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SCIENCE DIRECT
Experimental and Molecular Pathology 81 (2006) 92–99
Experimental and Molecular Pathology
www.elsevier.com/locate/yexmp

ATPases and lipid peroxidation in the rat sciatic nerve in the course of experimental neoplastic disease

Sławomir Michalak [a,b,*], Zofia Adamczewska-Goncerzewicz [a], Jozef Szczech [a]

[a] Department of Neurochemistry and Neuropathology, University of Medical Sciences, 49, Przybyszewskiego str., 60-355 Poznań, Poland
[b] Neuroimmunological Unit, Medical Research Center, Polish Academy of Sciences, Poznań, Poland

Received 6 September 2005, and in revised form 11 October 2005
Available online 28 December 2005

Abstract

Peripheral nerve involvement in the course of neoplastic disease represents a clinically significant complication, with clinical uncertainties raising questions as to its pathophysiology. The aim of this study was the analysis of ATPase activities, lipid peroxidation and sulfhydryl groups in the sciatic nerve of tumor-bearing rats. We investigated also morphometric features of the sciatic nerve of experimental animals. An increase was noted in Na^+/K^+-ATPase and Mg^{+2}-ATPase activities and elevation of conjugated diene and malonyldialdehyde contents, associated with a decrease in sulfhydryl groups in Morris-hepatoma-bearing rats. The morphometric evaluation revealed myelin sheath thickening, associated with an increase in axon cross-section area and degenerative changes in dorsal horns.

In this study, the moderate lipid peroxidation in experimental neoplastic disease was demonstrated to lead to depletion of sulfhydryl groups in the degenerating rat sciatic nerve which was associated with stimulation of ATPase activities.

Keywords: ATPases; Lipid peroxidation; Sulfhydryl groups; Morphometry; Sciatic nerve; Experimental neoplastic disease

Introduction

Peripheral neuropathy develops in the course of neoplastic disease as a result of chemotherapy, infiltration, metabolic disturbances, cachexia and as a paraneoplastic syndrome. The frequency of paraneoplastic neuropathy depends on diagnostic criteria and diagnostic methods used. According to McLeod (1993), 5% of cancer patients manifest neuropathies, 12% present abnormalities on quantitative sensation testing and 40% patients manifest electrophysiologic abnormalities. Neuropathies were observed most frequently in patients with small cell lung cancer (Camdessanche et al., 2002), prostate cancer (Camdessanche et al., 2002; Luengo-Marquez et al., 2003), monoclonal gammapathies, Hodgkin disease, lymphoma (Karup and Crone, 2002) and breast cancer (Peterson et al., 1994).

Peripheral nerve involvement in the course of neoplastic disease may be classified basing on distribution, pathomechanisms involved, pathological features and electrophysiological findings.

Focal nerve lesions develop as a result of neoplastic infiltration and radiation therapy, particularly in brachial and lumbosacral plexus, as an effect of vasculopathy with asymmetric lesions to axons and as a paraneoplastic reaction in brachial plexus or with asymmetric lesions of axons. On the other hand, polyneuropathies may result from chemotherapy, and, in such cases, they manifest as sensory or sensorimotor polyneuropathies or as paraneoplastic syndromes involving sensory and motor fibers, sensory ganglions, anterior horn motoneurons or autonomic nerve fibers (Karup and Crone, 2002).

Subacute sensory neuropathy, sensorimotor paraneoplastic neuropathy, paraneoplastic vasculitic neuropathy, acute and chronic inflammatory demyelinating polyneuropathy and mononeuritis multiplex are the most frequent clinical manifestations (Cher et al., 1998; Antoine et al., 1999).

Since 1965, when Wilkinson and Żeromski (1965) described anti-neuronal antibodies in lung cancer patient, who manifested sensory neuropathy, the pathomechanism believed to be responsible for the development of neurological

* Corresponding author. Department of Neurochemistry and Neuropathology, University of Medical Sciences, 49, Przybyszewskiego str., 60-355 Poznań, Poland. Fax: +48 61 8691 697.
E-mail address: slamic@yahoo.com (S. Michalak).

0014-4800/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2005.10.003

paraneoplastic syndromes has been thought to be immune-mediated. However, there exist groups of seronegative patients with true paraneoplastic neuropathies. In the study of Antoine et al. (1999), a group of 26 patients with paraneoplastic neuropathy was differentiated into 7 seropositive cases and 19 patients with no antibodies present. The observation of patients with absence of anti-onconeural antibodies and/or any other immunological markers raises the question of pathophysiology of paraneoplastic syndromes.

Furthermore, the unsatisfactory effect of immunomodulation treatment, even if a vigorous one (Keime-Guibert et al., 2000), brings doubts as to the pathomechanism involved in paraneoplastic neuropathy, indicating that not only irreversible neuronal degeneration but also various reactions of unknown nature are possible.

Morphological examination of peripheral nerve biopsies in patients with anti-onconeural antibodies revealed fiber loss and Wallerian degeneration, and thinning of myelin sheath in some cases (Antoine et al., 1999), in the patient with anti-disialosyl antibody, a decrease in mean diameters of large myelinated fibers was observed as well (Kobayashi et al., 2005). In seronegative patients, neuropathological examination showed fiber loss, Wallerian degeneration, mononuclear cell infiltrations in endoneurium, epineural vasculitis (Antoine et al., 1999) and reduction of the myelin sheath cross-section area per endoneural cross-section area in sural nerves (Wang and Schroder, 1998).

In subacute sensory neuropathy (SSN), neurophysiological examination revealed diminished sensory potentials, mildly reduced conduction velocities, no sensory responses, normal motor conduction velocities, amplitudes of the motor responses and normal EMG findings (Karup and Crone, 2002). In neurophysiological examination, sensorimotor neuropathy was characterized by denervation activity in EMG (Trojaborg et al., 1967) and by abnormalities in conduction in some cases (Lenman et al., 1981).

In our study, we used a transplantable Morris hepatoma 5123 which originated from the cell line obtained after treatment of Buffalo-strain rats with *N*-(2-fluorenyl)-phtalamic acid. The observations made by Morris and other authors revealed lung metastases 6 weeks after tumor inoculation with no infiltration in the central or peripheral nervous system. Microscopically, the tumor was identified to represent hepatocellular carcinoma (Morris et al., 1960).

Screening various potential experimental approaches, we have chosen Morris hepatoma because there are only few cases noticed in literature manifesting neuropathy in the course of hepatocellular carcinoma (Calvey et al., 1983; Phanthumchinda and Rungruxsirivorn, 1991; Nishiyama et al., 1993; Hatzis et al., 1998).

The aim of this study was to examine the effect of advanced experimental neoplastic disease on morphometric features, lipid peroxidation, sulfhydryl groups (as antioxidants mainly represented by glutathione) and on ATPase activities in the sciatic nerve in the rat. In contemporary literature, no data are available which would concern the problems.

Lipid peroxidation, antioxidants and ATPase activities were investigated in particular in studies on diabetic neuropathy (Vincent et al., 2004; Jain and Lim, 2001; Osawa and Kato, 2005). This common neurological complication leads to increased content of the indices of oxidative stress in peripheral nerves, in association with a reduction in glutathione level (Low et al., 1997). However, also the toxic neuropathy induced by dithiocarbamate and disulfiram was associated with lipid peroxidation even if it was not recognized if it is an event contributing to demyelinization or represents its consequence (Tonkin et al., 2004).

Basing on observations of the role of lipid peroxidation, antioxidants and ATPase activities in metabolic and toxic neuropathies, we undertook our study in experimental neoplastic disease.

Material and methods

Adult male Buffalo strain rats, 3½ months of age (300–350 g), were used in our experiments. The homogenate of Morris hepatoma 5123 was inoculated intramuscularly (0.5 mL) into the left hind limbs of the animals. Twenty one days after tumour transplantation, the rats were sacrificed using halothane anesthesia and perfused with a 4% neutral formalin solution. At this time, the animals presented clinical signs of cachexia, and their body mass was reduced by nearly 30%. Previous observations of the model demonstrated that at 21st day there were no metastases, which appeared at first after 6 weeks in lungs. The right sciatic nerve and spinal cord were removed and fixed by immersion in neutral formalin solution. The tumor was dissected and used for morphological examination.

The control and experimental groups each included ten rats.

A light microscope examination was performed using the JENAVAL (Carl Zeiss, Jena) instrument, a color Video Camera (CCD, Sony) and, for the image saving and quantitative morphometric examination, MultiScan software (Computer Scanning System 2, Poland). Among the morphometric parameters, we examined fiber circumference, fiber cross-section area, axon circumference, axon cross-section area and myelin cross-section area.

In addition, the following indirect ratios were calculated:

- fiber cross-section external shape ratio (squared fiber circumference/fiber cross-section area),
- fiber cross-section internal shape regularity ratio (axon cross-section area/fiber internal cross-section area),
- axon cross-section shape ratio (squared axon circumference/axon cross-section area),
- ratio of myelin to axon cross-section areas (g-ratio).

The results of morphometric studies were tested with Kolmogorov–Smirnov, Liliefors and Shapiro–Wilk tests for normality, and significance of respective differences was tested with the non-parametric Mann–Whitney test.

Spinal cord samples were stained using hematoxylin–eosin and Klüver–Barrera techniques.

Activities of Na^{+}/K^{+}-ATPase, Ca^{+2}-ATPase and Mg^{+2}-ATPase were estimated by means of the modified technique described by Samson and Quinn (1967) using adequate concentrations of sodium, potassium, magnesium and calcium ions and ouabain inhibition. The activity was estimated basing on the amount of the released inorganic phosphorus, analyzed with the technique of Bartlett (1959) and expressed in micromoles per minute per milligram of protein.

Lipid peroxidation was estimated with the use of Recknagel and Glende (1984) procedure for conjugated dienes and Ohkawa technique (Ohkawa et al., 1979) for malonyldialdehyde content. The content of each of them was expressed per milligram of protein, estimated according to Lowry et al. (1951). Sulfhydryl group content was analyzed basing on the spectrophotometric method with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) according to Ellman (1959) and expressed per milligram of protein.