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In vitro characterisation of calcium phosphate biomaterials loaded with lidocaine hydrochloride and morphine hydrochloride

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Abstract Calcium phosphate substitutes drug delivery systems are well known substances used in minor bone void-filling to release their therapeutic agent in situ. Few studies associating anaesthetics and analgesics have been performed to date. The aim of this work was to study the association of the analgesic, morphine, and the local anaesthetic, lidocaine, with a calcium deficient apatite matrix. Three types of biomaterials i.e. powders, granules and blocks, were prepared by isostatic compression, wet granulation and a combination of the two, evaluated and compared. The chemical structure of the associated therapeutic agent was studied and the characteristics of the drug delivery systems were appraised in terms of drug release. The integrity of the lidocaine hydrochloride structure, as determined by RMN ^{1}H, was confirmed regardless of the formulation technique used (isostatic compression or wet granulation). However, analyses of morphine hydrochloride by RMN ^{1}H revealed slight structural modifications. The association and formulation techniques that were used made it possible to obtain an in vitro release time varying from 1 to 4 days for lidocaine hydrochloride and from 1 to 3 days for morphine hydrochloride.

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1 Introduction

Bone void-filling is a frequent problem that occurs in osteoarticular pathology. Numerous substances can be used to fill bone voids, such as materials, or biomaterials, allo-transplants or autotransplants. The material must be well tolerated, well integrated in in situ osseous structures and must enable quality osseous neoformation with mechanical properties sufficient to support stress at the site of implantation.

Calcium phosphate substitute is a well known substance used in minor bone void-filling [1]. This material is also used as a drug delivery system for therapeutic agents such as antibiotics [2], growth factors (growth hormone, bone morphogenetic proteins, transforming growth factor-beta, insulin-like growth factor) [3, 4] osteoporotic drugs (alendronate) [5], anticancer drugs [6].

Few studies associating anaesthetics and analgesics have been performed to date [7–9]. However, this association could be beneficial in that it would simultaneously fill the bone void and alleviate pain. By allowing the in situ release of a therapeutic agent, the analgesic effect could be very much enhanced. Such a drug delivery system (DDS) could be of interest when releasing the drug locally during the first 72 post operative hours, when the pain is more intense. This technique could significantly reduce side effects and complications due to high doses and would improve patient comfort.

The aim of this work was to study the association of the analgesic, morphine, and the local anaesthetic, lidocaine, with a calcium phosphate matrix. These two analgesics are used as osseous pain killers in pathological conditions such as osteoporosis and Paget disease. The incorporation of these therapeutic agents into a calcium phosphate matrix would provide a twofold benefit, i.e., that of analgesia and of an osseous filler promoting bone growth.

The matrix was prepared by chemical synthesis and associated with lidocaine hydrochloride and morphine hydrochloride. Three types of biomaterials were prepared, evaluated and compared, i.e. powders, granules and blocks. The chemical structure of the associated therapeutic agent was studied and the characteristics of the drug delivery systems were appraised in terms of drug release.

2 Materials and methods

2.1 Preparation and characterization of CDA powder

Calcium deficient apatite (CDA) was synthesized in the laboratory using a previously described chemical hydrolysis reaction [10], purified by washing and then oven-dried at 40°C to a constant weight. A heat treatment was applied to the powder in a Vecstar furnace (Eurotherm, Switzerland) in order to transform the CDA into BCP (biphasic calcium phosphate) and to physicochemically characterize the powder (X Ray diffraction and Fourier Transformed Infra Red). The sintering protocol consisted of heating at 5°C/min to 1,000°C, holding for 4 h at 1,000°C and then slow cooling at 5°C/min to 20°C to avoid heat shock.

2.2 Preparation of therapeutic agent-matrix granules

CDA powder was first mixed with the therapeutic agent using a Turbula mixer (T2C, WAB, Switzerland): 5% (w/w) of lidocaine hydrochloride (lidocaine hydrochloride, Cooper, France) or 20% (w/w) of morphine hydrochloride (morphine hydrochloride, Cooper, France) mixed with CDA.

3 types of samples were prepared: granules, blocks and powder.

- *Wet granulation formulation*: The mixed powder was agglomerated by wet granulation using distilled water in a planetary blender (Krups, France). The mass obtained was forced through the mesh of two grids (1.040 and 0.630 mm) in an oscillated granulator (Erweka AR 400 Apparatebau GmbH, Germany). The granules obtained were oven-dried at 37°C to a constant weight. Granules were then mechanically sieved (Analysette 3 PRO, Fritsch, Germany) for 5 min to collect the 200–500 μm fraction.
- *Isostatic compression formulation*: Isostatic compression was performed using hyperbar equipment (Alstom, France). 4 g of the mixed powder were introduced to an elastomer mold under vacuum and then transferred into a high-pressure chamber containing water and compressed under 140 MPa for 5 min. Part of the blocks that were obtained was then ground in a mortar.
- *Wet granulation and isostatic compression formulation*: The mixed powder was granulated by wet granulation as previously described and then compressed using isostatic compression under 140 MPa for 5 min. Part of the blocks that were obtained was ground in a mortar.

For lidocaine—CDA samples, Granules A were prepared by wet granulation. Blocks B were prepared by isostatic compression, Blocks C were prepared by wet granulation and isostatic compression, Powder D was obtained after isostatic compression and crushing and Powder E by wet granulation, isostatic compression and grinding.

For morphine—CDA samples, Granules I were prepared by wet granulation, Blocks II were prepared by isostatic compression, Blocks III were prepared by wet granulation and isostatic compression, Powder IV was obtained after isostatic compression and crushing and Powder V by wet granulation, isostatic compression and grinding.

2.3 Complete release assays

A preliminary extraction test was performed in order to prove that the formulation techniques did not trap any of the incorporated therapeutic agents, preventing them from being released in the dissolution medium.

Tests were performed for morphine hydrochloride and lidocaine hydrochloride with different samples:

- Powders were ground in a mortar from the existing blocks, i.e., from isostatic compression (B–D and II–IV) and from wet granulation followed by isostatic compression (C–E and III–V).
- Powders were ground in a mortar from granules prepared by wet granulation (A and I).

200 mg of each of these samples were placed in 13 ml of distilled water and mixed for 3 h. The different solutions were then filtered (Millipore filter of 0.22 μm). The amount of each therapeutic agent was then measured by a UV spectrophotometric assay at their maximum absorption wavelength to determine the quantity released from the CDA.

2.4 Nuclear magnetic resonance analysis of the associated therapeutic agent

The chemical structure of lidocaine hydrochloride and morphine hydrochloride associated with CDA in granules by wet granulation and isostatic compression was studied by ^{1}H NMR (400 MHz spectrometer, Bruker, France) on D_2O solution samples:

Lidocaine hydrochloride alone

Lidocaine hydrochloride after isostatic compression

Lidocaine hydrochloride associated with CDA by wet granulation

Lidocaine hydrochloride associated with CDA by isostatic compression.

^{1}H NMR analysis of morphine hydrochloride was performed on samples prepared similarly to lidocaine hydrochloride samples.

2.5 Therapeutic agent release profiles

A culture chamber dissolution test was performed in order to establish the release profiles of the therapeutic agents from the biomaterials. The proportion of therapeutic agent released from the CDA was measured by a UV–Visible spectrophotometer assay.

Lidocaine hydrochloride and morphine hydrochloride release profiles were assessed for each type of material, block and powder (Granule A, Blocks B, Blocks C, Powder D, Powder E and Granule I, Blocks II, Blocks III, Powder IV and Powder V), using a previously described dissolution test [11]. Two hundred and fifty milligrams of granules were deposited in a Millicell culture chamber fitted with a Biopore® membrane. The chambers were immersed in six-well culture plates containing 13 ml of distilled water that were subsequently placed in an oven at 37°C on a 3D rocking platform (Stuart Scientific, STR 9, UK) (5 rpm). Aqueous solutions were removed and replaced with fresh distilled water at 3, 6, 9, 24 and 48 h and then on a daily basis. The amount of lidocaine hydrochloride and morphine hydrochloride released into aqueous solutions was determined by a UV–Visible spectrophotometer assay (Shimadzu UV-1605 UV Visible spectrophotometer, Roucaire, France) at 262.4 and 284 nm respectively. All experiments were performed in triplicate. The results are expressed as the percentage of lidocaine hydrochloride and morphine hydrochloride released over time ± SD.

3 Results

3.1 Preparation and characterization of CDA powder

X ray diffraction analysis clearly showed peaks specific for HA and β-TCP. HA and β-TCP percentages were determined as equal to 16/84 and the Ca/P ratio was equal to 1.52. FTIR analysis showed the absence of impurities and pyrophosphate.

3.2 Nuclear magnetic resonance analysis of the associated therapeutic agent

3.2.1 Lidocaine hydrochloride analysis

The spectral data of the lidocaine hydrochloride powder provided the reference for analyses as it had been subjected neither to association in CDA nor to wet granulation nor isostatic compression. The NMR spectra of lidocaine hydrochloride did not introduce a major difference to the reference spectra as it had been subjected to isostatic compression. The NMR spectra of lidocaine hydrochloride did not introduce a difference to the reference spectra as it had been subjected to a wet granulation and linked to CDA, itself having also been subjected to an isostatic compression.

3.2.2 Morphine hydrochloride analysis

The spectral data of the morphine hydrochloride powder provided a reference for analyses as it had been subjected neither to association in CDA nor to wet granulation nor isostatic compression. The NMR spectra of morphine hydrochloride had no major effect on the reference spectra as it had been subjected to an isostatic compression. On the contrary, the NMR spectra of the morphine hydrochloride linked to CDA by an isostatic compression and linked to CDA by a wet granulation had an effect on the reference. As such, slight structural modifications were noticed, i.e., the loss of a hydroxyl group (phenolic hydroxyl) and the loss of a hydrochloride function.

3.3 Complete release assays

Table 1 provides the percentages of lidocaine hydrochloride and morphine hydrochloride observed after extraction, as measured by a spectrophotometric assay in different test conditions. This table demonstrates that, on average, the amount of lidocaine hydrochloride extracted was 99.20 ± 1.16% when linked to CDA by wet granulation, isostatic compression or wet granulation followed by isostatic compression, and that on average the amount of morphine hydrochloride extracted was 98.84 ± 1.29% when linked to CDA by wet granulation, isostatic compression or wet granulation followed by isostatic compression.

3.4 Therapeutic agent release profiles

3.4.1 Kinetics of lidocaine hydrochloride release

Figure 1 shows the kinetics of lidocaine hydrochloride release from the CDA grains prepared by wet granulation, from blocks obtained by isostatic compression and by wet granulation followed by isostatic compression and from powders obtained from blocks. In addition, the figure provides the mean percentage of lidocaine hydrochloride released from the biomaterials over time (in hours).

Table 1 Percentages of lidocaine hydrochloride and morphine hydrochloride extracted from the granules of wet granulation, the powder of these granules, the powder of the blocks acquired by isostatic compression and the powder of blocks acquired by wet granulation followed by isostatic compression

	Percentage of lidocaine hydrochloride found after extraction in comparison with initial quantity	Percentage of morphine hydrochloride found after extraction in comparison with initial quantity
Wet granulation (granules)	99.61% (A)	98.60% (I)
Wet granulation (powder)	100.72%	98.76%
Granulation compression (powder)	98.96% (C and E)	100.21% (III and V)
Compression (powder)	97.52% (B and D)	101.81% (II and IV)

Percentages of lidocaine hydrochloride and morphine hydrochloride extracted from the granules of wet granulation, the powder of these granules, the powder of the blocks acquired by isostatic compression and the powder of blocks acquired by wet granulation followed by isostatic compression

3.4.1.1 Release kinetics for granules prepared by wet granulation (Granules A) Figure 1a shows the profiles of lidocaine hydrochloride release from granules prepared by wet granulation. One can note that 50% of lidocaine hydrochloride was released in approximately 10 h and that 101 ± 7.60% was released in 72 h.

3.4.1.2 Release kinetics for blocks and powders prepared by isostatic compression Figure 1b shows the release of lidocaine hydrochloride from blocks prepared by isostatic compression (Granules B) and from powder obtained by grinding these blocks in the mortar (Granules D). The release profiles obtained for Granules B showed that 50% was released in 17 h and 99.70 ± 1.76% was released in 24 h. The release profiles obtained for Granules D showed that 50% was released in about 16 h and 100.67 ± 4.15% was released in 120 h. The release profiles obtained for blocks (Granules C) and powders (Granules E) prepared by wet granulation followed by isostatic compression are described in Fig. 1c. The release profiles obtained for wet granulation followed by an isostatic compression (Powder E) showed that 50% was released in about 2 h and 102.75 ± 1.75% was released in 24 h. For Granules E, 50% of lidocaine hydrochloride was released in 16 h and 100.77 ± 0.2% of was released in 96 h.

3.4.2 Kinetics of morphine hydrochloride release

The amount of morphine hydrochloride released from Granules I to V over time was determined from the results of in vitro dissolution tests (Fig. 2).

The profiles of morphine hydrochloride release from granules prepared by wet granulation (Granules I) are presented in Fig. 2a. The kinetics show that 50% of morphine was released in 17 h and 99.38 ± 0.85% was released in 48 h.

The release profiles obtained from II (Fig. 2b) showed that 50% of morphine hydrochloride was released in 28 h and 91.73 ± 5.06% was released in 72 h with the profile reaching a plateau. For Granules IV, 50% of morphine hydrochloride was released in 18 h and 95.38 ± 1.60% was released in 72 h. The release kinetics (Fig. 2c) of the morphine hydrochloride obtained from the existing blocks of a wet granulation followed by a compression (Granules III) showed that 50% of morphine was released in 5 h and 99.78 ± 1.80% was released in 24 h. The release profiles of Powder V showed that 50% of morphine hydrochloride was released in 15 h and 90.64 ± 4.07% was released in 72 h, with the profile reaching a plateau.

4 Discussion

In this study, a calcium phosphate matrix of CDA was prepared by hydrolysis in an ammoniacal medium. The classical methods of physicochemical characterization (X Ray Diffraction and infrared spectroscopy (FTIR)) showed that the CDA prepared was pure and devoid of impurities (carbonates or HPO_4 groups) that could influence CDA dissolution during in vitro experiments or during the material degradation after in vivo implantation. There are no crystalline stages other than HA and β-TCP. The HA/β-TCP ratio was determined as equal to 16/84 with a Ca/P ratio of 1.52. Given that the CDA was unsintered, its specific area was higher than that of ceramics. This property allows for an increase in cell contacts in vivo. Similarly to bone, CDA is a non stoichiometric apatite. It also introduces a structure, a chemical reactivity facing cells, and a crystal size, approaching that found in normal bone. However, given that sintering increases the inflexibility of the material by granule joint formation, it is less rigid than a ceramic structure.

In vivo implantation studies of the CDA matrix in rabbits [12] showed that bone colonization was more

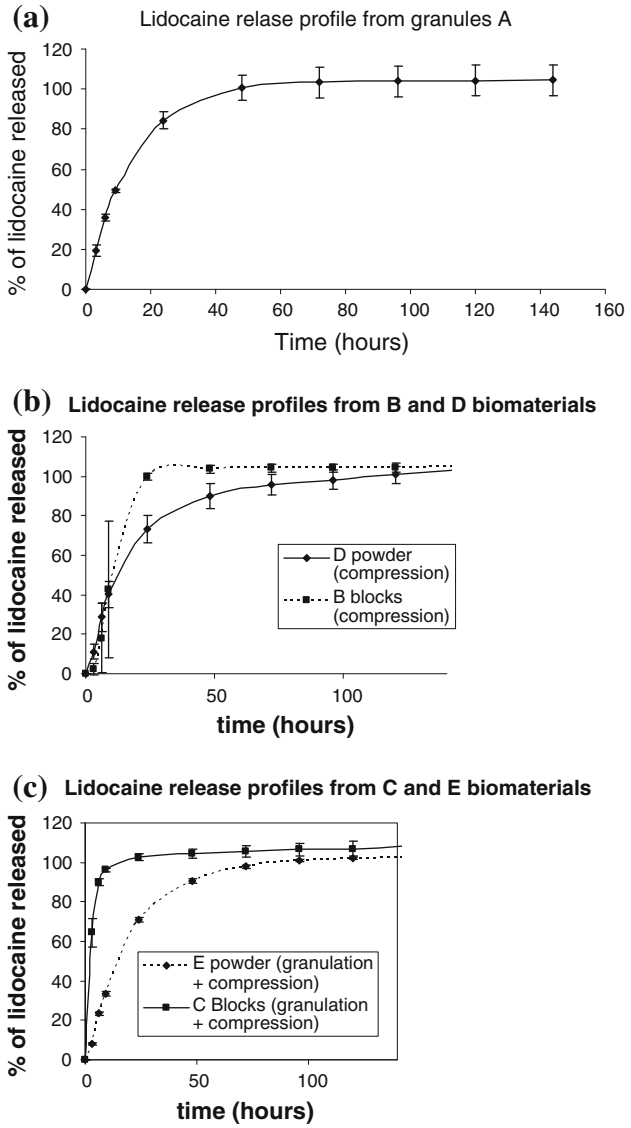

Fig. 1 Percentage of lidocaine released in function of time from calcium phosphate biomaterials ($n = 6 \pm$ SD)

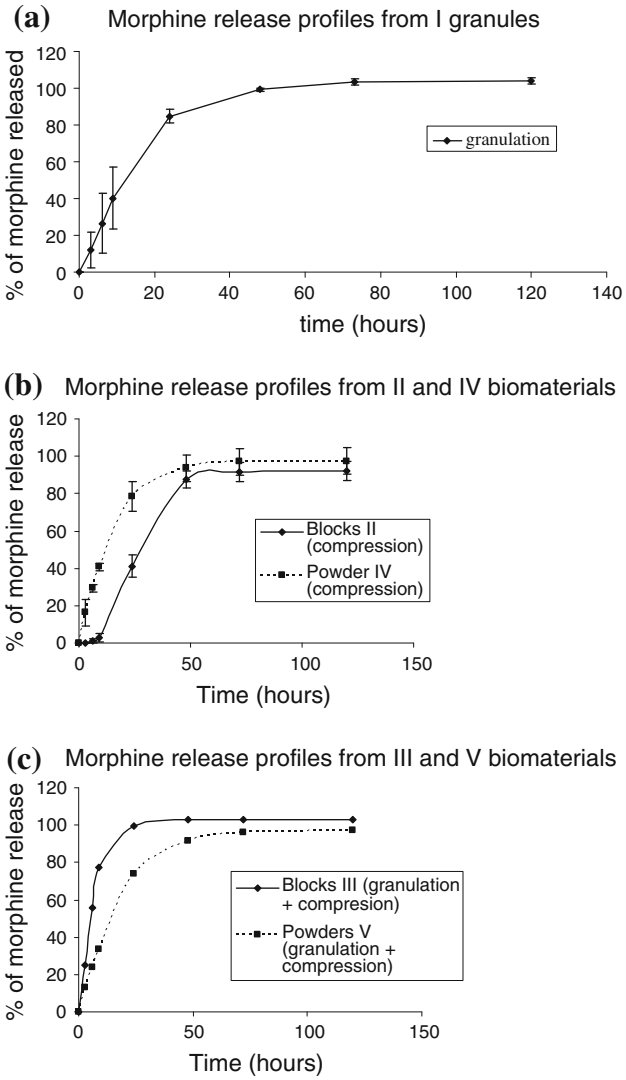

Fig. 2 Percentage of morphine released in function of time from calcium phosphate biomaterials ($n = 6 \pm$ SD)

significant with CDA than with BCP. This property seems to be linked to the high solubility of CDA in comparison with BCP, which seemingly increases the biological response. Moreover, CDA can be linked to a therapeutic agent to form a drug delivery system (DDS) [13].

In this study, the objective was to associate a local anaesthetic or a sedative analgesic with a bone substitute, so as to fill a bone void and to alleviate bone pain locally. By releasing the therapeutic agent in situ, the analgesic effect should be significantly increased [14].

Such a drug delivery system should make it possible to release the therapeutic agent in situ, notably during the first 72 post-operative hours, when the pain is more intense. This technique would considerably curb the risks of side effects and complications due to high doses, and would enhance patient comfort [15]. From this perspective, the lidocaine hydrochloride (local anaesthetic) and morphine hydrochloride (analgesic) were selected and linked to a calcium phosphate matrix.

Lidocaine hydrochloride is a local anaesthetic currently used in the clinic. The nervous C fibres involved in the perception of pain are the most sensitive to local anaesthetics. The presence of these fibres in the bone appears to enable lidocaine hydrochloride to provide local analgesia. The percentage of lidocaine hydrochloride incorporated into the CDA was set at 5%. A review of the literature showed that the percentage of lidocaine hydrochloride incorporated into a biomaterial can vary between 2 and 10% [7, 16, 17]. The release of lidocaine has been studied with other types of materials (other than bone substitutes)

which enabled us to estimate the percentage of lidocaine that should be incorporated into our calcium phosphate matrix. One group reported on a preliminary in vitro study on the association of local anaesthetics (prilocaine, bupivacaine and lidocaine) with a polymethylmethacrylate (PMMA) cement [17]. The latter study demonstrated that this association led to a slow release during the first 72 h (1.19% of lidocaine release). It should be noted that the study was performed for only 72 h as it is well known that PMMA releases the therapeutic agent slowly. Another study was conducted focussing on prolonging epidural anaesthesia and consisted in preparing and implanting a paste of biodegradable copolymer containing 10% of lidocaine into the epidural space of a rabbit. The release of the lidocaine decreased in comparison to a simple administration of a 10% lidocaine solution and the effective time of anaesthesia was nine times longer [17]. The release of the lidocaine (2% incorporated) was also studied with an injectable hydrogel (poloxamer 407) in a rat model. In this case, following injection close to the sciatic nerve, the analgesia was effective for 8 h [16].

Morphine hydrochloride, the other therapeutic agent studied here, is generally used as an analgesic and administered by the oral or parenteral route. Both routes of administration lead to the therapeutic agent being released into the systemic circulation. Consequently, the risk of side effects (nausea, constipation, respiratory depression and vomiting) increases and the targeted treatment site may receive an insufficiently analgesic dose. In the case of in situ administration, the side effects would be expected to be reduced given the amount of the therapeutic agent that could reach the blood circulation relative to the dose required for an analgesic effect specific to the site of treatment.

Three types of opioid receptor (mu, delta and kappa) are expressed by peripheral nerves and are responsible for transmitting the analgesic effect. The transmission of the nociceptive message is therefore inhibited in the presence of morphine. Morphine blocks opioid receptors present on C nerve fibre endings, which decreases the perception of pain [18]. As a result, these receptors on nervous fibres near to bone could be a target for morphine. In this study, the percentage of morphine hydrochloride incorporated into CDA was set to 20%. According to the literature, the concentration of morphine in biomaterials varies from 20 to 25% [19–21]. Studies associating morphine with biomaterials for the purpose of bone void filling are relatively rare as other materials are usually used. For this reason, 20% of morphine was incorporated to provide an uninterrupted oral release in a SPILA system (swelling polymer enlistment layer system). All of the morphine was released within 8 h [20]. An in vitro study using a poly (lactic acid co-glycolic) (poly (lactic-co-glycolic acid), PLGA) loaded with 25% of hydromorphone (a morphine agonist) implanted subcutaneously [21] showed that 70% of the therapeutic agent was released in 12 days.

The association of these therapeutic agents with a biomaterial therefore makes it possible to consider in situ therapeutic treatment so as to avoid treatment by the parenteral route. Another advantage would be to achieve a release that extends to proximity of the bone over several hours or even several days. From this perspective, two techniques of association were chosen that are increasingly used in the preparation of materials: isostatic compression and wet granulation.

Isostatic compression is a formulation technology that strengthens a material by applying even and homogeneous pressure, which then spreads the liquid in all directions. Therapeutic agents can be directly linked to the powder during compression. This process has been used for food sterilization. It has been described in the literature for material consolidation and is not thought to denature chemical bindings [22, 23]. By destroying non covalent bonds, hydrophobic and ionic bonds, the high pressure also has a virucide, bactericidal action, thus explaining its application in sterilization. This technique is also frequently used for preparing calcium phosphate materials such as BCP and CDA [11, 24].

Wet granulation is a densification technique very widely used in the pharmaceutical industry for manufacturing tablets. The aim of this technique is to obtain a homogeneous blend of each granule constituting a powder mixture. A granular form can be used to fill a bone defect. The advantage is that the technique fills the space perfectly and obviates the need to cut up a block. The links formed between the therapeutic agent and the calcium phosphate matrix by wet granulation are stronger than those formed by simple mixing.

The combination of both methods (isostatic compression preceded by wet granulation) was studied here to determine if it would increase the release-time for morphine and lidocaine hydrochlorides.

However, several precautions must be taken when associating a therapeutic agent with a calcium phosphate matrix. In general, therapeutic agents are stable under certain conditions but they can degrade due to changes in temperature, humidity, etc. It is therefore necessary to choose non-destructive methods for the therapeutic agents that allow for an extension of the release-time. NMR analyses were thus performed to ascertain whether or not each method causes degradation of the therapeutic agents.

The structural analyses of lidocaine hydrochloride by nuclear magnetic resonance of the proton showed that neither isostatic compression, nor wet granulation engenders changes in its conformation. These formulation techniques (wet granulation and isostatic compression)

therefore do not change the physicochemical characteristics of the therapeutic agents. In a previous study, it was shown that these two formulation techniques did not change the physicochemical structure of vancomycin (BCP loaded with an antibiotic) [24].

Nevertheless, in the structural analyses of morphine hydrochloride, light structural modifications were noticed when it was linked to CDA by isostatic compression and by wet granulation. This was not observed when morphine hydrochloride only had been subjected to isostatic compression. The modifications of the phenolic and hydrochloride function could be observed on RMN spectra when morphine hydrochloride was linked to CDA by isostatic compression or by wet granulation. Other analyses by ^{13}C NMR or mass spectrometry would enable a quantification of the definite structure of the morphine complex that had been formed. However, this would not predict possible modifications in its activity. Lidocaine hydrochloride, which remains chemically identical after formulation with calcium phosphates, presents a short-term clinical interest compared to morphine where biological activity will have to be checked.

Preliminary assays performed for each therapeutic agent (stability and complete release assays) showed that the therapeutic agent did not degrade at 37 or 4°C and that it could be released over a period of 5 weeks (for morphine) or 8 weeks (for lidocaine hydrochloride).

Complete release assays showed that all therapeutic agents were found in samples and that the formulation operations did not change the amounts of associated therapeutic agent.

The release kinetics of morphine and lidocaine from CDA were measured using a room chamber dissolution test prepared in the laboratory. This system provides a comparison of the release kinetics of the different biomaterials.

The different methods of formulation and association led to the acquisition of three forms of bone substitute: powders, blocks and granules. These different forms of bone substitute can be used in different types of osseous void fillings, or according to the preference of the surgeon. The block can therefore be cut up according to the size of the treatment site whereas powders and granules are more applicable to minor defects. These can be linked to a hydrogel to facilitate their establishment in situ. For this reason, they can be linked to hydrosoluble co-polymers allowing for the elaboration of a resorbable and injectable compound [25].

As regards the association of CDA with the lidocaine hydrochloride, blocks obtained by isostatic compression and isostatic compression preceded by wet granulation gave the quickest release profile in that the lidocaine hydrochloride was totally released in 24 h. Powders, acquired by grinding of each of these two types of blocks,

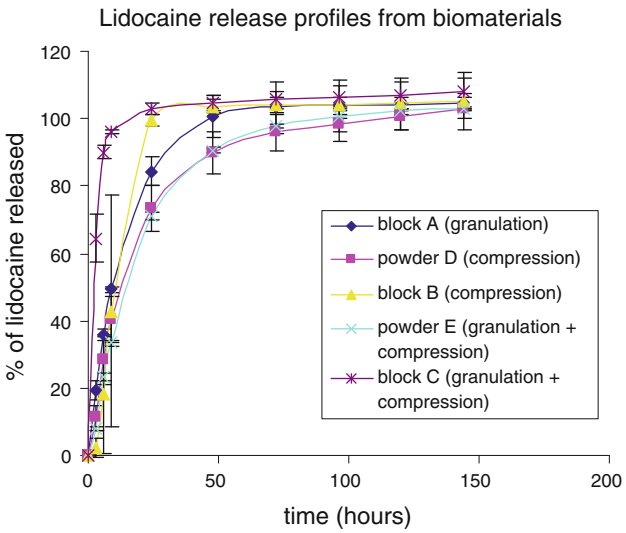

Fig. 3 Lidocaine release profiles from biomaterials ($n = 6 \pm$ SD)

gave a longer release of lidocaine over approximately 4 days. The release kinetics acquired with the grains of wet granulation were in between the two others, with lidocaine being completely released in 72 h. Consequently, the different formulation techniques led to the release of lidocaine hydrochloride linked to CDA over a period of time ranging from 1 to 4 days. Granulation preceding isostatic compression did not seem to provide a change in the release-time since both profiles were quite similar. In addition, both release kinetics were similar when both types of block were used. A comparison of the different association and formulation techniques (Fig. 3) led us to establish a scale of release speed for lidocaine (from the quickest to the slowest):

Isostatic compression (block)/granulation and isostatic compression (block) > granulation (granules) > isostatic compression and mortar grinding (powder)/granulation and isostatic compression and mortar grinding (powder).

It seems that when the biomaterial is in block form, the therapeutic agent is released quicker than when the biomaterial is in granule or powder form. This tendency could be explained by the effect of the specific surface of each form. Given that the surface was larger for the powder than for the block, the lidocaine released by granules into the middle of the powder mass could be again picked up by other surrounding granules. This suggests a chemical reactivity between the lidocaine hydrochloride and CDA which would lead to a controlled release. Consequently, the release would depend on the strength of the link between the lidocaine hydrochloride and the CDA, which is yet to be identified. However, given the release-time, the correlation is not likely to be too tight. The release-time was comparatively short (from 1 to 4 days) but could be relevant in the case of postoperative analgesia. Isostatic

compression and wet granulation were previously studied in the laboratory during the association of an antibiotic (vancomycin) with a calcium phosphate ceramic (BCP). The results showed that isostatic compression led to vancomycin release for up to 7 versus 3 days for wet granulation [11]. The difference in results between vancomycin and lidocaine could firstly be explained by the size difference between both molecules: vancomycin is a molecule of 1,450 Da whereas lidocaine hydrochloride is a molecule of 288.8 Da. The larger size of vancomycin would delay its release. In addition, as the solubility of CDA is greater than that of BCP, this favours the dissolution of lidocaine hydrochloride and therefore increases its release.

During the association of the hydrochloride morphine with CDA, the results obtained varied according to the formulations. Blocks obtained by isostatic compression only provided the slowest release. The release was very slow during the first few hours (up to 9 h), which corresponds to the period necessary to wet the block in order to release the morphine. Release was quick when the curve reached a plateau after 72 h, corresponding to 91.73 ± 5.06% of morphine hydrochloride release. This method seemed to allow for a slow release of morphine over time. When blocks were prepared by wet granulation followed by isostatic compression, the release of morphine hydrochloride was quicker. In fact, release was complete in 24 h (99.78 ± 1.80% of release). It seems that with isostatic compression, pre-granulation increased the release-time. The comparison of the result of grinding of both types of blocks (isostatic compression and wet granulation followed by isostatic compression) showed that the release kinetics were similar: quick release in 24 h (75.83 ± 3.04 and 73.08 ± 0.82%) then a slowing down to reach a plateau after 72 h (95.38 ± 1.38 and 90.64 ± 4.07%). When the biomaterial was in powder form, both production techniques induced morphine hydrochloride release in 72 h. As for wet granulation, a quick release was observed during the first 24 h, then the amount of morphine hydrochloride released remained smaller for up to about 72 h, equivalent to a release of 103.41 ± 0.72%. The different formulation techniques thus led to the release of morphine hydrochloride over a period of 1–3 days. By comparison of these results (Fig. 4), a classification of the rapidity of morphine release was established (ranging from the quickest to the slowest):

Wet granulation and isostatic compression (blocks) > granulation (granules) > isostatic compression ground (powder)/wet granulation and isostatic compression ground (powder) > isostatic compression (blocks).

During our study of vancomycin linked to BCP [7], isostatic compression was also found to provide the longest release-time. This tendency was observed with morphine hydrochloride. However, in comparison to vancomycin,

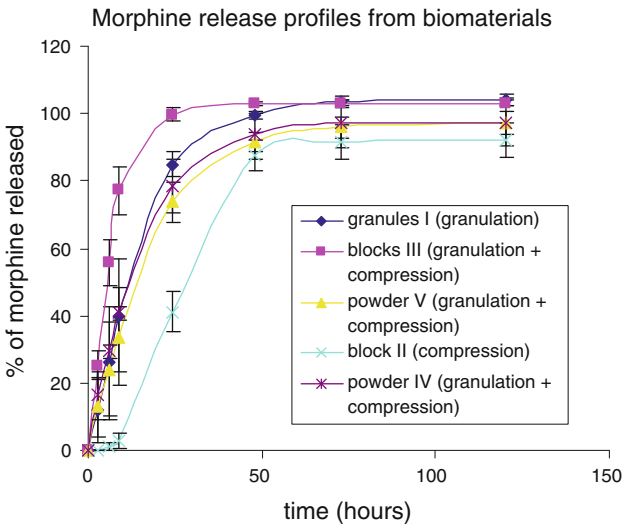

Fig. 4 Morphine release profiles from biomaterials ($n = 6 \pm$ SD)

morphine hydrochloride introduced a shorter release-time. This could also potentially be explained by the difference in size between the molecules (1,450 vs. 375.8 Da) and by the greater solubility of CDA versus BCP. Release therefore relates to the strength of link for the time being not identified between the morphine hydrochloride and the CDA.

The results obtained with lidocaine hydrochloride and morphine hydrochloride show that the two molecules do not have the same kinetics because morphine hydrochloride is released in a shorter time than lidocaine hydrochloride. The release peaks observed in the early hours of release are due to the dissolution of molecules located at the surface of blocks, granules or powders. However, for morphine and lidocaine hydrochlorides, the block wetting-time obtained following isostatic compression was slightly longer.

The aim of this implantation is to offer to the patient a complementary local analgesia associated to an osseous filler promoting bone growth. These release delays of respectively 1–4 days for lidocaine hydrochloride and 1–3 days for morphine hydrochloride seems to be interesting in post operative analgesia. Administration of others analgesics is necessary for the patient comfort but high doses won't maybe be required. Moreover, an oral administration will maybe be sufficient, comparatively to systemic administration that is more complex.

5 Conclusion

The objective of this study was to implement techniques of association and formulation of two therapeutic agents (lidocaine and morphine hydrochloride) with a calcium

phosphate matrix (calcium deficient apatite) with the intention of acquiring a biomaterial able to both play its part in bone substitution (to fill a bone void and to favour bone regrowth) and to release an analgesic locally at the site of treatment. The methods used were isostatic compression, wet granulation and a combination of the two. The materials were evaluated in terms of the release kinetics of their therapeutic agents.

The physicochemical characterization of the synthesized powders of CDA provided satisfactory results. The integrity of the lidocaine hydrochloride structure, as determined by RMN ^{1}H, was confirmed regardless of the formulation technique used (isostatic compression or wet granulation). However, analyses of morphine hydrochloride by RMN ^{1}H revealed slight structural modifications. Supplementary analyses will make it possible to ascertain whether these modifications bear an influence on the pharmacokinetic parameters and on pharmacokinetic activity.

The association and formulation techniques that were used made it possible to obtain an in vitro release-time varying from 1 to 4 days for lidocaine hydrochloride and from 1 to 3 days for morphine hydrochloride.

To date there have been no publications addressing the association of calcium phosphates with lidocaine hydrochloride and morphine hydrochloride. The study goes a step further towards providing knowledge on how to improve the mechanical properties of biomaterials. In addition, a gain in knowledge of correlations between CDA and pharmacologic substances would make it possible to elaborate effective associations between therapeutic agents and filling materials.

A pre-clinical evaluation could be envisaged for different formulations prepared in this way. These studies would notably provide insight into the optimum dose of analgesic that should be incorporated into the biomaterial and would additionally help to define their release kinetics for effective in situ treatment. As a result, according to the nature of the implant, the type of analgesia and the release-time for the different therapeutic agents, surgeons will be able to choose the most appropriate biomaterial (blocks, grains or powders) for their patients.

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