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Research Report

The effect of Mannose-6-Phosphate on recovery after sciatic nerve repair

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ABSTRACT

We have determined the effect of applying Mannose-6-Phosphate (M6P), a scar reducing agent, to a site of sciatic nerve repair. In anaesthetised C57-Black-6 mice, the left sciatic nerve was sectioned and repaired using 4 epineurial sutures. Either 100 μl of 600 mM Mannose-6-Phosphate (29 animals), or 100 μl of phosphate buffered saline as a placebo control (29 animals), was injected into and around the nerve repair site. A further group acted as sham-operated controls. After 6 or 12 weeks of recovery the extent of regeneration was assessed electrophysiologically and the percentage area of collagen staining at the repair site was analysed using picrosirius red and image analysis. Gait analysis was undertaken pre-operatively and at 1, 3, 6, 9 and 12 weeks postoperatively, to assess functional recovery. At 6 weeks the compound action potentials recorded from the regenerated nerves in the M6P group were significantly larger than in the placebo controls ($P=0.015$), and the conduction velocities were significantly faster ($P=0.005$), but there were no significant differences between these groups at 12 weeks. Gait analysis suggested better early functional recovery in the M6P group. In both repair groups there was a significant reduction in collagen staining between 6 and 12 weeks, suggestive of scar remodelling. We conclude that the normal scar remodelling process aids long term recovery in repaired nerves. Administration of 600 mM M6P to the nerve repair site enhances nerve regeneration and functional recovery in the early stages, and may lead to improved outcomes.

1. Introduction

Microsurgical suture repair is the current gold standard in clinical practise for transected peripheral nerves that can be re-apposed without undue tension (Millesi, 1979; Lundborg, 2000). However, the level of recovery from surgical repair is very variable and often disappointing (Robinson et al., 2000; Kim et al., 2003). The variable recovery results from a series of factors such as the nature and severity of the injury, the degree of neuronal cell death, and the extent of scarring and neuroma formation at the injury site. The present study focuses on the role of intra-neural scarring, which occurs in both surgically repaired nerves and damaged but unrepaired nerves (Mathur et al., 1983), and acts as a mechanical barrier to

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regenerating axons (Koopmans et al., 2009). In addition, the branching of regenerating axons obstructed by the scar tissue may result in the formation of neuroma (Foltán et al., 2008), which can be the source of injury-induced pain (Devor, 2001; Yates et al., 2004).

In a previous study we showed that the extent of nerve regeneration across a repair site is inversely proportional to the extent of scar formation, thereby supporting the hypothesis that scar reducing agents could enhance recovery (Atkins et al., 2006a). In subsequent studies we manipulated the transforming growth factor-beta (TGFβ) pathway, as this plays a major role in wound healing and scar formation (O'Kane and Ferguson, 1997) and others had reported that inhibition of TGF-βs could reduce intra-neural scarring (Nath et al., 1998; Davison et al., 1999). We initially found that application of antibodies to TGF-β1 and TGF-β2 at a site of nerve repair resulted in a reduction in scar formation but no improvement in regeneration (Atkins et al., 2006b). However, using an alternative approach, we found that application of Mannose-6-Phosphate (M6P) to a nerve repair site did enhance regeneration (Ngeow et al., 2011). M6P is a TGF-β inhibitor that works by interfering with the conversion of latent forms of the TGF-βs into their active form (O'Kane and Ferguson, 1997). In rodent skin, exogenous addition of M6P has a marked antiscarring effect (McCallion and Ferguson, 1996). We found that intra-neural and peri-neural application of 600 mM M6P to a site of sciatic nerve repair in mice resulted in larger compound action potentials and faster conduction velocities than in placebo controls, six weeks after repair. To assess further the potential therapeutic value of this approach we have now quantified regeneration after a longer recovery period and have also evaluated functional recovery.

Most studies on the effect of applying a scar reducing agent to a nerve repair site have assessed the outcome after recovery for only a few weeks (Atkins et al., 2006a, 2007; Davison et al., 1999; Lee et al., 2007; Nachemson et al., 1985; Ozay et al., 2007; Ozgenel and Filiz, 2003; Petersen et al., 1996; Wehling et al., 1992; Zou et al., 2006), whereas the long-term outcome is of most clinical significance. A study by Indar and Fry (1958) followed the changes in scar tissue between the 20th and 100th day after peripheral nerve suture. They found that a fibroblastic reaction still persisted at the 100th day but showed evidence of maturation and remodelling of the scar over this period. This process could potentially permit ongoing improvements in nerve regeneration and functional outcome. We have compared the outcome at 6 and 12 weeks using electrophysiological recordings and quantification of the collagen scar, in addition to gait analysis throughout the recovery period.

2. Results

All the mice recovered quickly from the nerve repair, with no evidence of autotomy (Wall et al., 1979). For the mice that underwent gait analysis, all groups showed an increase in weight over the 12 week period ($P<0.001$, all comparisons; Wilcoxon signed-rank test) and there was no significant difference between groups in body weight pre-operatively ($P=0.929$, Kruskal–Wallis test) or at post-operative week 12 ($P=0.495$).

2.1. *Electrophysiological recordings*

Examples of compound action potentials recorded in these experiments are shown in Fig. 1 and comparisons between the compound action modulus ratios in each group of animals are shown in Fig. 2. In the uninjured sham control groups, the median CAP modulus ratio was 0.91 at 6 weeks and 0.93 at 12 weeks, in each case indicating that slightly smaller compound action potentials were evoked by stimulation at the distal site. As expected, there was no significant difference between the two recovery periods in the sham controls. For both repair groups, after 6 weeks of recovery the median CAP modulus ratio was significantly lower than that for the sham uninjured controls (PBS, $P<0.001$; M6P, $P=0.001$; Kruskal–Wallis test with Conover–Inman pairwise comparisons). However, the median CAP modulus ratio in the M6P group (0.45) was significantly higher than in the PBS placebo group (median 0.29; $P=0.015$).

There was an improvement in the CAP evoked by distal stimulation in both repair groups by 12 weeks. In the PBS placebo group, the median CAP modulus ratio had increased from 0.29 to 0.73 by 12 weeks, and this difference was significant ($P<0.001$, Mann–Whitney *U* test). Similarly, in the M6P group the

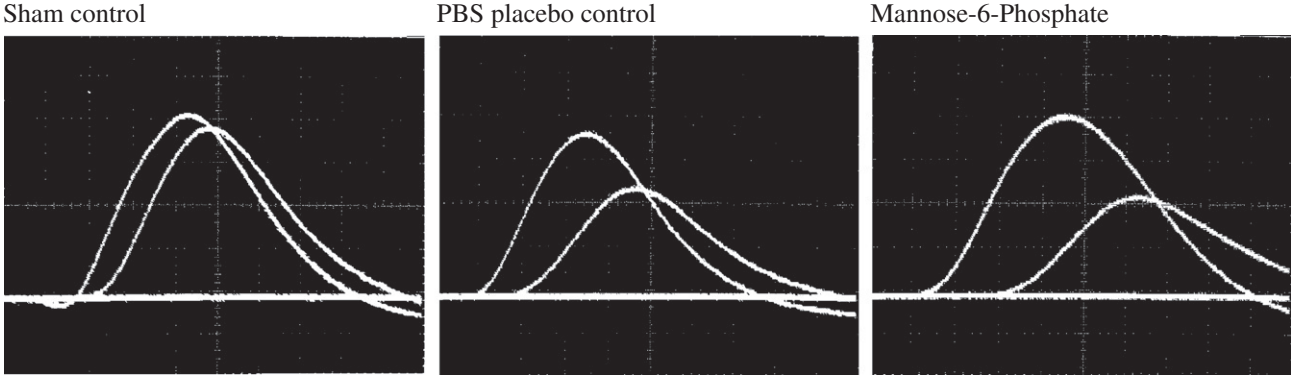

Fig. 1 – Examples of the recordings used for calculation of the compound action potential (CAP) modulus ratio for each group at 12 weeks, as seen on the oscilloscope. In each case the larger, shorter latency response (10 superimposed sweeps) was evoked by stimulation proximal to the repair and the smaller response by stimulation distal to the repair.