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Slow-release effect of pH-adjusted bupivacaine: In vitro demonstration

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Summary

Commercial solutions from the same lot of 0.25% bupivacaine (pH = 5.69 ± 0.02) were studied before, and at 3 min and 12 h after alkalinization with 0.4 mEq. sodium bicarbonate/20 ml (final pH = 7.39 ± 0.01). An in vitro dissolution method was modified from a previously described technique used to measure the slow-release effect of insulin suspensions. Alkalinization resulted in a significant increase in the number of crystals of bupivacaine base and in the prolongation of bupivacaine release from the crystals. The results are in agreement with several clinical reports in which prolonged duration of the sensory block was observed. The extent of adsorption on the walls of glass bottles and of plastic syringes increased with the duration of contact after alkalinization. The adsorbed fraction of bupivacaine therefore could not participate in producing the expected clinical effect and might explain several unsuccessful attempts at pH adjustment.

Introduction

Previous clinical studies have shown that pH adjustment of bupivacaine may reduce the latency period of the sensory block (Hilgier, 1985; Melman et al., 1985; Douglas et al., 1986; McMorland et al., 1986; Coventry and Todd, 1989). The most common explanation for this effect is that the increase in the unionized lipophilic form of the local anesthetic favors the transfer through the lipid membrane of the nerve and accelerates the onset of action. The above-mentioned investigations as well as others (Bavoux et al., 1988) have also demonstrated an increase in the duration of the sensory block. The underlying mechanism of this phenomenon remains unclear and this laboratory report is aimed at demonstrating a slow-release effect of crystals resulting from alkalinization of bupivacaine. On the other hand, several studies have been unable to demonstrate any clinical benefit from alkalinization (Bedder et al., 1988; Benhamou et al., 1989; Stevens et al., 1989). The roles of crystallization and adsorption of such crystals of bupivacaine base were also investigated in an effort to explain the failures of pH adjustment.

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Materials and Methods

Bottles (20 ml) from the same lot of 0.25% bupivacaine (pH = 5.69 ± 0.02) were studied. The control situation consisted of bottles of bupivacaine to which was added 0.8 ml of water (final pH = 5.69 ± 0.03) (group 1). Alkalinized solutions were prepared by adding 0.8 ml of 4.2% sodium bicarbonate (0.4 mEq.) to 20 ml of 0.25% bupivacaine (final pH = 7.39 ± 0.01). The dosages of bicarbonate were chosen in order to reproduce the conditions of a previous study in which pH adjustment led to increases in duration of the sensory block (Bavoux et al., 1988). pH was measured in 10 bottles of each group with a pH-meter (Microcomputer solution analyser Consort®) calibrated before each measurement with standard solutions at pH 4.01 and 6.87. The solutions were stored at 37 °C for 30 min (group 2) or 12 h (group 3).

Dissolution method

The in vitro dissolution method was modified from that of Graham and Pomeroy (1984). 18 ml of each solution were pumped into a disposable plastic syringe placed in an electrical pump (Vial Medical SE 200, Becton Dickinson®) programmed at a rate of 1.6 ml min^{-1}. The syringe was connected to a 0.22 μm membrane filter (Miller-GS, Millipore SA®, Molsheim, France) which was connected to a 2 ml delivery tubing. The filtrates were collected in 20 ml glass tubes. When the automatic syringe became empty (10 min), the 2 ml delivery tubing was renewed whereas the 0.22 μm membrane filter was kept in place. The syringe was filled with 8 ml of isotonic phosphate-buffered saline (pH 7.35) previously warmed to 37 °C. The rate of delivery of the automatic pump was maintained at 1.6 ml min^{-1} so that 8 ml of the filtrate were collected in a glass tube after 5 min. This rinsing procedure (aimed at collecting the bupivacaine stored in the filter) using the same amount of phosphate buffer was repeated four times in an effort to achieve the maximum dissolution effect.

Measurement of bupivacaine concentration

Baseline concentrations of bupivacaine were determined immediately after the addition of water or sodium bicarbonate. Concentrations in the filtrates at 10, 15, 20, 25 and 30 min were also measured using high-performance liquid chromatography (HPLC; Shimadzu® LC6A) with a UV spectrophotometric detector (Shimadzu® SPD6A) coupled to a recorder integrator (Shimadzu® CR6A). The wavelength was set at 240 nm. Fresh amounts of mobile phase were prepared daily by mixing acetonitrile (599 ml), water (400 ml) and 1 N NaOH (1 ml). The mobile phase was pumped out at a constant flow rate of 1.5 ml min^{-1}. All determinations of concentration were performed in triplicate.

Counts of particles

Counts of particles were evaluated as previously described (Bonhomme et al., 1988). Briefly, different sizes of particles of bupivacaine base were separated using a multichannel particle counter (Coulter Counter model TA II, Coultronics®, France SA 95 Margency) according to size (maximal diameter accepted in each channel being 2.5, 5, 10, 25 and 50 μm).

Adsorption of alkalinized bupivacaine

The amount of crystals of bupivacaine base adsorbed on the walls of either bottles or plastic syringes was determined using the following procedure: bottles from groups 1–3 (control and alkalinized bupivacaine) were emptied with disposable plastic syringes of 20 ml (at 30 min and 12 h) in order to mimic the clinical setting in which bicarbonate is added and mixed with bupivacaine in the glass bottles and then pumped in the syringe for injection into the epidural space. The empty bottles were then filled with 8 ml of acetonitrile and gently agitated to dilute the crystals of bupivacaine adsorbed on the walls. 100 μl of this aliquot were sampled in order to determine the bupivacaine concentration as described previously. The same procedure was performed for the evaluation of the amount of bupivacaine base which had been deposited within the plastic syringes and was thus unavailable for producing a clinical effect.

Statistics

Results are expressed as means ± SD and statistical analysis of data from the three groups was

performed by using analysis of variance followed when necessary by a Student's test a post hoc test.

Results

Dissolution

Baseline concentrations of bupivacaine before filtration were not significantly different whether sodium bicarbonate or water was added in the bottles (group 1, $C_0 = 2.33 \pm 0.03$ mg/ml; group 2, $C_0 = 2.30 \pm 0.01$ mg/ml; group 3, $C_0 = 2.35 \pm 0.05$ mg/ml). The dissolution profiles of each solution after the initial filtration and rinsing with phosphate buffer are shown in Fig. 1. Briefly, a significantly faster rate of dissolution was observed in non-alkalinized as compared with alkalinized solutions, at every time point during the procedure ($p < 0.05$). Moreover, the dissolution rate of the 12 h crystals was slower than that of the 30 min crystals ($p < 0.05$).

Count of particles

The numbers of particles before and after alkalinization are summarized in Fig. 2. In the control situation, both the total number and the redistribution of the different sizes are in agreement with the data of a previous investigation (Bonhomme et al., 1988). Addition of bicarbonate produced a dramatic increase in the absolute number of particles ($n_{final} = n_{initial} \times 3.7$) but did not modify the relative redistribution of the numbers of crystals of each size. Small particles of less than 2.5 μm in fact represented 70% of all particles in the control situation and 69.4% after alkalinization (NS). The same applied to particles with a diameter between 2.5 and 5 μm which represented 21.3% before and 25.3% after alkalinization (NS). That is, among every 100 new particles created by alkalinization, about 70 were less than 2.5 μm in diameter and 25 were less than 5 μm.

Adsorption of crystals

The results are summarized in Fig. 3. For a given initial amount of bupivacaine, the longer the duration of contact between soluble bupivacaine, crystals of bupivacaine base and alkali, the greater the amount of bupivacaine crystallized and adsorbed on the walls of both bottles and syringes.

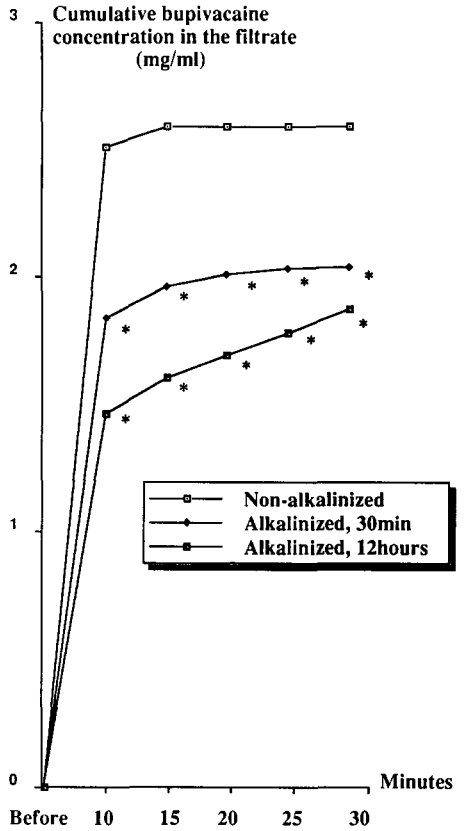

Fig. 1. Typical results obtained from one set of measurements using the modified dissolution method and describing the cumulative bupivacaine concentration in the filtrate expressed in mg/ml as a function of time. * $p < 0.05$ vs non-alkalinized solutions.

Discussion

The data presented in this article demonstrate that the crystallization of bupivacaine base results in a slow-release effect and adsorption, both of which may have important implications for its clinical use. On consideration of the parenteral route for the administration of the drug, the slow-release effect may be achieved by employing the three main procedures which may be com-

bined when attempting to improve the clinical efficacy of the drug: (1) the addition to the drug of an adjuvant – which merely as a consequence of its properties alone – may reduce the degree of diffusion, catabolism or elimination (e.g., in the use of oily vehicles); (2) the modification of the active principle of the drug either via the addition of base (e.g., slow-release penicillin), acid (e.g., steroids), or alcohol or through combination with a metal or protein (e.g., insulins); and (3) the preparation of suspensions containing crystals of the drug. The latter galenic technique has thus far been the most widely used method and has been extensively employed in the investigation of rapidly acting, and intermediate- and slow-release insulin forms (Brange, 1987). In suspensions of crystalline insulins, the size of the crystals is proportional to the duration of the clinical action: the larger the crystals, the slower the release and the longer the duration of action (Graham and Pomeroy, 1984; Brange, 1987). In this context, it appeared logical to expect that crystals of bupivacaine may have a slow-release effect. To demonstrate this effect, an in vitro dissolution method was adapted from studies on insulin crystallization (Graham and Pomeroy, 1984). Phosphate buffer was chosen since its pH is close to both physiological pH and the pH of alkalinized bupivacaine. A membrane filter of very small pore size (0.22 μm) was used in order to ensure that even very small crystals were retained. It has previously been shown that raising the temperature of pH-adjusted solutions of local anesthetics increases the process of crystallization (Bourget et al., 1990). All the procedures were performed at 37 °C to maximize the extent of crystallization and to mimic physiological conditions. Our results show that pH-adjusted solutions of bupivacaine have a slower rate of dissolu-

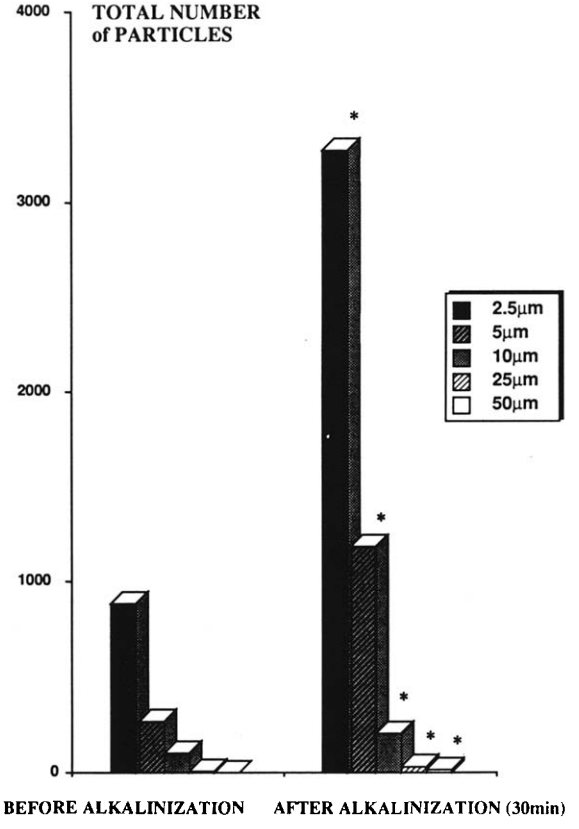

Fig. 2. Counts of particles before and after alkalinization. * $p < 0.05$ vs non-alkalinized solutions.

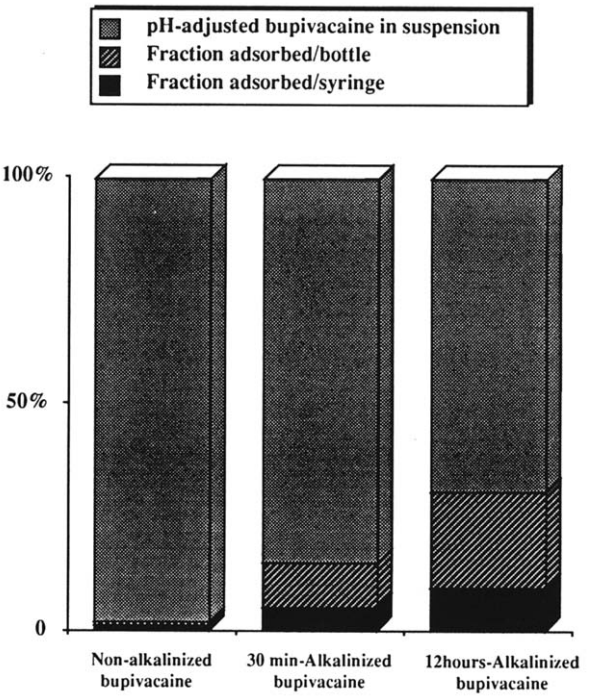

Fig. 3. Relative proportions of soluble and adsorbed bupivacaine in the three groups.

tion than control solutions. A slow-release effect may be enhanced by increasing either the total number or the size of these particles. In addition, this report shows that alkalinization created a larger number of particles but did not change the relative size of the particles. Since the majority of the particles are small in diameter, a significantly prolonged duration of this effect is not expected to occur. This is the most probable explanation for the slow-release effect, in the clinical setting, observed in earlier studies using highly alkalinized bupivacaine (Hilgier, 1985; Bavoux et al., 1985).

Adsorption of the crystals onto the walls of bottles and syringes may also be of considerable importance regarding its clinical use, since the amount of adsorbed drug that is available for injection is less than the total applied due to the proportion undergoing adsorption on the walls, this explanation also being indicated by the variability in the data on pH adjustment published thus far which may well be related, at least partially, to the uncontrolled adsorption of bupivacaine. Recalling the comparison with insulin (Brange, 1987), the literature indicates that the major determinants of adsorption are as follows: (1) the concentration of drug; (2) the physical nature of the wall; (3) the duration of contact; and (4) the temperature – adsorption being greater in extent at 37 °C than at 20 °C. Since the amount of adsorbed insulin is difficult to predict, it is a safer practice to adjust the rate of administration according to the clinical effect (blood sugar determination) rather than on the basis of a speculative calculated rate of adsorption. The same would apply to alkalinized bupivacaine for which the extent and the quality of the sensory block should guide the administration of eventual top-ups.

In conclusion, this study has shown that the process of alkalinization generates crystals containing molecules of bupivacaine base. Such crystals might prolong the duration of the sensory block through a slow-release effect as demonstrated by an in vitro technique; however, these crystals adsorb on the walls of their container and might reduce the amount of the drug available for action.

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