

Method Development and Optimization for Intrathecal Injection in Dogs

Purpose

Administered drugs frequently encounter challenges in penetrating brain regions due to the presence of the blood-brain barrier and the clearance of drugs by the mononuclear phagocyte system. Although intracerebral or intraventricular administration can rapidly deliver drugs to the brain, these methods are invasive and infrequently employed in clinical application. Intrathecal injection, on the other hand, facilitates the direct delivery of drugs into the cerebrospinal fluid (CSF), presenting an effective strategy for intracerebral drug administration. Unlike other methods, intrathecal injection bypasses the blood-brain barrier. Furthermore, it is safe, minimally invasive, and constitutes a practical option for treatment. For instance, the intrathecal route has been effectively utilized to deliver therapeutic proteins to brain regions. Intrathecal injection via lumbar puncture is a crucial component in the investigation of neurological diseases.

Methods

To facilitate dosing, animals were briefly anesthetized. Both male and female dogs were fasted overnight. A sufficiently large area of the surrounding skin was shaved (approximately from L4 to L7, centered on the spinous processes) and cleansed with an aseptic solution. The operator wore sterile gloves. The dosing was administered via an intrathecal injection between the lumbar segments L5 and L6. If administration between L5 and L6 was not feasible, the segments L4 and L5 or L6 and L7 were used as alternatives. The injection was delivered gradually, with a total volume of 500 μ L of normal saline administered over the course of one minute.

The spinal needle was inserted at the caudal aspect of the L6 spinous process, slightly lateral, and directed cranioventrally, advancing into the vertebral canal through the L5-L6 interarcuate space. Upon penetration of this ligament and entry into the vertebral canal, resistance decreased markedly; often, twitching of the tail or pelvic limb was observed as the needle irritated the cauda equina nerve roots. The needle was advanced until it contacted the bone of the ventral aspect of the spinal canal to access the ventral subarachnoid space; subsequently, the stylet was removed to determine the presence or absence of CSF fluid and to collect it. If CSF was not observed, the needle was slowly readjusted, with rotation and modification of the bevel orientation. If CSF flow was still not evident, the needle was gradually withdrawn and rotated while remaining within the vertebral canal until CSF became visible.

Results

No procedure-related body weight, food consumption, and body temperature changes were noted. Procedure-related findings, such as transient salivation, tremors, abnormal gait, and decreased activity, were noted in both male and female dogs only on Day 1 and fully resolved by Day 2. No gross abnormalities were observed in the nervous system tissues.

The following microscopic changes were interpreted as related to the experimental procedure:

Spinal Cord, Lumbar, and/or Sacral

- Needle Track: Degeneration, Dystrophy, Axonal, Gliosis, Mononuclear Cell Infiltration, Hemorrhage, Microgliosis, and/or Tissue Loss

Specific changes directly associated with inadvertent spinal cord penetration were diagnosed under the site "Needle Track."

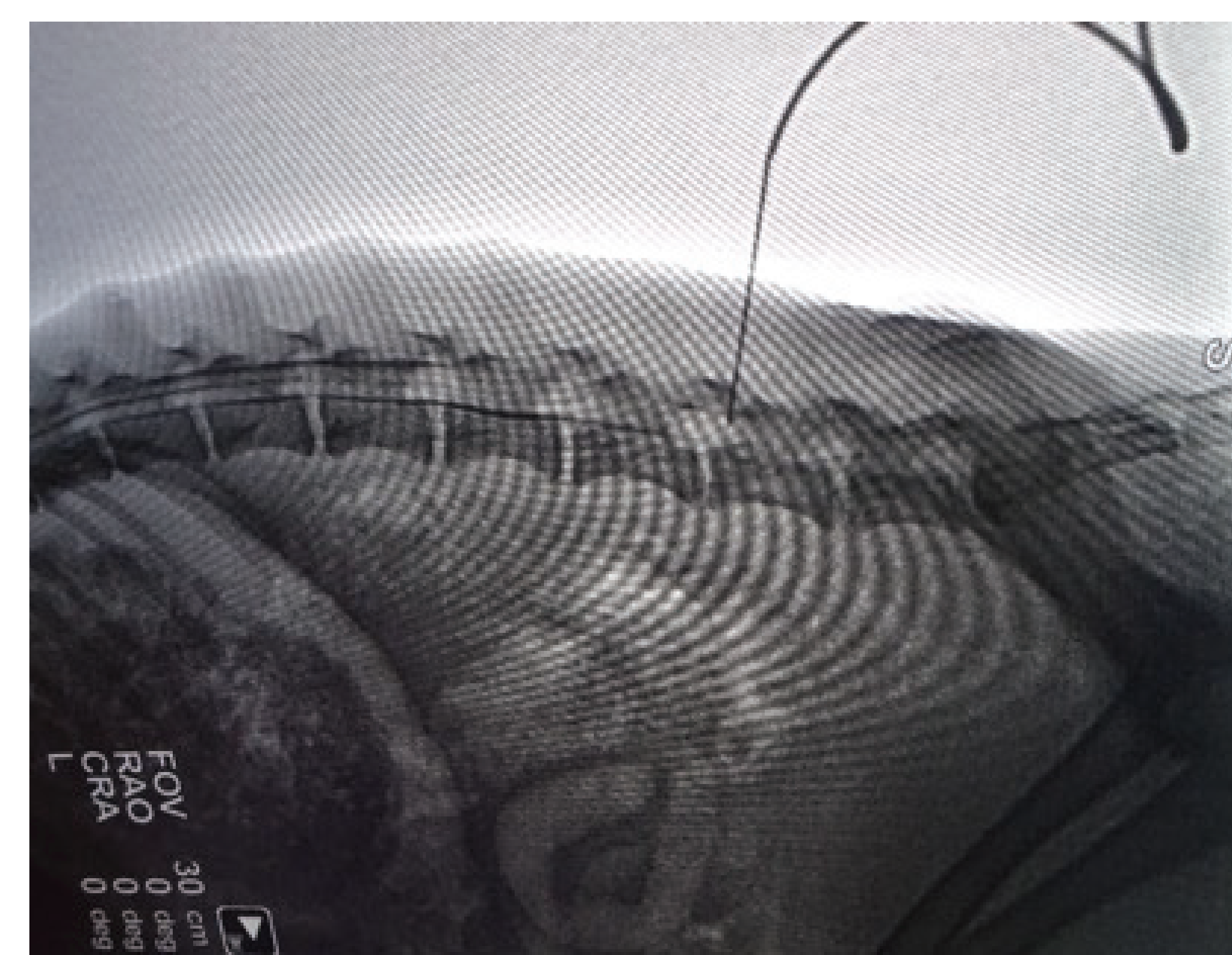
- Tracts, Dorsal, Lateral, and/or Ventral: Degeneration, Dystrophy, Axonal, and/or Microgliosis, Locally Extensive

Occasionally, one or more low-grade, locally-extensive changes were observed in sections of the sacral spinalcord that did not contain discrete needle tracks.

- Nerve Root, Spinal: Degeneration, Nerve Fiber and/or Decreased Staining, Locally Extensive

Occasionally, locally-extensive changes were observed in portions of the spinal nerve roots included in sections of the sacral spinal cord. In some instances, these spinal nerve root changes were located adjacent to spinal cord needle tracks.

Figure 1 Digital Subtract Angiography (DSA) of caudal spine. Lateral radiograph showing adequate spinal needle placement at L5-L6.



Conclusions

All the above findings indicate that, with refinements, the intrathecal injection method is considered suitable for the evaluation of drugs in dogs using this route of administration.

