Injectable Corticosteroid Preparations: An Embolic Risk Assessment by Static and Dynamic Microscopic Analysis

BACKGROUND AND PURPOSE: Transforaminal CS injections have been associated with severe adverse CNS events, including brain and spinal cord infarction. Our purpose was to describe the static and dynamic microscopic appearances of CS preparations, with an emphasis on their potential to cause adverse central nervous system events by embolic mechanisms during transforaminal injection.

MATERIALS AND METHODS: Pharmaceutical preparations of nondilute injectable CSs were used after appropriate mixing: MPA (40 mg/mL), TA (40 mg/mL), and DSP (8 mg/2 mL). For dynamic imaging, a novel methodology was devised to replicate the flow of crystals within spinal cord arterioles. In addition, CS preparations were mixed with plasma to assess for changes in crystal size, morphology, and tendency to aggregate.

RESULTS: The CS preparations MPA and TA are composed of crystals of varying sizes. MPA crystal size range was 0.4–26 μm (mean, 6.94 μm), TA crystal size range 0.5–110 μm (mean, 17.4 μm), and DSP did not contain any significant crystals or particles. There was no change in the crystal morphology or propensity to aggregate after mixing with local anesthetic. After mixing with plasma, the crystals also were unchanged; however, there was a significant reduction in the size of aggregates. On dynamic imaging, these aggregates were proved to maintain their integrity and to act as potential embolization agents.

CONCLUSIONS: MPA and TA have a substantial risk of causing infarction by embolization if inadvertently injected intra-arterially at the time of TFESI. DSP is completely soluble and microscopically has no potential to obstruct arterioles. When performing cervical TFESI procedures, the administration of insoluble CSs should be avoided.

ABBREVIATIONS: CNS = central nervous system; CS = corticosteroid; DSP = dexamethasone sodium phosphate; LA = local anesthetic; MPA = methylprednisolone acetate; RBC = red blood cell; TA = triamcinolone acetonide; TFESI = transforaminal epidural spinal injection.
Materials and Methods

Agents
The analyzed CS preparations were limited to 40 mg/mL MPA (Depo-Medrol; Pfizer, New York, New York), 40 mg/mL TA (Kenalog; Bristol-Myers Squibb, New York, New York), and 4 mg/mL DSP (Hospira, Lake Forest, Illinois). The LA preparation used was 0.25% bupivacaine hydrochloride (Marcaine; AstraZeneca, London, United Kingdom). Human plasma was obtained by centrifuging whole blood at 3000 rpm for 10 minutes. Plasma was obtained by using a disposable pipette.

Materials
At all stages standard syringes and needles were used to replicate clinically performed CS preparation and administration techniques. The smallest caliber needle used was a 25-gauge needle. We used the μ-Slide I Luer flow kit (Ibidi, Munich, Germany) to perform dynamic microscopic analysis. This allows microscopic images to be obtained as CS preparations flow through a 200-μm-depth channel (Fig 1).

All for static imaging, measurements were obtained with an Axioskop 40 microscope (Carl Zeiss Microimaging, Jena, Germany) fitted with an OptiScan motorized stage (Prior Scientific, Cambridge, United Kingdom) controlled by the Image-Pro Plus 6 image analysis program (Media Cybernetics, Bethesda, Maryland). Images were collected by using an Achromplan ×10, ×20, and ×40 phase contrast objective with a ProgRes C10+ digital camera (Jenoptik Ag, Jena, Germany). The images were calibrated for each objective on the microscope by using the stage movement controlled by the Image-Pro Plus Scope Pro plug-in. These calibrations were checked against a slide micrometer.

For all dynamic imaging, an AxioCam HR charge-coupled device camera in black-and-white mode (Carl Zeiss Microimaging) was used at 1388 × 1040 resolution, 12 images/s, and videos recorded via Axiovision software (version 4.7; Carl Zeiss Microimaging).

Methods
All agents were shaken as to manufacturer’s instructions. Nine separate samples were prepared for analysis: MPA only, TA only, DSP only, MPA + LA, TA + LA, DSP + LA, MPA + LA + plasma, TA + LA + plasma, and DSP + LA + plasma (Table). A mixture ratio of 1:1 or 1:1:1 was used for the prepared samples. For every 1 mL (mil) of CS, 1 mil of LA was mixed (1:1). All samples were prepared immediately before imaging (delay of no >2 minutes between preparation and imaging).

For static imaging, a single drop of prepared sample was placed on a microscope slide via a standard 25-gauge needle, and a coverslip was applied. High resolution (2080 × 1542) color RGB digital images were then obtained at ×100, ×200, and ×400 magnification after calibration had been performed. For dynamic imaging, the prepared samples were manually injected via a short low-pressure tube attached to the μ-Slide device (Fig 1). Injection speed was similar to standard clinical technique. Gray-scale digital video clips by using MPEG4 encoding at a resolution of 1360 × 1040, 10 frames/s, and data rate of 2648.59 kbits/s were then obtained. All video clips were calibrated and annotated. All dynamic imaging was performed at ×200 magnification only.

For static measurements, random fields of view of each preparation were used. From the high magnification (×400) images, individual crystals were measured to establish minimum, maximum, and mean sizes. Lower power magnification (×100) images were used to measure minimum, maximum, and mean sizes of the crystal aggregates. On dynamic imaging, random frames from the video clip were used to obtain the minimum, maximum, and mean crystal aggregate measurements.

A size cutoff of 10 μm was used for group analysis. This number was picked because particles >10 μm have been demonstrated to occlude capillaries in vivo. It is also of note that the average diameter of a RBC is 6–8 μm.

StatPlus (AnalystSoft, Alexandria, Virginia) was used for statistical analysis. The Student t test was used to test for statistical significance. This study was exempted from Institutional Review Board approval.

Results

MPA
MPA was confirmed to be a crystalline preparation (On-line Fig 1). Crystals tend to be oval or round and relatively uniform in size (mean, 6.9 μm; range, 0.5–26 μm). In any random field of view, most crystals were less than the size of a RBC (On-line Fig 2). A mean of 16.7% of crystals were >10 μm (Table). On static imaging the crystals tended to aggregate (On-line Fig 1). Approximately 84.7% of crystals were aggregated into larger particles. Aggregates ranged from 5 to 200 μm, with a mean size of 59 μm, when formed on a glass slide. Mixing with LA had no significant effect on crystal sizes or aggregation (On-line Fig 3). Mixing with plasma, however, caused a reduction in the size of the visualized aggregates (mean, 21.6 μm; range, 5–55 μm: On-line Fig 4). There was no change in the size of individual crystals after mixing with plasma (On-line Fig 5).

On dynamic imaging, the aggregation of crystals was confirmed to be real and not an artifact of using glass slides. The aggregates visualized flowing through the channel ranged from 5 to 195 μm, with a mean of 42.9 μm. These aggregates maintained their integrity during injection and hence effectively act as large particles (On-line Fig 6). Similar to the results of static imaging, there was no change in aggregation or crystal size after mixing with local anesthetic (On-line Video 1). After mixing with plasma, aggregates significantly reduced in size. These microaggregates ranged from 5 to 50 μm, with a mean size of 21.6 μm (Fig 2). The injectate appeared denser.
Comparison of the crystals and aggregates found in MPA and TA at high-powered microscopy

| Crystal size | MPA | MPA + LA | MPA + LA + Plasma | TA | TA + LA | TA + LA + Plasma |
|--------------|-----|----------|-------------------|----|---------|------------------|
| Mean (μm)    | 6.94| 7.1      | 6.91              | 17.4| 17.1    | 17.9             |
| Range (μm)   | 0.5–26| 0.4–26  | 0.5–25            | 1–110| 0.5–108 | 0.3–110          |
| % >10 μm³    | 16.7| 15.9     | 17.1              | 38  | 41      | 40               |
| % aggregated³ | 85² | 83      | 70²,*            | 85² | 81      | 10²,*            |
| Aggregate size | Mean (μm) | 42.9⁴ | 45.1            | 21.64⁴,*  | 14.3⁵ | 15.1            | 9.6⁵,*          |
| Range (μm)   | 5–195| 4.1–186 | 3.2–50           | 2–115| 3–111   | 5–25             |
| % >10 μm³    | 88   | 82      | 80               | 28⁶ | 22      | 40⁶,*            |

Note—DSP is not included in this table as there were no crystals or aggregates identified in DSP.
³ Percentage of crystals >10 μm and therefore with embolic potential.
² Percentage of crystals that are formed into aggregates.
¹ Denoting where there were statistically significant differences in measurements between plasma and nonplasma mixed corticosteroid (*P < .05).
⁴ Denoting where differences in measurement were statistically significant between MPA and TA.

Due to the more diffuse dispersion of individual crystals and smaller aggregates (On-line Video 2). Approximately 80% of aggregates, after mixing with plasma, were >10 μm as visualized on dynamic imaging.

**TA**

TA is also a crystalline preparation; however, the crystals are morphologically different to MPA (On-line Fig 7). The largest crystals tended to be rectangular (On-line Fig 8). There is also a greater range in the size of TA crystals compared with MPA. Individual crystals ranged in size from 1 to 110 μm, with a mean size of 17.4 μm. Approximately 85% of crystals were components of aggregates; however, the largest crystals did not tend to aggregate together. As a result, aggregates measured no >15 μm. Most aggregates were much smaller than this and composed of small crystals. Like MPA, there was no effect after mixing with LA. After mixing with plasma, crystal aggregation reduced to an even greater degree than that seen with MPA (On-line Fig 9).

On dynamic imaging, the integrity of the large individual crystals and crystal aggregates was unchanged. There was no change on mixing with LA (On-line Fig 10 and On-line Video 3). After mixing with plasma, very few aggregates were visualized (Fig 3 and On-line Video 4). Those that were present had a range of sizes from 5 to 25 μm, with a mean of 9.8 μm. Approximately 40% of aggregates measured >10 μm. Overall, approximately 50% of the “particulates” (ie, crystals or aggregates) in TA when mixed with plasma are >10 μm.

**DSP**

DSP is not a crystalline preparation (On-line Fig 11). Because the environment in which the DSP samples were prepared was not perfectly clean, small skin cells and other tiny particles (submicrometer in size) were occasionally seen on high magnification. Otherwise, no crystals or particulates >1 μm were identified at high magnification on static imaging. Certainly, there is no constituent of DSP close to, or larger than, a RBC. Mixing with LA had no effect, and no particulates were precipitated. Mixing with plasma revealed the presence of a few RBCs remaining after centrifuging of whole blood but no change to the DSP preparation.

On dynamic imaging, there was no change to these results (On-line Video 5 and Fig 4). The residual RBCs were visible flowing through the channel (On-line Video 6).

**Discussion**

The recent reporting of severe adverse CNS events, including death, occurring during or immediately after TFESI proce-
Commercially available CSs can be divided into 3 groups: insoluble (includes MPA and TA), soluble (includes DSP), and a formulation composed of a mixture of insoluble and soluble CSs.\(^1\) In general, injectates containing insoluble CSs have been preferred over soluble CSs during TFESI procedures because a medium- to long-term relief of symptoms is desired.\(^1,16,17\) This long-term benefit principally derives from the fact that insoluble CSs are esters and hence require cellular esterases for the active steroid moiety to be released. In addition, insoluble CS preparations are supplied as a crystalline powder in aqueous suspension that is less likely to be systemically absorbed soon after injection compared with soluble preparations.\(^17\) It is this crystalline nature of insoluble CSs, with the potential to occlude arteries, that is now regarded as the leading cause of the reported CNS adverse events occurring during TFESI. It is important to note that there are no case reports of events occurring with the use of soluble CSs.

Researchers have previously examined CSs by microscopy to evaluate their crystalline appearance.\(^18-20\) The clarity of the images obtained and the analysis of only static preparations, however, limited the ability to fully clarify their embolization potential. Our study has used higher powered, higher resolution microscopes in combination with close replication of clinical procedures and the dynamic intra-arterial environment to analyze the propensity of CSs to cause embolization events.

The results of this study demonstrate that the 2 most commonly administered insoluble CSs—MPA and TA—are composed of individual crystals that range in sizes significantly larger than RBCs; 16.7% of MPA crystals and 38% of TA crystals are \(>10\) \(\mu\)m. In addition, these crystals do not dissolve or change in morphology after mixing with LA or plasma.

Importantly, as has been suggested in other papers, insoluble CS crystals can form larger aggregates when dropped onto a static microscope slide.\(^18\) We have demonstrated that these apparent aggregates maintain their integrity when flowing through an arteriole simulator and therefore act as a larger particulate in the arterial system. Interestingly, after mixing with plasma, these aggregates significantly decrease in size. The mean sizes of MPA crystal aggregates before and after mixing with plasma were 42.9 and 21.64 \(\mu\)m, respectively \((P < 0.05)\). Similarly for TA, the mean sizes of crystal aggregates before and after mixing with plasma were 14.3 and 9.8 \(\mu\)m, respectively \((P < 0.05)\). Crystal aggregation in TA was so inhibited after mixing with plasma that it was thought unlikely to play a major role in any potential embolization events. TA crystals tend to be quite large (maximum size of 110 \(\mu\)m) and are thus capable of occluding tiny vessels. It is not clear why crystal aggregation is inhibited when mixed with plasma but it is possible similar to the mechanism by which calcium oxalate crystal aggregation in urine is inhibited by serum albumin and globulins.\(^21\) Plasma contains albumin and globulins that may have inhibitory effects on individual intercrystal adhesion, thereby reducing the size of the aggregates that form.

DSP was confirmed to be a completely soluble form of CS without evidence of any crystalline structures at high-powered microscopy. In addition, there was no change to the appearance of DSP after mixing with LA and plasma.

The clinical significance of these results is readily evident. Insoluble CSs are composed of crystals and larger aggregates, capable of occluding the small end arteries supplying the CNS; thus, they present an extremely high risk of causing infarction. Indeed, the fact that insoluble CSs, when mixed with plasma, are composed of smaller aggregates (still \(>10\) \(\mu\)m) than reported previously, increases the risk of infarction as arterial injection will generate a shower of small particulates that will obstruct the most distal arterioles where collateral vessels are less likely to be present.\(^22\) DSP cannot cause embolization events because it does not contain structures that are larger than the size of RBCs. It is important to note that these results are not only applicable to scenarios involving paraspinal injections but also relevant to any scenario where inadvertent injection of CSs is possible, especially at sites with limited ability to collateralize or cope with short-term distal microembolization (eg, injections at the wrist or ankle).

These results are compatible with an in vivo study performed on pigs examining the effect of direct vertebral artery injection of insoluble CSs versus soluble CSs.\(^23\) We demonstrated that DSP had no effect on pigs as assessed by functional examination, imaging, and histologic examination. Conversely, the administered insoluble CS (MPA) induced visible ischemic changes in the CNS on MR imaging, with eventual death in all cases. These results reinforce the concept that insoluble CSs are extremely dangerous if they enter an artery supplying the CNS.

The most common preservative and drug vehicle present in steroid injectates is benzyl alcohol and polyethylene glycol, respectively. These have been postulated by many studies to be sources of significant toxicity.\(^24-26\) Neurotoxic effects ascribed to benzyl alcohol include demyelination and neural degener-
ation. Polystyrene latex particles administered intravenously to rats—A collaborative study. J Pharmacol Exp Ther. 1983;226:124–30

References

1. MacMahon PJ, Eustace SJ, Kavanagh EC. Injectable corticosteroid and local anesthetic preparations: a review for radiologists. Radiology 2009;252:647–61
2. Rathmell JP, April CF, Bogduk N. Cervical transforaminal injection of steroids. Anesthesiology 2004;100:1395–600
3. Rosenkranz M, Greyska U, Niesen W, et al. Anterior spinal artery syndrome following periradicular cervical nerve root therapy. J Neurol 2004;251:1299–31
4. Brouwers PJ, Kottink EJ, Simon MA, et al. A cervical anterior spinal artery syndrome after diagnostic blockade of the right C6-nerve root. Pain 2001;91:397–99
5. Ruppen W, Högli R, Rosas S, et al. Neurological symptoms after cervical transforaminal injection with steroids in a patient with hypoplasia of the vertebral artery. Acta Anaesthesiol Scand 2008;52:165–66
6. Muro K, O’Shaughnessy B, Ganju A. Infarction of the cervical spinal cord following multilevel transforaminal epidural steroid injection: case report and review of the literature. J Spinal Cord Med 2007;30:385–88
7. Suresh S, Berman J, Connell DA. Cerebellar and brainstem infarction as a complication of CT-guided transforaminal cervical nerve root block. Skeletal Radiol 2007;36:449–52
8. Baker R, Dreyfuss P, Mercer S, et al. Cervical transforaminal injection of corticosteroids into a radicular artery: a possible mechanism for spinal cord injury. Pain 2003;103:211–15
9. McLellan MR, Crumpton C. Cortical blindness and neurologic injury complicating cervical transforaminal injection for cervical radiculopathy. Anesthesiology 2003;99:509–11
10. Dawley JD, Moeller-Bertram T, Wallace MS, et al. Intra-arterial injection in the rat brain: evaluation of steroids used for transforaminal epidurals. Spine (Phila Pa 1976) 2009;34:1638–43
11. Macky TA, Helmy D, El Shazly N. Retinal toxicity of triamcinolone’s vehicle (benzyl alcohol): an electrophysiologic and electron microscopic study. Graefes Arch Clin Exp Ophthalmol 2007;245:817–24
12. Hahn AF, Feasby TE, Gilbert JJ. Paraparesis following intrathecal chemotherapy. Neurology 1983;33:1032–38
13. Hetherington NJ, Dooley MJ. Potential for patient harm from intrathecal administration of preserved solutions. Med J Aust 2000;173:141–43
14. Rasmussen N, Selk P. Lumbar nerve root pain: what works and what doesn’t? Int Musculoskeletal Med 2009;33:166–71
15. Gesler RM, Garvin PJ, Klamer B, et al. The biologic effects of polystyrene latex particles administered intravenously to rats—a collaborative study. Bull Paracervical Pharm 1973:2101–13
16. Dent PB, Walker N. Intracartilagenous corticosteroids in the treatment of juvenile rheumatoid arthritis. Curr Opin Rheumatol 1998;10:475–80
17. Cole BJ, Schumacher HR Jr. Injectable corticosteroids in modern practice. J Am Acad Orthop Surg 2005;13:37–46
18. Tiso RL, Cutler T, Catania JA, et al. Adverse central nervous system sequelae after selective transforaminal block: the role of corticosteroids. Spine J 2004;4:468–74
19. Derby RS, Snell SH, Date ES, et al. Size and aggregation of corticosteroids used for epidural injections. Pain Med 2008;9:227–34
20. Benzon HT. Epidural steroid injections for low back pain and lumbosacral radiculopathy. Pain 1986;24:277–95
21. Grover PK, Moritz RL, Simpson RJ, et al. Inhibition of growth and aggregation of calcium oxalate crystals in vitro—a comparison of four human proteins. Eur J Biochem 1998;253:637–44
22. Sliwa JA, Maclean IC. Ischemic myelopathy: a review of spinal vasculature and related clinical syndromes. Arch Phys Med Rehabil 1992;73:365–72
23. Okubadejo GO, Talcott MR, Schmidt RE, et al. Perils of intravascular methylprednisolone injection into the vertebral artery. An animal study. J Bone Joint Surg Am 2008;90:1932–38
24. Younis HS, Shaver M, Palacio K, et al. An assessment of the ocular safety of inactive excipients following sub-tenon injection in rabbits. J Ocul Pharmacol Ther 2008;24:206–16
25. Chang YS, Wu CL, Tseng SH, et al. In vitro benzyl alcohol cytotoxicity: implications for intravitreal use of triamcinolone acetonide. Exp Eye Res 2008;86:942–50
26. Toyooka K, Fujimura H. A cervical anterior spinal artery syndrome after diagnostic blockade of the right C6-nerve root. Pain 2001;91:397–99
28. Craig DB, Habib GG. Facedic paraparesis following obstetrical epidural anesthesia: possible role of benzyl alcohol. Anesth Analg 1977;56:219–21
29. Ray CE. Pain Management in Interventional Radiology. Cambridge, United Kingdom: Cambridge University Press; 2008
30. Spaccarelli KC. Lumbar and caudal epidural corticosteroid injections. Mayo Clin Proc 1996;71:169–78
31. Yin W, Bogduk N. Retrograde filling of a thoracic spinal artery during transforaminal injection. Pain Med 2009;10:689–92
32. Verrills P, Nowesenitz G, Barnard A. Penetration of a cervical radicular artery during a transforaminal epidural injection. *Pain Med* 11:229–31.

33. Huntoon MA. Anatomy of the cervical intervertebral foramina: vulnerable arteries and ischemic neurologic injuries after transforaminal epidural injections. *Pain* 2005;117:104–11

34. Houten JK, Errico TJ. Paraplegia after lumbosacral nerve root block: report of three cases. *Spine J* 2002;2:70–75

35. Kennedy DJ, Dreyfuss P, Aprill CN, et al. Paraplegia following image-guided transforaminal lumbar spine epidural steroid injection: two case reports. *Pain Med* 2009;10:1389–94

36. Thefenne L, Dubecq C, Zing E, et al. A rare case of paraplegia complicating a lumbar epidural infiltration. *Ann Phys Rehabil Med* 2010;53:575–83

37. Lenoir T, Deloin X, Dauzac C, et al. Paraplegia after interlaminar epidural steroid injection: a case report. *Rev Chir Orthop Reparatrice Appar Mot* 2008;94:697–701