

Safety and Efficacy of Substance P-SAP

(continued from page 1)

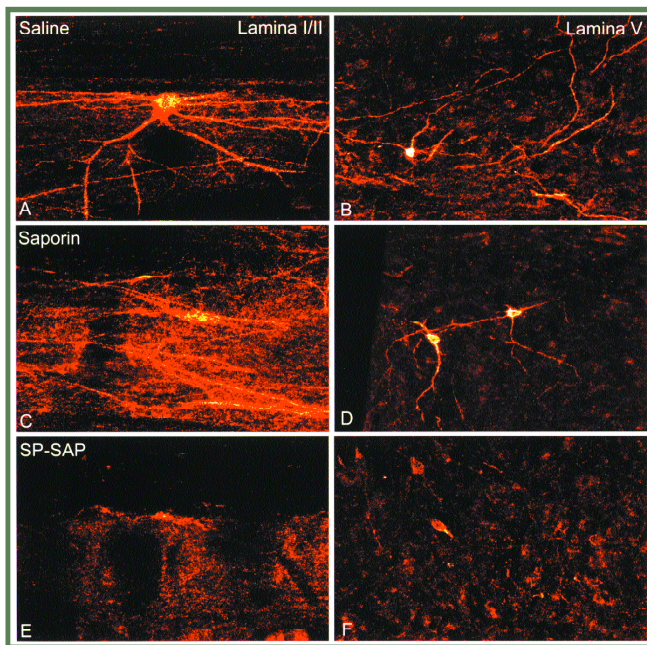


Figure 2. Immunostaining with an antibody to SPR of dog spinal cord after treatment with PBS (panels A and B), SAP (panels C and D), and SP-SAP (panels E and F). Thirty days after injection, animals were sacrificed and cords were removed and processed as described in Mantyh et al.

Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.

Spinal histopathology was assessed at approximately 7, 28 or 90 days. Spinal cords and brain were processed for hematoxylin and eosin (H&E) staining and were evaluated by Dr. Marjorie Grafe in the Department of Pathology at Oregon Health Science Center in Portland. Immunohistochemical staining of the spinal cord for NK-1 receptors, and various markers for cell types and neuropeptides was performed in the laboratory of Dr. Patrick Mantyh, University of Minnesota, Minneapolis. Additional immunohistochemistry of selected brains was done in the laboratory of Dr. Yaksh.

SP-SAP bolus doses of 15, 45 or 150 μg produced an essentially equivalent and significant reduction in the number of NK-1 receptors in the lumbar spinal cord at 28 or 90 days following dosing. Given previous studies it is believed that this reduction was due to death of these neurons, not simply a loss of the receptor protein. No changes were seen in the total number of neurons or the amount of glial fibrillary acidic protein (GFAP), a marker for astrocytes, suggesting a lack of diffuse neuronal loss. Staining for various neuropeptides, including substance P, was unchanged demonstrating a lack of effect of SP-SAP on primary afferent neurons. H&E staining showed no evidence of any histological damage in any animals. Spinal cord sections from SP-SAP treated dogs were indistinguishable from animals receiving either PBS or SAP.

Importantly, all effects of SP-SAP on NK-1 receptor neurons were limited to the lumbar region near the site of injection. This lack of widespread distribution was also seen when examining SAP levels in spinal cord tissues. This

localization of effect may be due not only to the relatively large molecular weight of the molecule (33kDa) but also to the short intrathecal half-life of between 30-60 minutes of the active compound.

There was no sign of pain or discomfort during or immediately following injection. In animals receiving either SP-SAP or the control compound SAP, a transient series of behavioral and physiological signs including an increase in blood pressure, tactile allodynia, neck and truncal rigidity with mild rhythmic tremors and bradykinesia occurred. These signs required butorphanol or buprenorphine analgesia in some animals and were generally dose-related with the time of onset being shortest (3-4 hours) at the 150- μg doses. These signs completely resolved over 1-3 days and by 7 days the dogs were indistinguishable from those who received only PBS. It is hypothesized that this acute constellation of signs was a non-specific reaction to the presence of saporin since it was seen in both the SP-SAP and the SAP groups. Since there were no differences in the total neuronal numbers, GFAP levels or H&E histopathology in any of the SAP dogs, it appears these effects were not due to any permanent cellular toxicity.

In summary, these studies demonstrated that a single intrathecal bolus of 15, 45 or 150 μg SP-SAP produced ablation of greater than 80% of the neurons containing the NK-1 receptor in dogs. This suggests a safety factor of at least 10-fold between a fully effective dose, 15 μg , and a dose, 150 μg , that produced unacceptable acute behavioral signs. Behavior quickly returned to normal, and as evidenced by the lack of pathological signs, there appeared to be no long-term detrimental effects associated with SP-SAP administration at the doses tested. The SP-SAP-induced lesioning was spatially limited to the area of the catheter tip without spread to other spinal regions. These studies, for the first time, demonstrate both safety and efficacy, as defined by loss of NK-1 receptors, on intrathecal SP-SAP in a large animal producing the preliminary data necessary to advance SP-SAP to a clinically available compound.

This article is a summary of material presented by the author in Allen JW, Mantyh PW, Horais K, Tozier N, Rogers SD, Ghilardi JR, Cizkova D, Grafe MR, Richter P, Lappi DA, Yaksh TL (2006) Safety evaluation of Intrathecal Substance P-Saporin, a targeted neurotoxin, in dogs. *Toxicol Sci* 91(1):286-298.