Clinical actions of subarachnoid sevoflurane administration

in vivo: a study in dogs

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Background. Halogenated ethers produce clinical effects at spinal sites. Nevertheless, in vitro and in vivo studies have not determined whether the immobilizing effect in the spinal cord is due to inhibition of nociceptive or motor transmission or both. Our goal was to characterize the clinical effects of direct spinal sevoflurane administration.

Methods. Five adult beagle dogs completed the study. In a randomized and blinded manner each animal received placebo (saline 0.1 ml kg⁻¹) and three concentrations of pure sevoflurane administered intrathecally (0.05, 0.075 and 0.1 ml kg⁻¹) by means of a permanent spinal catheter. Sensory and motor block and state of consciousness were determined at baseline and at predetermined regular intervals until at least 2 h after total recovery.

Results. None of the dogs presented a decrease in consciousness with either 0.05 or 0.075 ml kg⁻¹ of sevoflurane. Administration of 0.1 ml kg⁻¹ produced light sedation (2 on a four-point sedation scale) in three of the five dogs. A comparison of the duration of the sensory and motor blocks among the three sevoflurane dosages shows a significant dose-dependent increase that is greater in all cases than that for the saline solution.

Conclusions. Spinal administration of pure sevoflurane resulted in a dose-related and totally reversible motor and sensory regional block without any signs of clinical neurotoxicity or significant decrease in consciousness. Therefore the model allows us to comment on the analgesic effects at the spinal level in addition to the direct immobilizing effects of sevoflurane.

Keywords: anaesthetic–analgesic regimens; anaesthetic techniques, subarachnoid; anaesthetics volatile, sevoflurane; model, dog

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The spinal action of halogenated ethers produces several important clinical effects during general anaesthesia. While hypnosis and/or amnesia are caused by the cerebral action of halogenated ethers, immobility despite surgical stimulus and blockade of the adrenergic response to painful stimuli are facilitated by the action of halogenated ethers on spinal motor neurones and the posterior column of the spinal cord.1–3 Thus halogenated ethers have been recognized (both in vitro and in human reports) as immobilizing and analgesic agents through their effects on the spinal cord.4–6 Consequently, the cerebral action of halogenated ethers may also be enhanced by a spinal action that depresses ascending sensory information.7

Existing in vitro and in vivo studies of the spinal effects of halogenated ethers have not determined whether the immobilizing effect at the spinal cord is due to inhibition of nociceptive transmission acting on dorsal neurones or inhibition of the motor neurones or a combination of both effects.8–11 This study presents a novel in vivo experimental model in dogs that has not been used before. Sevoflurane is directly administered to the spine instead of through the more traditional route of systemic inhalation. The main objective of our study with this model was to characterize the effects of sevoflurane, administered directly to the spine in pure liquid form for the first time, and to study the clinical effects of increasing concentrations of the drug on consciousness and on superficial and deep sensitivity, as well as on the motor response to a painful stimulus and the possibility of motor block. The study was designed to evaluate sensory effects...
separately from motor effects. In addition, we also assessed the reversibility and duration of clinical spinal effects and whether administration produces signs of medullar lesions.

Materials and methods

Eight adult beagles, five males and three females, were used in this study. The age range was 2–6 years and the weight range was 12–18 kg. The animals were acquired, inspected by a veterinarian and underwent an acclimatization period of 1 week before experiments were started. All experimental procedures were performed according to European Union and Spanish Government regulations, and were supervised and approved by the Complutense University Animal Care Facility.

Catheter placement

Catheters were placed under general anaesthesia (premedication with medetomidine, induction with propofol and maintenance with isoflurane and fentanyl) with standard monitoring and mechanical ventilation. A 3 cm vertical incision was made in the medial lumbar region between L5 and L6 creating a tobacco sac to house the catheter connected to an injection site cap with a latex membrane through which the anaesthetic could be administered with a transdermic needle.

The subarachnoid space was located with a puncture between the L4 and L5 vertebrae. An epidural Tuohy calibre 20 epidural needle (Perisafe®, Becton Dickison, Bidford-on-Avon, UK) was inserted using the loss of resistance technique and, once the epidural space was located, advanced until a free flow of cerebrospinal fluid (CSF) was obtained. At this point the catheter was introduced into the subarachnoid space. Catheter placement was checked using a myelographic scope, introducing 0.5 ml of a low concentration of iodinated contrast (240 mg ml⁻¹) (Omnipaque®, Amersham Health, Cork, Ireland). The distance between the insertion point and placement in the spinal space was calculated, and another 5 cm was added to place the cathetor at L2.

The catheters were left to stabilize for 1 week. Proper catheter placement and function were confirmed 72 h before beginning the studies by administering a test dose of lidocaine 0.1 mg kg⁻¹. If there were doubts as to proper catheter function, myelography was repeated.

Sevoflurane administration

Each animal received three sevoflurane doses (Sevorane®, Abbot Laboratories, Queenborough, Kent, UK): 0.05 ml kg⁻¹ (0.076 mg kg⁻¹), 0.075 ml kg⁻¹ (0.114 mg kg⁻¹) and 0.1 ml kg⁻¹ (0.152 mg kg⁻¹), and saline solution 0.1 ml kg⁻¹ (0.9%) as control. The specific gravity of sevoflurane is 1.52 g l⁻¹ and its molecular weight is 200.05. The clinically relevant dose of sevoflurane is ~0.4 mM (~1 MAC). We used a priori calculation to design the study based on the theoretical distribution of CSF volume in the dog (2.5–3 ml kg⁻¹). According to this, the maximum dose of 0.1 ml kg⁻¹ used in our study corresponds to 0.24 mM, which is <1 MAC, the medium sevoflurane dose of 0.075 ml kg⁻¹ corresponds to 0.18 mM, which is ~0.5 MAC and the lowest dose of 0.05 ml kg⁻¹ corresponds to 0.12 mM (<0.33 MAC). These doses were assigned randomly and blindly. To ensure blinding, two people randomly chose the doses and administrated each dose in one room, and a third person (the same observer for the entire study) made the evaluations in another room. The animals were dosed at intervals of at least 72 h and after each administration the catheter was flushed with 0.3 ml saline solution. Immediately after administration, the animal was allowed to walk freely.

Data collection

We used four clinical tests following a modification of the method described by Feldman and colleagues (Table 1). The first test evaluated the level of consciousness on a four-point sedation scale, the second test evaluated motor function on a three-point motor block scale, and the third and fourth tests evaluated sensation. The painful stimulus test evaluated the response to a deep noxious sensory stimulus (ungual base pressure with a Halstead clamp) on the dog’s four legs on a three-point scale. The other sensory block test was the prick test or panniculus reflex exploration, which evaluated the response to a superficial sensory stimulus (skin pricking by piercing the skin with a needle) on a two-point scale (Table 1). All four tests were performed on all animals at predetermined regular intervals (0, 5, 15, 30, 45, 60, 75, 90, 105 and 120 min, and then every 30 min for as long as necessary until 2 h after the recovery was complete, or for a minimum of 2 h). The maximum degree of blockade of each dose was graded on a three-point scale (1=no effect;
for a range of sevoflurane doses were analysed by one-way administration and the immediately preceding control was excluded from the study.

These measures allowed determination of the following times for motor and sensory tests: blockade onset (time needed between drug administration and the start of any degree of block), total duration of blockade (time during which the animal presents any level of block), duration of complete blockade (time during which animal presents complete or total block) and time to recovery (time elapsed between maximum block and a return to basal sensory values).

Deep sensory response was evaluated by observing the response to pinching the space between the toes on both the front and hind paws using a Halstead clamp protected with plastic sheaths (painful stimulus test). Absences of vocalization or head movement toward the area being pinched were taken as indicating deep analgesia. The prick test was performed bilaterally using vertebral dermatome distribution to determine whether the reflex was present, but was grouped into lumbar block (lumbar and sacral) or purely sacral block, since either region was considered blocked when at least two of its dermatomes were blocked. This sensitivity was measured by skin pricking, i.e. superficially piercing the skin with a 25 gauge needle (skin prick test) in a caudocranial direction. We also checked that the reaction of the animal was a response to a nociceptive stimulus and not a habituation phenomenon. For this purpose, we checked that the animal did not respond similarly to an painless stimulus, such as a pat on the hind leg. Motor blockade was evaluated by assessing gait and the ability to stand on four legs.

Exclusion criteria

Exclusion criteria were as follows: dogs presenting with any alteration in neurological status before initiating drug dosing; dogs showing no symmetrical motor blockade of the hind legs after the test administration with lidocaine; dogs with any sign of alterations due to catheter malpositioning that might affect the results; dogs in which it was not possible to perform all four experiments with the same catheter.

Statistical analysis

The difference between the value found during sevoflurane administration and the immediately preceding control was analysed for each variable considered. Differences obtained for a range of sevoflurane doses were analysed by one-way analysis of variance (ANOVA with repeated measures) with Dunnett post hoc tests on raw data using an iterative procedure with commercial software. Two-way ANOVA was used to compare pairs of curves (Graph-Pad Prism 3.0, Graph-Pad Software, San Diego, CA, USA). The Friedman test was used to compare the degrees of intensity of maximum blockade at different doses.

Results

Three of the eight dogs studied were excluded for the following reasons: a slight limp after catheter placement (necropsy detected medullar cone injury from the catheter), non-homogeneous distribution of the myelography contrast and an asymmetric blockade with lidocaine (necropsy revealed a congenital medullar membranous malformation) and because it was necessary to change the catheter during the study making it impossible to perform the complete study with the same catheter.

Level of consciousness

None of the dogs displayed a decrease in consciousness with the saline solution, sevoflurane 0.05 ml kg$^{-1}$ or sevoflurane 0.075 ml kg$^{-1}$. Administration of sevoflurane 0.1 ml kg$^{-1}$ produced light sedation (2 on a four-point sedation scale) in three of the five dogs. None of the sevoflurane doses produced a state of moderate sedation or of general anaesthesia in any of the five dogs evaluated.

Motor blockade

The saline solution did not produce any degree of motor block. Blockade onset, total duration of blockade and recovery times for each sevoflurane dose are shown in Table 2. None of the dogs showed maximum motor blockade of the hind legs with 0.05 ml kg$^{-1}$ and none presented any blockade whatsoever of the front legs. With 0.075 ml kg$^{-1}$ a complete motor blockade was obtained in the hind legs of one dog, which also had partial front leg blockade, but there was only partial blockade in the other four dogs. At the highest dose (0.1 ml kg$^{-1}$) all dogs had a complete motor blockade of their hind legs, three had a partial blockade of the front legs and the other two had complete blockade of all four legs. Comparison of the degree of intensity of maximum motor blockade achieved with the different doses showed significant differences between the maximum,
medium and minimum doses (Friedman test, P<0.05), without a statistically significant difference for maximum blockade intensity between the medium and minimum dosages (post-hoc difference of Friedman test, P>0.05). Comparison of the total duration of the motor blockade among the three sevoflurane doses shows a significant dose-dependent increase between doses (post-hoc difference of Friedman test, P=0.003) that is greater than the effect of the saline solution at any of the three sevoflurane doses (post-hoc difference of Friedman test, P=0.004).

Sensory blockade

Painful stimulus test
Saline solution did not produce any grade of sensory block. The onset and total duration of blockade and recovery times for each sevoflurane dose are shown in Table 2. The lowest dose of sevoflurane (0.05 ml kg\(^{-1}\)) produced a partial block in the hind legs only in five dogs. The medium dose (0.075 ml kg\(^{-1}\)) produced a partial block in the hind legs of four dogs and a total block of both hind legs in one dog. The highest dose (0.1 ml kg\(^{-1}\)) produced a total block in the hind legs in all the dogs, and a partial block in the front legs of two of the dogs. The post-hoc difference of Friedman test comparing the degree of intensity of the maximum sensory blockade at the different doses showed significant differences between the maximum, medium and minimum doses (P<0.05) but no significant differences in block intensity between the medium and minimum doses (P>0.05). A comparison of the duration of the total sensory block between the three sevoflurane doses shows a significant dose-dependent increase (Friedman test P=0.002) that is greater in all cases than that for the saline solution.

Prick test in the sacral region
The saline solution did not produce any grade of sensory block. Blockade onset, total duration of blockade and recovery times for each sevoflurane dose are shown in Table 2. A comparison of the duration of the sensory block in the sacral region among the three sevoflurane doses shows a significant dose-dependent increase (one-way ANOVA with repeated measures, P=0.03) that is always greater than that for the saline solution (one-way ANOVA with repeated measures, P=0.001).

Prick test in the lumbar region
The results for the sensory block of the lumbar region are shown in Table 2. Comparison of the duration of the lumbar region sensory block among the three sevoflurane doses shows a significant dose-dependent relationship between dose and duration of block (one-way ANOVA with repeated measures, P=0.01) that is always greater than that for the saline solution (one-way ANOVA with repeated measures, P=0.002) (Table 2).

Ratio of motor block to sensory block (painful stimulus test)
The duration of the motor block was compared with that of the sensory block at the different doses by determining the ratio between the motor block and the sensory block as measured by the prick test in which the response is independent of the degree of motor block. The ratio was 1.31(0.5) at 0.05 ml kg\(^{-1}\), 1(0.2) at 0.075 ml kg\(^{-1}\) and 1.2(0.2) at 0.1 ml kg\(^{-1}\). The average was 1.17(0.12).

Discussion
This study is the first to administer sevoflurane directly via the subarachnoid space in an experimental situation employing live animals in order to analyse clinical regional effects. Therefore it is difficult to compare our method, dosages and clinical findings with earlier studies. This model is also the first to study the clinical signs of sensory block independently from those of motor block. The doses employed here were chosen by administering different sevoflurane doses to three dogs in an earlier preparatory study to determine the lowest dose with a clinical effect. This was found to be 0.05 ml kg\(^{-1}\).

Subarachnoid sevoflurane did not alter the level of consciousness at doses of 0.05 or 0.075 ml kg\(^{-1}\), and had only a slight sedative effect in three of the five dogs at the highest dose of 0.1 ml kg\(^{-1}\). This made it possible to evaluate the animal’s response to a painful stimulus clinically. The response took the form of a head movement towards the stimulated area, although the animal could not move its legs because of the motor blockade produced by the sevoflurane. Thus it was possible to discriminate whether the drug-induced blockade of the motor response to a painful stimulus was the result of motor blockade, sensory blockade or, as shown in this study, both blockades simultaneously.

Sevoflurane administration via the spinal route always produced an effect that followed the homogeneous distribution in a caudocranial distribution, and the duration and intensity of the blockade increased with the increase in drug dose. Thus the ad integrum recovery from the clinical effects in all dogs without neurological sequelae supports the validity of the method employed here as well as the results obtained for the spinal effects of sevoflurane.

Sensory block was dose dependent in duration, intensity and extension, allowing confirmation of the analgesic effects of sevoflurane administration to the spinal medulla. The hypothesis of an analgesic effect by general anesthetics\(^{216-18}\) has been widely documented in many experiments. Our model, in which the level of consciousness is unaffected, allows analgesia and not interruption of nociceptive transmission to be noted. This was not possible in earlier studies in which the subjects were unconscious.\(^{\text{}^{11}}\)

The duration, intensity and extension of the motor block were dose dependent, in agreement with the theory that the immobilizing clinical effect of sevoflurane is produced by the spinal action of the drug. Several studies have
demonstrated that inhalational anaesthetics have a direct effect on motoneurone excitability and hyperpolarize motoneurones.\textsuperscript{9,19,20} Other studies have observed an inhibition of the F wave (directly related to motoneurone excitability) with the suppression of movement in response to surgical stimulus.\textsuperscript{10,21–25} Our study has shown that the clinical effect of the motor block produced by the intradural administration of sevoflurane does not alter the conscious awareness of the dog.

The average intensity and duration of the motor and prick test sensitivity/pannicular reflex blocks was similar for each of the sevoflurane doses, and so we believe that the clinical effects of sevoflurane on sensory transmission and the effect on the motoneurone are equally strong.

In summary, subarachnoid sevoflurane administration in dogs produces a reversible dose-dependent sensory and motor block that does not affect the level of consciousness.

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