**Injection procedure for conventional ankle block**

Ropivacaine was given in five injections at the ankle in the isoflurane-anesthetized animal, using the malleoli as landmarks. For the first and second injections, the needle was inserted posterior to the medial and lateral malleoli until bone was contacted, the needle withdrawn slightly, and the drug injected into the small fossa formed by the skin, the malleoli and the Achilles tendon. The third and fourth injections were infiltrated in a fan shaped manner subcutaneously from the anterior borders of the malleli towards the midline across the dorsum of the paw adjacent to the ankle. The fifth injection was given deeper, with perpendicular needle insertion in the middle of the dorsum of the paw adjacent to the ankle until bone was contacted, the needle withdrawn slightly and drug infiltrated in a fan shaped manner.

**Preparation of Magnetic Nanoparticles/Ropivacaine complexes.**

Sixty- four mg of the MNPs were mixed with 128 mg ropivacaine (12.8 ml of 1% Naropin®) in 20 mL water and stored at 4°C for 48 hours. The free drug was then removed by centrifugation (at 10,000 rpm for 20 min) and the MNPs washed three times with deionized water. Finally, the drug-loaded MNPs (MNP/Ropiv) were dispersed in 8 ml deionized water. The Fe3O4 content in the complexes was determined by thermogravimetric analysis.

To determine the thermal responsiveness of the MNP/Ropiv complexes, ropivacaine content and release characteristics were determined *in vitro* by UV-Vis spectroscopy. Ropivacaine-loaded MNPs were dispersed in 1 ml water containing 0.2% w/v nanogels and 0.11% w/v ropivacaine and placed in dialysis tubes. The tubes were placed in 50 ml water in a beaker at 0, 18 and 37°C because the lower critical solution temperature (LCST) for these MNPs is 25.10C based on previous findings. LCST is the temperature above which the MNPs shrink (and release any drug loaded into them). In a previous study,1 the formed nanogels had an average diameter around 287 nm at 18°C, 154 nm at 37°C and127 nm at 50°C. The dialysis membrane used was standard semi-permeable regenerated cellulose membrane (Spectrum Laboratories Inc., Rancho Dominguez, CA). Its molecular weight cutoff is 1,000 Daltons, making it permeable to water and drug molecules but impermeable to nanogels (> 200 nm). The osmotic pressure across the membrane was not determined. The dialysis process was driven by the ropivacaine concentration gradient across the membrane. The released ropivacaine passed through the membrane into the water, in which their concentration was determined. In order to maximize the diffusion rate of ropivacaine, the water in the beaker was changed after every measurement. The UV absorbance of ropivacaine at 263 nm follows Beer’s law when its concentration in water is lower than 0.1 mg/mL. The molar absorptivity is 1.15 mL/mg\*cm. The concentration of ropivacaine was determined using the equation:

Absorbance (at 263 nm) = absorptivity x 1 cm x concentration.

Although the MNP/Ropiv complexes used *in vivo* were at a higher concentration to maximize the drug content and release efficiency of the complexes, the ratio of nanogels to ropivacaine molecules was similar in both preparations, and thus, the *in vitro* drug release kinetics are expected to be similar for both concentrations. Although highly stable at room temperature, the complex could release small amounts of ropivacaine into the aqueous phase of the suspension during storage. To minimize this possibility, the final complex was prepared on the day of use and kept on ice until immediately prior to IV injection. The suspension was not sterilized before use.

**Ropivacaine assay of plasma and ankle tissue.**

The investigator overseeing and performing the analysis of plasma and tissue drug levels (R.V.) was blind to the experimental group of the samples.

To extract ropivacaine from plasma, 60 µl of plasma was mixed with 20 µl perchloric acid and bupivacaine, the internal standard. The mixture was vortexed well and centrifuged at room temperature. To extract ropivacaine from ankle tissue, the tissue was pulverized and extracted with methanol by shaking at room temperature for 4 hours. Aliquots of the methanol were evaporated. An aliquot of plasma was added to dissolve the content. Perchloric acid and the bupivacaine internal standard were added and the content vortexed well and centrifuged at room temperature. Five µl of the solution was injected into the HPLC-MS-MS system.

HPLC conditions: A symmetry C18 column (3.5 um; 2.1 x 50 m) and two mobile phases were used in a gradient manner to separate various components: Buffer A -2 mM ammonium acetate, 0.1% formic acid and 5% methanol in water; Buffer B-2mM ammonium acetate, 0.1% formic acid and methanol. The retention time of ropivacaine was 1.4 minutes and that of bupivacaine was 2.15 minutes.

Mass spectrometry (MS) conditions: The capillary voltage was 3 kV; source temperature, 1200C; desolvation temperature, 6000C, and the cone gas flow 50 l/hour. Transition ions were 275 > 126 (cone voltage of 40, collision 22) for ropivacaine and 289.2 > 140.1 (cone voltage of 40, collision voltage of 22) for bupivacaine. The assay was linear from 0-1000 ng/ml and the interday and intraday CV was less than 10% at 10, 100 and 500 ng/ml.

References.

1. Dong H, Mantha V, Matyjaszewski K. Thermally Responsive PM(EO)2MA Magnetic Microgels via Activators Generated by Electron Transfer Atom Transfer Radical Polymerization in Miniemulsion. Chem Mater 2009; **21**: 3965-72