Supplementary data

Materials and methods

Measurement of mechanical allodynia and thermal sensitivity

Sensitivity to mechanical and thermal stimulation were measured at times indicated post-SCI by two independent examiners in a blinded manner. Mechanical allodynia was measured using the Dynamic Plantar Aesthesiometer (Ugo Basile, Comerio, Italy) (Martucci et al., 2008). The withdrawal threshold (expressed in grams) was measured five times and the reported value represents the mean of these five evaluations. Thermal sensitivity of the plantar hindpaws was measured according to the method described previously (Hargreaves et al., 1998) (Plantar Test Apparatus, Ugo Basile). The mouse was placed unrestrained in a clear plastic compartment (11 cm * 17 cm* 14 cm). When the mouse was stationary and not attending to the tester or stimulus, an infrared radiant heat source was applied through a glass floor to the middle of the plantar surface of the hind paw, between the foot pads. A photocell automatically stopped the heat source and the timer when the mouse lifted its paw. Each mouse was tested for five trials on each hind paw, with at least 1 minute between trials, and where the application of the heat source was randomized in order to minimize windup or avoidance behaviors. Latency (in seconds) to withdrawal from the heat source was recorded, together with any other behavior indicating attendance to the stimulus, including sniffing, licking, looking at the affected paw, or attacking the stimulus. High and low latencies were dropped for each paw, and the remaining six latencies were averaged for each mouse (Hoschouer et al., 2010; Watanabe et al., 2015).

References

Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77–88.

Hoschouer EL, Basso DM, Jakeman LB (2010). Aberrant sensory responses are dependent on lesion severity after spinal cord contusion injury in mice. Pain 148: 328–342.

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Watanabe S, Uchida K, Nakajima H, Matsuo H, Sugita D, Yoshida A, et al. (2015). Early transplantation of mesenchymal stem cells after spinal cord injury relieves pain hypersensitivity through suppression of pain-related signaling cascades and reduced inflammatory cell recruitment. Stem Cells 33:1902-1914.

Table S1. Effects of treatment with DBHB (0.4, 0.8, and 1.6mmol/kg/day) on locomotor function and neuropathic pain in mice exposed to SCI.

	Time after trauma (weeks)								
	-1	0	1	2	3	4	5	6	7
Sham	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0
Sham+DBHB (0.4mmol/kg/day)	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0
Sham+DBHB (0.8mmol/kg/day)	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0
Sham+DBHB (1.6mmol/kg/day)	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0	9±0
SCI	9±0	0.4±0.1*	1.6±0.4*	1.9±0.3*	2.3±0.4*	3.1±0.6*	3.4±0.5*	3.8±0.4*	4.0±0.5*
SCI+DBHB (0.4mmol/kg/day)	9±0	0.4±0.1	1.8±0.5	2.1±0.5	2.5±0.6	3.1±0.7	3.5±0.8	3.9±0.7	4.3±0.8
SCI+DBHB (0.8mmol/kg/day)	9±0	0.3±0.1	1.9±0.5	2.3±0.6	2.8±0.7	3.6±0.8	3.8±0.8	4.4±0.8	4.9±0.7#
SCI+DBHB (1.6mmol/kg/day)	9±0	0.3±0.1	2.2±0.7	3.0±0.6#	4.0±0.5#	4.6±0.6#	4.9±0.7#	5.5±0.6#	5.7±0.6#

The time courses of locomotor recovery were evaluated by the nine-point Basso Mouse Scale (BMS) scoring. DBHB, D-β-hydroxybutyrate; SCI, spinal cord injury; Data were presented as the mean ± SD of eight mice per group. * $P < 0.05$ compared with the sham-operated mice; # $P < 0.05$ compared with the SCI mice.