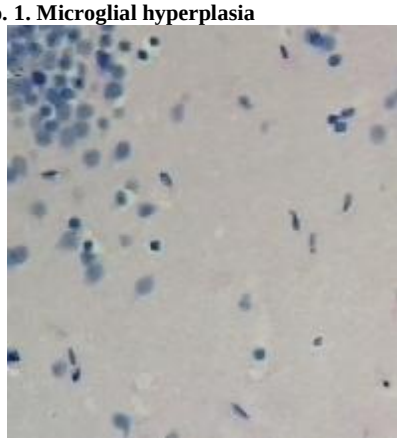
Statistical analysis

The results are expressed as mean ± standard deviation and a p value of <0.05 was considered statistically significant, calculated in Microsoft Excel using two tailed Student t test.

RESULTS

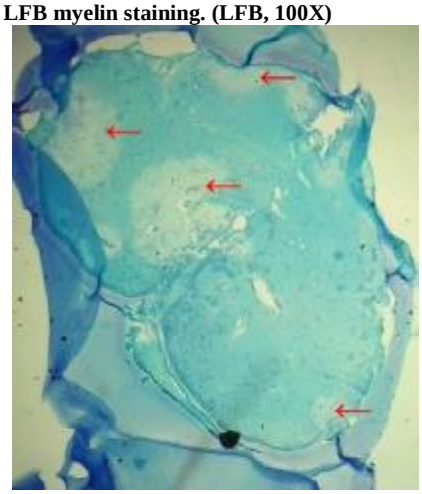
Microglial hyperplasia (figure no.1) was absent in the control lot. The lot exposed to low dose CNS extract showed microglial hyperplasia in 75% of the examined sections, while the lot exposed to high dose CNS extract showed microglial hyperplasia in 66.6% of the examined sections.

Figure no. 1. Microglial hyperplasia



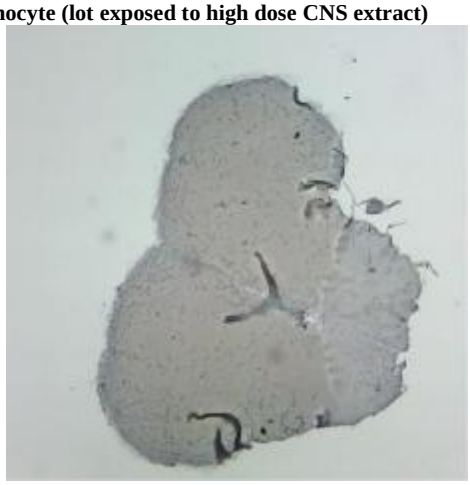
Prevalence of hypochrome areas (figure no.2) was 16% in the control lot. The lot exposed to low dose CNS extract presented a prevalence of hypochrome areas of 62.5%, and the lot exposed to high dose CNS extract presented a prevalence of hypochrome areas of 66.6%, both significantly higher than the control.

Figure no. 2. Clearly demarcated, asymmetrical hypochrome areas in LFB myelin staining. (LFB, 100X)



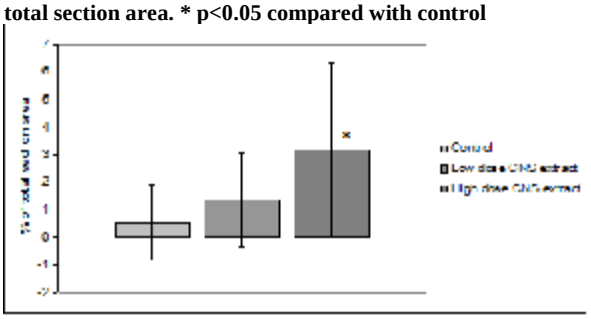
Neither the control lot, nor the lot exposed to low dose CNS extract, or the one exposed to high dose CNS extract presented any histiocytes, inflammatory infiltrate, oligodendroglial depletion or axonal degeneration. CD_3 immunohistochemistry for the identification of T cell lymphocyte infiltrates was "0" negative (figure no. 3).

Figure no. 3. D. Rerio brain, "0" negative for CD3 lymphocyte (lot exposed to high dose CNS extract)



The control lot had a percentage of hypochrome area compared to total area of the examined sections of 0.56±1.37%. The lot exposed to low dose CNS extract had a percentage of hypochrome area compared to total area of the examined sections of 1.37±1.77%, while the lot exposed to high dose CNS extract had a percentage of hypochrome area compared to total area of the examined sections of 3.18±3.16%, which was significantly higher compared with the control (p=0.035) (figure no. 4).

Figure no. 4. Percentage of hypochrome areas compared to total section area. * p<0.05 compared with control



DISCUSSIONS

In the reviewed literature, one of the methods used to highlight demyelination is the identification of hypochrome areas in Luxol Fast Blue.(7,8,9) In the current study, exposure to CNS extract caused the appearance of hypochromic areas in over 60% of individuals independent of the amount of antigen, suggesting an autoimmune mechanism.(10)

In terms of lesion extension, the group exposed to high dose CNS extract presented hypochrome areas on 3.18 ± 3.16% of the total area of the examined sections, significantly higher compared to the control (0.56 ± 1.37%). Examined rodent studies obtained values of 2.3-30.7% of the corpus callosum surface area for cuprizone (11) and 12% of the surface area of the white matter in medullary sections for experimental autoimmune encephalitis.(12) Although the present study

obtained values at the lower limit of the interval, species differences and methodology on quantification of lesions do not allow a thorough comparison with these studies.

In the present study, the administration of salmon SNC emulsion to zebrafish resulted in the appearance of microglial hyperplasia in over 60% of the experimental animals. Although hypochrome areas do not consistently coincide with microglial hyperplasia, both are encountered in experimental allergic encephalomyelitis.(13)

The obtained results suggest an experimental model of demyelination by autoimmune mechanism in zebra fish. This possibility is supported by the documentation in previous studies of autoimmune processes in zebra fish.(14)

CONCLUSIONS

Administration of salmon CNS extract at doses of 1 and 2 µl caused the appearance of hypochrome areas in Luxol Fast Blue stained slides and microglial hyperplasia, suggestive of demyelination. However, further research is needed to validate the model.

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