Plasma concentrations of bupivacaine after brachial plexus administration of liposome-associated and plain solutions to rabbits

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Bupivacaine has been associated to multilamellar liposomes with the aim of altering circulating plasma concentrations after injection into the rabbit brachial plexus. Plasma concentrations of bupivacaine have been compared after administration of free drug (BP) or bupivacaine associated to multilamellar liposomes (BP-MLV) made of phosphatidylcholine and cholesterol (molar ratio 4:3). Under light general anaesthesia, one group of six rabbits received an axillary injection of 2.5 mg BP (1 ml, 0.25%), and a second received the same dose of BP-MLV. In both groups ^3H bupivacaine was used as a marker. The brachial plexus was located using a nerve stimulator. Injection of the anaesthetic solutions invariably prevented the motor response of the paw. The arterial plasma concentrations of bupivacaine were determined after 5 to 240 min and after 24 hr by beta counting. In the MLV population, additional measurements were performed after 48 and 72 hr. The two plasma curves showed a plateau ($0.2 \mu\text{g} \cdot \text{ml}^{-1}$) which was reached after five minutes in the BP group and after 90 min using BP-MLV. In the BP-MLV group, the plasma concentrations of bupiva-

caine were lower during the first ten minutes ($P < 0.05$), and higher after 24 hr ($P < 0.05$). Radioactivity decreased between 4 and 24 hr in the BP group and between one and two days in the BP-MLV population. It is concluded that elevated plasma drug concentrations were maintained for longer with BP-MLV than with BP. This could prolong the action of the local anaesthetic through a slow release.

La bupivacaine a été associée à des liposomes multilamellaires, composés de phosphatidylcholine et cholestérol (rapport molaire 4:3) dans le but de modifier les concentrations plasmatiques de l'anesthésique local après administration au niveau du plexus brachial du lapin. Sous anesthésie générale, un premier groupe (BP) de six lapins a reçu une injection plexique de 1 ml de bupivacaine commerciale à 0,25% sans adrénaline. Le second groupe (BP-MLV) a reçu 1 ml de bupivacaine liposomale à 0,25%. De la bupivacaine tritiée a été utilisée comme marqueur. Le plexus brachial a été repéré à l'aide d'un stimulateur de nerf. L'injection des solutions anesthésiques fit disparaître les réponses musculaires dans la patte. Les concentrations artérielles de bupivacaine ont été mesurées à des temps différents (de 5 à 240 minutes et après 24 heures) par comptage de la radioactivité plasmatique et exprimées en $\mu\text{g} \cdot \text{ml}^{-1}$. Dans le groupe MLV, des prélèvements supplémentaires ont été pratiqués après 48 et 72 hr. Les deux courbes plasmatiques montrent un plateau identique ($0,2 \mu\text{g} \cdot \text{ml}^{-1}$), qui est atteint après cinq minutes dans le groupe BP et après 90 min dans le groupe BP-MLV. Dans ce dernier groupe, la concentration plasmatique de bupivacaine était plus basse durant les dix premières minutes ($P < 0,05$) et plus élevée après 24 hr ($P < 0,05$). La concentration plasmatique de bupivacaine diminuait entre 4 et 24 hr dans le groupe BP et après un à deux jours dans le groupe BP-MLV. L'association de la bupivacaine à des MLV pourrait prolonger la durée d'action de la bupivacaine administrée au niveau du plexus brachial par le biais d'une libération progressive de l'anesthésique local.

Key words

ANAESTHETIC TECHNIQUES: regional, axillary block;
ANAESTHETICS, LOCAL: bupivacaine;
PHARMACOKINETICS: local anaesthetics;
PHARMACOLOGY: liposome association.

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Liposomes have been used as drug carriers in an attempt to control the delivery of biologically active agents which can lead to prolonged effect and reduced toxicity.^{1,2} Specifically, local anaesthetics have been encapsulated into liposomes with the aim of obtaining a longer duration of action or a reduction in circulating plasma concentrations.³⁻⁷

Bupivacaine, a long-acting amino-amide local anaesthetic, has been associated to multilamellar liposomes (MLV).^{5,7} Preliminary animal studies indicated that epidurally injected MLV act as a depot formulation, releasing bupivacaine in a controlled fashion and ensuring a low, constant and prolonged plasma concentration.⁷ Boogaerts *et al.* have shown that the liposomal formulation of 0.5% bupivacaine increases the duration of postoperative analgesia in humans without motor blockade or adverse side effects.⁵

The present study was undertaken to compare the plasma concentrations of bupivacaine after injection of plain and liposomal formulations of bupivacaine into the rabbits' brachial plexus. The axillary route was selected because it represents the easiest technique of brachial plexus blockade in the rabbit.

Methods

Liposomal encapsulation of bupivacaine

The method of bupivacaine association to liposomes has been described in detail elsewhere.⁸ Bupivacaine was inserted into liposomes made of egg phosphatidylcholine (Egg Pc, Fraction VII, Sigma, St. Louis, MO) and cholesterol (Ch, Sigma) in a molar ratio 4:3. The total weight of lipids was 60 mg. Lipophilic bupivacaine was obtained from the chloride solution (bupivacaine hydrochloride, Marcaine®, Astra Nobel Pharma) by adding KOH to a pH > 10. The precipitate was filtered and dissolved in chloroform. The chloroform/bupivacaine solution containing 2.5 mg of drug was mixed homogeneously in a glass vial with Egg Pc and Ch, both dissolved in the same organic solvent and 0.001 mCi ³H-bupivacaine in ethanol (³H-bupivacaine, 0.1 mCi · ml⁻¹, Institut des Radio-Éléments, Fleurus, Belgium) were added as a marker. Tritium labelling of bupivacaine was performed on a methyl group associated to the benzoyl lipophilic moiety of the molecule. Evaporation of the solvent under a nitrogen atmosphere resulted in the formation of a lipid film of Egg Pc, Ch, lipophilic bupivacaine and its radionuclide. This film was kept overnight in a vacuum lyophilizer to remove the last traces of chloroform.

One milliliter phosphate buffer, pH 8.1, brought to an osmolality of 280 mOsm · kg⁻¹ H₂O by the addition of NaCl, was added to the lipid film, and multilamellar vesicles (MLV) were formed by vortexing until the film

completely disappeared. The measured osmolality and viscosity of both plain and liposomal solutions were identical. The liposomal preparations were kept at 4°C until use.

Injection into the brachial plexus of rabbits

After approval of the Institutional Animal Investigation Committee, New Zealand male albino rabbits (Rechtspersoonlijkheid Rijksstation, Ghent, Belgium) weighing about 2.5 kg were anaesthetized by *im* injection of 0.1 mg · kg⁻¹ Hypnorm®, an association of fluanisone and fentanyl (Janssen Pharmaceutica, Beerse, Belgium). Anaesthesia lasted for about two hours and if a longer duration was required further Hypnorm 0.05 mg · kg⁻¹ was administered.

Two groups of six rabbits were studied. The first group received an injection of 1 ml bupivacaine 0.25% without adrenaline (Marcaine®, Astra Nobel Pharma) with 0.001 mCi ³H bupivacaine as a marker and the second group received the same dose of bupivacaine associated to MLV.

The axillary region was prepared with rabbits in the supine position. A ground electrode was attached to the opposite paw and connected to the output pole of a peripheral nerve stimulator (Stimuplex®, B. Braun-Melsungen AG, FRG). A 23-gauge insulated needle (Pole Needle®, Equip Medical, Holland) was connected to the other pole. The brachial plexus was located using the axillary approach. The needle was used as a seeking electrode to elicit motor movement in the paw. Flexion of the distal part of the paw was the defined end point. The output from the stimulator was set at the lowest milliamperage (0.1 mA at 1 Hz) and the seeking needle was then advanced until motor activity was evident. Once the nerves were located, the local anaesthetic solution was injected.

Bupivacaine plasma concentrations were determined by counting the radioactivity (Rad) in arterial blood in both groups. A 24-gauge needle was inserted into the rabbit ear artery. Two milliliters of blood were collected in heparinized tubes at various times following injection for 24 hr. In the MLV bupivacaine group, two additional samples were obtained after 48 and 72 hr. The blood samples were centrifuged at 3,500 g for 15 min using a bench centrifuge (Beckman GPR; Palo Alto, CA) and 0.5 ml plasma samples were mixed with 1 ml tissue solubilizer (Soluene®, Packard) in Packard glass vials. Ten milliliters scintillation cocktail (Instafluor®, Packard) were added and beta counting was performed using a Rackbeta® Counter (Pharmacia, Uppsala, Sweden). Plasma concentrations of bupivacaine expressed in µg · ml⁻¹ were calculated as follows:

$$\left(\frac{\text{Rad in 1 ml plasma}}{\text{total Rad injected}} \right) \times \text{total } \mu\text{g bupivacaine injected.}$$

The method was sensitive from cpm countings corresponding to $0.02 \mu\text{g} \cdot \text{ml}^{-1}$ and reproducible from 0.05 to $0.7 \mu\text{g} \cdot \text{ml}^{-1}$.

After awakening from general anaesthesia, the animals were put on the floor and an attempt was made to encourage them to walk.

Statistical analysis

The results are expressed as the mean $\pm$ SD. Statistical significance between groups was determined using ANOVA. $P < 0.05$ was considered statistically significant.

Results

The axillary injection prevented the motor response of the paw produced by nerve stimulation within a few seconds in using BP and BP-MLV.

Arterial plasma bupivacaine concentrations are shown in the Figure. The highest concentrations averaged $0.2 \mu\text{g} \cdot \text{ml}^{-1}$ in both experimental groups. Radioactivity was detected in plasma after five minutes in both groups but the plasma concentration profiles were different. In the BP group, the plasma curves showed a plateau which was reached after five minutes. For the BP-MLV group the plasma concentration increased gradually during the first 90 min and was followed by a prolonged plateau phase.

During the first ten minutes, plasma concentrations were higher after administration of the plain solution ($P < 0.05$). The mean plateau levels were $0.214 \pm 0.031 \mu\text{g} \cdot \text{ml}^{-1}$ in the BP group and $0.204 \pm 0.014 \mu\text{g} \cdot \text{ml}^{-1}$ in the BP-MLV group (NS).

The duration of the plateau phases was different: in the BP group, the plasma concentration decreased between four and 24 hr after administration. After 24 hr, the plasma concentrations were higher in the BP-MLV group ($P < 0.05$). Plasma drug concentrations decreased thereafter to $0.118 \pm 0.009 \mu\text{g} \cdot \text{ml}^{-1}$ after 48 hr and $0.085 \pm 0.012 \mu\text{g} \cdot \text{ml}^{-1}$ after 72 hr in this group.

After awakening from general anaesthesia, no motor blockade of the paw was observed in either group. Sensory blockade was not evaluated.

Discussion

Liposomal bupivacaine prevented the nerve motor response of the paw within a few seconds of injection which indicated that the anaesthetic effect of this formulation was preserved. The minimum stimulating current (0.1 MA) used for location of the plexus using insulated needle confirmed that the injections were made very close to the nerves in the axillary sheath.¹⁰ The low concentration of bupivacaine used (0.25%) explains the absence of motor blockade at the time of awakening of the animals. Association of bupivacaine to multilamellar liposomes modified its pharmacokinetic profile after injection

FIGURE Time course (Log. min) of plasma concentrations of bupivacaine ($\mu\text{g} \cdot \text{ml}^{-1}$) in two groups of six rabbits after injection into the axillary brachial plexus of plain (BP) or liposome-associated bupivacaine (BP-MLV). Mean $\pm$ SD. * $P < 0.05$.

tion into the rabbit brachial plexus. The plasma plateau phase, commonly observed following this mode of administration, occurred later using BP-MLV than with BP.¹¹ This might be attributed to a delay in the vascular resorption of bupivacaine molecules released from the phospholipid vesicles.

The rate of local anaesthetic absorption from an injection site is dependent upon the site of injection, the dose of local anaesthetic and the pharmacological profile of the drug.^{11,12} In the present experiments, the same doses of bupivacaine, free or associated to liposomes, were administered.

The low vascularity and the presence of varying amounts of adipose tissue in the brachial plexus region account for the rather slow absorption of local anaesthetics from this site.¹³ The adipose tissue may act as a depot for these drugs and will tend to delay their vascular resorption.^{14,15} It is likely that a portion of the administered dose was absorbed by the fat¹⁶ and this could explain the low plasma concentrations of bupivacaine. The maximal concentrations averaged $0.2 \mu\text{g} \cdot \text{ml}^{-1}$ in the plateau phase encountered with the BP and BP-MLV solutions.

Only the free fraction of bupivacaine is able to diffuse in the blood vessels. A progressive and sustained release of bupivacaine from MLV could account for an interstitial constant free fraction of local anaesthetic which ensured delay in duration of plasma levels. Liposomes would form an artificial local depot of bupivacaine releasing their content slowly. This property could account for the prolonged plateau phase to 48 hr with the liposomal formulation.

The present plasma measurement of bupivacaine was based upon total ^3H -bupivacaine radioactive counting. Tritium was chosen because it represents a stable isotope with a half-life of 12 yr. Circulating bupivacaine is rapidly

catabolized into more polar compounds by the liver.⁹⁻¹¹ The radioactivity measured in the rabbit plasma thus involved bupivacaine and catabolic products, and did not represent a true mirror of local anaesthetic concentration. Tritium labelling of bupivacaine was performed on a methyl group associated to the benzoyl lipophilic moiety of the molecule. Hepatic biotransformation of the local anaesthetic does not alter this part of the molecule,¹¹ as 2,6-Xylidine represents one of the final catabolites of bupivacaine, and the tritium radioactivity counted in the plasma necessarily involves this biotransformation product. Further specification of the chemical composition of plasma radioactivity needs studies using qualitative chromatography detection of circulating bupivacaine and known metabolites. The main purpose of this experiment was to compare the plasma levels of bupivacaine using the two formulations of bupivacaine and not a pharmacokinetic study. The feasibility of a stable isotope method has been demonstrated in a similar experiment.¹⁷

In conclusion, association of bupivacaine to MLV maintains elevated plasma concentrations of bupivacaine for longer than after a plain solution after axillary administration to rabbits. This raises the possibility of prolonging local anaesthetic action by slow release of the drug.

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