Sodium Bisulfite

Scapegoat for Chloroprocaine Neurotoxicity?

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Background: Neurologic deficits after apparent intrathecal injection of 3% Nesacaine-CE intended for epidural administration created concern about the potential toxicity of chloroprocaine and the preservative sodium bisulfite. Although bisulfite-free formulations of chloroprocaine were subsequently introduced into clinical practice, the relative toxicities of this anesthetic and preservative were never clearly established. The current studies used a relevant functional and histologic model to investigate the intrathecal neurotoxicity of these two compounds.

Methods: In the first experiment, rats implanted with intrathecal catheters were given one of two commercially available solutions of chloroprocaine, one of which contained sodium bisulfite; control animals received saline. Animals were assessed for sensory impairment 7 days after administration using the tail-flick test and were killed to obtain histologic specimens to quantify nerve injury. In the second experiment, identical methodology was used to investigate the effects of freshly prepared solutions of chloroprocaine, chloroprocaine with sodium bisulfite, sodium bisulfite, and saline.

Results: The two experiments yielded similar results. In experiment 1, tail-flick latencies and nerve injury scores after administration of plain chloroprocaine were significantly greater than those of chloroprocaine containing bisulfite. Injury scores for animals receiving chloroprocaine with bisulfite were elevated compared with those for animals given saline. In experiment 2, animals receiving plain chloroprocaine developed elevations in tail-flick latencies and nerve injury scores that were significantly greater than those for all other groups. Nerve injury scores with chloroprocaine containing bisulfite were greater than with saline or bisulfite alone. Tail-flick latencies and nerve injury scores with bisulfite alone were similar to those with saline.

Conclusions: Clinical deficits associated with unintentional intrathecal injection of chloroprocaine likely resulted from a direct effect of the anesthetic, not the preservative. The data also suggest that bisulfite can reduce neurotoxic damage induced by intrathecal local anesthetic.

CHLOROPROCAINE is an ester anesthetic that was introduced into clinical practice over 50 yr ago. It is particularly well suited to epidural anesthesia, having a fast onset, producing excellent sensory block, and undergoing rapid hydrolysis in the bloodstream by pseudocholinesterase. These characteristics account for its early popularity, particularly in obstetrics, where its short plasma half-life eliminated concern for systemic toxicity and fetal anesthetic exposure.

Unfortunately, in the early 1980s, reports of neurologic injury associated with the use of Nesacaine-CE (a chloroprocaine solution containing sodium bisulfite, 0.2%) tempered enthusiasm and led to its near abandonment. Review of these cases suggested that injury was likely due to intrathecal delivery of anesthetic intended for the epidural space. However, some of these cases did not have definitive evidence for intrathecal injection, and several were complicated by hypotension or total spinal anesthesia. It was also postulated that relatively large volumes of fluid injected into the subarachnoid space might have elevated subarachnoid pressure and impaired perfusion of neural tissue. Nonetheless, most investigators concluded that injury resulted from a direct neurotoxic effect of the anesthetic solution, and attention became focused on the relative contribution of its components, chloroprocaine and the antioxidant bisulfite. Unfortunately, the ensuing laboratory work generated data that are incomplete, fragmented, and often contradictory.

Modifying well-validated techniques for intrathecal catheterization, drug delivery, and sensory assessment in the rat, our laboratory has developed a relevant in vivo model suitable for investigation of intrathecal neurotoxicity. The model provides for quantitative assessment of functional impairment and histopathology. Although the obvious differences in anatomy and physiology between rats and humans must be considered, the model parallels recent cases of clinical injury. Importantly, in contrast to previous in vivo models used for evaluation of the spinal toxicity of chloroprocaine and bisulfite, histologic damage has consistently correlated with functional impairment. The current studies used this model to evaluate the neurotoxic potential of chloroprocaine and bisulfite and their potential interaction.

Materials and Methods

The Committee on Animal Research of the University of California at San Francisco approved this study. All experiments were conducted on male Sprague-Dawley rats (200–250 g).

Surgical Procedure

The animals were anesthetized by intraperitoneal injection of methohexital (40–60 mg/kg), and catheters...
were introduced into the subarachnoid space using previously described modifications\(^7,8\) of the method of Yaksh and Rudy\(^13\); 32-gauge polyurethane catheters (Micor, Allison Park, PA) were passed through a slit in the atlantooccipital membrane and advanced 11 cm to lie with their tip caudal to the conus medullaris. The rats were allowed to recover for 24 h before the study began.

**Measurement of Sensory Function**
To assess sensory function, the tail-flick test was performed at the proximal, middle, and distal portions of the tail, as previously described.\(^7,8\) To prevent tissue damage, the heat stimulus was terminated if no response occurred by 8 s (cutoff).

**Test Solutions**

**Experiment 1.** Rats were divided into three groups. One group (n = 33) received 3% Nesacaine-MPF (Astra Pharmaceutical, Westboro, MA), a commercially available, preservative-free, solution of chloroprocaine containing 0.33% NaCl. A second group (n = 33) received a commercially available solution of 3% chloroprocaine HCl containing 0.18% sodium bisulfite and 0.21% NaCl (Abbott Laboratories, North Chicago, IL). The third group (n = 15) received preservative-free normal saline (Abbott Laboratories). The pHs for these solutions were 3.32, 2.86, and 3.01, respectively.

**Experiment 2.** Four solutions were administered: 3% chloroprocaine HCl with 0.4% NaCl (n = 31), 3% chloroprocaine HCl with 0.2% sodium bisulfite and 0.2% NaCl (n = 32), 0.2% sodium bisulfite and 0.2% NaCl (n = 29), and 0.4% NaCl (n = 29). These solutions were prepared immediately before injection by dissolving crystalline chloroprocaine HCl, sodium bisulfite, and NaCl (Sigma Chemical, St. Louis, MO) in preservative-free sterile water (Abbott Laboratories). They were pH adjusted by titration of HCl and NaOH; the pHs were 3.03, 3.03, 3.02, and 3.01 for the chloroprocaine, chloroprocaine with bisulfite, bisulfite, and saline solutions, respectively.

**Experimental Protocol**
The rats were placed in a horizontal acrylic restraint, and baseline tail-flick latency was assessed immediately before infusion. Test solutions were administered at a rate of 1 µl/min for 2 h using a mechanical infusion pump. A segment of calibrated polyethylene tubing was inserted between the syringe and the intrathecal catheter, and infusion was monitored by observing the movement of a small air bubble within the tubing. The animals were evaluated for sensory impairment by determining tail-flick latency 7 days after infusion.

**Tissue Preparation**
After sensory assessment, the animals were killed by injection of an overdose of pentobarbital and were perfused intracardially with a phosphate-buffered glutaraldehyde–paraformaldehyde fixative. The spinal cord and nerve roots were dissected out, immersed in the same glutaraldehyde solution used for perfusion fixation, and embedded in glycol methacrylate. The embedded tissue was sectioned using a JB-4 microtome (1-µm transverse sections; Energy Beam Sciences, Agawam, MA). The tissue was treated with 4% osmium tetroxide and stained with toluidine blue. Histopathologic evaluation was performed using light microscopy by a neuropathologist blinded to the intrathecal solution received and to the results of sensory testing.

**Statistical Analysis**

**Functional Assessment.** Tail-flick latencies at the proximal, middle, and distal portions of the tail were averaged to give a mean tail-flick latency. To assess whether the three groups were equivalent before administration of the test solutions, raw baseline latencies were compared using one-way ANOVA. To assess sensory function 7 days after infusion, tail-flick latencies were converted to the percent maximal possible effect, calculated as ((tail-flick latency − baseline)/[cutoff − baseline]) × 100. These data were compared using one-way ANOVA and the Student–Newman–Keuls test.

**Histologic Analysis.** Quantitative assessment of nerve injury was determined by examination of cross sections obtained 12 mm caudal to the conus medullaris. There were ∼ 25 fascicles per cross section, and each was assigned an injury score of 0–3, where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe (table 1). The injury score for each animal was then calculated as the average score of all fascicles present in the cross section. These data were compared using the Kruskal–Wallis test and Dunn test. To assess whether bisulfite might have a subclinical effect on spinal cord morphology, sections were obtained 6 mm rostral to the conus from animals in the second experiment that had received either bisulfite alone or saline.

Analyses were performed with StatView 4.01 (SAS Institute, Inc., Cary, NC). For all comparisons, \( P < 0.05 \) was considered significant.

**Results**

**Experiment 1**
Seven animals receiving chloroprocaine containing NaCl and six animals receiving chloroprocaine contain-

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<tr>
<td>0</td>
<td>Normal</td>
<td>No edema; no injured nerve fibers</td>
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<tr>
<td>1</td>
<td>Mild</td>
<td>Edema; little or no nerve fiber degeneration or demyelination</td>
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<td>2</td>
<td>Moderate</td>
<td>&lt; 50% of nerve fibers with degeneration and demyelination</td>
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<td>3</td>
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<td>&gt; 50% of nerve fibers with degeneration and demyelination</td>
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Fig. 1. Sensory function 7 days after intrathecal administration of a commercially available, preservative-free solution of 3% chloroprocaine HCl (Chloro) containing 0.33% NaCl (n = 27); a commercially available solution of 3% chloroprocaine HCl containing 0.18% sodium bisulfite and 0.21% NaCl (n = 26); or preservative-free normal saline (n = 14). Tail-flick latency values were calculated as the average of latencies for the proximal, middle, and distal portions of the tail and are expressed as the percent maximum possible effect (%MPE): (tail-flick latency − baseline)/[cutoff − baseline] × 100. Data represent mean ± SEM. *P < 0.05 versus all other groups.

Fig. 2. Nerve injury score for sections obtained 7 days after intrathecal administration of a commercially available, preservative-free solution of 3% chloroprocaine HCl (Chloro) containing 0.53% NaCl (n = 27); a commercially available solution of 3% chloroprocaine HCl containing 0.18% sodium bisulfite and 0.21% NaCl (n = 26); or preservative-free normal saline (n = 14). Nerve injury scores were based on all fascicles present in a cross section 12 mm caudal to the conus medullaris. Each fascicle was assigned an injury score of 0–3, where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe. The injury score for each animal was calculated as the average score for all fascicles in the section. Data represent mean ± SEM. **P < 0.05 versus all other groups; ***P < 0.05 versus saline.

Greater than with saline or bisulfite alone (fig. 4). Injury with bisulfite-free chloroprocaine was significantly greater than with chloroprocaine containing bisulfite. Nerve injury with bisulfite alone was similar to that with saline. The more rostral sections from bisulfite- and saline-treated animals were indistinguishable. The only notable finding was some mechanical impingement of the spinal cord by the adjacent catheter in some animals that was associated with a variable degree of pericatheter fibrosis without significant parenchymal extension.

Histopathologic Findings. Nerve injury scores with either formulation of chloroprocaine were significantly greater than with saline, and injury with chloroprocaine containing NaCl was significantly greater than that with chloroprocaine containing sodium bisulfite and NaCl (fig. 2).

Experiment 2
Three animals receiving chloroprocaine and five animals receiving chloroprocaine with bisulfite failed to develop anesthesia and were not studied. One animal given saline was excluded from analysis because the intrathecal catheter had advanced out an intervertebral foramen.

Neurologic Function.
There was no significant difference in baseline tail-flick latencies for the four groups. The group receiving bisulfite-free chloroprocaine developed elevations in tail-flick latency that were significantly greater than those for all other groups (fig. 3).

Histopathologic Findings. Nerve injury scores with either solution of chloroprocaine were significantly greater than with saline or bisulfite alone (fig. 4). Injury with bisulfite-free chloroprocaine was significantly greater than with chloroprocaine containing bisulfite. Nerve injury with bisulfite alone was similar to that with saline. The more rostral sections from bisulfite- and saline-treated animals were indistinguishable. The only notable finding was some mechanical impingement of the spinal cord by the adjacent catheter in some animals that was associated with a variable degree of pericatheter fibrosis without significant parenchymal extension.
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Fig. 4. Nerve injury score for sections obtained 7 days after intrathecal administration of freshly prepared 3% chloroprocaine (Chloro) with 0.4% NaCl (n = 29), 3% chloroprocaine with 0.2% sodium bisulfite and 0.2% NaCl (n = 27), 0.2% sodium bisulfite and 0.2% NaCl (n = 32), or 0.4% NaCl (n = 28). Each fascicle was assigned an injury score of 0–3, where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe. The injury score for each animal was calculated as the average score for all fascicles in the section. Data represent mean ± SEM. *P < 0.05 versus all other groups; **P < 0.05 versus bisulfite or saline.

Discussion

In the current experiments, intrathecal administration of 3% chloroprocaine induced substantial neural injury. In contrast, we found no significant functional impairment or morphologic damage with bisulfite administered without chloroprocaine. However, it is possible that failure to detect a difference could reflect a lack of statistical power. In addition, morphologic changes were assessed only by light microscopic examination, and bisulfite might have an effect detectable by more sensitive methods. Nonetheless, such effects would have questionable clinical significance given their subtlety and lack of associated functional impairment. Even more relevant, these findings would be meaningless compared with the profound injury induced by chloroprocaine. Further, far more than being benign or innocuous, bisulfite lessened both sensory dysfunction and histologic damage induced by chloroprocaine.

Other investigators have evaluated the neurotoxicity of chloroprocaine and bisulfite with widely divergent results. In a model paralleling clinical injury, Ravindran et al.14 evaluated the toxicity of 3% Nesacaine-CE administered intrathecally in dogs at a sufficient volume to induce apnea and hypotension. Only one animal receiving bupivacaine and one receiving low pH normal saline developed unilateral limp. In contrast, 7 of the 20 animals given Nesacaine-CE developed hind limb paralysis. Although these studies substantiated concerns regarding the neurotoxicity of this anesthetic solution, they did not examine bisulfite-free chloroprocaine or bisulfite alone.

Using an isolated rabbit vagus nerve model, Gissen et al.6,15 found that exposure to 3% chloroprocaine with 0.2% sodium bisulfite at pH 3 produced irreversible conduction failure, whereas the same solution buffered to pH 7.3 resulted in recovery. Irreversible block also occurred with exposure to bisulfite without chloroprocaine but only at a low pH, leading these investigators to suggest that liberation of sulfur dioxide was the etiology of the injury. Despite the critical problems extrapolating from an isolated segment of nerve to clinical injury, these experiments became the most widely cited, and their findings remain almost universally accepted.16

Evidence that bisulfite can be neurotoxic was also demonstrated by Wang et al.17 using indwelling catheters to facilitate spinal injection in rabbits. Animals receiving repetitive injections of 0.1–0.2 ml 2% chloroprocaine to a total dose of 30–50 mg fully recovered, whereas all animals receiving 0.6 ml sodium bisulfite (pH 5.3) developed hind limb paralysis. However, the same total dose of bisulfite by smaller repetitive injection did not induce impairment. Interestingly, histologic examination failed to reveal damage even in animals that remained paralyzed.

A second study of intrathecal toxicity in rabbits performed by Ready et al.18 reported similar findings. Both chloroprocaine and chloroprocaine with 0.2% bisulfite failed to produce consistent dysfunction, but bisulfite at concentrations of 0.4% or greater produced significant impairment. Although histologic changes were seen in all three groups, the correlation between function and histology was poor, leading these investigators to suggest that histologic examination provided “insufficient information to justify continuing the practice.”

Experiments conducted by Ford and Raj19 again demonstrated that bisulfite can induce conduction failure and histologic damage. These experiments also provided support for an interaction with pH, because a 10% bisulfite solution at pH 4.4 was well tolerated but induced conduction failure and histologic damage if the pH was reduced to 2.8. Similarly, Hersh et al.20 observed irreversible block of a monosynaptic reflex when 0.6% bisulfite was administered in a solution adjusted to pH 3.

In contrast, other investigators failed to find evidence for specific toxicity of either chloroprocaine or bisulfite.21,22 For example, Rosen et al.22 administered large volumes spinally to sheep and monkeys and found that neither Nesacaine nor the low pH Nesacaine carrier was more toxic than other anesthetics.

Adding to the confusion, studies conducted by Barsa et al.23 substantiated the neurotoxicity of Nesacaine-CE but did not support a role for pH in injury. Unlike the other anesthetics tested, conduction was impaired, and histologic examination revealed abnormalities in nerves exposed to Nesacaine-CE. Raising the pH from 3.3 to 7.0 did not diminish these effects.

Studies conducted in cell culture seemed to provide additional evidence to substantiate chloroprocaine toxicity.24 Using membrane fusion as the marker of toxicity, Seravalli et al.24 found chloroprocaine to be fusogenic, whereas sodium bisulfite and two chloroprocaine me-
tabolites (chloroaminobenzoic acid and dimethylaminooethanol) were not.

Further evidence favoring chloroprocaine as the etiology of injury was obtained by Kalichman et al.,25 who used endoneural edema as a measure of toxicity. All solutions containing chloroprocaine produced more edema than sodium bisulfite, whereas edema with sodium bisulfite (even at pH 3) was similar to that with saline. Although these studies approximated clinical reality more closely than those of Gissen et al.6,15, significant limitations still exist that diminish confidence in the clinical relevance of these findings. First, the relationship between edema and nerve injury is imperfect, particularly with respect to clinically evident damage and reversible effects, and this uncertainty is compounded by lack of functional assessment. Second, as the investigators note, extraneural application is analogous to epidural administration, and the data from intraneural injections (which more closely model intrathecal injection) failed to produce significant differences among study solutions. Finally, although intraneural injection is somewhat analogous to intrathecal injection, it is by no means equivalent, and substantial differences exist that must be considered when extrapolating from these data to spinal toxicity.

The current data thus serve to reinforce and extend the findings of Ravindran et al.,14 Barsa et al.,24 Seravalli et al.,24 and Kalichman et al.,25 but appear to conflict or challenge the data of several investigators, including Wang et al.17, Ready et al.,18 Ford and Raj19, Hersh et al.,20 and, most notably, Gissen et al.6,15 Although some of the discrepancies might reflect species susceptibility, we believe that it is possible to reconcile these seemingly incompatible findings by appreciating the relative toxicities of chloroprocaine and bisulfite, the nature of the laboratory models, and the experimental designs used by these investigators.

Chloroprocaine, like all local anesthetics, will induce neurotoxic damage if administered at a sufficient dose or concentration. Similarly, although we found bisulfite to be neuroprotective, higher doses are almost certainly toxic. This should not be unexpected—as expressed by Paracelsus in the 15th century, “All substances are poisons...the right dose differentiates a poison from a remedy.”26 When viewed in this light, but all but one of the studies suggesting bisulfite has greater toxicity can be dismissed because injury was induced with an excessive amount of bisulfite.17–20 For example, in the study by Wang et al.17, 0.2% bisulfite was compared with 2% chloroprocaine rather than 3% (the concentration associated with clinical injury). In addition, chloroprocaine was administered only by small repetitive injection, and bisulfite did not produce neurotoxicity when administered in this fashion. Ready et al.18 also found clinically relevant concentrations of bisulfite to be well tolerated, with an estimated EC50 for persistent block of toe spread of 0.4 - 0.8%. Similarly, in two electrophysiologic studies, Ford and Raj19 and Hersh et al.20 both found conduction to be unaffected by exposure to 0.2% bisulfite, and it became impaired only when bisulfite was administered at a concentration at least threefold higher.

There remains one set of experiments, from Gissen et al.6,15, that cannot be dismissed on the basis of disproportional administration of bisulfite. However, the profound differences that exist between the experimental conditions of these in vitro studies and clinical practice present substantial limitations that, for the most part, have never been adequately considered. First, experiments were conducted on isolated segments of nerve that lack a cell body, a blood supply, and normal physiologic defenses. Second, the model is, by nature, unstable, and conduction will deteriorate and fail without intervention within a few days. Third, conduction failure is an imperfect surrogate endpoint, and factors that affect conduction in this model may not necessarily produce impairment or histologic damage in an intact animal. Fourth, it is difficult to know relevant concentrations in an in vitro system devoid of normal physiologic processes. For example, the IC50 for tetraeceaine in this model is an order of magnitude lower than that for bupivacaine,27 despite their near clinical equivalence. Fifth, nerves are exposed to a bath containing undiluted bisulfite and a pH equivalent to that of the test solution, an exposure that is not likely to occur in vivo, in which cerebrospinal fluid dilution and cellular and cerebrospinal fluid buffers are present. This artificially low pH might preferentially favor bisulfite toxicity by encouraging release of sulfur dioxide while minimizing intracellular movement of chloroprocaine. Sixth, the composition of the in vitro bath remains relatively constant over time because it lacks redistribution or any appreciable uptake. Finally, activity of sulfite oxidase, the protective enzyme that catalyzes oxidation of sulfites to less toxic sulfates,28,29 is likely to be severely depressed in experiments conducted on segments of disrupted nerve exposed to an excessively low pH at room temperature.

We found bisulfite to limit injury induced by intrathecal chloroprocaine, a rather unexpected finding given the prevailing view of bisulfite as the etiology of Nesa-caine-induced injury. However, substantial data support a beneficial effect of antioxidants in various settings such as ischemic injury. The results of the current study extend this concept, suggesting an important role for oxidative injury in anesthetic neurotoxicity. These findings may provide direction for the development of effective strategies to reduce risk, attenuate damage, or promote recovery from injury.

The current findings have potential and perhaps urgent relevance to the current practice of spinal anesthesia. Over
the past decade, reports of neurologic deficits and transient neurologic symptoms associated with intrathecal lidocaine have stimulated interest in an alternative anesthetic. Although never gaining popularity, chloroprocaine has been investigated as a spinal anesthetic and appears to have a duration of effect suitable for replacing lidocaine. Taken together, this short duration of action and the perception that it is not neurotoxic have rekindled interest in chloroprocaine as a spinal anesthetic, while the availability of preservative-free epidural formulations enables spinal administration as an off-label use of these solutions. The current data raise a cautionary flag regarding this potential practice and indicate the need for additional laboratory and clinical studies.

The current study was not undertaken to assess the toxicity of chloroprocaine relative to other available anesthetics. However, we evaluated the neurotoxicity of 2.5% lidocaine in previous studies using similar methodology. Under these conditions, 2.5% lidocaine induced moderately less dysfunction (maximal possible effect of 48%) and slightly greater nerve injury (1.22) than the 3% chloroprocaine solutions used in the current experiments. Although historical and single-dose comparisons must be used with caution, these data suggest that the toxicities of chloroprocaine and lidocaine are roughly comparable on a milligram-for-milligram basis. Although additional studies are required to draw firm conclusions, the current findings should at least encourage restraint in dosing of this agent as a spinal anesthetic.

In summary, intrathecal administration of chloroprocaine induced significant functional impairment and histologic damage, whereas bisulfit did not. These data suggest that clinical injuries after apparent inadvertent intrathecal injection of epidural doses of Nesacaine-CE resulted from a direct effect of the anesthetic. In addition, coadministration of bisulfit reduced injury induced by chloroprocaine. This finding may provide insight into mechanisms of anesthetic neurotoxicity and strategies to reduce risk or promote recovery.

The authors thank Ricardo Ciriaces, S.R.A. (Department of Anesthesia and Perioperative Care, University of California at San Francisco), for excellent technical assistance.

References


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