Intrathecal Ropivacaine in Rabbits: Pharmacodynamic and Neurotoxicologic Study

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Background: Ropivacaine is available for spinal or intrathecal use in humans, although data on neurotoxicity after spinal injection are not yet available. The authors experimentally determined the relationship between doses of intrathecal ropivacaine and spinal effects and local neurotoxic effects.

Methods: Eighty rabbits equipped with an intrathecal lumbar catheter were studied. Sixty were randomly assigned to receive 0.2 ml of intrathecal solutions as a sole injection of: 0.2%, 0.75%, 1.0%, and 2.0% ropivacaine (doses from 0.4-4.0 mg; groups R0.2 to R2.0), 5.0% lidocaine (10 mg; group L), or 0.9% NaCl as control (group C). Twenty other rabbits received either repeated injections of 0.2 ml of 0.2% ropivacaine every 2 days during 2 weeks (total dose of 2.8 mg; group RINT); or a continuous intrathecal infusion of 0.2% ropivacaine at the rate of 1.8 ml/h over 45 min (2.7 mg; group RCONT). Injection rate was 30 s in all groups except RCONT. Time to onset, duration and extent of motor block, and variations of mean arterial blood pressure were recorded in all groups. Somatosensory evoked potentials were also recorded in group RCONT and RINT. Seven days after the last intrathecal injection spinal cord and nerves were sampled for histopathologic study.

Results: In groups R0.2 and RINT, the lowest dose of ropivacaine induced a clinically visible spinal block in only 50% of rabbits, but SEPs recorded in group RINT were decreased by 70% in the lumbar dermatome. Complete motor block was observed with doses greater than 1.5 mg of ropivacaine (group RCONT and R0.75 to R2.0). Onset time was shorter and duration of block increased as doses of ropivacaine increased. Significant hypotension was observed only with 4.0 mg of ropivacaine (concentration of 2.0%). Complete paralysis and hypotension were observed with 5.0% lidocaine. No neurologic clinical lesion was observed in rabbits receiving saline or ropivacaine within the 7 days after the last intrathecal injection, and histopathologic study revealed no sign of neurotoxicity in these groups. In contrast, intrathecal lidocaine induced clinical and histopathologic changes.

Conclusion: Ropivacaine induced dose-dependent spinal anesthesia, and did not induce any neurotoxicologic lesion in this experimental animal model.

LIDOCAINE has been shown to produce local neurotoxicity when intrathecally applied in humans1,2 and animals.3 Several cases of cauda equina syndrome have also been reported after intended epidural anesthesia, leading to the diagnosis of unexpected intrathecal insertion of the catheter.4,5 Ropivacaine, which is routinely used epidurally, has been proposed for intrathecal use as an alternative to lidocaine. Ropivacaine is a piperidolactid local anesthetic, which differs from bupivacaine by the presence of a propyl group on the aromatic ring instead of a butyl group and is used as a single enantiomer. With special regard to the cardiovascular6 and central nervous systems7,8, ropivacaine offers more systemic safety. No local neurologic complication has been reported with this drug so far. However, the small number of patients included in human studies with intrathecal ropivacaine does not permit us to draw conclusions about its safety.9–13 To date, there is no available published report about the local neurotoxicity of ropivacaine.

To test the local neurotoxicity of intrathecal ropivacaine in an experimental model, we first determined in rabbits the relationship between dose and motor and hemodynamic effects of ropivacaine, then we studied the clinical and histopathologic changes that ropivacaine might induce on the spinal cord and nerves of these animals.

Materials and Methods

Eighty albino New Zealand rabbits weighing 2.5–3.0 kg were included in the study, which was performed in accordance with French Ministry of Agriculture laws and guidelines for laboratory animal experiments, and approved by our Institutional Animal Investigation Committee.

The rabbits were chronically instrumented as follows: under general anesthesia and sterile conditions a laminectomy was performed at the caudal level to insert an intrathecal catheter. After dural incision, a 23-gauge catheter (Periquick®, Gamida Lab., Ebubonne, France) was gently inserted 7 cm cephalad into the intrathecal space to set the tip of the catheter at the L5 level. The right position of the catheter was certified by cautious aspiration of cerebrospinal fluid (CSF). The system was tunneled and secured, and implanted subcutaneously on the back of the rabbit. The deadspace of the catheter was 0.15 ml. Then, an arterial catheter was inserted via the femoral artery and heparinized.

After the intrathecal catheter had been inserted, rabbits were housed individually in standard cages with free movement.
access to food and water and with a natural light-dark cycle. They were included in the study the day after catheter implantation, only if they had a normal behavior, i.e., no allodynic reactions and symmetric walking.

Protocol of Intrathecal Injections

Rabbits equipped with catheters were randomly assigned to receive intrathecal solutions as follows:

- 0.2 ml of 0.2% ropivacaine (0.4 mg), over 30 s, in group R₀.₂ (n = 10);
- 0.2 ml of 0.75% ropivacaine (1.5 mg), over 30 s, in group R₀.₇₅ (n = 10);
- 0.2 ml of 1.0% ropivacaine (2.0 mg), over 30 s, in group R₁.₀ (n = 10);
- 0.2 ml of 2.0% ropivacaine (4.0 mg), over 30 s, in group R₂.₀ (n = 10);
- 0.2 ml of 0.9% NaCl as control, over 30 s, in group C (n = 10);
- 0.2 ml of 5.0% lidocaine (10.0 mg), over 30 s, in group L (n = 10);
- repeated injection of 0.2 ml of 0.2% ropivacaine (total of 2.8 mg), over 30 s, every 2 days within 2 weeks, in group RINT (n = 10);
- continuous injection of 0.2 ml of 0.2% ropivacaine at the rate 1.8 ml/h over 45 min (total of 2.7 mg) in group CONT (n = 10).

After each injection the intrathecal catheter was flushed with 0.2 ml of 0.9% saline solution.

Assessment of Effects

Motor block was recorded every minute until maximal intensity was reached, and then every 10 min until complete recovery from spinal anesthesia. The motor block was scored by using a 4-point scale as follows: 0 indicated that the rabbit had free movement of hind limbs without any limitation; 1 indicated that limited or asymmetrical limb movement for spontaneous body support or walking occurred; 2 indicated inability to achieve spontaneous support of the back of the body on hind limbs; and 3 indicated a total limb paralysis.

Onset time was defined as the time elapsed from the end of intrathecal injection until reaching the maximal score of the block. Total duration time was comprised between the end of injection and complete recovery from the block.

Because in the initial part of the study we observed no obvious clinical effects in most rabbits with the lowest dose of ropivacaine (0.4 mg), we decided to record the somatosensory evoked potential (SEPs) in group RCONT and RINT. SEPs were elicited by stimulation of the tibial nerve at the ankle with surface electrodes. Stimulus strength, adjusted above the motor threshold was usually 5–10 mA. The stimulation rate was 2.9 Hz and the duration of stimuli 0.1 ms. Recording was performed using subcutaneous needle electrodes located at the thoracolumbar level (active electrode facing the spine, referenced to an electrode set at the belly) and at the cortex (Cz active electrode in the midline of the scalp 2 cm behind Cz, referenced to a midfrontal Fpz electrode).

The amplifier bandpass (−6 dB/oct) was 2 Hz to 3,000 Hz and analysis time was 100 ms. Three hundred sweeps were averaged at each time, and latency and amplitude of both spinal and cortical responses were measured in each condition. Measurements were performed every 3 min during the first 30 min after intrathecal injection of ropivacaine.

Mean arterial blood pressure was continuously monitored from a femoral artery catheter (Sirecust 401.1, Siemens, Erlangen, Germany). Baseline value was recorded after a steady state period of 15 min after arrival of unsedated animals in the operative theater. After intrathecal injection, an hypotensive episode was defined as a drop in pressure to less than 30% from the baseline value, and was treated by continuous intravenous administration of dopamine at an initial rate of 10 μg · kg⁻¹ · min⁻¹. When blood pressure returned to the baseline value and remained stable for at least 5 min, the rate of administration was decreased by 2 μg · kg⁻¹ · min⁻¹ from its current level (8, 6, 4, 2, and 0 μg · kg⁻¹ · min⁻¹). The total dose and duration of dopamine administration were recorded.

Neurologic Postanesthetic Follow-up

Animals were observed after intrathecal injection, and then examined daily by an investigator unaware of the injected solutions. Any weight loss or abnormalities in food intake, urinary or fecal incontinence, and limb weakness or paralysis were noted when they occurred.

Histopathologic Study

In all groups, 7 days after the last planned injection, rabbits received an intravenous injection of 15 ml Evans blue, 2%, then 45 min later the whole spinal cord and nerves were sampled by laminectomy after injection of a lethal dose of thiopental. At that time the intrathecal position of the catheter was checked. The spinal nervous structures were immersed in a fixative solution containing glutaraldehyde and formaldehyde and stored at 4°C until histopathologic examination. Spinal cord and nerves were embedded in paraffin, then cut with a microtome in 6-μm section slices at thoracic and lumbar levels. Examinations were performed on 6 slices in each segment by a neuropathologist unaware of the injected solutions. Hematoxylin- and cosin-stained slides were examined by using light and six other uncolored slides in fluorescent microscopies, and were rated as normal (no histopathologic changes) or pathologic (loss of myelin area, necrosis, vacuolization, meningeal thickening, hemorrhage within intrathecal or epidural spaces, fibrin deposit and presence of inflammatory cells, loss of vessel outline with large diffusion of dye). Lesions were con-
considered pathologic if they were homogeneous in a minimum of two different slices of the spinal cord. An isolated lesion was not considered to be drug related.\textsuperscript{15}

**Statistics**
Results of spinal effects were expressed as mean ± SD. The relationship between doses of ropivacaine and durations (onset and recovery times of motor block) were studied using the sigmoid E\textsubscript{max} model or linear regression with the software package WinNonlin version 1.51 (S.C.I., Apex, NC). Changes of mean arterial blood pressure and of SEPs values were studied by analysis of variance (ANOVA) for repeated measures. The significance level was set at $P < 0.05$.

**Results**
Intrathecal injections did not induce writhing or squeaking in any animal. Postmortem examinations confirmed that intrathecal catheters were all in the intrathecal space, in posterior position in 70% of cases, facing the L\textsubscript{5} vertebra corpus.

**Spinal Anesthesia Effects**
Saline solution did not induce any change in motor function or in blood pressure. Ropivacaine induced a dose-dependent motor block (fig. 1). With the lowest dose (0.4 mg), 50% of rabbits presented with motor block, and complete motor block was seen in all animals with higher doses. The relationship between dose and onset time was linear, i.e., onset time was shorter with increasing doses of ropivacaine. A sigmoidal relationship was found between duration of motor block and dose, with an E\textsubscript{max} of 95 min (IC 95% 58–132 min) and an E\textsubscript{50} of 0.95 mg (IC 95% 0.24–1.73 mg) ($P < 0.01$) (fig. 1).

Somatosensory evoked potentials latency did not change at cortical and lumbar levels in groups R\textsubscript{CONT} and R\textsubscript{INT}. SEPs amplitude was affected by ropivacaine in both groups (fig. 2). However in group R\textsubscript{INT}, the lowest value was observed 6 min after injection at the cortical level (−35%), and at 9 min at the lumbar level (−55%). In group R\textsubscript{CONT}, the lowest value occurred 9 min after infusion onset at the cortical level (−35%), and 27 min at the lumbar level (−65%).

Hemodynamic changes were not significant after intrathecal ropivacaine 0.4–2.0 mg, but a significant hypotension was observed after 4.0 mg injection (group R\textsubscript{2.0}) (fig. 3). Dopamine administration was required for a median duration of 45 min (range 30–90 min).

Lidocaine induced immediate and complete motor block. The mean duration of motor block lasted 85 min...
(range 70–100 min). Significant hypotension was observed immediately after the injection, requiring the use of dopamine at the same rate of infusion as in group R2.0, and for a median duration of about 40 min (range 35–70 min).

Complete recovery of intrathecal anesthesia was observed in all groups receiving local anesthetics. Within the 7-day examination period after the last injection, no disturbance in behavior, no weight loss and no obvious neurologic deficit was observed in groups receiving ropivacaine. In contrast, two rabbits that received lidocaine presented behavioral or drink or food intake disturbances, and allodynia at the touch of flanks.

**Histopathologic Study**

In all groups of rabbits, nonspecific histopathologic changes were seen. Polynuclear proliferation in the intrathecal space associated with meningeal thickening and epidural hematoma were found (fig. 4). Perivascular infiltration and polynuclear proliferation in Virchow-Robin spaces were also observed (fig. 4). When such lesions occurred, they were almost localized around the intrathecal catheter insertion site, and were attributed to surgery or implantation of the catheter. Incidence of such effects have been summarized in the table 1.

In all ropivacaine groups, histopathologic changes were similar to those observed with saline solution (table 1); i.e., hematoma in the epidural space (fig. 5) or polynuclear proliferation and meningeal thickening. No other histopathologic changes in optical as well as in fluorescent microscopic examination were observed at the lumbar and thoracic levels in both the spinal cord and the nerves (fig. 5).

In contrast, rabbits receiving intrathecal 5% lidocaine presented with signs of local neurotoxicity. Some areas with red blood cell infiltration were also found in the anterior horn of the spinal cord. Two rabbits presented with areas of loss of myelin or with necrosis in spinal cord, and two others presented with axonal degeneration, endoneuronal edema, and perivascular lymphocytosis infiltration in spinal nerves (fig. 6). Rabbits presenting lesions were not those requiring the highest dose of vasoconstrictors.

**Discussion**

The major result of this study is that intrathecal ropivacaine, in the dose-ranging paradigm tested, does not induce any neurotoxic effect after single or repeated injections, while lidocaine induced clinical and histopathological changes in this experimental model.
We measured only motor effects, because it is not possible to obtain a reliable assessment of sensory block in rabbits. However, as all nervous fibers share the same site of action with local anesthetics, we speculated that sensory and motor block had a similar time and intensity profile. Moreover, by using an electrophysiologic technique (SEPs), we found that ropivacaine blocked sensory fibers at root and lumbar spinal cord levels. We found a dose-dependent spinal anesthesia with ropivacaine. Onset time of motor block was linearly shortened with increasing doses of ropivacaine and total duration increased after a sigmoidal curve. This is in agreement with the physiology of spinal anesthesia: onset time is directly related to injected doses, whereas numerous processes are involved in the clearance of anesthetics from their sites of action. As a consequence of sympathetic block, hypotension occurred with ropivacaine, but only with the highest dose, which has already been reported by others.

We chose to inject solutions in the lumbar region to avoid the total spinal anesthesia induced by intracisternal administration of local anesthetics. Postmortem examination displayed that intrathecal catheters were set in the posterior intrathecal space in most cases, and anesthesia was restricted to the posterior hind limbs with limited hemodynamic effects and respiratory changes even when large doses of ropivacaine (up to 2.0 mg) were injected. Only with the highest dose of ropivacaine (4.0 mg) or with lidocaine, vasoconstrictors were needed to correct hypotension. Histopathologic changes observed might have been the consequence of arterial hypotension-induced decreased spinal cord blood flow or might be related to the effects of vasoconstrictors on the spinal circulation. However, both lidocaine and ropivacaine induced these hemodynamic changes and required vasoconstrictors, but histopathologic changes were observed only after intrathecal lidocaine injection.

Most models used for the assessment of neurotoxicity of local anesthetics are derived from Yaksh and Rudy’s technique, where the intrathecal catheter is introduced in the CSF through the atlanto-occipital membrane with the tip placed in regard to distal lumbosacral nerves. This consistently produces a restricted block in hind limbs, but severe lesions in the conus can occur with this technique, probably reflecting the mobility of the distal tip of the catheter and its contact with the part of the spinal cord. We used a model of intrathecal catheterization allowing a CSF access distal to the conus. An advantage of this technique is that no catheter-induced spinal cord lesion was observed in our control group. Moreover, a volume of less than 0.5 ml injected in the lumbar intrathecal is likely atraumatic, as previously reported after cisternal administration of similar volumes. However, a drawback of any study in which intrathecal catheters are implanted is that they are invariably associated with some degree of catheter-related histopathologic changes. Inflammatory cells and minor meningeal thickening in the area close to the catheter tip were indeed only observed in the lumbosacral epidural

<table>
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<th>C (n = 10)</th>
<th>R_{0.2} (n = 10)</th>
<th>R_{0.75} (n = 10)</th>
<th>R_{1.0} (n = 10)</th>
<th>R_{2.0} (n = 10)</th>
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<th>R_{INT} (n = 10)</th>
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<tr>
<td>Inflammatory changes</td>
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<td>Meningeal thickening</td>
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Frequency (number of rabbits in each group) of nonspecific changes observed within groups. Inflammatory changes correspond to polynuclear proliferation in any area of the spinal cord and of meningeal thickening in the area of catheter insertion.

![A](image1.png) ![B](image2.png)

Fig. 5. Hematoxylin- and eosin-stained slide in rabbits after intrathecal ropivacaine: (A) (500 × magnification) a posterior meningeal thickening can be seen in an animal receiving 1.0% ropivacaine (group R_{1.0}), and (B) (125 × magnification) no specific lesion was observed after 2.0% ropivacaine.

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space and around few spinal nerves in the group receiving saline solution.\textsuperscript{14,21–23} Epidural hematomas were also found in some rabbits in the current study that were presumably related to surgery or catheter implantation.

Local anesthetics have been reported to induce axonal lesions in peripheral nerves of rats, with a rate that increases exponentially as the dose increases.\textsuperscript{24} In contrast to what happens after peripheral nerve administration, the CSF dilutes and decreases concentration of injected solutions. However, physicochemical properties of CSF and those of injected solutions are quite different. This may lead to maldistribution of local anesthetics in the CSF, explaining why cauda equina syndrome may occur after intrathecal lidocaine in experimental models\textsuperscript{25} and in humans.\textsuperscript{1} Maldistribution also maintains spinal nerves in contact with highly concentrated local anesthetic solutions, especially after repeated injections through small diameter catheters.\textsuperscript{25} In the current study, we observed that 5.0% lidocaine induced neurotoxic lesions that are in accordance with previous clinical cases.\textsuperscript{1,4,5} We have previously reported similar histopathologic changes with 1.0% lidocaine in rabbits.\textsuperscript{15} The fact that lidocaine induced neurotoxic lesions in our model, and can be associated with neurologic permanent sequelae in specific clinical circumstances\textsuperscript{1,4,5} enhances the robustness of our neurotoxic evaluation. As the volume of CSF is small in rabbits, using highly concentrated local anesthetic solutions results in high concentrations in direct contact with spinal cord and nerves. To date, there is no published study assessing the neurotoxic potential of intrathecal ropivacaine. The aim of these preclinical data were also to determine the maximum tolerable concentration and hence the maximum tolerable dose to adequately assess the neurotoxicity of ropivacaine. We thus designed a dose-ranging paradigm and injected intrathecal ropivacaine from 0.2% to 2.0% with various modes of injections and did not find any neurotoxic effects of ropivacaine under such experimental conditions. The histologic changes observed after ropivacaine or saline were catheter-related, in contrast to those observed with 5.0% lidocaine. Our experimental model strongly suggests that ropivacaine is safe for spinal anesthesia with concentrations of up to 2%.

We were unable to determine the doses producing neurotoxic lesions in the rabbit spinal cord, and this represents a weakness of our study. Similarly, it would have been interesting to use ropivacaine at the 2% concentration in group R\textsubscript{CONT}, while the maximal commercial concentration of ropivacaine is 1%. We must keep in mind that there are no safe drugs, only safe doses, because all local anesthetics in high concentrated solutions

\begin{figure}
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\includegraphics[width=\textwidth]{fig6.png}
\caption{Hematoxylin- and eosin-stained slide (500 × magnification) in rabbits after intrathecal 5\% lidocaine. (A) An area with hemorrhage in anterior horn can be seen; (B) a meningeal thickening and axonal degenerations with edema; and (C) an endoneuronal edema and perivascular infiltration.}
\end{figure}
could produce significant pathologic changes in nerves. Because we could not determine the ratio between doses and concentrations useful for therapy and those producing neurotoxic lesions, further studies with rabbits or other species, using higher doses or longer periods of exposure, are required to draw firm conclusions with ropivacaine. Nevertheless, in the current study both local anesthetics were used at equipotent doses in regard to motor effects. This is an important point, underlying that doses of ropivacaine tested appeared safer than those of lidocaine in our experimental model.

We report the first neurotoxicologic study with intrathecal ropivacaine and did not find any specific signs of local neurotoxicity in our animal model. Our results suggest that ropivacaine would be a suitable agent for intrathecal anesthesia in humans.

References

23. Yuksh TL, Nouichet BY, Durzant PA: Studies of the pharmacology and pathology of intrathecally administered 4-anilinopiperidine analogues and morphine in the rat and cat. Anesthesiology 1986; 64:54-66