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SCIENCE DIRECT
Experimental Neurology 198 (2006) 65–71
Experimental Neurology
www.elsevier.com/locate/yexnr

Early paranodal myelin swellings (tomacula) in an avian riboflavin deficiency model of demyelinating neuropathy

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Received 18 July 2005; revised 17 October 2005; accepted 25 October 2005
Available online 5 December 2005

Abstract

Introduction: Disruption of the complex architectural and molecular organization of the paranodal region of myelinated peripheral nerve fiber may initiate the evolving time dependent process of segmental demyelination. In support of this notion was the finding of focal paranodal myelin swellings (tomacula) due to redundant folding of myelin sheaths, early in the time course of an avian riboflavin deficiency model of demyelinating neuropathy.
Methods: Newborn broiler meat chickens were maintained either on a routine diet containing 5.0 mg/kg riboflavin (control group) or a riboflavin-deficient diet containing 1.8 mg/kg riboflavin. Riboflavin concentrations in the liver were measured at postnatal day 11. Peripheral nerves were morphologically examined at days 6, 11, 16 and 21 using light and electron microscopy and teased nerve fiber techniques.
Results: Riboflavin-deficient chickens showed signs of a neuropathy from days 8 and pathological examination of peripheral nerves revealed a demyelinating neuropathy with paranodal tomacula formation starting on day 11. Paranodal tomacula consisted of redundant myelin infoldings or outfoldings, increased in size and frequency after day 11. After day 16, the paranodal swellings showed prominent degenerative changes accompanied by an increased frequency of myelinated fibers showing demyelination.
Conclusion: Tomacula due to redundant myelin folds are generally considered a remyelination phenomenon, yet in this avian riboflavin deficiency model of demyelination, the paranodal tomacula occurred early in the course of demyelination.

Keywords: Riboflavin; Demyelinating neuropathy; Tomacula; Paranode

Introduction

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine), a water-soluble vitamin (vitamin B2), is a precursor of flavin mononuleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD are coenzymes for numerous oxidases and dehydrogenases in eukaryotic cells (Rivlin, 2001). Riboflavin deficiency leads to impaired β-oxidation of fatty acids with preservation of the tricarboxylic acid cycle and electron transport chain (Ross and Hansen, 1992). It has long been recognized that chickens fed a riboflavin-deficient diet develop a peripheral neuropathy characterized by segmental demyelination with endoneurial edema, hypertrophic Schwann cells and lipid deposition in the cytoplasm of Schwann cells (Johnson and Storts, 1988; Jortner et al., 1987; Phillips and Engel, 1938a,b; Wyatt et al., 1973). It is a characteristic feature of this model that chickens start to recover spontaneously after 3 weeks probably due to the endogenous riboflavin produced by intestinal bacteria and a declining riboflavin requirement with increasing age and decreasing growth (Johnson and Storts, 1988; Jortner et al., 1987). Similar riboflavin-deficient peripheral neuropathies have been reported in the dog (Street et al., 1941), rat (Norton et al., 1976; Shaw and Phillips, 1941), pigeon (Wada et al., 1996) and humans (Lane et al., 1964, 1975).

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0014-4886/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2005.10.028

Materials and methods

Studies were conducted on broiler meat chickens (Cobb 500, Cobb-Vantress Inc, Arkansas, USA) supplied by Inghams Enterprises Pty Ltd., Adelaide, South Australia. Day-old chickens were maintained ad libitum either on a routine diet containing 5.0 mg/kg riboflavin (Starter Ration, Ridley Agriproducts, Murray Bridge, South Australia) or a riboflavin-deficient diet containing 1.8 mg/kg riboflavin (Specialty Feeds, Perth, Western Australia). Except for riboflavin levels, these two diets were complete and had the same composition. The normal riboflavin requirement for young, rapidly growing broiler (meat-type) chickens is 3.6 mg/kg of feed (Nutrient Requirements of Poultry, 1994. National Academy of Sciences, National Academy Press, Washington, DC, USA). Chickens were clinically evaluated and weighed daily. They were killed on postnatal (PN) days 6, 11, 16 and 21 (Table 1).

Peripheral nerves were fixed by transcardiac perfusion with 4% paraformaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Sciatic and brachial nerves were collected and processed according to a published method for light and electron microscopy and teased nerve fiber studies (Cash and Blumbergs, 1995). Dorsal root ganglia, spinal cord and brain were also collected for routine histopathological evaluation.

Ten chickens (5 fed a conventional diet and 5 the riboflavin-deficient diet) were killed on PN11 and fresh livers collected for riboflavin analysis (Division of Laboratory Medicine, Royal Perth Hospital, Perth, Western Australia).

This experimental protocol was approved (83/04) by the Animal Ethics Committee of the Institute of Medical and Veterinary Science, Adelaide, and conformed to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Results

Clinical manifestations and riboflavin concentrations

No neurological dysfunction was observed in the first week. On PN8d, 5/31 riboflavin-deficient chickens had an unsteady gait and, although alert, were reluctant to move unless driven. By PN11d (11/25) and PN16d (9/11), chickens became progressively more paretic and showed signs previously reported in naturally-occurring riboflavin deficiency (Klasing and Austic, 2003; Summers et al., 1995). Some birds were unable to rise from their hocks, limbs were extended and toes often turned inward. The latter clinical sign, termed “curled toe paralysis”, is characteristic of avian riboflavin deficiency (Johnson and Storts, 1988; Jortner et al., 1987; Phillips and Engel, 1938a,b; Wyatt et al., 1973). The wings often drooped and were frequently used as an aid in walking. A reluctance or inability to move to feed resulted in progressive growth retardation. Incomplete recovery was noted in 3/5 remaining chickens starting on PN18d. No neurological signs were found in control birds fed a conventional diet.

Table 1
Control and treated chickens in this study

	PN6d	PN11d	PN16d	PN21d
Control animals on conventional diet	6	8	5	5
Treated animals on VB2-deficient diet	6	14	6	5

The average body weight of riboflavin-deficient chickens was significantly lower than that of control birds and this trend persisted until the completion of this study (data not shown here). The riboflavin concentration in the liver of PN11d riboflavin-deficient chickens (mean ± SD = 23.94 ± 1.49 μg/g) was significantly (student t test, $P < 0.01$) lower than that of control birds (mean ± SD = 32.36 ± 1.96 μg/g) fed a conventional diet.

Light microscopy

The results of transverse plastic section studies of peripheral nerves are summarized in Table 2. No pathological alterations were detected in peripheral nerves from control birds. The myelinated fiber (MF) density, size and appearance in PN6d riboflavin-deficient chickens (Fig. 1B) were comparable to those in age-matched control chickens (Fig. 1A).

Focal myelin swellings were a characteristic finding in riboflavin-deficient chickens, and were first detected in sciatic and brachial nerves at PN11d (Fig. 1D). Myelin swellings increased in size and frequency by PN16d (Fig. 1F). The focal expansions of myelin were due to asymmetrical myelin infolding or outfolding (Figs. 1D and F). Complex myelin folds were often associated with myelin splitting and degeneration (Fig. 1F). These changes became more severe with increasing age of the birds, and by PN16d and PN21d tended to obscure the original redundant myelin folds in some fibers. Longitudinal sections revealed that the complex myelin foldings were located in paranodal (including juxtaparanodal) regions with preservation of the internodal myelin sheath (Fig. 2). Complex myelin infolding or outfolding was not found in internodal regions when lengths of up to 1 mm were examined. Simple myelin infolding or outfolding (Figs. 1A and C) was also found in control birds, but complex folding with myelin splitting and degeneration was not detected.

Large axons not surrounded by a discernible myelin sheath under oil immersion (×1000) (naked axons suggestive of demyelination) were a common finding in riboflavin-deficient chickens. They were first noticed at PN11d, and with the passage of time became obvious at PN16d (Fig. 1F) and more prominent at PN21d (Fig. 1H). Remyelination, as evidenced by disproportionately thin myelin sheaths compared to the axonal diameter, was first detected at PN16d and became more apparent at PN21d (Fig. 1H).

Increased endoneurial space between individual MFs, suggestive of endoneurial edema, lipid deposition in Schwann cell cytoplasm and hypertrophic Schwann cells were first observed at PN11d, and became more apparent at PN16d and PN21d.

No lesions were found in dorsal root ganglia, spinal cord or brain.