http://dx.doi.org/10.2106/JBJS.18.00162


## Appendix

### *Tissue Harvest for Histological Analysis and Blinding*

Animals were killed at random, and tissue was harvested for histomorphometric analyses by blinded investigators after compression (day 0) and at 7 and 15 days after decompression. In the experiments, the mice were identified by a number assigned on the day of the nerve surgery and were not identified by treatment group except for dosing. Treatment (EPO, 4-AP, or saline solution) was administered daily as indicated by the protocol in an unblinded fashion by one investigator (or a pair to cover odd days and weekends). During that unblinded period to provide dosing, no data were acquired or analyzed. The animals underwent functional assessment (walking) just before dosing, and footprints were indexed by mouse number and date only. The tissue was harvested by number by another investigator, and the histological specimens were sent to the Center for Musculoskeletal Research Histology Core Facility (for standard histological analysis) and the Electron Microscopy Facility for toluidine-blue-based histomorphometric analysis. The slides were associated with the mouse number, and the analysis was done on the basis of the number alone. In this way, the animals were only identified by their treatment group in two instances: first, for treatment by an investigator who was not performing any analysis and, second, after the analysis was completed for tabulation of metrics. Harvested tissues were placed into 0.1-M sodium-cacodylate-buffered 4.0% paraformaldehyde/2.5-glutaraldehyde at 4°C for 48 hours, postfixed for 2 hours in darkness with 1.0% osmium tetroxide/1.5% potassium ferrocyanide, and dehydrated (30-minute exchanges) in graded ethanol to 100% (×3), 100% propylene oxide (×2), 1:1 propylene-oxide/EPON-Araldite resin, and 100% EPON-Araldite resin (60 minutes, and then in fresh resin overnight). Samples were then entered into resin-filled silicone molds and polymerized (48 hours, 65°C). One-micrometer sections in water droplets on glass slides were heated on hotplates and stained with 0.1% toluidine blue.

http://dx.doi.org/10.2106/JBJS.18.00162


TABLE E-1 Significant P Values for Figure 1

| Time | P Value* | |
|---|---|---|
| | EPO/EPO | 4-AP/4-AP |
| Baseline | NS | NS |
| Week 1 | 0.0107 | 0.0003 |
| Week 2 | 0.0007 | <0.0001 |
| Week 3 | <0.0001 | <0.0001 |
| Week 4 | 0.0018 | <0.0001 |
| Week 5 | 0.0002 | <0.0001 |
| Week 6 | <0.0001 | <0.0001 |

*Compared with untreated group. NS = not significant.

TABLE E-2 Significant P Values for Figure 2

| Parameter | P Value* | | |
|---|---|---|---|
| | Sham | EPO/EPO | 4-AP/4-AP |
| Axon diameter | <0.0001 | NS | NS |
| Fiber diameter | <0.0001 | NS | NS |
| G ratio | NS | NS | <0.0001 |
| Myelin thickness | <0.0001 | NS | <0.0001 |
| No. of myelinated fibers | <0.0001 | <0.0001 | NS |
| Proportion of myelinated fibers | | | |
| Small fibers | Measurement not performed | NS | NS |
| Medium fibers | Measurement not performed | 0.0488 | 0.0111 |
| Large fibers | Measurement not performed | 0.0016 | 0.0193 |

*Compared with untreated group. NS = not significant.

http://dx.doi.org/10.2106/JBJS.18.00162


TABLE E-3 Significant P Values for Figure 3

| Time | P Value* | | | |
|---|---|---|---|---|
| | Saline/EPO | EPO/EPO | Saline/4-AP | 4-AP/4-AP |
| Day 0 | NS | <0.0001 | NS | <0.0001 |
| Day 3 | NS | <0.0001 | 0.0011 | <0.0001 |
| Day 5 | 0.0218 | <0.0001 | <0.0001 | <0.0001 |
| Day 7 | 0.0012 | <0.0001 | <0.0001 | <0.0001 |
| Day 9 | 0.0001 | | <0.0001 | <0.0001 |
| Day 11 | 0.0326 | Measurement not performed | Measurement not performed | Measurement not performed |

*Compared with untreated group. NS = not significant.

TABLE E-4 Significant P Values for Figure 4

| Parameter | P Value* | | | | |
|---|---|---|---|---|---|
| | Sham | Saline/EPO | EPO/EPO | Saline/4-AP | 4-AP/4-AP |
| Axon diameter | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0003 |
| Fiber diameter | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| G ratio | NS | <0.0001 | NS | <0.0001 | <0.0001 |
| Myelin thickness | <0.0001 | 0.0002 | <0.0001 | <0.0001 | <0.0001 |
| No. of myelinated fibers | 0.0006 | NS | <0.0001 | NS | NS |
| Proportion of myelinated fibers | | | | | |
| Small fibers | Measurement not performed | <0.0001 | <0.0001 | 0.0434 | 0.0455 |
| Medium fibers | Measurement not performed | 0.0005 | 0.0424 | NS | 0.0251 |
| Large fibers | Measurement not performed | <0.0001 | <0.0001 | 0.0005 | 0.0001 |

*Compared with untreated group. NS = not significant.

TABLE E-5 Significant P Values for Figure 5

| Parameter | P Value* | | | | |
|---|---|---|---|---|---|
| | Sham | Saline/EPO | EPO/EPO | Saline/4-AP | 4-AP/4-AP |
| Axon diameter | 0.0180 | NS | NS | 0.0107 | NS |
| Fiber diameter | 0.0028 | NS | 0.0320 | <0.0001 | NS |
| G ratio | NS | NS | NS | <0.0001 | <0.0001 |
| Myelin thickness | 0.0029 | NS | 0.0290 | <0.0001 | <0.0001 |
| No. of myelinated fibers | NS | NS | NS | NS | NS |
| Proportion of myelinated fibers | | | | | |
| Small fibers | Measurement not performed | NS | NS | NS | NS |
| Medium fibers | Measurement not performed | NS | NS | <0.0001 | 0.0044 |
| Large fibers | Measurement not performed | NS | 0.0121 | 0.0001 | 0.0332 |

*Compared with untreated group. NS = not significant.