Apoptotic gene expression in neuropathic pain

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INTRODUCTION

Pain initiated or caused by a primary lesion or dysfunction in the nervous system is defined as neuropathic pain [1]. It results from direct injury to nerves in the peripheral or central nervous system and is associated with several clinical symptoms. Neuropathic pain treatment is extremely difficult, as it is a very complex disease, involving several molecular pathways [1]. Excitatory or inhibitory pathways controlling neuropathic pain development show altered gene expression, caused by peripheral nerve injury [2, 3]. This study used several experimental pain models to demonstrate the occurrence of programmed cell death in the centers controlling pain induction and maintenance, such as spinal cord and pre-frontal cortex.

MATERIALS AND METHODS

Surgery and behavioural tests

Mononeuropathy was induced by spared nerve injury (SNI) and/or chronic constriction injury (CCI). Mice were anaesthetized with sodium pentobarbital, the sciatic nerve was exposed at mid-thigh and freed of connective tissue, and the common peroneal and the tibial nerves were tight-ligated with 5.0 silk (for CCI), and sectioned distal to the ligation leaving the remaining sural nerve intact (for SNI). Sham mice were anaesthetised and the sciatic nerve exposed, but no ligatures or cuts were placed.

Thermal hyperalgesia was evaluated by using a Plantar Test Apparatus. The latency of nociceptive reaction was measured in seconds under basal conditions and at 3, 7, 14 and 21 days after sham operation or sciatic nerve injury.

Mechanical allodynia was measured with a Dynamic Plantar Anesthesiometer, which applies a linearly increasing mechanical force to the dorsum of the mice hind paw.

RNA extraction and RT-PCR

Total RNA was extracted from homogenized whole cortex or spinal cord using an RNA Trizol-Reagent. The total RNA concentration was determined by UV spectrophotometer. The mRNA levels of the genes under analysis were measured by RT-PCR amplification. Sequences for the rat mRNAs from GeneBank were used to design primer pairs for RT-PCRs. Each RT-PCR was repeated at least four times. A semiquantitative analysis of mRNA levels was carried out by the " GEL DOC 1000 UV SYSTEM" (Bio-Rad, Hercules, CA). The measured mRNA levels were normalised with respect to hypoxanthine-guanine phosphoribosyltransferase (HPRT), chosen as housekeeping gene, and the gene expression values were expressed as arbitrary units ± SE.

Western blotting

For the protein extraction, the cortex or the spinal cord was minced into small pieces with a blender, then was suspended in lysis buffer. The tissue was sonicated in ice. Sonication was carried out for two cycles of 15 s each. Each sample was loaded, electrophoresed in a 12% polyacrylamide gel and electroblotted onto a nitrocellulose membrane. Primary antibodies to detect casp-3 were used at 1:500. Immunoreactive signals were detected with a horseradish peroxidase-conjugated secondary antibody and reacted with an ECL system. Protein levels were normalised with respect to the signal obtained with anti-actin monoclonal antibodies.

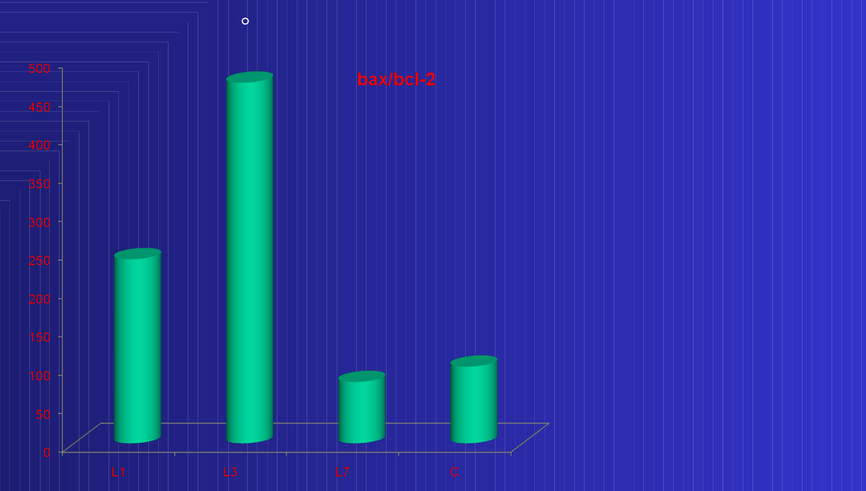
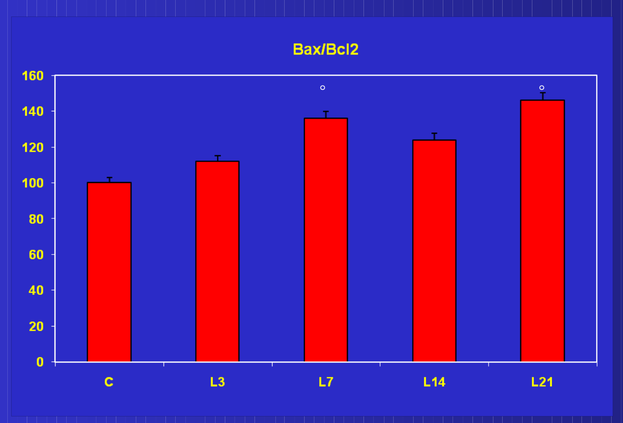
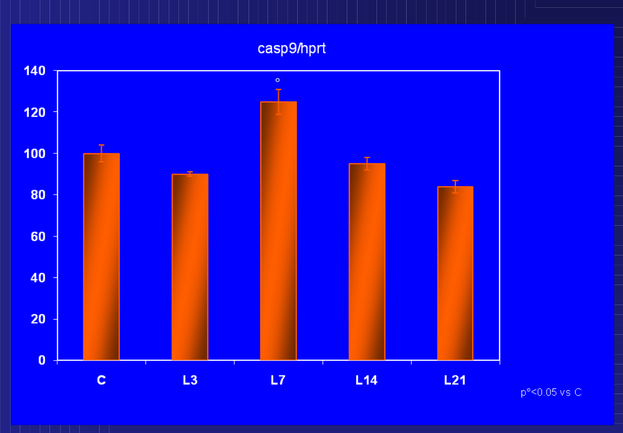


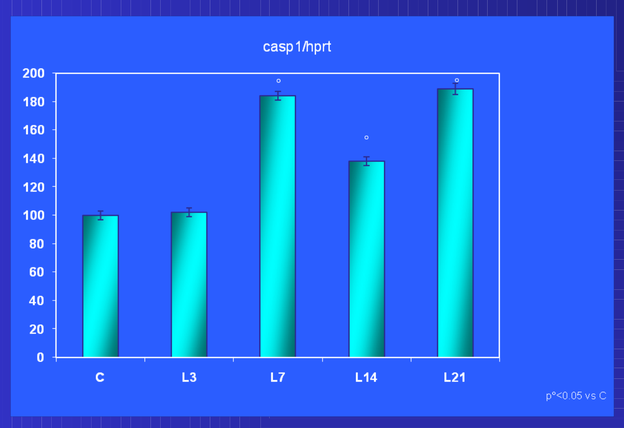
Fig. 1. Changes in the genic expressions in CCI-neuropathic mice. The graphs indicate the percentage of gene expression ratio. L: CCI mice. C: control mice. The numbers indicate the days of the sciatic nerve ligature. °p<0.05 vs C.

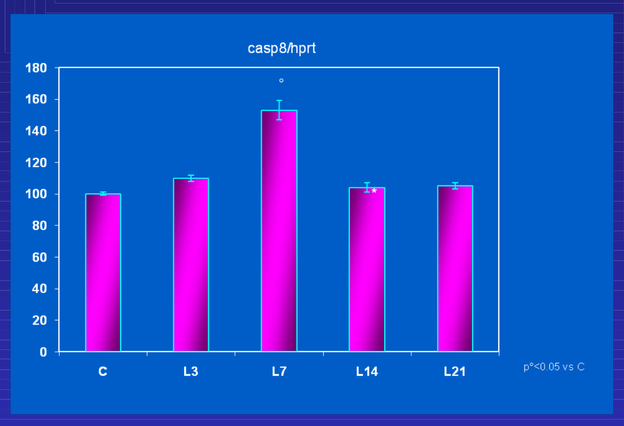
RESULTS AND CONCLUSIONS

We combined behavioural, molecular and morphological approaches to assess the involvement of bcl-2 gene family and caspases in neuropathic pain. Chronic constriction injury (CCI) and spared nerve injury (SNI) of rodent sciatic nerve induced the appearance of pain-like behaviours, such as hyperalgesia and allodynia. An early (2-3 days post-CCI) apoptosis appeared in the spinal cord neurons as the pro-apoptotic bax gene increased (320±19%). The incidence of apoptosis appeared to be limited to the first few days following nerve injury. Subsequently, increased expression of anti-apoptotic bcl-2 family genes may inhibit further neuron loss. SNI triggered apoptotic pathway and caspases activation in pre-frontal cortex 7, 14, and 21 days post-injury. Among the time-points analyzed, RT-PCR analysis showed increased expression of the bax/bcl-2 ratio (40±2%), bid (16±2%), caspase-1 (84±3%), caspase-8 (53±6%), caspase-9 (25±6%), caspase-12 (58±2%), TNF (32±2%) genes in the cortex by 7 days post-injury. Western blot analysis showed increased active Caspase-3 protein levels in the cortex at 3, 7, and 14 days post-surgery. This study shows that apoptotic genes could be an useful pharmacological target in neuropathic pain controlling.









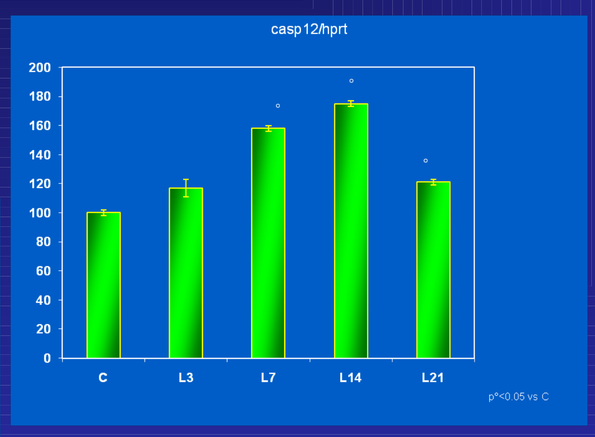


Fig. 2. Changes in the genic expressions in SNI-neuropathic mice. The graphs indicate the percentage of gene expression ratio. L: SNI mice with nerve ligature. °p<0.05 vs C.

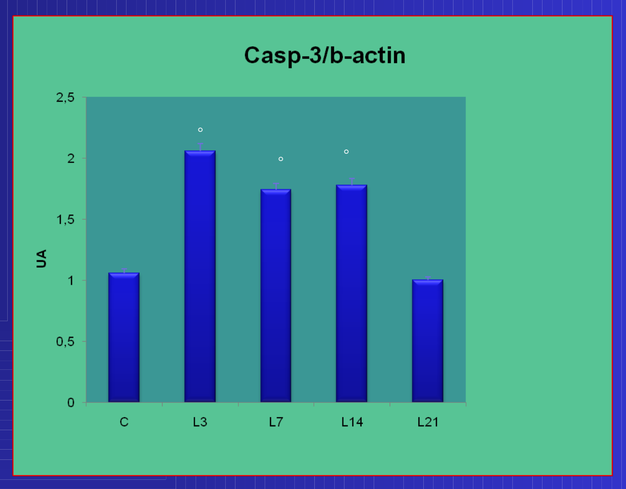


Fig. 3. Changes in the Caspase-3 protein levels in SNI-neuropathic mice. The graphs indicate the percentage of Caspase-3 active form. L: SNI mice with nerve ligature. °p<0.05 vs C.

REFERENCES

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