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Skilled reaching deterioration contralateral to cervical hemicontusion in rats is reversed by pregabalin treatment conditional upon its early administration

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Abstract

Introduction: Gabapentinoids are first-line treatments for painful traumatic and nontraumatic central nervous system disorders. Evidence from a large human study suggests that early use of gabapentinoids after spinal cord injury improves motor scores. The underlying mechanism is unknown.

Objectives: We sought to examine the effects of early pregabalin (PGB, a gabapentinoid) treatment on performance in a fine motor task (skilled reaching) after cervical hemicontusion. We also asked whether early PGB administration affected PGB responsiveness later on.

Methods: Rats received C4/5 cervical hemicontusions. Injury severities ranged from 80 to 150 kdyn. We monitored evidence of skin irritation (peri-incisional and elsewhere) and quantified food pellet retrieval using the Montoya staircase test. Behaviours were assessed in rats receiving early (for 3 weeks from injury induction) and/or late (resuming or beginning at week 8) PGB treatment in animals with 150-kdyn injuries.

Results: Contralateral skilled reaching waned in control animals with 150-kdyn injuries. This was prevented in animals, which received early PGB as long as treatment continued. Deterioration of skilled reaching was reversed by later (week 8) PGB only in animals that had received early treatment. Ipsilateral reaching impairment was not improved by PGB. Relief of skin irritation verified early PGB efficacy.

Conclusion: Hemicontusive spinal cord injury produces a contralateral motor phenotype evocative of on-going neuropathic pain. Early PGB preserves sensitivity to subsequent PGB treatment, indicating that motor function is impaired by neuropathic pain and can be improved indirectly by early PGB administration. Direct effects of PGB on motor circuitry cannot be excluded but are not supported by our data.

Keywords: Spinal cord injury, Pregabalin, Rats, Skilled reaching, Tonic pain

1. Introduction

Neuropathic pain is a concern for many people with spinal cord injury (SCI),[1] emphasizing a need for its study in animal models. Here, we report on a novel behavioural consequence of SCI, probably reflecting a state of on-going neuropathic pain, arising in a rat model of cervical spinal hemicontusion.[14] This injury results in incomplete destruction of ipsilateral gray and white matter, sparing contralateral spinal tissue.[14]

This work was motivated by results from a large clinical cohort showing that early gabapentinoid treatment led to improved muscle strength over the first year after injury.[6,29] The mechanism is unknown, but the simplest explanation would be a direct effect on injured motor circuitry.[20,26] We investigated the effects of early pregabalin (PGB, a gabapentinoid) treatment after cervical hemicontusion to model clinical findings, and as a first step toward linking its potential neuroprotective or regenerative effects to fine motor function (skilled forepaw reaching). The results, however, suggest that early PGB's effect on later (contralateral) motor improvement rely on preservation of its ability to modulate neuropathic pain.

2. Methods

2.1. Animals

All procedures conformed to guidelines of the Canadian Council on Animal Care and were approved by our institutional Animal Care Committee. We started with 40 Sprague–Dawley rats

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(282–395 g). Two died during surgery; another reached humane end-point 2 to 4 weeks later. Additional behavioural data from control arms of other studies in our centre were collected from 104 Sprague–Dawley rats of similar weights. All injuries were made using the Infinite Horizon Impactor (model IH-0400; Precision Systems and Instrumentation LLC, Fairfax Station, VA) as per Lee et al.[14] Contusions for the PGB study were delivered with a force of 150 kdyn. Other data were collected from 40 rats with identical injuries (20 each from 2 independent experiments), from 14 rats with 80-kdyn injuries, 16 rats with 100-kdyn injuries, and 34 rats with 120-kdyn injuries (20 and 14 from 2 independent experiments). In all cases, the injury was made on the rats' dominant sides (see below). All rats received identical perioperative care and monitoring.[21] An additional group of sham-operated rats (n = 9) was used to monitor surgery-associated skin irritation.

2.2. Pregabalin treatment

Rats received either filtered water or 34 mg/kg of PGB (TEVA Canada Limited, Toronto, ON, Canada) in filtered water through gavage at 12-hour intervals, beginning 1 hour after surgery. This is similar to that used to prompt axonal regeneration in mice (46 mg/kg).[26] Treatment ceased 12 to 24 hours before week 4 of behavioural testing. Pregabalin treatment resumed (or began) in all animals at the beginning of the eighth postoperative week.

2.3. Behavioural testing

All data were collected in a blinded fashion. Fine motor function was assessed with the Montoya staircase test.[14,19] Handedness was determined for each animal (on the penultimate of 12 training sessions over 6 weeks—the ultimate was considered baseline). Postoperative testing was performed at the end of weeks 2, 4, 6, 7 (PGB study only), and 8.

2.4. Statistics

Evidence of skin irritation (wet wounds or fresh scabs around the incision or elsewhere) was collected from monitoring charts and is presented as proportion of animals. Biweekly comparisons were made between vehicle and PGB-treated groups (χ^2 tests). Significant differences ($P < 0.05$) are indicated (**Fig. 1**).

Skilled reaching success is reported as percent of pellets retrieved (individually and mean ± SE). Comparisons were made in Prism 8 (GraphPad) between baseline and postoperative success rates using a one-way RM analysis of variance followed by Dunnett test. We compared retrieval success between PGB- and vehicle-treated animals at 2, 4, and 6 weeks using unpaired two-tailed *t* tests. We used a paired two-tailed *t* test to compare success values at 7 (all untreated) and 8 (all PGB-treated) weeks for (1) all animals and (2) separately for animals which had had early vehicle or early PGB. We also tested for linear trend over the entire postoperative period for all studies. Exact *P* values are shown (**Fig. 2**).

3. Results

3.1. Skin irritation

All spinal cord–injured animals showed evidence of peri-incisional scratching after SCI, irrespective of injury severity (**Fig. 1A**). This was also evident in sham-operated animals, but earlier and with greater prevalence than in animals with SCI, probably because of spared motor function. A small proportion of animals (<20%) with 150-kdyn injuries also had lesions around the ipsilateral ear, in the territory of the great auricular nerve (which projects centrally to the first 4 cervical segments[15]). Early PGB treatment significantly reduced the prevalence of peri-incisional scratching, in-line with known effects of PGB on postoperative pain[4] and pruritus.[9,17]

3.2. Montoya staircase pellet retrieval

Fifty-nine percentage of all rats were left-handed. After 150-kdyn injuries (60% lefties), but not 80-, 100-, or 120-kdyn injuries, there was a progressive decline in skilled reaching contralaterally (**Fig. 2**). Tests for linear trend showed statistical significance for 3 independent data sets (2 in **Fig. 2A**, and the vehicle arm of **Fig. 2B**, 59 animals in total).

Pregabalin treatment prevented the initial decline in reaching success (**Fig. 2B**), but its cessation (before week 4) resulted in worsened performance, statistically equivalent to that of vehicle-treated rats. Resuming or starting PGB treatment at the beginning of week 8 resulted in reaching success one week later that was significantly greater than that at week 7 ($P = 0.0085$, all animals, paired *t* test). Reaching success with late PGB treatment only improved in animals that had received early PGB (**Fig. 2C**, $P = 0.0084$, paired *t* test). Pregabalin had no effect on ipsilateral reaching (**Fig. 2D**).

4. Discussion

This study shows that after cervical hemicontusion: skilled reaching contralateral to injury worsens with time; early PGB treatment prevents the initial decline but only as long as treatment continues; and improvement is conferred by delayed PGB treatment only in animals which received early PGB. Relief of skin irritation by PGB confirmed efficacy. Our data argue that PGB does not cause sedation (increased pellet retrieval despite reduced scratching at week 2) nor does it motivate pellet retrieval by increasing appetite.[12] Although other motivational issues such as anhedonia or learned helplessness are possible, on balance, the data suggest that neuropathic pain interferes with execution of fine movement.

Anticonvulsants like PGB are first-line analgesic treatments for traumatic and nontraumatic central nervous system disorders. Pregabalin binds to $\alpha 2\delta$ subunits of presynaptic voltage-sensitive calcium channels,[3,25] but its analgesic effect probably relies on some other mechanism since its concentration in cerebral spinal fluid reaches maximum concentration ~8 hours after oral administration,[5] and yet, it takes several days for efficacy.[10] Furthermore, clinically relevant doses do not inhibit transmission acutely in the dorsal horn.[3]

Patients prescribed PGB within 3 months of SCI had a higher motor score 1-year after injury than those prescribed PGB later.[29] But how? By direct effects on ability to move, or indirectly by some other mechanism? Although the clinical study did not find any relationship between pain and motor function with early PGB, gabapentinoid status at the time of assessment was unknown, potentially masking effects we have uncovered here. In addition to its anticonvulsant and analgesic effects, recent data highlight PGB as neuroprotective in models of multiple sclerosis,[11] facial nerve injury[20] and epilepsy,[2] and as proregenerative for ascending dorsal column axons after SCI.[26]

That PGB cessation results in decreased skilled reaching capability suggest that its early administration is neuroprotective in the sense that PGB sensitivity remains even after treatment interruption. Because the injury spares contralateral tissue, and because ipsilateral skilled reaching is not improved, PGB probably protects crossed pathways such as the spinothalamic

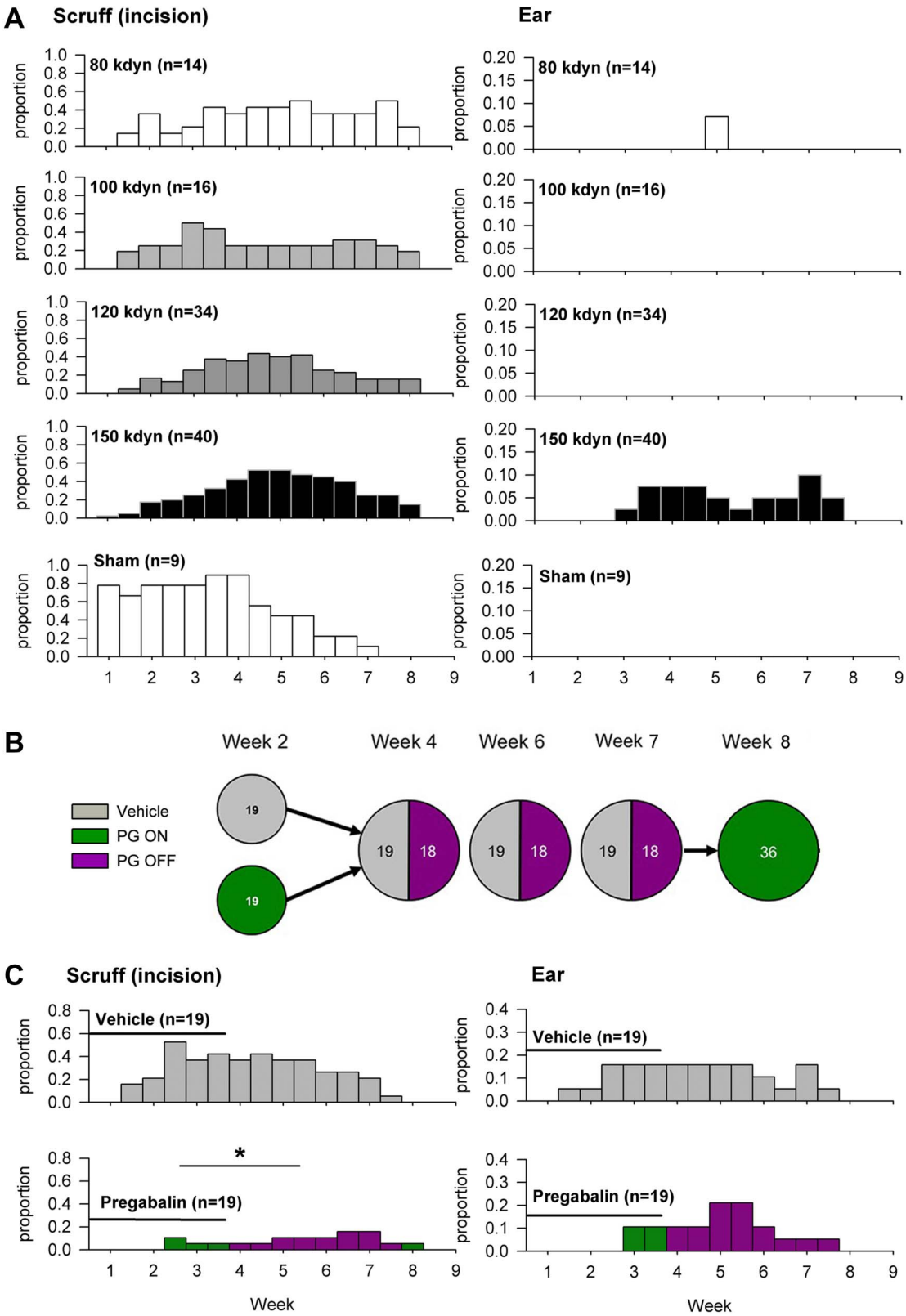

Figure 1. (A) Skin irritation after cervical hemicontusion. Scratching near the incision site occurred regardless of injury severity, while scratching behind the ipsilateral ear occurred in some animals with more severe injuries. In spinal-intact sham-operated animals, scratching was more prevalent and occurred earlier than in those with SCI. (B) Schematic indicating treatment groups and animal numbers. "PG off" (magenta) refers to animals that had had PGB treatment starting at the time of injury. (C) PGB delayed the onset and reduced the prevalence of peri-incisional scratching (*$P < 0.05$, χ^2 test). PGB, pregabalin; SCI, spinal cord injury.

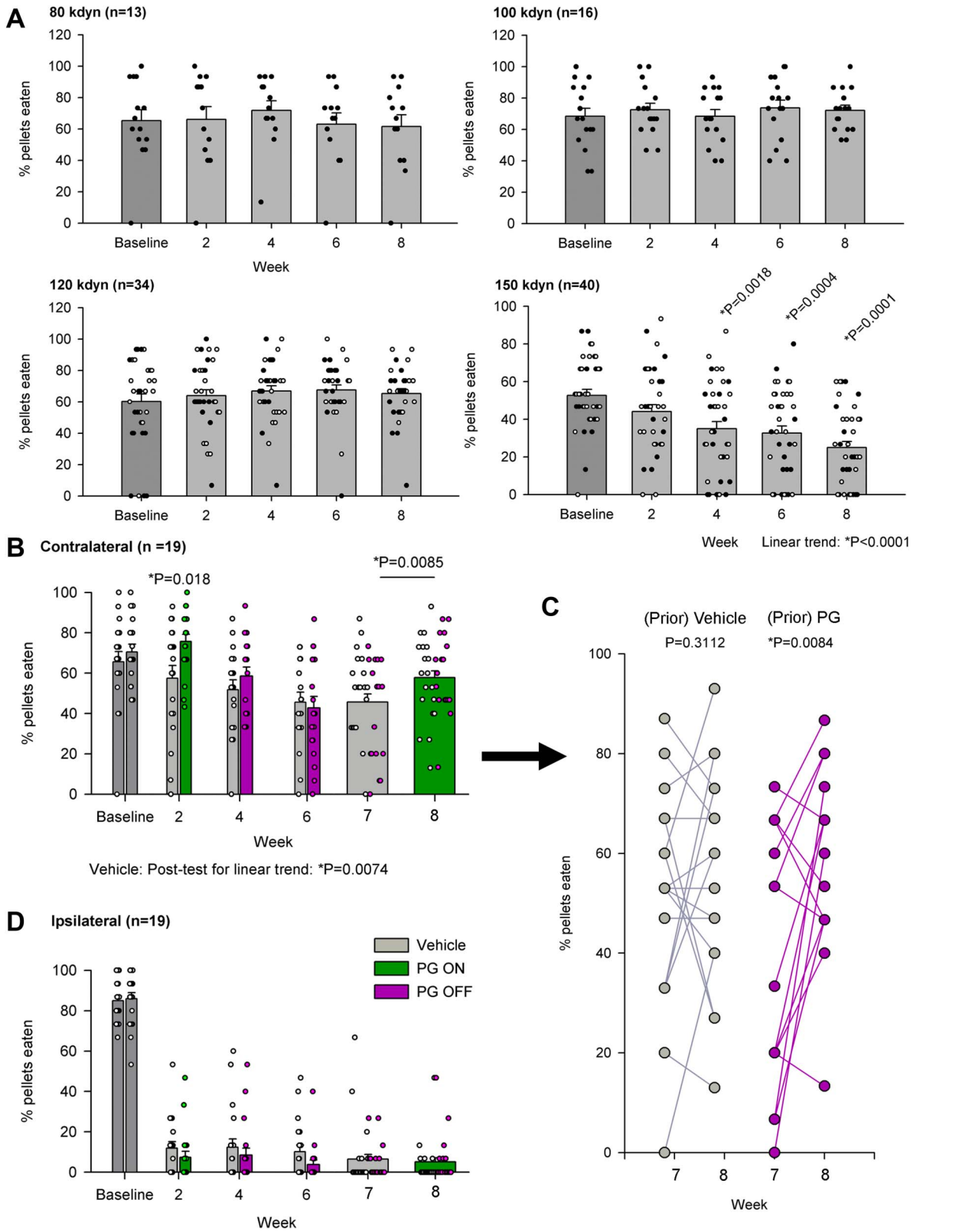

Figure 2. (A) Pellet retrieving ability contralateral to a cervical hemicontusion. With more severe injury, there was a progressive reduction in pellet retrieval success. Filled and open symbols represent 2 independent experiments each at 120 and 150 kdyn. *Significant differences from baseline (one-way analysis of variance followed by Dunnett test for multiple comparisons). Linear trends were $P < 0.0001$ for both 150-kdyn experiments. (B) Early PGB treatment (green), prevented the initial decline in contralateral pellet retrieving ability (asterisk at 2 weeks, two-tailed unpaired *t* test), but cessation abolished this effect. Later, PGB treatment improved retrieval ability (asterisk between weeks 7 and 8, two-tailed paired *t* test). Magenta-filled symbols indicate animals that had received early PGB but were removed from treatment between weeks 4 and 7. (C) Retrospective analysis of weeks 7 and 8 data revealed that only those animals that had received early PGB were responsive to later PGB treatment (two-tailed paired *t* test). (D) Neither early nor late PGB treatment affected ipsilateral pellet retrieving ability. PGB, pregabalin.

tract (STT): incomplete STT damage is believed to be a prerequisite for SCI-induced neuropathic pain.[7,8,30] Clinically, contusive STT-sparing hemicord lesions are rare but can result in contralateral pruritus[27] and neuropathic pain.[16] For these reasons, we suggest that progressive deterioration of contralateral reaching relies on residual but abnormal STT function, an idea that could be tested in the future using complete (STT-severing) spinal hemisection. The absence of any ipsilateral influence of PGB also argues against a direct effect on motor circuitry. A "basement" effect might be postulated, but we have previously shown that ipsilateral skilled reaching after 150-kdyn hemicontusions can be improved through therapeutic intervention.[23,24]

Studying SCI-induced neuropathic pain in experimental animals is notoriously difficult because of reliance on responses to peripheral stimulation.[13] Such measures are problematic because of hyper-reflexia,[28] and their questionable relevance to the clinical condition in which pain is most often spontaneous or tonic.[22] Attempts to assess stimulus-independent neuropathic pain after experimental SCI in rats have involved exploratory behaviour[18] or conditioned place preference.[31] The time course of declining reaching ability shown here corresponds well with gabapentinoid-sensitive decreases in exploratory behaviour,[18] further bolstering the idea that worsening skilled reaching reflects neuropathic pain. In people, symptoms are also progressive and persistent.[4,22]

In conclusion, cervical spinal hemicontusion reduces contralateral skilled reaching probably as a result of neuropathic pain associated with STT damage. Early PGB treatment not only prevents the initial decline in ability but also preserves sensitivity to PGB. Whether this relies on structural neuroprotection (ie, tissue sparing) or otherwise remains to be determined. In addition to stressing the importance of early pain intervention after SCI,[6] these data offer an explanation for beneficial effects of early PGB on clinically demonstrated improvements in motor scores.[29]

Disclosures

The authors have no conflict of interest to declare.

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