# Sciatic nerve block with bupivacaine-loaded microspheres prevents hyperalgesia in an inflammatory animal model

## *[Le bloc du nerf sciatique réalisé avec de la bupivacaïne encapsulée dans des microsphères prévient l'hyperalgésie dans un modèle inflammatoire animal]*

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**Purpose:** The aim of this study was to evaluate the effect of different durations of local anesthetic neural blockade on hyperalgesia after carrageenan infiltration in a rat model.

**Methods:** Inflammation was obtained by injection of carrageenan in the righ hind paw. Hyperalgesia was determined by measuring the threshold of response to increasing mechanical stimuli on the contralateral and on the ipsilateral paw. The development of edema was measured. After identification of the sciatic nerve by nerve stimulation, blockade was performed either one hour before or after carrageenan infiltration. Animals were randomly assigned into three groups: without sciatic nerve block (control group; $n = 20$), block with bupivacaine (B) and block with bupivacaine-loaded microspheres (B-Ms) injection before or after carrageenan infiltration ($n = 10$ for each group).

**Results:** Carrageenan infiltration in the control group induced a severe ipsilateral and contralateral hyperalgesia. After blockade with B (duration = 2 ± 0.5 hr) hyperalgesia was present and delayed only by the duration of the local anesthetic effect. A longer duration of block achieved with B-Ms (duration greater than five hours), was associated with the absence of development of both ipsilateral and contralateral hyperalgesia. No preemptive effect was recorded.

**Conclusion:** B-Ms as a drug delivery system prolongs the duration of neural blockade and avoids hyperalgesia phenomena in this rat model of inflammation.

***Objectif :*** *Le but de cette étude a été d'évaluer, sur un modèle d'inflammation de la patte de rat, l'effet de la durée du bloc sur les phénomènes d'hyperalgésie homo et controlatérale.*

***Méthode :*** *L'inflammation a été obtenue par une infiltration de carragénine. L'hyperalgésie des deux pattes à été évaluée par le test de retrait de la patte à une pression mécanique croissante. L'œdème a été mesuré. Le nerf sciatique a été repéré par neurostimulation, soit une heure avant, soit une heure après l'infiltration de carragénine. Les animaux ont été randomisés en trois groupes : sans bloc sciatique (groupe témoin ; n = 20), avec bloc sciatique à la bupivacaïne (B) ou à la bupivacaïne encapsulée dans des microsphères (B-Ms). Les blocs ont été réalisés soit avant soit après l'inflammation (n = 10 par groupe).*

***Résultats :*** *Dans le groupe témoin, l'hyperalgésie homolatérale et controlatérale ont été significatives. Après l'injection, de bupivacaïne, et malgré la durée du bloc de deux heures, la bupivacaïne n'a pas empêché l'apparition ou la réapparition des phénomènes d'hyperalgésie homo et controlatérale. Avec la B-Ms la durée de bloc d'environ cinq heures a permis de supprimer ces phénomènes. Il n'a pas été retrouvé d'effet préventif.*

***Conclusion :*** *La B-Ms permet non seulement de prolonger la durée du bloc mais aussi de prévenir la survenue des phénomènes d'hyperalgésie.*

RECENT animal studies suggest that prolongation of a nerve block for more than 12 hr, via a catheter or with experimental long-lasting local anesthetics may reduce contralateral and ipsilateral inflammatory hyperalgesia.[1,2] The aim of our study was to compare, with a single shot injection, the block obtained with

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bupivacaine (B) to the block prolonged by the use of bupivacaine-loaded microspheres (B-Ms) in the carrageenan inflammatory rat model.

## Methods

B-Ms were prepared by spray drying of bupivacaine and poly (lactide-co-glycolide) polymer.[3] Experiments were performed on male Sprague-Dawley rats weighing 265–325g. The Local Animal Research Committee approved the study. Inflammation was induced by injection of 0.1 mL of 2% carrageenan *sc* in the plantar area of the right hind paw under a brief general anesthetic (halothane 2–3%). The circumference of the paw was measured to the nearest mm at the metatarsal level.[4] The threshold of response (i.e., change in withdrawal threshold) to increasing pressure with the use of an Analgesy-Meter was evaluated. Ipsilateral and contralateral hyperalgesia were determined by positioning the paw under a pressure pad, the probe tip (diameter 1 mm) being applied to the dorsal, lateral and external aspects of the paw avoiding the territory innervated by the saphenous nerve. The choice of a cut-off value of 400 g was necessary to limit injury to the paw.[2]

Under general anesthesia (halothane: 2–3%), the sciatic nerve was identified using a nerve stimulator.[5] Injections were performed via an insulated needle (0.7-mm inner diameter for B; 1.3-mm inner diameter B-Ms); 0.5 mL were injected. Two minutes after the end of anesthesia the sciatic block could be evaluated by the rat's ability to hop and to place weight on its hind leg.

The animals were randomly assigned to one of three groups by computed list. The control group (group C; $n = 20$) received carrageenan injection without sciatic nerve block. In the second group, 1.25 mg of plain bupivacaine (0.5 mL of bupivacaine 0.25%) were used to block the sciatic nerve one hour before (group B/C; $n = 10$) or one hour after carrageenan injection (group C/B; $n = 10$). In the last group, 12.5 mg of bupivacaine administered as bupivacaine-loaded microspheres were used for sciatic nerve block one hour before (group B-Ms/C; $n = 10$) or one hour after carrageenan injection (group C/B-Ms; $n = 10$). The dose ratio of bupivacaine solution microspheres was determined in a previous study.[6]

Sample size calculation was performed based on a previous study using the same model ($n = 10$).[3] Data were analyzed using the analysis of variance following by the unpaired student's t test with Bonferroni correction for parametric data. The Mann-Whitney U and Kruskal-Wallis tests were used for nonparametric data. Statistical significance was defined as $P < 0.05$.

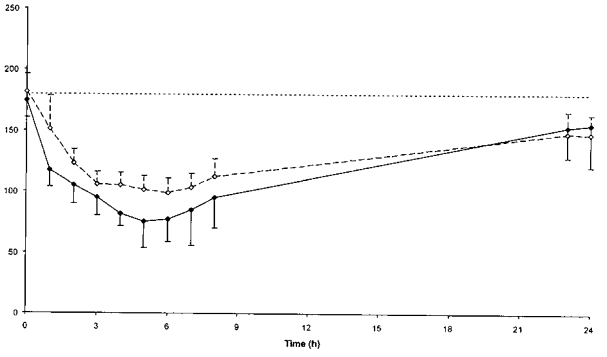


FIGURE 1 Time course of mean withdrawal threshold in the control group ($n = 20$). Ipsilateral hyperalgesia was evaluated on the right hind paw after carrageenan injection (• and continuous line). Contralateral hyperalgesia was evaluated on the left paw (O and discontinuous line). The dotted line identifies baseline.

## Results

In group C (Figure 1) the mean withdrawal threshold of the right hind paw (ipsilateral hyperalgesia) decreased significantly from one hour after carrageenan injection ($P < 0.005$) up to 24 hr ($P < 0.05$). For the left paw (contralateral hyperalgesia) the decrease of the mean withdrawal threshold was significant after carrageenan injection from two hours ($P < 0.03$) up to 24 hour ($P < 0.05$). The edema significantly increased after carrageenan injection from one hour to 24 hr ($P < 0.01$). Edema was never observed on the contralateral paw in any group.

For all bupivacaine groups (B and B-Ms) motor block was reported for each animal. The average duration of neural blockade was 2 ± 0.5 hr with B, and 5 ± 1 hr with B-Ms.

In group B, after recovery of the block, ipsilateral hyperalgesia appeared from five to 24 hr ($P < 0.05$; Figure 2). Contralateral hyperalgesia appeared from four to 24 hr ($P < 0.05$). When carrageenan injection was performed one hour before the sciatic block there was a significant decrease of the withdrawal threshold in the injected hind paw ($P = 0.002$). As in group C, edema of the injected hind paw increased from one to 24 hr after carrageenan injection ($P < 0.01$).

When B-Ms were injected on the sciatic nerve, both ipsilateral and contralateral hyperalgesia were not observed after recovery of the block (Figure 3). The withdrawal threshold of the injected hind paw ($P = 0.0002$), but not of the contralateral hind paw, decreased significantly when carrageenan injection was performed one hour before the sciatic block ($P =$

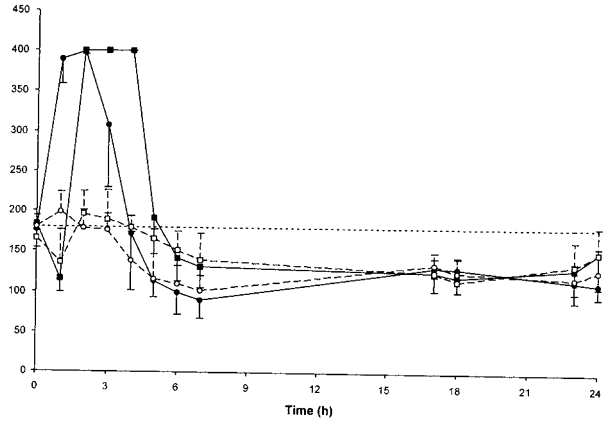


FIGURE 2 Time course of mean withdrawal threshold (cut-off 400 g) in the plain bupivacaine (B) group. Ipsilateral hyperalgesia was evaluated after sciatic nerve block with bupivacaine, one hour before carrageenan injection (● and continuous line: for group B/C; $n = 10$) or one hour after carrageenan infiltration (■ and continuous line: group C/B; $n = 10$). Contralateral hyperalgesia was evaluated (O and discontinuous line: for group C/B and ❑ and discontinuous line: for C/B group B/C). The dotted line identifies baseline.

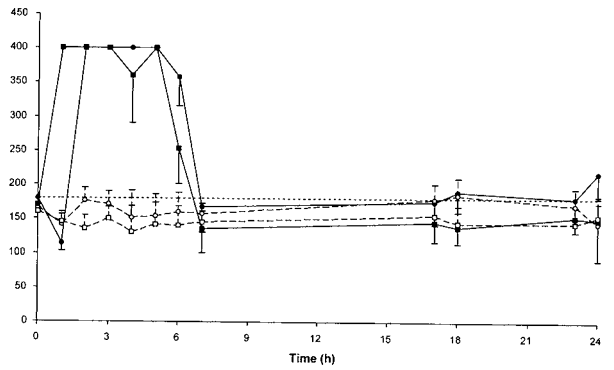


FIGURE 3 Time course of mean withdrawal threshold (cut-off 400 g) in the bupivacaine-loaded microsphere (B-Ms) group. Ipsilateral hyperalgesia was evaluated after sciatic nerve block with bupivacaine-loaded microspheres, one hour before carrageenan injection (■ and continuous line: group B-Ms/C; $n = 10$) or one hour after carrageenan injection (● and continuous line: group C/B-Ms; $n = 10$). Contralateral hyperalgesia was evaluated (❑ and discontinuous line: for group B-Ms/C and O and discontinuous line: for group C/B-Ms). The dotted line identifies baseline.

0.008). Edema of the hind paw was significantly increased from one to 24 hr after carrageenan injection ($P < 0.01$).

No significant preemptive effect was observed when sciatic blockade was performed before carrageenan infiltration.

## Discussion

Whereas in groups B hyperalgesia was only delayed (two hours), bupivacaine-loaded microspheres allowed a sustained nerve block (four to six hours) avoiding either the appearance (postcarrageenan group) or the reappearance (precarrageenan group) of ipsilateral and contralateral hyperalgesia in this rat model. The duration of local anesthetic blockade seems to be a determinant of the appearance or the reappearance of hyperalgesia.

With bupivacaine, all our data were similar to previous studies in the same carrageenan inflammatory animal model.[1,2,4,5] Prolongation of the block with bupivacaine-loaded microspheres also agrees with a previous study.[7,8]

Previous studies have demonstrated that hyperalgesia could be blocked when the block is prolonged (12 hr), but these results were obtained with multiple injections via a catheter[2] or with experimental long-lasting local anesthetics.[1] In the present study, we confirmed that only four to five hours of block are sufficient to allow the suppression of hyperalgesia in this rat model.

Nevertheless, the mechanisms by which hyperalgesia is prevented are not well defined and the role of local anesthetics on hyperalgesia through their action on peripheral nerve inputs remains unclear. We failed to demonstrate a preemptive effect but further studies must be performed to evaluate this point. Yet, bupivacaine-loaded microspheres appear to be a promising drug delivery system not only to prolong local anesthetic blockade[4] but also to avoid hyperalgesia phenomena.

In conclusion, plain bupivacaine was not sufficient to avoid the appearance or reappearance of hyperalgesia. Bupivacaine-loaded microspheres as a drug delivery system could prolong the block and avoid these hyperalgesia phenomena. These data obtained with mechanical stimuli must be confirmed with thermal stimuli and adapted to human nerve physiology to become useful for the treatment of postoperative pain when bupivacaine-loaded microspheres become available for clinical studies.

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