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# Perineural dexamethasone attenuates liposomal bupivacaine-induced delayed neural inflammation in mice *in vivo*

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## Abstract

**Background:** Liposomal bupivacaine (Exparel®) is a sustained-release formulation of bupivacaine for use in surgical infiltration anaesthesia. We analysed the histological nerve toxicity and clinical effectiveness of perineural Exparel® alone or with added dexamethasone in a mouse model.

**Methods:** We assigned 98 mice receiving a perineural sciatic nerve injection into seven groups: sham ($n$=14, perineural saline), B ($n$=14, perineural bupivacaine), BDIP ($n$=14, perineural bupivacaine + intraperitoneal dexamethasone), BDPN ($n$=14, perineural bupivacaine + perineural dexamethasone), E ($n$=14, perineural Exparel®), EDIP ($n$=14, perineural Exparel® + intraperitoneal dexamethasone), and EDPN ($n$=14, perineural Exparel® + perineural dexamethasone). The duration of thermoalgesic and motor block was evaluated in 49 mice (seven mice randomly selected by group) every 30 min until recovery. Mice were killed for sciatic nerve histological assessment at 14 or 28 days.

**Results:** The median duration of motor block was 90, 120, 120, 120, 180, and 180 min and the duration of thermoalgesic block was 240, 300, 360, 360, 360, and 420 min for groups B, BDIP, BDPN, E, EDIP, and EDPN, respectively. The B group mice showed mild neural inflammation at 14 days and the E group mice showed mild neural inflammation at 28 days. Addition (intraperitoneal or perineural) of dexamethasone reduced neural inflammation induced by bupivacaine, whereas only perineural dexamethasone reduced neural inflammation induced by Exparel®.

**Conclusions:** Perineural or systemic dexamethasone had a protective effect against the neural inflammation induced by bupivacaine, and perineural dexamethasone attenuated delayed inflammation induced by perineural Exparel®.

**Keywords:** bupivacaine; dexamethasone; liposomal bupivacaine; mouse; nerve block; neurotoxicity; regional anaesthesia; sciatic nerve

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### Editor's key points

- Liposomal bupivacaine, a sustained-release formulation of bupivacaine, is being used for regional anaesthesia, but its potential for neurotoxicity and its mitigation by adjuvants are unclear.
- This study investigated histological nerve toxicity and clinical effectiveness of perineural liposomal bupivacaine or bupivacaine alone or with added dexamethasone in a mouse model.
- Compared with bupivacaine, perineural sciatic nerve injection of liposomal bupivacaine prolonged the duration of motor and thermoalgesic block.
- Addition of perineural dexamethasone further prolonged motor and thermoalgesic block and protected against neurotoxicity seen at 28 days after perineural infiltration of liposomal bupivacaine in this mouse model.

Postoperative analgesia is a key component to enhance recovery after surgery.[1] Despite therapeutic advances, postoperative pain is often insufficiently controlled, resulting in an increase of morbidity and decrease in quality of life and patient satisfaction. As systemic opioids have many adverse effects, regional anaesthesia appears to be the best alternative against postoperative pain.[2,3] To prolong the effect of local anaesthetics, a number of strategies have been investigated, for example continuous perineural catheters. Although effective,[4] the catheters themselves have potential risks (infection, delayed mobilisation, increased nursing costs) and can be challenging in the setting of ambulatory surgery.[5,6] Furthermore, prolonged administration of local anaesthetics can cause nerve damage.[7]

Several studies have investigated the effect of adding adjuvants to local anaesthetic injections, for example midazolam, buprenorphine, clonidine, dexmedetomidine or magnesium, but results have been contradictory.[8,9] Dexamethasone is a synthetic long-acting glucocorticoid that prolongs the action of local anaesthetics when added locally.[10–16] However, there are concerns given the lack of safety data, the off-label use of dexamethasone as an analgesic adjuvant and the uncertain benefit are of concern.[17] Thus, many practitioners encourage systemic administration instead of perineural injection.[17,18]

A number of new local anaesthetic preparations have been developed in order to prolong their analgesic effect, such as polymeric microspheres (formulated by a solvent extraction method and supplied by Medisorb Technologies International, L.P., Cincinati, OH, USA),[19] microcrystals (e.g. Lecithin-coated tetracaine-HI microcrystals), liposomes (Pacira Pharmaceuticals, Inc., San Diego, CA, USA),[20–23] and lipospheres (vesicles made of triglyceride with a phospholipid outer covering). Even though they are effective in prolonging the action of local anaesthetics, they are not (yet) widely used, perhaps because there are no well accepted studies that address concerns about toxicity (nerve toxicity, inflammatory response, myotoxicity). One particular formulation (liposomal bupivacaine, Exparel®, Pacira Pharmaceuticals, Inc., San Diego, CA, USA) is commercially available and has been used in several clinical studies of local tissue infiltration for surgery.[24–26] After animal studies showed only mild inflammation and myotoxicity,[21] a clinical study in healthy human volunteers used perineural Exparel® for single-shot femoral nerve block.[27] In 2018, based on the results of a multicentre study,[28] the US Food and Drug Administration (FDA) approved use of Exparel® for nerve block analgesia after shoulder surgeries.[29] However, histological data are lacking concerning the potential neurotoxic effect of Exparel® delivered perineurally. We studied the neurotoxicity of perineural liposomal bupivacaine alone or with added dexamethasone over a period of up to 4 weeks in a mouse model, and compared this with injection of plain bupivacaine.

## Methods

All animal procedures were approved by the Toulouse Medical Area Standing Committee on Animals (CEEA122: 2014–31) and are consistent with the published guidelines for the humane use of laboratory animals.[30] Four-month-old male mice (C57BL6 wild-type; Laboratoires Janvier, St Berthevin, France; 25–35 g) were kept in plastic cages with soft bedding with a 12 h light–dark cycle and were fed *ad libitum*. Before each experiment, mice were allowed to acclimatise for 7 days with their environment. The absence of behavioural signs of stress (immobility, frequent defecation), and normal motor and thermoalgesic sensory responses were verified. All experiments were carried out in a quiet environment at room temperature.

### Surgical procedures

Surgical and anaesthetic procedures were performed under general anaesthesia (inhalation induction and maintenance with sevoflurane (SEVOran, Abbvie Laboratories, North Chicago, IL, USA) and an equal mixture of oxygen and air. Mice were placed in the prone position and the skin prepped three times with povidone–iodine. A longitudinal incision of 2 cm was made with a sterile N° 15 blade over the left thigh, the sciatic nerve was exposed through a posterolateral approach, and the previously prepared local anaesthetic mixture was injected into the perineural space using a 30 G catheter (Becton Dickinson and Co., Franklin Lakes, NJ, USA) and 1 ml tuberculin syringe (Pentaferte ITALIA, Campli, Italy). Care was taken to ensure injection into the perineural space under the fascia covering the nerve. The fascia was then closed carefully with a non-absorbable suture in order to be able to locate the injection site at a later time. Special attention was paid to avoid mechanical nerve damage. The skin was closed with two layers of polypropylene 5–0 sutures (Prolene; Ethicon, Somerville, NJ, USA). Peritoneal injections were performed under general anaesthesia in the lower right abdominal quadrant, away from the midline to avoid inadvertent injection into the bladder or caecum, using a 24 G needle (Becton Dickinson and Company Limited, Drogheda Co., Louth, Ireland) and 1 ml tuberculin syringe. A suction test was performed before administration. The animals were allowed to emerge, and 14 or 28 days later the sciatic nerve was exposed in the same fashion under general anaesthesia and a 1 cm segment excised for histological analysis. The animals were euthanised via cervical transection under general anaesthesia.

### Preparation of local anaesthetic solutions

The 98 mice were divided into seven groups of 14 each:

- Sham group: 0.15 ml perineural isotonic saline solution

- B group: 0.05 ml perineural bupivacaine 0.5% (0.25 mg, which is ~10 mg $kg^{-1}$) plus 0.1 ml perineural isotonic saline solution
- BDIP group: 0.05 ml perineural bupivacaine 0.5% plus 0.1 ml perineural isotonic saline solution plus 0.2 ml intraperitoneal dexamethasone 0.04% (8 μg, which is ~0.3 mg $kg^{-1}$)
- BDPN group: 0.05 ml perineural bupivacaine 0.5% plus 0.1 ml perineural dexamethasone 0.04% (4 μg, which is ~0.15 mg $kg^{-1}$)
- E group: 0.05 ml perineural Exparel® 1.3% (0.665 mg, which is ~25 mg $kg^{-1}$) plus 0.1 mL perineural isotonic saline solution
- EDIP group: 0.05 mL perineural Exparel® 1.3% plus 0.1 ml perineural isotonic saline solution plus 0.2 ml intraperitoneal dexamethasone 0.04% (8 μg, which is ~0.3 mg $kg^{-1}$)
- EDPN group: 0.05 mL perineural Exparel® 1.3 plus 0.1 ml perineural dexamethasone 0.04% (4 μg, which is ~0.15 mg $kg^{-1}$)

The mice were randomly assigned to these groups, and the local anaesthetic solutions were prepared by an individual who was not involved with either the nerve blockade or the subsequent neurobehavioural testing. For bupivacaine (bupivacaïne HCl 0.5%; Aguettant, Lyon, France), doses were calculated based on previously published data.[20,31–33] Dexamethasone (4 mg $ml^{-1}$; Mylan SAS, Saint Priest, France) was diluted to a concentration of 4 μg in 0.1 ml. At 22.5°C and 30 min after mixing the local anaesthetic solutions, pH values were 6.22 (0.03), 6.45 (0.16), 5.72 (0.15), and 5.60 (0.18) for the B, BDPN, E, and EDPN groups, respectively. The pH of isotonic saline solution was 7.02 (0.04) with the same conditions.

## Behavioural tests

After emergence from general anaesthesia, 49 mice (seven mice randomly selected per group) participated in the behavioural tests. Motor and thermoalgesic sensory functions were assessed at 30 min intervals (until complete thermoalgesic-motor block recovery) by an investigator who was blinded to the treatment group. An acrylic four-chamber container (Model 400; IITC Life Science Inc., Woodland Hills, CA, USA) was used to separate mice. Thermal pain response was assessed through paw withdrawal latency (PWL)[32,34] in response to a heat lamp stimulus applied to the plantar surface of the left rear paw (Hargreaves test, Plantar Analgesia, model 390; IITC Life Sciences Inc., Woodland Hills, CA, USA). The light beam focused on the top of the glass creates a 4×6 mm intense spot on the paw. The withdrawal reflex of the rear paw involves contraction of flexor muscles in the hip and is a polysynaptic reflex induced by noxious stimulation. PWL to the radiant heat stimulus was recorded with the infrared intensity set at 35%, and reaction time 0.1 s. A 12 s cut-off time for the heat source was set to avoid tissue damage.[34] The heat stimulation was repeated three times at an interval of 2–3 min for each paw, and mean values were then calculated. A PWL of more than 7 s was considered thermal pain response impairment and defined the thermoalgesic block.[20,32] Two successive PWL <7 s at a 30 min interval was considered as complete thermoalgesic block recovery at the time point where the first PWL was <7 s.

Concomitantly with thermoalgesic sensory function assessment, motor function of locomotion was assessed at 30 min intervals using the following scale: 0, normal motor function of locomotion; 1, normal dorsiflexion ability and the mouse walking with curled toes; 2, moderate dorsiflexion ability and the mouse walking with curled toes; 3, no dorsiflexion ability and the mouse walking with curled toes.[35] A score of 2 or 3 was considered impaired motor function of locomotion. At the same time, every 30 min, a thermoalgesic sensory and motor assessment of the contralateral paw was performed (control) for each mouse. Sensorimotor behavioural tests were repeated before the euthanasia.

## Histology

At 14 or 28 days after the surgical procedure, a segment of the sciatic nerve was harvested under general anaesthesia. This time interval was arbitrarily chosen in order to correspond to medium-range or longer-term nerve tissue damage.[33] The specimens were placed in 10% formalin for 48 h, washed with ethanol, and embedded in paraffin. Longitudinal sections of 5 μm thickness were prepared and stained with haemotoxylin–eosin in order to evaluate inflammation and Wallerian degeneration (Fig 1). A pathologist who was blinded to the treatment group evaluated each specimen using a score to quantify the degree of inflammation by judging the degree of nerve swelling and lymphocytic infiltration[33,36]: no inflammation, absence of any signs of inflammation; mild inflammation, ≤50% of the surface with swelling and lymphocytic infiltration; severe inflammation, >50% of the surface with swelling and lymphocytic infiltration.

Wallerian degeneration, reflecting abnormal myelination and axonal degeneration after nerve injury, was quantified and used to assess the neurotoxicity of the local anaesthetic mixtures.[33] The following score was used: no Wallerian degeneration, no lesions identified; mild Wallerian degeneration, ≤50% of nerve fibres with Wallerian degeneration; severe Wallerian degeneration, ≥50% of nerve fibres show Wallerian degeneration.

## Statistical analysis

Because of the lack of data about the toxicity of Exparel® with dexamethasone, it was not possible to perform a sample size

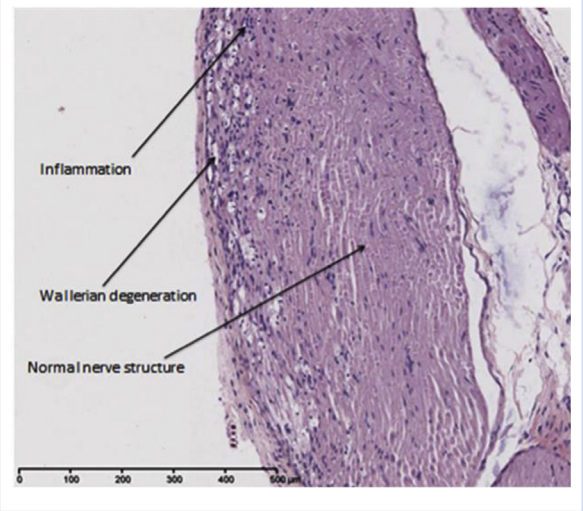



Fig. 1. Histological examination of a sciatic nerve showing mild Wallerian degeneration and mild inflammatory response. Neural inflammation is characterised by nerve swelling and lymphocytic infiltration (arrow), whereas the typical pattern of Wallerian degeneration is evident as ballooning (arrow).

calculation. The duration of motor and thermoalgesic block was treated as non-parametric data and expressed as median [range]. Differences between groups were tested using the non-parametric test of Kruskal–Wallis. If the Kruskal–Wallis test was positive, a *post hoc* analysis for pairwise comparisons of subgroups was performed. The scores for inflammation and Wallerian degeneration at 14 and 28 days were considered categorical variables (no, mild, severe) and were compared between groups using the $\chi^2$ test. Statistical analysis was carried out using MedCalc Statistical Software, version 17.2 (MedCalc Software bvba, Ostend, Belgium; 2017). A *P*-value <0.05 was considered statistically significant.

## Results

Of 98 mice included in the study:

- Forty-nine mice had an assessment of thermoalgesic sensory and motor function at 30 min intervals. Each mouse had normal motor and thermoalgesic sensory function with a PWL <7 s for both legs at the basal state. The Sham group mice showed no motor or thermoalgesic block (PWL <7 s), and their behavioural data are thus not listed below.
- All mice except one were histologically analysed after randomisation at 14 days ($n$=49) or 28 days ($n$=48). A muscle sample taken from a mouse of the EDIP group was randomly selected for sacrifice at 28 days and was therefore not included in the histological analysis. The 98 mice had a normal sensorimotor behavioural test with PWL <7 s before euthanasia.

### Histology

#### *Inflammation*

At 14 days mild neural inflammation was found in some mice in all groups (Table 1). All mice in the B group had mild neural inflammation. Inflammation was more frequent in the B group than in other groups. Differences between groups were statistically significant ($P$=0.04). At 28 days there was no inflammation in the Sham, B, BDIP, and EDPN groups, whereas five of seven mice in the E Group had mild inflammation (Table 1, $P$=0.001 for the comparison between groups). No severe inflammation had been observed at 14 or 28 days.

#### *Wallerian degeneration*

At 14 days, mild Wallerian degeneration was observed in some mice in all groups except the Sham group. The differences between groups did not reach statistical significance (Table 2, $P$=0.70). At 28 days, mild Wallerian degeneration was observed in all groups except the Sham group. These observations did not reach statistical significance (Table 2, $P$=0.70).

In summary, medium-range mild neural inflammation induced by bupivacaine was reduced by dexamethasone (perineural or intraperitoneal), and long-term mild neural inflammation induced by Exparel® was reduced by perineural dexamethasone (Table 1).

### Behavioural tests

#### *Motor block*

The duration of motor block was different between groups. B Group: 90 [60–120] min, BDIP group: 120 [90–150] min; BDPN group: 120 [120–150] min; E group: 120 [90–150] min, EDIP Group: 180 [150–210] min and EDPN Group: 180 [150–210] min ($P$=0.00001). For pairwise comparison between groups, see Fig 2.

#### *Thermoalgesic block*

The duration of the thermoalgesic block was different between groups. B group: 240 [210–270] min; BDIP group: 300 [240–360] min; BDPN group: 360 [270–390] min; E group: 360 [270–390] min level $P$=0.00002). For pairwise comparison between groups, see Fig 3.

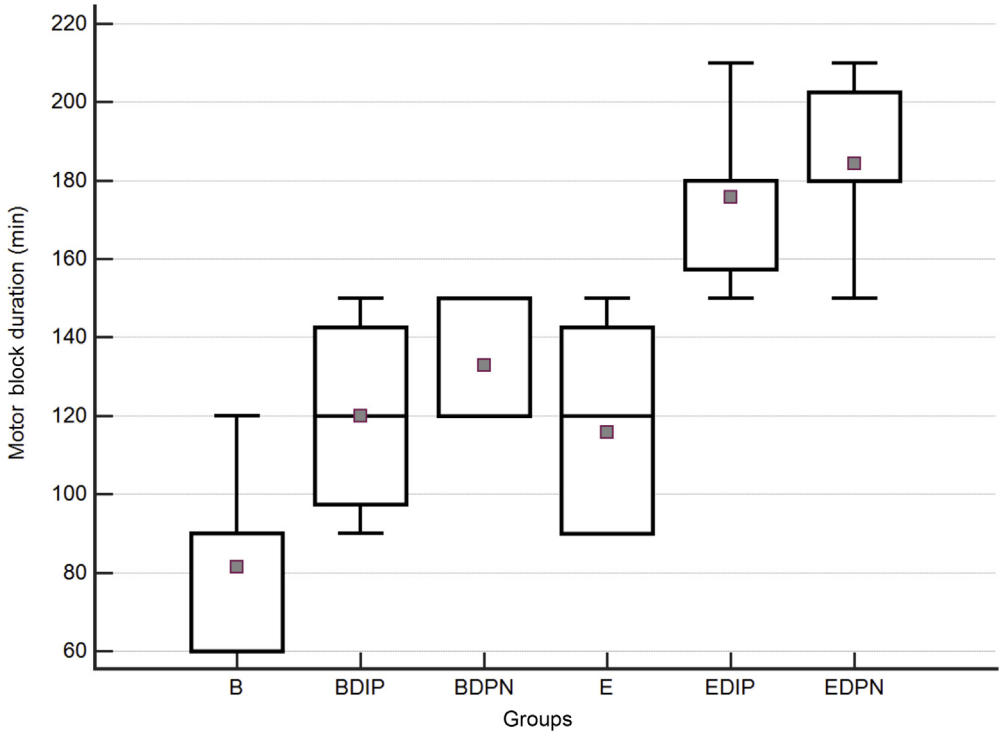
In summary, the duration of motor and thermoalgesic blocks in the Exparel® group was significantly longer than in the plain bupivacaine group. Perineural addition of

**Table 1** Histological neural inflammation at day 14 ($n$=49, seven mice randomly selected per group; $P$=0.04) and at day 28 ($n$=48, seven mice randomly selected per group minus one in the EDIP Group because of improper histologic sampling; $P$=0.001). SHAM, perineural isotonic saline solution; B, perineural plain bupivacaine; BDIP, perineural plain bupivacaine plus added intraperitoneal dexamethasone; BDPN, perineural plain bupivacaine plus added perineural dexamethasone; E, perineural Exparel®; EDIP, perineural Exparel® plus added intraperitoneal dexamethasone; EDPN, perineural Exparel® plus added perineural dexamethasone.

| Group | Neural inflammation at day 14 | | | Neural inflammation at day 28 | | |
|---|---|---|---|---|---|---|
| | None | Mild | Severe | None | Mild | Severe |
| SHAM | 5 | 2 | 0 | 7 | 0 | 0 |
| B | 0 | 7 | 0 | 7 | 0 | 0 |
| BDIP | 5 | 2 | 0 | 7 | 0 | 0 |
| BDPN | 6 | 1 | 0 | 6 | 1 | 0 |
| E | 3 | 4 | 0 | 2 | 5 | 0 |
| EDIP | 4 | 3 | 0 | 4 | 2 | 0 |
| EDPN | 4 | 3 | 0 | 7 | 0 | 0 |

**Table 2** Histological Wallerian degeneration at day 14 ($n$=49, seven mice randomly selected per group; $P$=0.70) and at day 28 ($n$=48, seven mice randomly selected per group minus one in the EDIP group because of improper histologic sampling; $P$=0.70). SHAM, perineural isotonic saline solution; B, perineural plain bupivacaine; BDIP, perineural plain bupivacaine plus added intraperitoneal dexamethasone; BDPN, perineural plain bupivacaine plus added perineural dexamethasone; E, perineural Exparel®; EDIP, perineural Exparel® plus added intraperitoneal dexamethasone; EDPN, perineural Exparel® plus added perineural dexamethasone.

| Group | Wallerian degeneration at day 14 | | | Wallerian degeneration at day 28 | | |
|---|---|---|---|---|---|---|
| | None | Mild | Severe | None | Mild | Severe |
| SHAM | 7 | 0 | 0 | 7 | 0 | 0 |
| B | 5 | 2 | 0 | 6 | 1 | 0 |
| BDIP | 4 | 2 | 1 | 5 | 1 | 1 |
| BDPN | 6 | 1 | 0 | 6 | 1 | 0 |
| E | 6 | 1 | 0 | 6 | 1 | 0 |
| EDIP | 4 | 2 | 1 | 3 | 2 | 1 |
| EDPN | 6 | 1 | 0 | 6 | 1 | 0 |




***Post-hoc* analysis**

| Factor | n | Average rank | Different ($P$<0.05) from factor number |
|---|---|---|---|
| (1) B | 7 | 6,43 | (2)(3)(4)(5)(6) |
| (2) BDIP | 7 | 16,79 | (1)(5)(6) |
| (3) BDPN | 7 | 20,79 | (1)(5)(6) |
| (4) E | 7 | 15,50 | (1)(5)(6) |
| (5) EDIP | 7 | 33,71 | (1)(2)(3)(4) |
| (6) EDPN | 7 | 35,79 | (1)(2)(3)(4) |

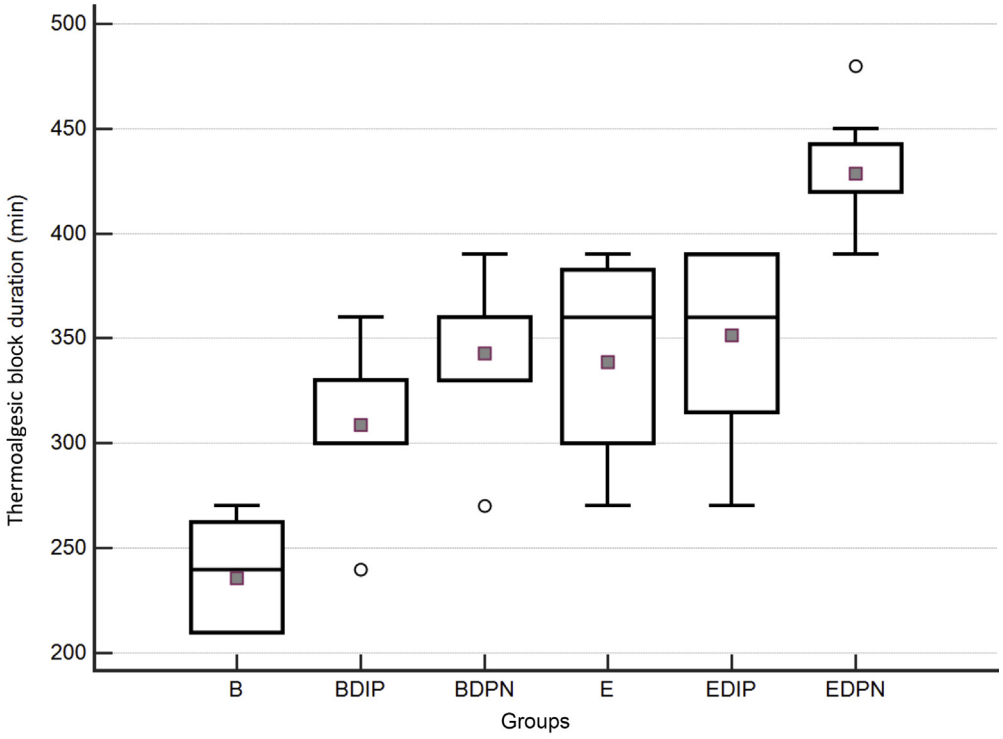
Fig. 2. Duration of motor block for the different groups. Median values shown as solid line within box of 25th and 75th percentile values ($P$<0.0001). Whiskers represent range values. Square markers represent mean values. SHAM, perineural isotonic saline solution; B, perineural plain bupivacaine; BDIP, perineural plain bupivacaine plus added intraperitoneal dexamethasone; BDPN, perineural plain bupivacaine plus added perineural dexamethasone; E, perineural Exparel®; EDIP, perineural Exparel® plus added intraperitoneal dexamethasone; EDPN, perineural Exparel® plus added perineural dexamethasone.

dexamethasone to Exparel® further prolonged the thermoalgesic and motor block.

## Discussion

We evaluated the neurotoxicity and effectiveness of perineural injection of liposomal bupivacaine alone or with perineural or systemic dexamethasone in an *in vivo* mouse model. Histological analysis showed the inflammatory effect of perineural bupivacaine at a medium-range time point. Adding perineural or systemic dexamethasone exerted a protective effect against this inflammatory response at 14 days when added to plain bupivacaine. The pro-inflammatory effect of perineural liposomal bupivacaine (Exparel®) was observed at a longer-range time point. The addition of perineural but not systemic dexamethasone exerted a protective effect against this inflammatory response at 28 days when added to Exparel®.

The duration of motor and thermoalgesic blocks in the Exparel® group was significantly longer than in the plain bupivacaine group. Addition of dexamethasone by either route to plain bupivacaine prolonged the motor and thermoalgesic blocks to reach duration comparable with Exparel®. Addition of perineural or systemic dexamethasone to Exparel® further prolonged motor block, whereas only addition of perineural dexamethasone to Exparel® prolonged thermoalgesic block.




***Post-hoc* analysis**

| Factor | n | Average rank | Different ($P<0.05$) from factor number |
|---|---|---|---|
| (1) B | 7 | 4,86 | (2)(3)(4)(5)(6) |
| (2) BDIP | 7 | 16,14 | (1)(5)(6) |
| (3) BDPN | 7 | 22,50 | (1)(6) |
| (4) E | 7 | 22,14 | (1)(6) |
| (5) EDIP | 7 | 24,79 | (1)(2)(6) |
| (6) EDPN | 7 | 38,57 | (1)(2)(3)(4)(5) |

Fig. 3. Duration of thermoalgesic block for the different groups. Median values shown as solid line within box of 25th and 75th percentile values (P<0.0001). Whiskers represent range values. Square markers represent mean values. SHAM, perineural isotonic saline solution; B, perineural plain bupivacaine; BDIP, perineural plain bupivacaine plus added intraperitoneal dexamethasone; BDPN, perineural plain bupivacaine plus added perineural dexamethasone; E, perineural Exparel®; EDIP, perineural Exparel® plus added intraperitoneal dexamethasone; EDPN, perineural Exparel® plus added perineural dexamethasone.

Conventional local anaesthetics for peripheral nerve blockade provide 12–24 h of analgesia, which often is insufficient to manage longer-lasting postoperative pain. Slow release of bupivacaine with new formulations is relatively attractive to prolong analgesic effect, especially in ambulatory surgery where sparing perineural catheters and related care is of major interest. The effectiveness of surgical site infiltration of Exparel® has been shown when compared with placebo.[24–26] Pharmacological data suggest a prolonged slow release from multivesicular liposomes over approximately 96 h.[37] In order to facilitate early recovery after surgery, some authors have suggested perineural administration of Exparel® to prolong the duration of sensory blockade.[27] Analysis of data from six controlled studies of perineural Exparel® showed that the safety profile of liposomal bupivacaine was comparable with that of plain bupivacaine, and that adverse effects were caused by opioid rescue or to the surgical procedure itself.[38] Liposomal bupivacaine administered perineurally has been used for postsurgical analgesia after total knee arthroplasty, and compared with placebo femoral nerve block with liposomal bupivacaine resulted in lower pain scores and reduced opioid requirements with a safety profile similar to placebo.[39] Contrary results have been shown in a database analysis of more than 88 000 patients who received a peripheral nerve block for total knee arthroplasty in which liposomal bupivacaine was used perineurally in 21.2% of patients and was not associated with a clinically relevant improvement in inpatient opioid prescription or opioid-related complications.[40] After the conduct of a Phase III brachial plexus nerve block study in patients undergoing major shoulder surgery where liposomal bupivacaine added to bupivacaine reduced pain and enhanced patient satisfaction,[41] Pacira[29] proposed to add nerve block as

an indication for Exparel® to produce regional analgesia, which was approved by the FDA in 2018 for nerve block after shoulder surgery.

Our results are consistent with the findings of McAlvin and colleagues,[20] who found that the duration of sensory block induced by Exparel® was significantly longer (median 240 [inter-quartile range, IQR=240–240] min) compared with bupivacaine 0.5% (median 120 [IQR=120–165] min, $P$=0.001) or bupivacaine 1.31% (median 210 [IQR=180–240] min, $P$=0.01).[20] Even though these findings seem promising, the dose–effect relationship after perineural Exparel® remains to be defined.[27]

We also showed that perineural addition of dexamethasone to Exparel® further prolonged thermoalgesic and motor block. Dexamethasone has been shown to prolong the action of local anaesthetics when added locally or intravenously as an adjuvant.[42] The exact mechanism of action remains to be defined because an effect of dexamethasone on nerve conduction and action potential propagation has not been shown. Possible mechanisms include local vasoconstriction leading to slow absorption of local anaesthetic, activation of inhibitory potassium channels, direct action on glucocorticoid receptors of nociceptive peripheral nerve fibres, or a generalised anti-inflammatory effect.[43] Future studies with antagonists of glucocorticoid receptors may help define the mechanism of action.

Our study supports existing concerns regarding a neurotoxic effect of bupivacaine with a pro-inflammatory effect and Wallerian degeneration with perineural bupivacaine. Although this difference was not statistically significant, the local neurotoxic effects of local anaesthetics is well known.[36] Indeed, increasing the dose or exposure time to local anaesthetics is correlated with the severity of neurotoxic effects. This local neurotoxicity may be attributable to alterations in cellular metabolism (involving calcium regulation, the oxidative pathway in mitochondria with generation of free oxygen radicals), ultimately leading to cell death.[44] One of the main findings in our study was the fact that medium-range inflammation was reduced by dexamethasone (perineural or systemic) for bupivacaine, and long-term inflammation induced by Exparel® was reduced by perineural dexamethasone.

The results in the animal literature show inconsistent findings regarding the inflammatory response after Exparel®. Doses up to 30 mg kg$^{-1}$ of Exparel® on the brachial plexus of rabbits and dogs led to minimal granulomatous inflammation in fatty tissue surrounding the nerve roots and without actual nerve damage, suggesting the relative safety of liposomal bupivacaine.[21] Perineural injection of 1.3% Exparel® with 0.5% or 1.3% bupivacaine to the sciatic nerve in a rat model found no signs of neurotoxicity at day 4 and day 14, but the inflammatory response was slightly higher in the Exparel® group at day 4.[20] The long-term inflammatory response (at 28 days) shown in the Exparel® group in our study could either be a result of the liposomal micro-encapsulation (the vehicle for bupivacaine) or the prolonged contact of released bupivacaine with the tissues.[20] Bupivacaine-loaded microparticles were associated with myotoxicity, whereas control particles were not, and even low concentrations of bupivacaine that were non-toxic over brief exposures became highly toxic after days or weeks of exposure.[45] A possible implication of the latter finding is that myotoxicity is an inevitable concomitant of sustained release of local anaesthetics.[45] The protective effect of dexamethasone against the toxic effects of local anaesthetics have also been described in a model of cell cultures in which pre-treatment of neuroblastoma cells with dexamethasone exerted a protective effect on bupivacaine-induced neuronal cell injury through a threonine–serine protein kinase B-dependent mechanism.[46] The effect of the addition of dexamethasone to bupivacaine on axonal degeneration and demyelination was also tested in an animal model in which perineural but not systemic dexamethasone prevented the toxic effects of bupivacaine.[31] We have shown that addition of perineural dexamethasone to ropivacaine did not increase sciatic neural toxicity *in vivo*.33 A systematic review of clinical studies did not report neural toxicity or serious adverse effects because of administration of perineural dexamethasone.[10] These results may point to a potential benefit of locally added dexamethasone, despite concerns about the local use expressed by some authors.[17,43] However, the optimal perineural dexamethasone dose to prolong analgesia remains to be defined. Future studies should investigate strategies – including the use of adjuvants alone or in combination – that may potentiate the analgesic effects of local anaesthetics, thus allowing a reduction in the amounts of local anaesthetics and thereby diminishing their toxic effects.

Our study has several limitations. Firstly, it is worth noting that the pharmacokinetic behaviour of intraperitoneal administration as a model of systemic administration in rodents is influenced by hepatic metabolism. Intraperitoneal drugs are absorbed by mesenteric vessels[47] with a pharmacokinetic profile similar to oral, but not intravenous, administration. Secondly, owing to the lack of data on the toxicity of Exparel® with dexamethasone, it was not possible to perform a sample size calculation, so our study might suffer from a lack of power. Thirdly, despite a cautious surgical approach, it is possible that inflammation resulted from surgical trauma and not to the adjuvant or local anaesthetics. Fourthly, periods of 14 and 28 days were chosen because they correspond to medium- and long-term periods in mice models. Because of the pharmacokinetics of Exparel® it is possible that, as bupivacaine-induced inflammation, lesions caused by Exparel® may regress in the longer term (more than 28 days). Fifthly, the pH of the local anaesthetic mixtures vary from group to group with Exparel® solutions more acidic than bupivacaine or isotonic saline solutions. Ideally, injectates should have been buffered at pH 5–6 before injection. However, transient acidic pH *per se* does not result in neural inflammation, but the effect of prolonged acidity on neural inflammation should be considered in further studies. Finally, because the histological inflammatory response has not been shown to have a clinical correlate, its clinical significance remains to be determined.

In summary, compared with the bupivacaine, perineural sciatic nerve injection of Exparel® prolonged the duration of motor and thermoalgesic block in a mouse model. Perineural dexamethasone added to Exparel® further prolonged motor and thermoalgesic block and exerted a protective effect against the inflammation seen at 28 days after perineural infiltration with Exparel®.

## Authors' contributions

Study design: MB, CD, MK, VM
Coordination: MK, VM
Statistical analysis: MB, CD
Performed all measurements: FF, AK, MS, PM
Histological evaluation: AB
Performed all surgical procedures: DA, AC, EC
Wrote the manuscript: FF, AK, MS, PM

Read and approved the final version of the manuscript: all authors.

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## Declarations of interest

The authors declare that they have no conflicts of interest.

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