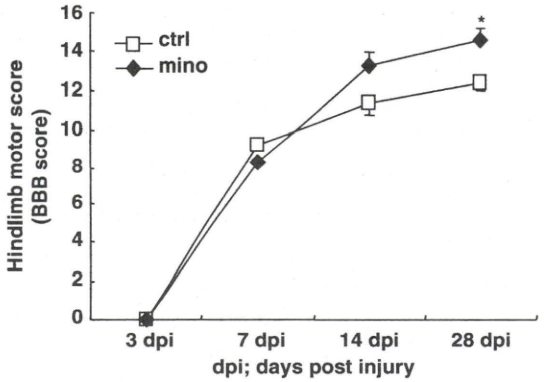


**Figure 1** The functional recovery of hind limbs after spinal cord injury in both the treated and control groups. Thoracic spinal cord injured rats were treated with either minocycline (treated group: mino) or saline (control group: ctrl). The Basso, Beattie and Bresnahan (BBB) scale was used to assess the motor function of the hind limbs of the injured rats. At 28 dpi, the treated group showed better motor function, and the difference between the two groups was statistically significant (values are shown as mean ± standard error of the mean; $*P<0.05$).

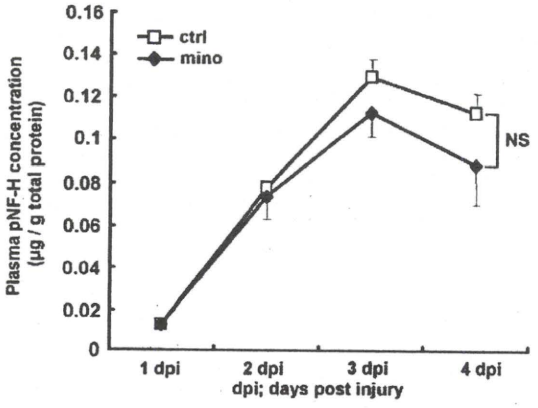


**Figure 2** Changes in plasma pNF-H levels induced by minocycline treatment. The levels of plasma pNF-H in serial samples obtained from injured rats in both the treated (mino) and control (ctrl) groups were analyzed by an enzyme-linked immunosorbent assay. The absolute pNF-H values were divided by the total protein concentration of each sample (values are shown as mean ± standard error of the mean). Even though the pNF-H level in the treated group was lower than that in the control group at 3 and 4 dpi, the difference was not statistically significant.

was not statistically significant (repeated-measure analysis of variance; $P=0.27$), the same trend was observed at 4 dpi. The reduction in the plasma pNF-H levels at 3 and 4 dpi may indicate the protective effects of minocycline against axonal damage in the treated group.

*Plasma pNF-H levels at 3 dpi correlate with locomotor function recovery*

As the plasma pNF-H level at 3 dpi was lower in the treated group than in the control group, we next examined whether the pNF-H level at 3 dpi can serve as a predictor of the recovery of hind limb function. Figure 3 shows a plot of

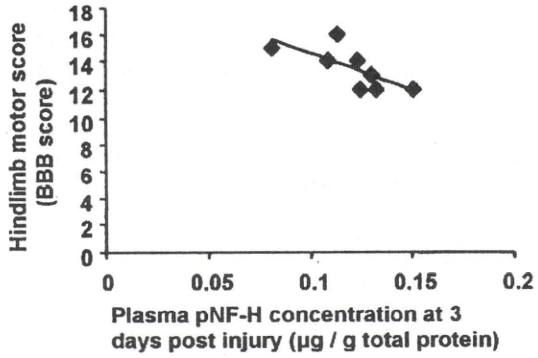


**Figure 3** Correlation between plasma pNF-H level and recovery of hind limb function after spinal cord injury. The plasma pNF-H concentration at 3 dpi was plotted against the hind limb functional score at 28 dpi for each sample (both the treated and control groups). The regression line indicates a negative correlation between these two parameters.

the plasma pNF-H level (3 dpi) and hind limb motor score (28 dpi) for each rat in both groups. Statistical analysis revealed a negative correlation between these two parameters (Spearman's rank correlation coefficient: $r_s=-0.78$; $P<0.05$), indicating that a subject with a low pNF-H level is more likely to achieve a higher motor score at 28 dpi.

## Discussion

This study is the first to assess whether blood pNF-H levels reflect the neuroprotective effects of minocycline against SCI. We found that minocycline treatment reduced plasma pNF-H levels at 3 dpi, and this reduction was correlated with the functional motor score; this finding is consistent with the reported neuroprotective effects of the drug. Therefore, we suggest that plasma pNF-H levels can serve as a biomarker, to some extent, for monitoring the amelioration of tissue damage by SCI treatments.

We observed a reduction in plasma pNF-H levels in the treated group at 3 dpi, but the difference between the two groups was not statistically significant. This marginal reduction in the pNF-H level could be caused by either a minimal protective effect on the axons induced by minocycline or a timing error in blood sample collection. Most of the studies on minocycline treatment for SCI describe the difference in the histological findings between the treatment and control groups at 7 or 14 dpi.[3] The plasma pNF-H level decreased after 3 dpi, and this finding was consistent with other reports.[9] Further, we speculate that the pNF-H level at later time points would be influenced by restoration of the blood–brain barrier. Therefore, the comparison of the pNF-H levels at 7 or 14 dpi would not be feasible for evaluating the neuroprotective effect of therapeutic interventions. On the basis of these findings, we consider that blood pNF-H levels would best reflect the effects of neuroprotective drugs if the drugs exert their function within 3 days after SCI.

The association between serum pNF-H levels and prognosis has already been reported in studies with subarachnoid

hemorrhage and amyotrophic lateral sclerosis patients.[10,16] To determine the utility of pNF-H as biomarker of clinical SCI, further studies with patients with varying degrees of SCI are required. Taking such biomarkers in clinical trials may reduce the number of the patients required, which accelerates the development of novel therapeutic approaches against the traumatic disorder.

## Conflict of interest

The authors declare no conflict of interest.

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