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Sustained release of bupivacaine from devices based on chitosan

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Abstract

Chitosan beads loaded with bupivacaine (16±3 μg of drug per milligram of beads) were prepared by cross-linking with glutaraldehyde. In vitro drug release at pH and temperature conditions similar to those of the biological systems were studied. Maximum release of bupivacaine was obtained between 100 and 120 h, depending on the presence of lysozyme in the release medium, since the enzyme facilitates the release process. A constant release rate of the drug, between 11 and 15 μg/h, was observed for 30 h. In order to prolong bupivacaine release, the drug-loaded chitosan beads were coated with a poly(DL-lactide-co-glycolide) film. The resulting device allows the drug to be released in a sustained form; a constant release rate between 28.5 and 29.5 μg/h was obtained for 3 days, and the maximum release of bupivacaine took place at day 9. The in vitro results indicate a possible application of these bupivacaine loaded chitosan systems as drug release devices with an analgesic action. Thus, they could be used in the treatment of dental pain in the buccal cavity, where drug release would be made easier by lysozyme of the saliva.

Keywords: Drug delivery; Chitosan beads; Poly(DL-lactide-co-glycolide); Bupivacaine

1. Introduction

Local anaesthetics are widely used in surgical, obstetric and dental pain, as well as in the therapy of chronic pain. Among these drug, bupivacaine plays a valuable role in the overall management of surgical and postoperative pain associated with dental care [1–4]. On the other hand, the therapeutic index of local anaesthetics can be improved by including them in drug delivery systems [5]. Thus, different devices based on liposomes [6], poly(D,L-lactide) and poly(lactide-co-glycolide) [7], as well as albumin microspheres [8,9] have been designed to obtain a sustained release of this kind of drugs.

Among the biodegradable materials used for controlled drug release, chitosan (poly[α(1→4)2-amino-2-deoxy-D-glucopyranose], a polysaccharide having structural characteristics similar to glycosaminoglycans, [10] has been extensively used [11,12]. A large number of biomedical applications of chitosan have been studied, thus it has been assayed as biomaterial for wound healing [13,14] and prosthetic material [15], since it can be biodegraded by enzyme action [16].

The purpose of the present study was to prepare bupivacaine-loaded chitosan beads that allow a sustained release of this drug, and to prolong the delivery of the drug by means of coating the beads with a poly(DL-lactide-co-glycolide) film, in order to obtain chitosan devices with a potential analgesic action, which can be used to control dental pain by placing them in the buccal cavity.

2. Materials and methods

2.1. Materials

Chitosan (minimum 85% deacetylated) (Sigma-Aldrich Quimica, S.A., Madrid, Spain), glutaraldehyde (25%) (Sigma-Aldrich Quimica, S.A.), lysozyme (50 000 U/mg prot) (Sigma-Aldrich Quimica, S.A.), poly(DL-lactide-co-glycolide) (50:50, $M = 40\,000–75\,000$) (Sigma-Aldrich Quimica, S.A.), di-potassium monohydrogen phosphate (K_2HPO_4) (Panreac, Barcelona, Spain), potassium di-hydrogen phosphate (KH_2PO_4) (Panreac), methanol (Panreac).

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Bupivacaine ($C_{18}H_{28}N_2O$) was kindly supplied by Inibsa Laboratories (Spain). Milli-Q® (Millipore, Madrid, Spain) water was used.

2.2. Preparation of bupivacaine-loaded chitosan beads

In order to prepare chitosan beads loaded with bupivacaine, chitosan was dissolved in 2% v/v acetic acid to obtain a solution of 15 mg/ml. Bupivacaine (50 mg) was dissolved in 5 ml of the chitosan solution. This bupivacaine–chitosan solution was blown into a 1:1 solution of NaOH (6% w/v) and methanol by a syringe with a nozzle of 0.13 mm in diameter to form beads. Then glutaraldehyde was added and, after 1 h of incubation at 25 °C, beads were washed with a 5% solution of sodium bisulphite to remove the glutaraldehyde excess. Bupivacaine-loaded chitosan beads were dried at room temperature over anhydrous $CaSO_4$ to constant weight.

2.3. Determination of bupivacaine included in chitosan beads

Bupivacaine-loaded chitosan beads (40 mg) were added to 57 ml phosphate buffer (1 mM, pH 7.5) containing 5 mg of lysozyme, and they were maintained under vigorous stirring for 120 h at 37 °C. Bupivacaine concentration was determined by HPLC (Spectra-Physics SP8800 HPLC pump, SP 100 UV absorbance detector and SP 4400 computing integrator). The stationary phase was Lichrosorb RP8 5 μm (15 × 0.46 cm; Teknokroma). The eluent was 0.01 M di-hydrogen sodium phosphate with acetonitrile (70:30 v/v) pH 2.1 [17]. The flow rate was set at 1.5 ml/min and the detector wavelength was 205 nm. Bupivacaine standards of 1–1000 μg/ml were run for external standardisation and linear curves with a correlation coefficient of 0.999 were generated from the area under the peak measurements. The bupivacaine retention time was 5.5 ± 0.2 min.

2.4. Inclusion of bupivacaine-loaded beads in a poly(lactide-co-glycolide) film

Poly(DL-lactide-co-glycolide) (75 mg) was dissolved in chloroform (2 ml); 1 ml of this solution was spread on a teflon mould (1.3 × 1.6 cm). The film was allowed to set at 25 °C until it was dried. Then, 300 mg of drug-loaded beads were dispersed on the film. Finally, 1 ml of the poly(DL-lactide-co-glycolide) solution was added to the beads and the chloroform was completely evaporated. The chitosan based device was dried at 25 °C over anhydrous $CaSO_4$ to constant weight. The dimensions of the devices were 1.1 × 1.4 × 0.2 cm.

2.5. Bupivacaine stability in solution

Bupivacaine stability in solution at 37 °C was studied. Bupivacaine (1 mg/ml) in phosphate buffer (1 mM, pH 7.5) was maintained at constant stirring rate (500 rpm) for 21 days. At intervals, 100 μl samples were withdrawn from the solution in order to follow the change in bupivacaine concentration by HPLC. A similar experiment was carried out to determine the stability of bupivacaine in the presence of lysozyme. The drug (25 mg) was dissolved in phosphate buffer (25 ml, 1 mM, pH 7.5) with lysozyme (5 mg) and maintained at 37 °C for 21 days. Aliquots of 100 μl were taken at intervals, and bupivacaine concentration was determined by HPLC.

2.6. Bupivacaine release studies

Drug-loaded chitosan beads (40 mg) were added to 7 ml of phosphate buffer 1 mM pH 7.5 inside a dialysis bag (Spectra/Por membrane MWCO: 6-8000), which was placed in a vessel containing 50 ml phosphate buffer at constant temperature (37 °C) and stirring rate (500 rpm). At intervals, 100 μl samples were withdrawn from the solution in order to follow the change in bupivacaine concentration by HPLC. The removed volume from the vessel was replaced with phosphate buffer.

In order to study the effect of lysozyme on bupivacaine release from the beads, a similar experiment was carried out. In this case, 5 mg of lysozyme were added to the dialysis bag.

Bupivacaine release experiments from the device formed by drug-loaded chitosan beads in a poly(DL-lactide-co-glycolide) film were carried out in the absence and in the presence of lysozyme. In the first case, a device was placed inside a dialysis bag with 10 ml of phosphate buffer (1 mM, pH 7.5). This bag was submerged in a vessel with 90 ml of buffer at constant temperature (37 °C) and stirring rate (500 rpm). At intervals, 100 μl samples were drawn from the solution to determine bupivacaine concentration by HPLC. The removed volume was replaced with the phosphate buffer. In the second case, 10 mg of lysozyme was placed inside the dialysis bag with the chitosan device. The conditions of the experiment were maintained. All the experiments were carried out in triplicate.

2.7. Scanning electron microscopy

The morphology of bupivacaine-loaded chitosan beads and the drug-loaded chitosan device was studied by scanning electron microscopy (SEM) (JEOL JSM-6400 Electron Microscope). The beads were fixed on a rigid support and coated with gold. The device was fixed on a rigid support and coated with gold.

3. Results and discussion

The surface morphology of bupivacaine-loaded chitosan beads, determined by SEM, is shown in Fig. 1A. The beads were 0.99 ± 0.05 mm in size and spherical in overall shape. Beads of similar characteristics have been obtained in different studies, thus chitosan beads loaded with aspirin [18] were also spherical and their size was about 900–1100 μm. When bupivacaine-loaded chitosan beads were included in a poly(DL-lactide-co-glycolide) film, the beads were not alone, but were surrounded by the polymer and grouped together (Fig. 1B). Chitosan has been used to prepare different drug delivery systems, thus co-matrix systems of aspirin-loaded chitosan beads incorporated in a poly(ethylene vinyl acetate) matrix with heparin have been described [18]. On the other hand, bupivacaine has been incorporated in an albumin microspheres/poly(lactide-co-glycolide) co-matrix [8].

When the stability of bupivacaine in phosphate buffer at 37 °C was studied (Fig. 2), degradation of the drug as a function of time was observed, and the amount of bupivacaine is 75% at 200 h. In the presence of lysozyme the same result was observed, thus the enzyme had no effect on bupivacaine degradation. Lysozyme is an enzyme with chitosan activity that is widely distributed in mammalian, it is present in different fluids as saliva or vaginal fluid, and also in cells as macrophages. The amount of bupivacaine loaded in chitosan beads was determined in phosphate buffer in the presence of lysozyme and it was 16 ± 3 μg of drug per milligram of beads.

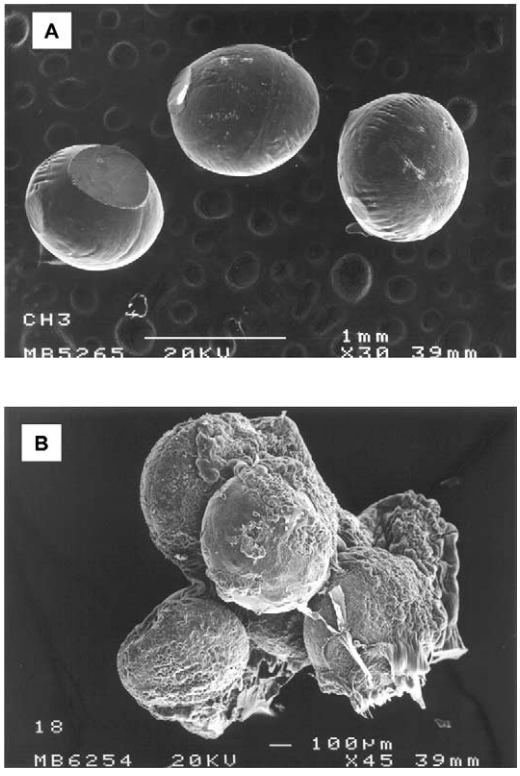

Fig. 1. SEM of (A) bupivacaine-loaded chitosan beads; (B) bupivacaine-loaded chitosan beads included in a poly(DL-lactide-co-glycolide) film.

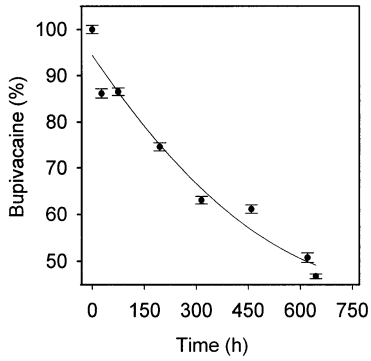

Fig. 2. Percentage of bupivacaine as a function of time of incubation at 37 °C. The drug is dissolved in phosphate buffer 1 mM, pH 7.5.

The release of bupivacaine from chitosan beads is shown in Fig. 3A. The drug is slowly released, thus a constant drug release rate of 11.1 μg/h is obtained for the first 30 h, a time at which about 66% of the maximum drug released is detected. The maximum drug release (10 ± 2 μg/ml = 570 ± 114 μg drug) takes place at 120 h. When release experiments are carried out in the presence of lysozyme (Fig. 3B) the release rate in the first 30 h is 15.3 μg/ml, thus the enzyme increases bupivacaine release rate as a consequence of its hydrolytic action on chitosan bonds, and 79% of the drug is released at this time. The maximum amount of drug released also increases in the presence of the enzyme, thus 627 ± 114 μg (11 ± 2 μg/ml) of the drug is released from 40 mg of beads, and it takes place at 100 h. Thus, lysozyme not only increases the release rate of bupivacaine but also the maximum amount released. This indicates that a part of the loaded drug only can be released when some bonds of the polymer are broken, which probably facilitates the release of the drug placed in the more internal part of the beads.

One characteristic of these drug release kinetics is the absence of a burst effect in the first hour of the process, where only 9% of the drug has been released. The burst effect has been described for the release of bupivacaine from albumin microspheres, where about 60% of the drug is released after 1 h, [8], and for cytarabine release from chitosan microspheres [19], where 36% of the drug is released during the first hour. On the other hand, the maximum drug concentration in the release medium (Fig. 3), in the absence and in the presence of lysozyme, is maintained constant for 250–300 h, which indicates that the degradation effect of the release medium on the drug concentration (Fig. 2) is balanced by a slow release of bupivacaine from chitosan beads, if this were not so, a decrease of bupivacaine concentration would be observed.

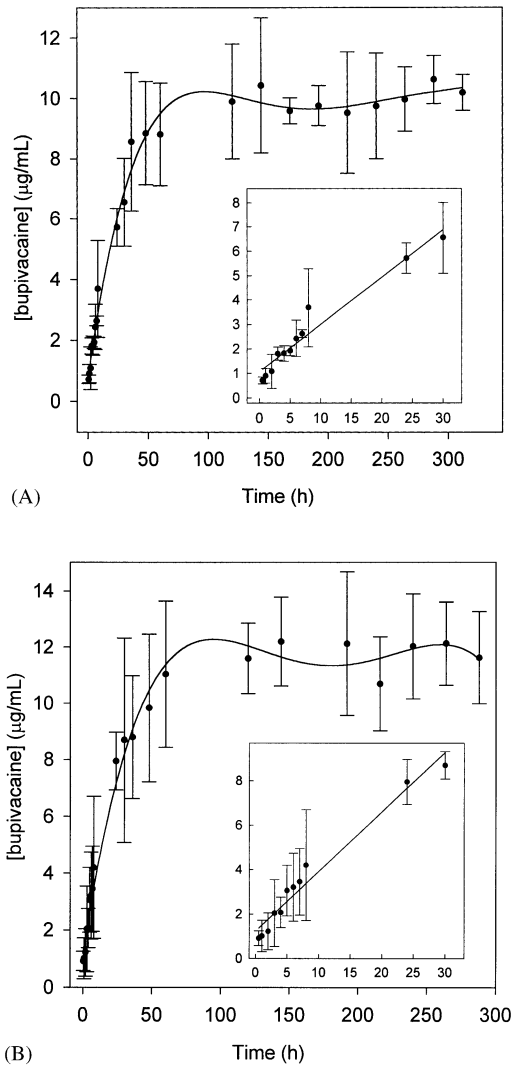

Fig. 3. Bupivacaine released from chitosan beads as a function of time: by swelling (A); in the presence of lysozyme (B). Inset: Initial stage of the bupivacaine release. Phosphate buffer 1 mM, pH 7.5, 37 °C.

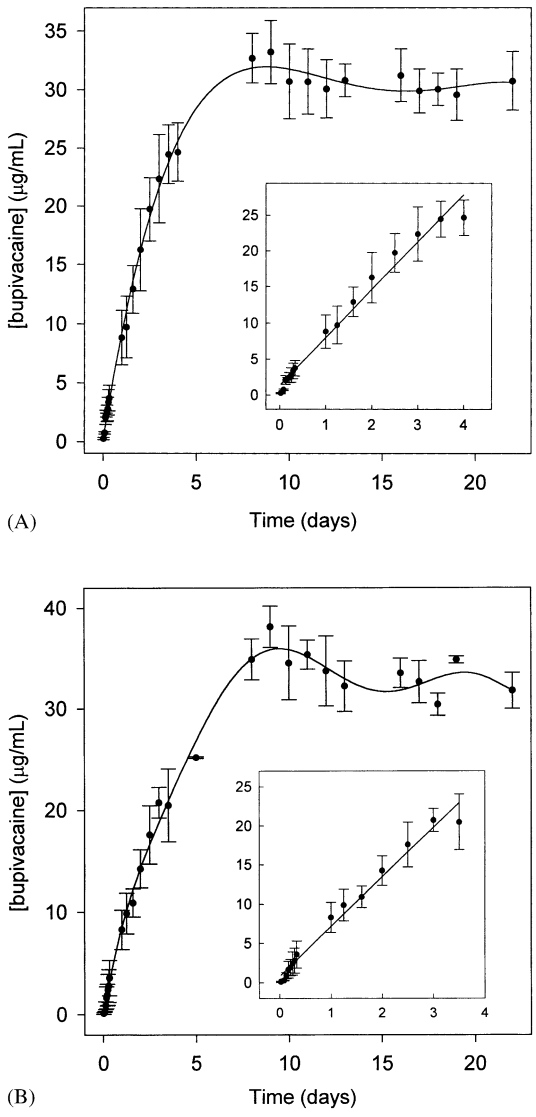

Fig. 4. Bupivacaine released from chitosan-based devices: by swelling (A); in the presence of lysozyme (B). Inset: Initial stage of the bupivacaine release. Phosphate buffer 1 mM, pH 7.5, 37 °C.

Bupivacaine release from chitosan-based devices is shown in Fig. 4. In the absence of lysozyme in the solvent medium, a constant release rate of the drug (29.5 μg/h) is observed up to 3.5 days, and the maximum amount of released drug (33 ± 3 μg/ml = 3.3 ± 0.3 mg bupivacaine) is obtained at day 9. When the release kinetic is studied in the presence of lysozyme a constant drug release rate of 28.5 μg/h for 3 days is observed, and the maximum amount of bupivacaine is 3.8 ± 0.2 mg (38 ± 2 μg/ml) at day 8 (Fig. 4B). In this case, chitosan beads are coated with a poly(DL-lactide-co-glycolide) film, which makes the hydrolytic action of lysozyme more difficult; and as a consequence, the release rate of the drug during the first days is almost the same in the absence and in the presence of the enzyme.

The effect of lysozyme on drug release is observed in the maximum concentration of bupivacaine in the release medium, which is larger in the presence of the enzyme, and also in the time at which it takes place. That effect is due to the fact that the enzyme breaks chitosan bonds and facilitates the drug release. Furthermore, the coating of chitosan beads with poly(DL-lactide-co-glycolide) increases the time at which the maximum release of bupivacaine is obtained. This is because the polymer makes contact between the solvent medium and the chitosan beads difficult, and also because bupivacaine-loaded chitosan beads in the device are in contact with each other, thus decreasing the surface exposed to the dissolution medium. The amount of bupivacaine of chitosan-based devices is 4.5 mg, however, the maximum amount released was 3.8 ± 0.2 mg. Thus, some bupivacaine was slowly released, and thus it balanced the degradative effect of the solvent medium on the drug. In the absence of lysozyme, when a higher percentage of the loaded drug was not detected in

the release medium, an evident constant drug concentration was observed, which only can be explained by the slow release of bupivacaine for a long period of time. When lysozyme was in the medium, a greater amount of released bupivacaine was detected at day 8, and from this time a constant drug concentration, which was lower than the maximum one, was maintained for a long period of time. Thus, only a part of the degradative effect of the medium on the drug was balanced by bupivacaine release in the presence of lysozyme.

This study has shown that bupivacaine can be included in chitosan beads, that sustained release of the drug in conditions of pH and temperature similar to that of the mammalian organisms takes place, and that a maximum release of the drug is obtained at 120 h. The drug release is facilitated in the presence of lysozyme due to its hydrolytic effect on chitosan bonds, and maximum concentration of bupivacaine is observed at 100 h. When bupivacaine-loaded chitosan beads are capped with poly(DL-lactide-co-glycolide), the time needed to obtain the maximum drug release increases up to 8–9 days. Thus, chitosan microspheres as well as the devices formed by these microspheres covered by poly(lactide-co-glycolide) can be considered suitable for in vivo release of bupivacaine, since chitosan as well as poly(DL-lactide-co-glycolide) are biodegradable materials whose degradation products can be metabolised in the organism. These systems could be used for the control of dental pain in the buccal cavity, where lysozyme would acts by facilitating bupivacaine release.

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