Original Article

COMPARISON OF THE MORPHOLOGICAL FINDINGS IN TWO RAT MODELS OF NEUROPATHIC PAIN

Lachezar K. Surchev[1], Slavina K. Surcheva[2], Nikolay S. Yanev[2], Lubomir N. Kasakov[3], Mila V. Vlaskovska[2]

[1]Department of Anatomy, Histology and Embryology
[2]Department of Pharmacology and Toxicology
Medical Faculty
Medical University Sofia
[3]Institute of Neurobiology
Bulgarian Academy of Sciences
Sofia, Bulgaria

Corresponding Author:
Slavina Surcheva
Department of Pharmacology
Faculty of Medicine
Medical University Sofia
2 Zdrave str.
Sofia, 1431
Bulgaria
e-mail: slavina_s@yahoo.com

Summary

Up to now the mechanism of neuropathic pain appears to be enigmatic. The progress in its discovery as well as in its problematic treatment requires study of experimental animal models. Therefore, in this study we aimed to compare the morphological and behavioural changes induced by two models of neuropathic pain. The chronic constriction injury model is a model of neuropathic pain caused by unilateral loose ligation of the sciatic nerve. Streptozotocin-induced (70 mg/kg, i.p.) diabetes (hyperglycemia ≥ 14 mmol/l) is a model of diabetic neuropathy. The experiments were carried out on adult male Wistar rats (200-250g) and electron microscopy was applied to reveal the changes. The diabetic neuropathy was characterized by partial separation of the axolemma from the myelin sheath, primary demyelination, signs of Schwann cell dysfunction, hypertrophy of the basal lamina. Furthermore, the chronic constriction injury was accompanied by small or large focal destruction of the myelin and an increased number of mitochondria in the axonal profiles of the spinal cord. Therefore, application of combined models is probably better suited to unravel the key mechanisms causing neuropathic pain.

Keywords: chronic constriction injury, diabetic neuropathy, analgesia, streptozotocin, pain.

Introduction

According to the most conventionally accepted definition the neuropathic pain is initiated or caused by a primary lesion or dysfunction in the nervous system [1]. Neuropathic pain results from damage to or dysfunction of the peripheral or central nervous system, rather than stimulation of pain receptors [2]. There are different forms of neuropathic pain which correspond to a variety of disease states and are demonstrated in the clinical practice with a variety of symptoms [3]. This pain represents a very serious worldwide unresolved clinical problem due to its severity, chronicity and resistance to simple analgesics [2]. Therefore, it is widely investigated. Rodent models of neuropathic pain are used to unravel the respective pain mechanisms involved in the injury to peripheral nerves. The streptozotocin (STZ)-induced diabetic rat model demonstrates many of the abnormalities observed in humans [4]. Chronic constriction injury (CCI) model is an old and well characterized model of post-traumatic painful peripheral neuropathy. Both light and electron microscopic studies based on these animal pain models present morphological data in an attempt to disclose a structural basis of neuropathic pain. However, there are

controversial reports in the literature on this topic. There are statements for a lack of myelinated fibre loss or any change in the myelinated fibre size [5-7]. Other studies, however, claim that the number of fibers is diminished [8-10]. Recently we published an investigation comparing the morphological equivalents in these two models of neuropathic pain by studying only the mixed (sensory and motor) sciatic nerve [11]. The aim of the present study is to extend this work by additional investigation of a purely sensory nerve in the streptozotocin induced model and in the chronic constriction injury model of neuropathic pain.

Materials and Methods

All experiments were approved by the Ethics Committee of the Medical University of Sofia. Male Wistar rats (200-220g) were used. Animals were housed in groups of six under a 12 h light/dark cycle (lights on at 07.00 h) with food and water *ad libitum*. Each experimental group consisted of 6 - 8 animals.

Peripheral diabetic neuropathy was induced by single injection of streptozotocin (STZ, 70 mg/kg, i.p.). Rats with plasma glucose concentrations 14 mmol/l in addition to polyuria and other diabetic features were considered to have type 1 diabetes.

Experimental painful peripheral neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve by loose ligation at mid-thigh level with two silk sutures. During operation rats were anaesthetized with calypsol (50 mg/kg, i.p.) and nembutal (12 mg/kg, i.p.). Thereafter the animals were allowed to recover for 10 days. The development and presence of neuropathic allodynia and hyperalgesia was established by using von Frey filament test, paw pressure test, hot plate test and heat plantar test.

Thirty five to fourty days after injection of streptozotocin or nerve ligation, 3 animals per group were anesthetized with intraperitoneal injection of sodium pentobarbital (40 mg/kg). Then the animals were perfused intracardially with half-strength Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde) in 0,1M phosphate buffer pH 7,4 for 20 min. Small tissue samples from the sciatic and sural nerves were additionally fixed for several hours in the same fixative at 4°C. After rinsing in buffer they were postfixed with 1% osmium tetroxide for 1 hour. Following a second wash the tissue pieces were dehydrated through a graded ethanol series and were infiltrated using a mixture of one-half propylene oxide and one-half durcupan before embedding for fourty eight hours in durcupan. Ultrathin sections were cut on a an ultramicrotome (LKB, Stockholm-Bromma, Sweden), stained with lead citrate and uranyl acetate and photographed in Hitachi H-500 electron microscope (Hitachi, Tokyo, Japan).

Results

Under electron microscope the sciatic and sural nerves from rats with STZ-induced diabetes display heterogeneous structure (Fig. 1). Unchanged profiles of myelinated and unmyelinated nerve fibers are readily seen. However, some nerve fibers' axolemma is partially detached from the myelin sheath as a sign of initial nerve alteration. Profiles of nerve fibers can be seen which myelin sheath shows focal destructions of the lamellar structure. They are small or larger and sometimes occupy a considerable part of the myelin sheath. At these sites the myelin lamellae do not display their paralleled appearance and uniformed distance but are either fused at places or even more distanced and form vortex figures. The basal lamina of Schwann cells is often thickened. However, in some instances it can be thinner. Although not available in each nerve fiber these fine structural alterations clearly contrast with the normal ultrastructure of the sciatic and sural nerves of control animals.

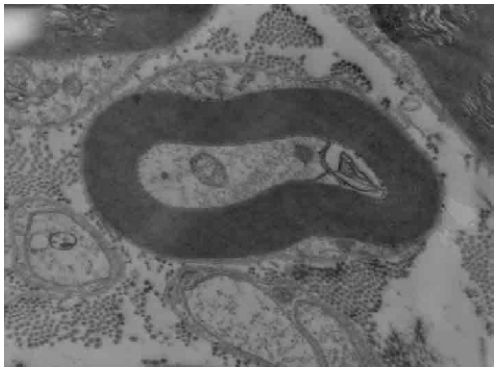

Fig. 1. STZ-induced diabetes. Sural nerve. Separation of the axolemma from the myelin and myelin defects. X 13000

When viewed under electron microscope, the alterations in the sciatic nerve of rats with chronic constriction injury are more prominent (Fig. 2). Distal to the ligature the sciatic nerve fibers display various appearances. Many myelinated profiles show unaffected myelin. Their axoplasm has normal electron density but sometimes can be locally detached from the myelin sheath and could contain individual vacuoles. There are nerve fibre profiles, especially larger ones, which show local destructions of the myelin and electron denser axoplasm with many vacuoles. The unmyelinated nerve fibers are usually in almost unaltered fascicles. Nevertheless, there are often fascicles containing unmyelinated profiles with a damaged structure - electron dense axoplasm containing vacuolar profiles. When investigating the sural nerve, the same alterations were found as already described distal to the ligature of the sciatic nerve.

Under electron microscope, the fine structure of the sciatic nerve proximal to the ligature appears to be normal. Individual changes of some unmyelinated profiles - swelling and light changes of the usual oval outlines - could be established.

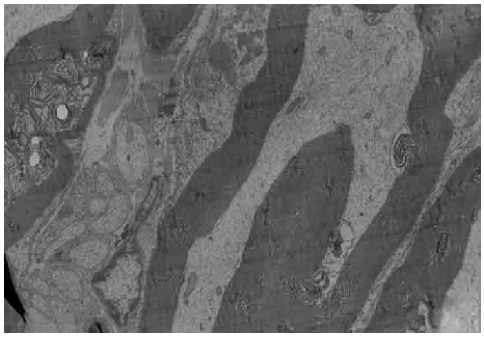

Fig. 2. CCI rat model. Sciatic nerve. Injury of myelinated and unmyelinated nerve fibers. X 16000

Discussion

Several key points are characteristic for the current statement of the problem with the neuropathic pain. Firstly, approximately 3% of the world human population is affected by that type of pain [12]. Secondly, the neuropathic pain has largely unknown mechanism(s) although knowledge of the cellular and molecular mechanisms of neuropathic pain has now advanced [13]. Thirdly, that is why as compared to other types of pain the pharmacological treatment of the neuropathic pain has at present little success. Therefore, the neuropathic pain is a very serious and complicated problem in the clinical medicine that is far from being resolved.

Obviously, a prerequisite for a clear understanding of the pathophysiological mechanisms of neuropathic pain appearance and treatment is to know if there are definite structural changes of the peripheral nerve fibers and to what extent they exist in the patients as well in the experimental animal models. Two of them are widely used in research laboratories for their simplicity and easy performance - STZ-induced diabetic rat model and CCI rat model. However, surprising controversies exist in the literature when using these models to evaluate the qualitative or the quantitative changes in the peripheral nerves [5-10]. Most probably, the sources of these contradictory data could be the different methods and experimental conditions used. The difference in criteria for evaluation of the results is another possibility. Recently, we attempted to clarify these controversies by careful investigation of eventual fine structural changes in the nerve fibers using transmission electron microscopy [11]. However, in that work the mixed sciatic nerve was investigated having motor nerve fibers besides the sensory ones. Therefore, in the present study in addition to the sciatic nerve the purely sensory sural nerve was examined. As the data on the structure changes in both nerves are identical, obviously they are characteristic of the sensory nerve fibers, which are related to the generation of neuropathic pain. We found that the diabetic neuropathy demonstrates partial separation of the axolemma from the myelin sheath, primary demyelination, signs of Schwann cell dysfunction, hypertrophy of the basal lamina. The chronic constriction injury was additionally accompanied by small or large focal destruction of the myelin. Although the changes are not very severe as they are manifested in man [14], they can be found by careful electron microscopic observation. Furthermore, these ultrastructural changes are almost the same in both models being lightly more expressed in the CCI model. Additionally the myelin is more damaged by CCI.

Conclusion

In conclusion, both models investigated display nearly the same ultrastructural changes. The reported light morphological differences point out that it may be better to combine different models in order to better approximate the mechanisms that contribute to neuropathic pain.

References

1. Merskey H, Bogduk N. Classification of chronic pain: description of chronic pain syndromes and definitions of pain terms, 2nd edn. Seattle: IASP Press; 1994. p. 212.
2. Gilron I, Watson CPN, Cahill CM, Moulin DE. Neuropathic pain: a practical guide for the clinician. CMAJ. 2006;175(3):265-75.
3. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet. 1999;353(9168):195964.
4. Zimmermann M. Pathobiology of neuropathic pa-in. Eur J Pharmacol. 2001;429(1-3):23-37.
5. Sharma AK, Thomas PK. Peripheral nerve struc-ture and function in experimental diabetes. J Neurol Sci. 1974;23(1):1-15.
6. Brown MJ, Sumner AJ, Greene DA, Diamond SM, Asbury AK. Distal neuropathy in experimental diabetes mellitus. Ann Neurol. 1980;8(2):168-78.
7. Wright A, Nukada H. Sciatic nerve morphology and morphometry in mature rats with streptozocin-induced diabetes. Acta Neuropathol. 1994;88(6):571-78.
8. Jakobsen J. Axonal dwindling in early experimen-tal diabetes. I. A study of cross sectioned nerves. Diabetologia. 1976;12(6):539-46.
9. Zemp C, Bestetti G, Rossi GL. Morphological and morphometric study of peripheral nerves from rats with streptozotocin-induced diabetes mellitus. Acta Neuropathol. 1981;53(2):99-106.
10. Thomas PK. The pathogenesis of diabetic neuropathy: current problems and prospects. In: Ward JD, Goto Y, editors. Diabethic neuropathy. Chichester: Wiley; 1990. p. 3-14.
11. Surchev L, Surcheva S, Janev N, Kasakov L, Vlaskovska M. Comparative assessment of the morphological equivalents in two experimental rat models of neuropathic pain. C R Acad Bulg Sci. 2008;61(8):1079-86.
12. Sandroni P, Benrud-Larson LM, McClelland RL, Low PA. Complex regional pain syndrome type I: incidence and prevalence in Olmsted county. A population-based study. Pain. 2003;103(1-2):199-207.
13. Walker K, Fox AJ, Urban LA. Animal models for pain research. Mol Med Today. 1999;5(7):319-21.
14. Thomas PK, Tomlinson DR. Diabetic and hypoglycemic neuropathy.In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, editors. Peripheral neuropathy. Philadelphia: Saunders; 1993. p. 1219-50.