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Delayed acetyl-L-carnitine administration and its effect on sensory neuronal rescue after peripheral nerve injury☆

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Summary Protection of sensory neurons after peripheral nerve injury is clinically crucial since inadequate sensory recovery is seriously affected by the death of up to 40% of sensory neurons. Immediate acetyl-L-carnitine (ALCAR) treatment eliminates this cell loss, but may not always be clinically feasible, hence we studied the effect of delaying the initiation of ALCAR treatment.

Five groups of rats ($n = 5$ per group) underwent unilateral sciatic nerve axotomy. ALCAR treatment (50 mg/kg/day) was initiated immediately, or after delays of 6 h, 24 h or 7 days after injury. A sham-treated group served as control. L4 and L5 dorsal root ganglia were harvested bilaterally 2 weeks after injury and stereological sensory neuron counts were obtained.

☆ This work has been presented as detailed below:
Acetyl-L-carnitine eliminates sensory neuron loss after peripheral nerve injury: dose—response relationship and effect of delay in administration. Wilson ADH, Brannstrom T, Wiberg M, Terenghi G. Peripheral Nerve Society, Banff, Canada, 2003.
Delayed acetyl-L-carnitine administration & neuronal survival after peripheral nerve injury. Mr ADH Wilson, Dr T Brannstrom, Prof M Wiberg, Prof G Terenghi. B.A.P.S. Summer Conference, Newport, July 2003.

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Immediate sham treatment provided no neuroprotection (25% loss). Cell loss was eliminated when ALCAR was commenced within $\leq$24 h of axotomy. No statistically significant neuroprotective effect (18% loss) was evident compared to sham when ALCAR administration was initiated 7 days post-axotomy.

When commenced within a clinically applicable time frame ALCAR treatment remains highly neuroprotective, potentially improving clinical outcome following peripheral nerve trauma.

Despite considerable advances in surgical technique, sensory function does not normalise after peripheral nerve trauma.[1–3] Neuronal survival is a prerequisite for axonal regeneration,[4] but irrespective of how well the surviving neurons regenerate the death of primary sensory neurons ensures that normal innervation density, and thereby quality of sensation, can never return to normal. Hence the elimination of sensory neuronal death, which is initiated within 24 h of injury and extends to ~40% with time from injury,[5] could lead to improvement in outcome. The peptide and predominant acyl-carnitine in human tissues, acetyl-L-carnitine (ALCAR), has given very encouraging results in this regard. ALCAR increases nerve growth factor (NGF) binding[6] and has important roles in facilitating mitochondrial oxidative metabolism[7,8] both of which may go some way to explain its neuroprotective effect *in vitro*[9–11] and in a dose-dependent fashion[12,13] *in vivo*. Unlike other exogenous neurotrophic factors which may offer neuroprotection,[14] ALCAR is suitable for use in clinical trials, being safe when administered orally,[15–19] intramuscularly[18,20] or intravenously.[21]

Previous experimental studies have examined ALCAR's neuroprotective effect when administered at the time of injury, but this design does not reflect the clinical situation. Even though it is generally accepted that open wounds are best treated within 6–8 h of injury, there is commonly a delay of up to 24 h before the patient either presents to a casualty department/hand surgery unit, or has his/her wound explored in theatre.[22,23] In the majority of hand injuries, over half of which involve nerve injuries,[23,24] most of this delay occurs after admission[23] thus providing the opportunity to instigate neuroprotective treatment prior to formal exploration in the case of an obvious nerve injury. Because of these clinical constraints, it would be of interest to know whether delayed initiation of ALCAR treatment would offer a similar neuroprotective effect as immediate administration. This study investigates the effect of delays of up to 1 week from the time of injury in the initiation of systemic ALCAR administration on the magnitude of neuronal protection.

Materials and methods

All work performed in accordance with the terms of the Animal (Scientific Procedures) Act 1986 (project no. 70/4210) and the number of animals used was kept to a minimum.

Under halothane anaesthesia (May & Baker Ltd., UK) (2 ml/min in oxygen), five groups of young adult Sprague–Dawley rats (250–300 g body weight) underwent unilateral left sciatic nerve division at the upper border of quadratus femoris ($n = 5$ per group). To prevent spontaneous regeneration, both proximal and distal nerve stumps were ligated and secured into blind-ending silicon caps with 9/0 Ethilon sutures.

All animals received 50 mg/kg/day of acetyl-L-carnitine (ALCAR; SigmaTau Pharmaceuticals, Italy) parenteral systemic therapy dissolved in normal saline, via a 1-ml intraperitoneal injection. Each group was randomly selected to receive the initial dose after various delays from the time of operation as follows: 0 h, 6 h, 24 h and 1 week after the operation. Daily administration was then continued until harvesting at 2 weeks. A fifth 'sham treatment' group received a daily 1 ml injection of normal saline starting immediately after the operation.

Harvesting of the ipsilateral axotomised and contralateral, non-axotomised, control L4 and L5 dorsal root ganglia was performed under terminal anaesthesia (pentobarbitone, 240 mg/kg i.p.) after transcardiac perfusion with 300 ml of phosphate-buffered saline (PBS) followed by perfusion fixation with 400 ml of 4% (w/v) paraformaldehyde. The ganglia were then post-fixed in 4% paraformaldehyde at 4 °C overnight before equilibration in cryoprotectant solution (PBS containing 15% sucrose and 0.1% sodium azide). The ganglia were subsequently blocked in O.C.T. compound (BDH Laboratory Supplies, UK) and stored at −40 °C ready for sectioning. Each ganglion was cut in its entirety into