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Note

Prolonged local anesthetic effect of bupivacaine liposomes in rats

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Abstract

The purpose of the present study was to develop a sustained release local anesthetic formulation that would localize the local anesthetic drug at target sites and produce a prolonged sensory block by a single usual dose. Bupivacaine aqueous solution was encapsulated in multilamellar vesicles (MLV) of liposomes by mechanical shaking method. Wister rats (240 ± 30 g body weight) were given an injection of 0.2 ml liposomal bupivacaine (5 mg/ml), bupivacaine solution (5 mg/ml) or blank liposome. The duration of sensory analgesia was quantified using the tail flick test, and compared among the three preparations. Blank liposomes did not show any sensory block action. The duration of sensory block and the antinociceptive index (AI) in the rats treated with liposomal bupivacaine were 447 ± 19.6 min and $54 \pm 5\%$, respectively, and that in the rats treated with bupivacaine solution was 87 ± 6.7 min and $30 \pm 8\%$, respectively. Liposomal bupivacaine significantly ($p < 0.01$) prolonged the duration and enhanced the intensity of local anesthesia as compared with bupivacaine solution. The results suggest that liposomes are candidates for the formulation of ultra-long lasting local anesthetics with enhanced effect in a single normal dose.

Keywords: Antinociceptive index; Bupivacaine; Liposomes; Local anesthetics; Prolonged action; Tail flick test

Anesthetics are increasingly using local and regional techniques to provide postoperative pain relief. However, the duration of action of the drugs available is relatively short compared with the potential duration of pain. Local anesthetics are expected to localize in target sites with little systemic absorption. To approach the ideal, the

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conventional method is to add vasoconstrictors such as adrenaline to the local anesthetic solution for the purpose of delaying systemic absorption. However, the adjuvant is not without adverse effects, and they do little to prolong further the duration of the longer acting agents (Buckley et al., 1978).

Liposomes have been used as drug carriers in an attempt to control the delivery or targeting of biologically active agents which can lead to controlled release, prolonged effect and reduced toxicity. Liposomes have several desirable properties to serve as drug delivery vehicles in humans. The most common constituents of liposomal lipids are phosphatidylcholine and cholesterol which are non-toxic and non-immunogenic because they are components of human tissue (Langer, 1990).

Bupicvacaine, a long acting local anesthetic of amide group, is widely used mainly for regional nerve blocks, particularly when a prolonged effect is required (Reynolds, 1996). The purposes of the present study was to prepare a liposome formulation of bupivaciane and to evaluate the local anesthetic efficacy of the formulation. Multilamellar vesicle (MLV) was considered. The basic rational for the use of MLV-liposome as a means for controlled delivery of the drug is that the liposomes may serve as a drug depot at the injection site, and the drug in the inner vesicle should diffuse through several layers of concentric vesicles prior to being released to the body fluid.

Bupivacaine hydrochloride crystalline powder was a generous gift from Astra (Sweden) via Taipei Branch. Cholesterol (CH), egg yolk phosphatidylcholine (PC) and phosphatidic acid (PA) were purchased from Sigma (St. Louis, MO, USA). Liposomes were prepared by mechanical dispersion method (New, 1990). Briefly, a chloroform solution containing PC, CH and PA in a weight ratio of 1:0.4:0.1 were evaporated in a rotary evaporator under a stream of nitrogen gas to yield a thin lipid film, and then put in a vacuum desiccator overnight to assure complete removal of traces of organic solvent. The dried lipid film was hydrated by vortexing with 1.5% bupivacaine solution to produce multilamellar vesicles (MLVs) while entrapping bupivacaine solution. The MLVs were collected by centrifugation at $2500 \times g$ for 10 min. Plain liposomes were prepared by the same procedure but using normal saline solution instead of bupivacaine solution. The size of liposomes was analyzed by a particle analyzer (Courter N4 Plus, Submicron Particle Sizer). The average size of liposomes was 1.2 ± 0.5 (S.D.) μm.

Bupivacaine concentration was determined by spectrophotometric method. In brief, to 0.02 ml liposomal bupivacaine was added 4.98 ml hydrochloric acid (0.1 N) and vortexed with methylene dichloride to remove the liposomal lipids. Bupivacaine hydrochloride in the aqueous layer was measured by a spectrophotometer (Hitachi U2000) set at 220 nm. The recovery of bupivacaine from liposome was complete. The concentration of bupivacaine hydrochloride was then adjusted to 5 mg/ml for local anesthetic study.

Wister rats (Experimental Animal Center, College of Medicine, National Taiwan University) weighing 240 ± 30 g (S.D.) were used. Each preparation (bupivacaine solution, bupivacaine liposomes or plain liposomes) was studied in five rats. The rat was fixed on a tail-flick-test apparatus (Tail Flick Model DS20, Shinohara Electric Institute, Japan) with the portion of the tail 3 cm from its tip exposed to heat from a projector lamp. A single control switch simultaneously activated the light and a timer, and the timer stops automatically when the exposed rat's tail flicks. The time interval between switching on the light to flick of the tail was recorded as tail flick latency (TFL). The TFL measured before drug treatment was defined as baseline-TFL (BL). The intensity of the heat was adjusted to produce a baseline-TFL between 5.0 and 7.0 s. A 15-s cut-off time (COT) was used to avoid thermal injury. Failure to flick the tail by this time was taken to indicate sensory block. A dose of 0.2 ml of bupivacaine solution (5 mg/ml), bupivacaine liposome (5 mg/ml) or plain liposome was injected at the root of the tail on midline and its opposite side (0.1 ml each side). Tail flick test was started 15 min after injection, and the test was done every 15 min during the first 2 h followed by hourly tests for 5 h, then tests every 15 min thereafter until the TFL fell to baseline.

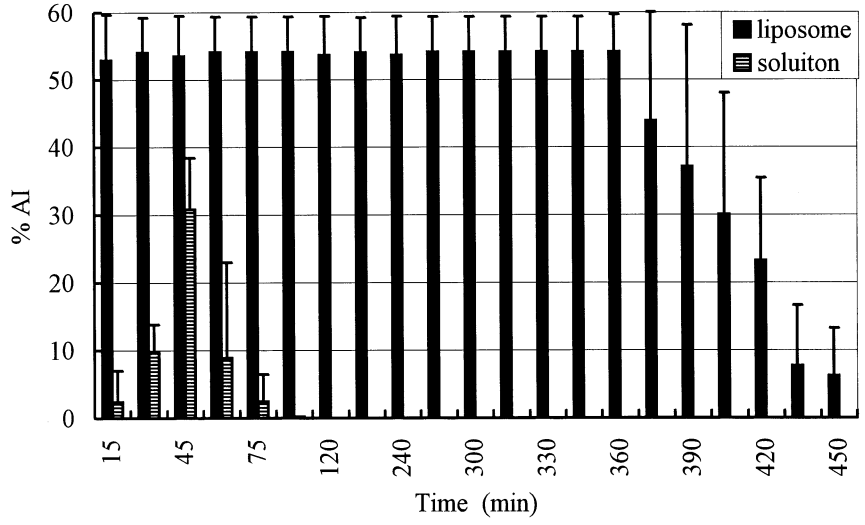

Fig. 1. Duration and %AI of liposomal bupivacaine and bupivacaine solution in rats' tail flick test. Each column with vertical bar represents mean with S.D. of five determinations. The %AI was significantly different ($p < 0.01$) between liposomal bupivacaine and bupivacaine solution at each time point.

The antinociceptive index (AI) (Raffa, 1996) was calculated as follows:

$$\%\mathrm{AI} = \frac{\mathrm{TFL} - \mathrm{BL}}{2\mathrm{BL}} \times 100\% \text{ when } \mathrm{TFL} > \mathrm{BL} \qquad (1)$$

Plain liposomes did not produce sensory block in any animal. Fig. 1 displays the %AI of bupivacaine solution and bupivacaine liposome. Bupivacaine liposome not only significantly ($p < 0.01$) prolonged the duration of sensory block, but also demonstrated significantly ($p < 0.01$) higher %AI than bupivacaine solution did through anesthetic period. The duration of sensory block after injection of bupivacaine solution and bupivacaine liposome was 87 ± 7 and 447 ± 20 min, respectively. The rapid onset (within 15 min) was the same, and the time to reach maximum %AI (30 and 45 min, respectively) was comparable with liposomal bupivacaine and bupivacaine solution.

Local anesthetics block nerve conduction by preventing the transient increase in the permeability of excitable membranes in Na^+. Their primary site of action is the cell membrane. Quaternary analogs of local anesthetics act in their charged form only from the inner surface of the membrane. They are relatively ineffective outside the cell membrane (Strichartz and Ritchie, 1987). Therefore, local anesthetics must first cross the membrane before they can exert a blocking action. At physiological pH only 5–10% of bupivacaine is in the basic form (Catterall and Mackie, 1996) which can permeate through cell membrane into nerve cells, then equilibriate to charged form and act on the inner surface of the membrane. The rapid onset and the higher %AI of liposomal bupivacaine than bupivacaine solution observed from this study suggests that liposomes may facilitate the transmembrane of associated bupivacaine. The results also suggest that the elimination of free bupivacaine from injection site owing to diffusion in interstitial fluid and absorption by capillary bed may be very rapid; and the absorption rate and the absorbed dose fraction of free bupivacaine by tissue capillary bed might be higher than that by the target nerve. Liposome encapsulation can restrict the diffusion and systemic absorption of bupivacaine at injection site.

Our previous experiments assessed that serum levels of lidocaine after topical application of liposomal lidocaine to rabbits' femoral and iliac vessels were lower than that after lidocaine solution (Hou and Yu, 1994). Intra-articular injection of liposomal lidocaine to rabbits also showed lower serum level of lidocaine than that of

lidocaine solution did (Hou and Yu, 1997). These results imply that entrapped in liposomes, the clearance of bupivacaine by systemic absorption, and consequently the elimination of the drug, at injection site should be decreased, maintaining a higher local drug concentration which might contribute to the increased local anesthetic intensity.

The bupivacaine concentration used in the present experiment (0.5%) is the clinical usual concentration of bupivacaine for peripheral nerve block (Reynolds, 1996). Nearly a 5-fold increase in duration of sensory block with liposomal bupivacaine compared to the same dose of bupivacaine solution was observed from this experiment. The mechanism may be ascribed to the gradual release of bupivacaine from the liposome depot which produces a sustained drug level in the extra-liposomal environment. A multilamellar vesicle (MLV) consists of several concentric spheres of lipid bilayers separated by aqueous compartments. Entrapped in the aqueous space of MLV, the diffusion, and consequently the elimination of bupivacaine from injection site should be retarded. Liposomes form an artificial local depot of bupivacaine releasing their content slowly. A progressive and sustained release of bupivaciane from MLV could account for a local interstitial constant level of free bupivacaine which ensures prolonged duration of local anesthesia.

Bupivacaine is known to induce central nervous system and cardiovascular toxic effects (Bloci and Covino, 1981; Reynolds, 1996). Such toxic effects could be avoided by limiting systemic absorption of bupivacaine from liposome formulation. Lowering of the plasma level of locally applied liposomal lidocaine has been assessed. (Hou and Yu, 1994, 1997). The prolonged duration and increased intensity of bupivacaine by liposomal formulation makes it possible to provide an effective ultralong anesthesia by a single normal dose. And consequently, the risk of toxic effects can be minimized.

In conclusion, the present study demonstrated that encapsulation of local anesthetic agent in MLV liposomes provides a longer duration and higher intensity of local anesthesia than commonly used solutions.

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