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Calcium Phosphate Carrying Simvastatin Enhances Bone Regeneration: A Systematic Review

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Several studies have aimed to develop alternative therapeutic biomaterials for bone repair. The purpose of this systematic review was to evaluate how statins carried by calcium phosphate affect the formation and regeneration of bone tissue in animal models when compared to other biomaterials or spontaneous healing. This systematic review followed the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions, the PRISMA guidelines, and the Preclinical Systematic Review & Meta-analysis Facility (SyRF). The protocol of this systematic review was registered in PROSPERO (CRD42018091112) and in CAMARADES. In addition, ARRIVE checklists were followed in order to increase the quality and transparency of the search. An electronic search was performed using the MEDLINE/PubMed, Scopus, SciELO, and PROSPERO library databases. The authors used a specific search strategy for each database, and they also conducted a search in the grey literature and cross-references. The eligibility criteria were animal studies, which evaluated bone repair treated with calcium phosphate as a simvastatin carrier. The selection process yielded 8 studies from the 657 retrieved. All manuscripts concluded that locally applied simvastatin carried by calcium phosphate is biocompatible, enhanced bone repair and induced statistically greater bone formation than cloth or calcium phosphate alone. In conclusion, the pertinent pre-clinical studies evidenced the calcium phosphate biocompatibility and its effectiveness in delivering SIM to improve the repair of bone defects. So, clinical trials are encouraged to investigate the impact of SIM associated with calcium phosphate bone graft in repairing bone defect in humans.

Key Words: statins, simvastatin, calcium phosphate, bone repair, bone regeneration

Introduction

Congenital diseases, infections, traumas and neoplasia can cause bone defect, which is a major problem in medicine and dentistry. Autologous bone grafts is the gold standard for bone regeneration in terms of osteogenesis, osteoconduction, and osteoinduction (1). However, these grafts have disadvantages, such as donor-site morbidity, risk of infection, hemorrhage, and chronic pain (2). For that reason, several studies have aimed to develop alternative therapeutic biomaterials for bone repair (1,3,4).

Tissue engineering is based on the following three pillars: biomaterial (scaffold), cells, and bioactive molecules (5). However, because of challenges related to the regulation and translation of cell therapy into clinical practice (6,7)including minimal manipulation and homologous use, may be subjected to a standards-based approach under the Safety of Human Cells, Tissues and Organs for Transplantation Regulations. The manufacture and clinical testing of cell and gene therapy products (CGTPs, there is growing interest in acellular therapy based on drug delivery biomaterials. Consequently, calcium phosphate has been studied as a biomaterial capable of carrying molecules such as bone morphogenetic proteins (BMPs) (8), platelet-rich plasma (9), strontium ranelate (10) and statins (11). These molecules can stimulate endogenous cells to promote the production of functional bone, resulting in a relatively low-cost product for tissue regeneration therapy (12).

Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors that are widely used in treating patients with hypercholesterolemia (13). They have also been studied because of their anabolic effects on bone tissue (14-18). Statins increase the expression of BMP-2 (16,17,19) and vascular endothelial growth factor (VEGF) (19). The growth factors BMP-2 and VEGF are of important scientific interest in bone regeneration therapy, but they are expensive and have a short half-life. Therefore, a molecule promoting the endogenous production of BMP-2 and VEGF would be valuable for the field of tissue engineering(12).

Statins can be administered systemically or locally within a vehicle or carrier. Studies using systemic administration indicate that severe muscular side effects are rare; however, mild side effects, such as myopathic symptoms, are common (20). For this reason, topical administration is recommended for bone regeneration,

since local application allows a controlled, gradual release of the molecule, increasing its bioavailability and effectiveness (12,14). Therefore, for local delivery strategy calcium phosphate can be used as a carrier. In addition, this material is biocompatible, osteoconductive, act as a space maintainer for bone formation and exhibit varying degrees of resorbability (3,21). For that reason, the aim of this systematic review (SR) is to evaluate the influence of calcium phosphate as a statin carrier on bone repair in animal studies, to provide information on potential clinical application. The following question was stated: How do statins carried by calcium phosphate affect the formation and regeneration of bone tissue in animal studies?

Material and Methods

The protocol of this systematic review (SR) was recorded in PROSPERO database under number CRD42018091112 in CAMARADES at http://syrf.org.uk/protocols/. This SR followed the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions (22). In order to increase the quality and transparency of the study, the PRISMA (23) and ARRIVE checklists (22) were followed.

Focused Question (Based On PICO Criteria)

How do statins carried by calcium phosphate (I) affect the formation and regeneration of bone tissue (O) in animal models (P) when compared to other biomaterials or spontaneous healing (C)?

Outcome Measures

The primary outcome measures were bone formation through histological and histomorphometrical analysis. The secondary outcome variables were the presence of biomaterial and biocompatibility.

Search Strategy

An electronic search was carried out in the MEDLINE/PubMed, Scopus, SciELO, and PROSPERO library databases up to October 2018. There was no date restriction, but only studies in English, Portuguese, Spanish, or French were included. In addition, a manual electronic search of some periodical journals was performed. Unpublished studies (i.e., grey literature) were searched for in the Grey Literature Report and OpenGrey databases. Searches in the references of the included studies (i.e., cross-referencing) were also conducted. A specific search strategy was used for each database, according to its characteristics (Box 1). Aiming for in vivo studies, a search filter was developed (23) that could detect 7% more records than the regular method, the PubMed Limit: Animals.

Eligibility Criteria

Inclusion Criteria

Pre-clinical and experimental studies that evaluated bone repair through calcium phosphate biomaterial as a carrier for statins compared to other biomaterials or blood clots

Exclusion Criteria

The exclusion criteria were manuscripts involving in vitro studies, syntheses, characterization of biomaterials, and reviews; studies in animals with systemic disorders or infections, such as osteoporosis or osteopenia; studies involving biomaterial associated/coating implant and alloy

Box 1. Search strategy

Databases	Keywords
PubMed	Developed filter (in vivo studies)20 AND ((("Hydroxymethylglutaryl-CoA Reductase Inhibitors" [Mesh] OR "Rosuvastatin Calcium" [Mesh] OR Simvastatin [Mesh]) AND ("Bone Remodeling"[Mesh] OR "Bone Development"[Mesh] OR "Bone Regeneration"[Mesh] OR "Osteogenesis"[Mesh] OR "Bone Resorption" [Mesh] OR "Fracture healing" [Mesh])) OR ((Statins [tiab] OR Rosuvastatin [tiab] OR Simvastatin [tiab] OR Fluvastatin [tiab]) AND ("bone formation" [tiab] OR "bone repair" [tiab] OR "osseoinduction" [tiab] OR "bone augmentation" [tiab] OR "bone regeneration" [tiab] OR "bone healing" [tiab] OR "bone induction" [tiab] OR osteogenesis [tiab] OR "fracture healing" [tiab] OR "bone defects" [tiab] OR bioresor*[tiab] OR biocompatibility[tiab]))))
Scopus	TITLE-ABS-KEY ((statins OR "Hydroxymethylglutaryl-CoA Reductase Inhibitors" OR simvastatin OR fluvastatin OR rosuvastatin) AND ("bone formation" OR "bone repair" OR "osseoinduction" OR "bone augmentation" OR "bone regeneration" OR "bone healing" OR "bone induction" OR "bone remodeling" OR "fracture healing" OR osteogenesis OR bioresorption OR biocompatibility) AND ("in vivo" OR animal OR rabbit OR mice OR rat OR sheep OR dog OR monkey) AND NOT implant) AND (LIMIT-TO (DOCTYPE,"ar")) AND (LIMIT-TO (LANGUAGE, "English"))
SciELO	(Statins OR Rosuvastatin OR Simvastatin OR Fluvastatin) AND ("bone formation" OR "bone repair" OR "osseoinduction" OR "bone augmentation" OR "bone regeneration" OR "bone healing" OR "bone induction" OR osteogenesis OR "fracture healing" OR "bone defects" OR bioresor* OR biocompatibility)
PROSPERO	Statin AND Bone
Grey Literature	Statin AND Bone

surface, systemic administration of statins, biomaterial associated with substances other than statins, cells or growth factors, statin ectopic injection; or studies having an outcome unrelated to bone formation, osteoconduction, bioresorption, or biocompatibility.

Study Selection, Screening Process and Data Extraction

The process of searching and selecting the studies was conducted in duplicate by two authors (M.D.C.M. and R.C). First, titles and abstracts were carefully evaluated. After the first evaluation, potential articles were carefully assessed according to the eligibility criteria of this review. Possible disagreements were resolved through a consensus between the authors. When necessary, the authors of the included studies were contacted by email for clarification.

The following data were extracted from the papers by two reviewers (G.P. and R.C.): DOI, authors, year, animal model, sample size, experimental period, biomaterials used, location of biomaterial implantation, analysis method, outcome, and main conclusion.

Assessment of Quality and the Risk of Bias

The quality assessment was done by two reviewers (R.C. and M.D.C.M.), using the ARRIVE guideline checklist (22). According to these guidelines, a checklist consisting of 26 items was developed; based on a previous study (24), each criterion was graded as "0" (not reported or not performed) or "1" (reported). A final score was then recorded for each study.

The risk of bias (ROB) was performed by two reviewing authors (M.D.C.M. and R.C.) using the risk-of-bias tool developed by the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) (25). The goal of this tool is to assess the quality of the applied methodology by classifying the papers as having a low, high, or unclear risk of bias.

Statistical Analysis

Means and standard deviations were extracted from the selected studies, and descriptive analyses were conducted. The software Microsoft Office Excel (2010) was used for descriptive statistics. A pairwise meta-analysis could not be conducted because of the heterogeneity between studies, which used different animal models, carrier biomaterials, administration sites, doses, and follow-up durations.

Results

Literature Search

The initial search resulted in 657 articles, comprising 371 titles from MEDLINE/PubMed, 12 from SciELO/Bireme, 270 from Scopus, three from PROSPERO, and one from cross-referencing. The search in the grey literature did not yield the inclusion of any additional studies. Duplicates were removed, and after screening the titles and abstracts, 14 studies were selected. Following the screening of the full text of the 14 selected studies, 6 manuscripts were excluded because they did not meet the eligibility criteria. As a result, 8 studies (11,26-32) were included in this systematic review (Fig 1).

Assessment of Quality and the Risk of Bias

With regard to quality assessment, the mean and median score of the studies were 11.75 (±2.05) and 12.5, respectively. The highest score was 15 points (11), and the lowest was 9 (28,30). None of the studies presented sample size calculations or baseline animal health data, and none mentioned blinding of accessor. In addition, none of the manuscripts described the implications of experimental methods for replacement, refinement, or reduction (the 3Rs) or discussed the relevance of the study to human biology. Regarding statistical methods, one study was qualitative, so it did not present statistics (26). All of the studies clearly defined the outcomes assessed and the study design. However, only one study mentioned parameters such as biomaterial implant randomization (27) and house and husbandry (11) (Table 1).

With respect to ROB, in 75% of the manuscripts all animals were included in the analysis. The baseline condition was reported to be the same for every group in half of the studies, considering that there was no disease induction. Seven out of eight studies reported all of the expected outcomes. None of the manuscripts were described as low risk for blinding in detection bias or sequence generation in selection bias. However, one study claimed that the allocation to different groups was done randomly (27).

Study Characteristics

In the eligible manuscripts, the experimental animal models used were rabbits(27,31,32) and rats (11,26,28-30), with a total of 291 animals (72 rabbits and 219 rats). The sample size varied from 3 to 9 (Fig 2). The experimental period was from 3 to 56 days. The calcium phosphate used in the eligible manuscripts was tricalcium phosphate lysine (26); alpha-tricalcium phosphate (α-TCP)(28,29); α-TCP, beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) (30); macroporous calcium phosphate bone cements (32); hydroxyapatite fiber (HAF) (27); biphasic calcium phosphate (BCP) (11); and nanostructured-hydroxyapatite (nHA) (31). In this study, the presentation form of the biomaterials used was a cylinder (26); particles 500-700 μm(28,29); particles 500-750 μm (30); cement paste and cylinders (32); fibers (27); particles (11), and powder (31). The biomaterial grafts were inserted into the region of the

femur, with perforation around 2 mm (26), 3 mm (31), and 5 mm (32); the muscle (32); and the bilateral calvaria 5 mm defects (11,27-30).

Plasma statin concentration was not analyzed in all studies. The method of analysis most often applied was histological analysis (11,27-32) and histomorphometrical evaluation (11,26-30,32), followed by micro-CT evaluation (27,29-31) and radiological and thermograph analysis (31), considering overlapping parameters. In addition, the parameters considered were newly formed bone (11,26-32); bioresorption (30,31); biocompatibility (29,31,32); bone mineral content and bone mineral density (29,32); simvastatin (SIM) release in vitro (27,28,32); morphology of the vital organs (26); BMP, TGF, and VEGF gene expression (28); and bone labeling with calcein at 7 days and tetracycline at 1 day (30).

Biocompatibility

The biocompatibility studies used rabbits as their animal models (31,32) . Yin et al. used macroporous

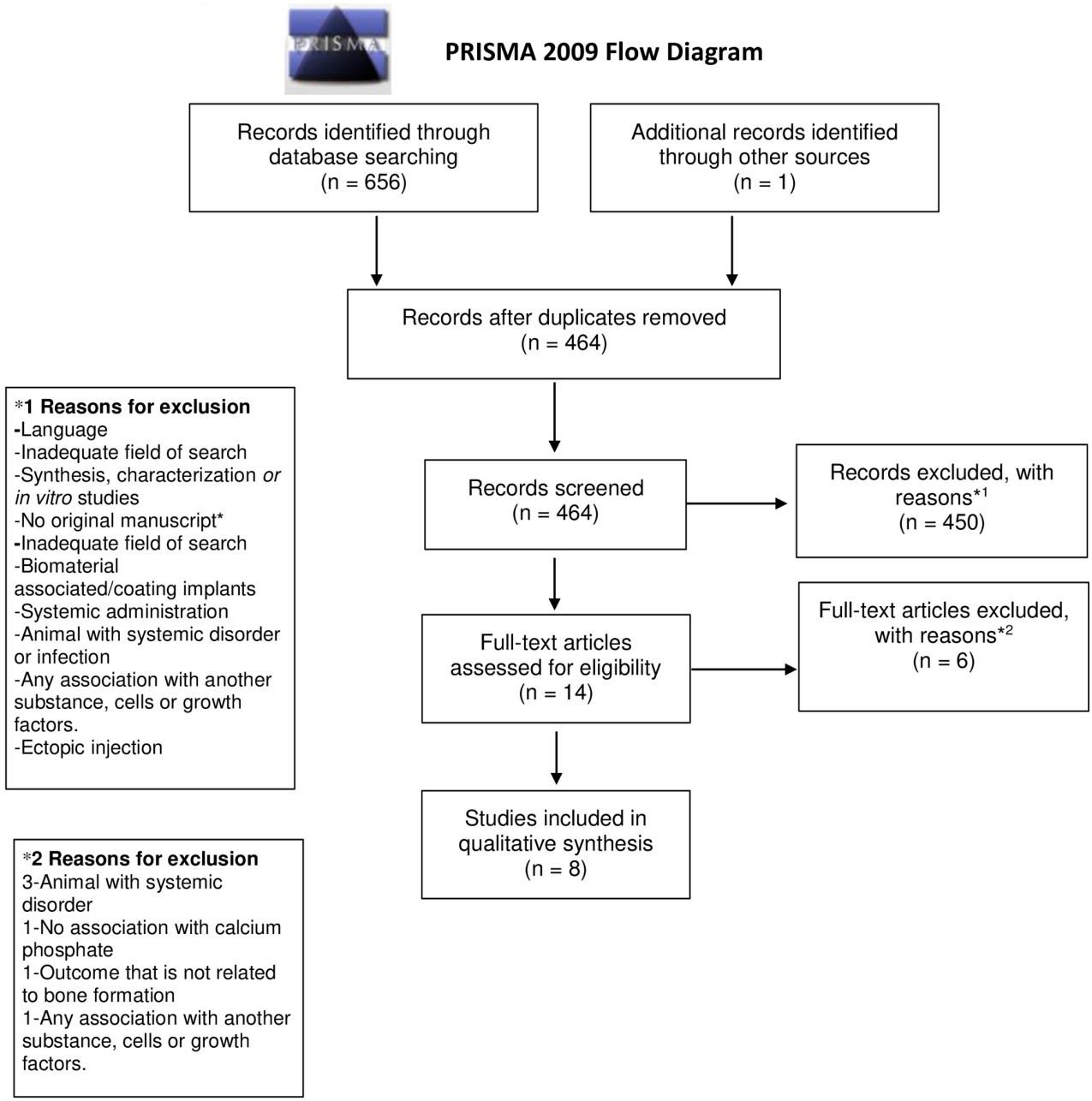

Fig. 1. Flow diagram of literature selection process. * E.g. reviews, letters and comments.

calcium phosphate cement (CPC), to be compared with CPC associated to simvastatin (32), and Shahrezaie et al. used nanostructured hydroxyapatite particles with and without simvastatin (31). The reported results indicate that small amounts (1 wt%) of SIM associated with the CPCs did not affect the physico-chemical properties or

Table 1. Quality assessment – Arrive-based score

References	Adah et al. 2006	Nyan et al. 2009	Nyan et al. 2010	Rojbani et al. 2011	Yin et al. 2012	Gao et al. 2013	Santana et al. 2016	Shahrezaie et al. 2017
1.Title	1	1	1	1	1	1	1	1
2.Abstract	1	1	1	1	0	1	1	1
3.Introduction								
Background information	0	1	0	0	0	1	0	0
Objectives	1	1	1	1	1	1	1	1
4.Methods								
Ethical statement	1	0	0	0	1	0	0	1
Study design	1	1	1	1	1	1	1	1
Experimental procedures	1	0	0	0	1	1	1	1
Experimental animals	1	0	0	1	0	0	1	1
Housing and husbandry	0	0	0	0	0	0	1	0
Sample size	0	0	0	0	0	0	0	0
Allocating animals	0	0	0	0	1	1	0	1
Anesthesia	1	0	0	0	1	1	1	1
Antibiotics	1	0	0	0	1	0	0	1
Analgesia	1	0	0	0	0	0	1	1
Blinding of assessor	0	0	0	0	0	0	0	0
Implant random.	0	0	0	0	0	1	0	0
Experimental outcomes	1	1	1	1	1	1	1	1
Statistical methods	0	1	1	1	1	1	1	1
5.Results								
Baseline data	0	0	0	0	0	0	0	0
Std error/ conf	0	1	0	1	1	1	1	0
6.Discussion								
Interpretation	1	1	1	1	1	1	1	0
Study limitations	0	0	1	0	1	0	1	0
Adverse events	0	0	0	0	0	0	0	0
3 Rs reported	0	0	0	0	0	0	0	0
Gen/trans	0	0	0	0	0	0	0	0
Funding	0	1	1	0	0	0	1	0
Total Score	12	10	9	9	13	13	15	13

Note: Implant random. =implant randomization; Std error/conf. =Standard error/confidence interval; Gen/trans= Generalizability and translation

biocompatibility (32), and nHA and SIM particles, when used to fill bone defect, were biocompatible with and beneficial to bone healing (31). Manuscripts that didn't focus on biocompatibility but that evaluated the inflammatory response described no or slight inflammatory reaction with low concentrations of SIM (11,27-32), which decreased over the experimental period in all studies, regardless of the implantation site. For SIM at 30 and 60 days after implantation in bone defects, they were completely degraded and replaced by new bone with significantly superior morphology and mineral density, as compared with the control group.

Newly Formed Bone

All manuscripts concluded that simvastatin associated with calcium phosphate enhanced bone repair (Box 2). Although one article provided only descriptive histological analysis (26), seven studies showed improved bone formation with statistically significant results (11,27-32). One article compared calcium phosphate alone and with SIM, without a control group (cloth) (27), and seven studies showed that this association enhanced bone repair when compared with the control groups (cloth) (11,26,28-32).

At two and eight weeks, 14 mg of tricalcium phosphate carrying 0.1 mg of SIM showed significantly higher bone volume than groups having 0, 0.01, 0.25, or 0.5 mg of SIM. In addition, the percentage of defect closure at eight weeks was significantly greater in the TCP-0.1 mg (97.86%±1.49) group than in the control group (41.67%±10.78) or the TCP-0.5 mg group (63.57%±9.3) (29). However, another study using 40 mg HA fiber (HAF) showed that 0.45 mg of SIM induced significantly higher bone formation than lower amounts at four weeks and eight weeks. In this same study, the percentage of new bone volume (for each SIM/HAF mg ratio) was: 20.22%±5.53 (0/40 mg), 11.72%±3.53 (0.15/40 mg), 42.14%±4.43 (0.45/40 mg), and 31.22%±8.58 (0.75/40 mg), having significant differences between the 0/40 mg and the 0.45/40 mg groups (p=0.17) and between the 0.15/40 mg and the 0.45/40 mg groups (p=0.002) at eight weeks (27).

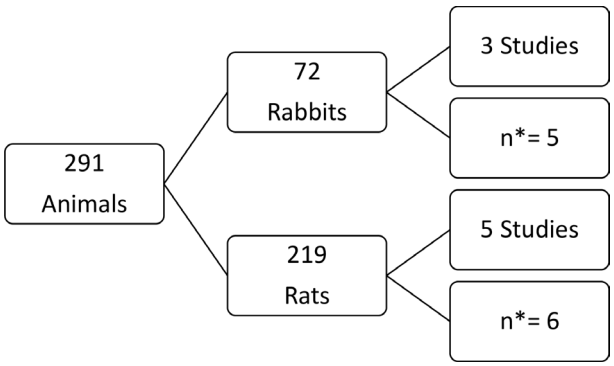

Fig. 2. Study characteristics. n*= sample size median per animal model.

According to Nyan et al, in the α-TCP group carrying 0.1 mg of SIM, most of the defects showed a complete bridging of new bone at the end of the experimental period due to a continuous regeneration of bone at the center. In addition, new bone was observed inside the remaining α-TCP particles (29). In Nyan et al, a similar response was shown: 21 days after surgery, new bone deposition was observed in the middle of the α-TCP particles, which were almost completely surrounded by the regenerated bone (28).

Most of the studies used TCP as biomaterial and presented positive results. Nyan et al. claimed that the percentage of new bone area was significantly higher in the α-TCP-SIM group (65.3%±6.3) as compared with the control group (15.8%±3.5) or the α-TCP-alone group (31.4%±3.8) at the end of experimental period (p<0.01) (28). Another study used β-TCP with 0.1 mg of SIM and showed in the SIM group a significantly greater amount of newly formed bone at six weeks (p<0.001) and at eight weeks (p<0.05) (30). Furthermore, biphasic calcium phosphate (BCP) associated with SIM showed a higher area of newly formed bone tissue (12.71%±1.21) as compared with control group (0.00%±0.00) and BCP alone (9.04%±0.86) (11). However, no significant results were observed using statins in association with HA particles (30). Yin et al. (32) used macroporous calcium phosphate cement, and through a histomorphometrical evaluation showed that the newly formed bone of the SIM group (7.4%±3.3) was significantly greater than in the non-SIM group (3.6%±1.4) (p<0.05).

Simvastatin (5 mg) in association with nanohydroxyapatite (nHA) had a synergistic effect, since the matrix of newly formed bone showed upper morphological characteristics compared with either nHA or SIM alone (31). Furthermore, another study also observed a positive effect in bone repair using 5 mg of SIM (26).

Discussion

Regarding bone regeneration, calcium phosphate as a statin carrier can be a valuable alternative to stem cells or growth factors. For that reason, this association (calcium phosphate and statin) can be recommended for clinical applications. This systematic review sought to provide information about how statins carried by calcium phosphate affect new bone formation and biocompatibility.

For bone healing, the effective dose of systemically administered simvastatin is higher than the routine dose in clinical applications (20-40 mg/day). For that reason, locally administered statin can be an alternative for achieving therapeutic concentrations for bone regeneration and avoiding the side effects of systemic doses (33). Given the possibility of a reduced inflammatory response with topical

Box 2. The effects of SIM carried by calcium phosphate on bone repair.

Reference	A.M /L.I.	Exp. periods	Biomaterial	SIM	Biomaterial preparation	Main conclusion	Data
Adah et al. 2006	Rats n=5 Femur (2mm)	30 days	TCP Lysine	5 mg	N.A.	SIM improved bone repair	Qualitative
Nyan at al. 2009	Rats n=7/ calvaria (5mm)	2, 4, 8 wks	14 mg α-TCP (particles)	0; 0.01; 0.1; 0.25; 0.5 mg	SIM was dissolved in ethanol and applied to the α-TCP	SIM (0.1 mg) improved bone repair	% NBFA (8 wks) Control: 41.67±10.78 α-TCP: 74.29±9.48 α-TCP+0.1SIM: 97.86±1.49] p<0.01 Bone volume (8 wks) TCP-0.1 group presented significantly greater bone volume than TCP alone (p<0.001)
Nyan et al. 2010	Rat n=6/ Calvaria (5mm)	3, 7, 10, 14 and 21 days	14 mg α-TCP (particles)	0.1 mg	SIM was dissolved in ethanol and applied to the α-TCP	SIM improved bone repair at 2 and 3 wks	% NBFA (21 days) Control: 15.8±3.5 α-TCP: 31.4±3.8 α-TCP+0.1SIM: 65.3±6.3] p<0.01] p<0.01
Rojbani et al. 2011	Rats n=6/ Calvaria (5mm)	6, 8 wks	14 mg α-TCP, β-TCP e HA (particles)	0.1 mg	SIM was dissolved in ethanol and incorporated into the biomaterial	SIM improved bone repair for TCPs, but not for HA	% NBFA (8wks) *Control: ±36 *α-TCP: ±60 *α-TCP+0.1SIM: ±71] p≤0.05] p<0.001
Yin et al. 2012	Rabbits n=8 / Femur (5x10mm)	4 wks	Macroporous CPC	1 % wt.	SIM was homogenized with the solid phase of the cement	SIM improved bone repair	% NBFA (4 wks) Control: N.A. CPC: 3.6±1.4 CPC+1%SIM:7.4±3.3] p<0.05
Gao et al. 2013	Rabbits n=5/ chamber- calvaria (5 x 3 mm)	4, 8wks	HA Fiber (40 mg)	0. 0.15, 0.45, 0.75 mg	SIM was dissolved in ethanol and applied to the HA	SIM (0.45mg) improved bone repair	% NBFV (4 wks) No cloth HAF: 22.45±4.04 HAF+0.45SIM:41.11±11.79] p=0.004
Santana et al. 2016	Rats n=6 / Calvaria (5mm)	8 wks	BCP (14g)	0.1 mg	SIM was dissolved in ethanol and BCP were soaked in SIM solution	SIM improved bone repair	% NBFA (8wks) Control:0.00±0.00 BCP: 9.04±0.86 BCP+0.1SIM: 12.71±1.21] p<0.05] p<0.05
Shahrezaie et al 2017	Rabbits n=4 / Femur (3 x1.5 mm)	30 days	nHA (0 and 1 mg) (powder)	0. 5 mg	N.A.	1 mg nHA+ 5mg SIM improved bone repair	% NBFV (30 days) Control: NA nHA: NA SIM: NA nHA + SIM: NA] p<0.05] p<0.05] p=0.001

Note: A.M.= Animal model; L.I.= Local of implantation; Exp.= Experimental; wks = weeks; SIM=Simvastatin; HA= Hydroxyapatite; TCP= Tricalcium Phosphate; nHA= nanoHydroxyapatite; %NBFA: percentage of new bone formation area; * approximately value obtained through the graph since the authors did not provided the numerical values; CPC=Calcium phosphate cement; %NBFV: percentage of new bone formation volume; HAF= Hydroxyapatite fiber; BCP: Biphasic calcium phosphate; N.A.: Not available .

application, it is important to analyze the tissue response to locally administered statin. Shahrezaie et al. claimed that nHA and SIM improved the scaffold biocompatibility (31), corroborating with another in vivo study which demonstrated that SIM significantly decreased pro-inflammatory cytokines and increased anti-inflammatory cytokine levels (34). Although simvastatin presented topical anti-inflammatory properties, this response changes at higher doses. This fact can be demonstrated in Stein's study, in which locally applied 2.2 mg of simvastatin caused significant inflammation in soft tissue in a rat mandible compared with lower doses (35). In addition, a large dose (10% wt) can produce an inflammatory response and induce a severe muscle necrosis around the biomaterial (32). Most of the manuscripts analyzed in this systematic review evaluated the inflammatory response and described no or a slight inflammatory reaction with low concentrations of SIM (0.1 to 0.45 mg) (11,27-30,32), which decreased over the experimental period in all studies.

In several studies, one of the main goals was to determine the optimal dose of simvastatin for inducing bone formation while minimizing the inflammatory response. Stein et al. stated that 0.1 mg of simvastatin in methylcellulose gel in a polylactic acid membrane resulted in minimal local inflammation, but it failed to stimulate significant bone growth (35). Nevertheless, when 0.1 mg of SIM (approximately 0.71% wt) was used with calcium phosphate carriers, it induced greater bone formation and caused only slight inflammation (11,28-30). Furthermore, another study used different SIM doses (0, 0.15, 0.45, 0.75 mg of SIM in 40 mg of calcium phosphate), finding 0.45 mg of SIM to be the ideal concentration because it induced significantly greater bone formation than lower amounts but had no inflammatory response (27). Proportionally, 0.45 mg of SIM in 40 mg of carrier is nearly 1.1% wt; therefore, this study corroborates the findings of Yin et al, which showed that 1% wt SIM had no inflammatory reaction and improved bone formation (32).

Several studies suggest that the anabolic bone effect of statins is dose-dependent at a certain level (27,29,36), since higher doses showed more intense and prolonged inflammation. The inflammatory response under normal conditions is self-limited, contributing to the helping process by removing necrotic tissue, debris, and bacterial contaminants, as well as recruiting and activating fibroblasts (37). However, higher doses may provoke an excessive inflammatory reaction, which could in turn stimulate the production of inflammatory cytokines and prevent the healing process (27,37).

SIM positively affects bone metabolism because it increases BMP-2 expression and suppresses osteoblast apoptosis, inducing osteoblastic differentiation, osteogenesis, and new bone formation. In addition, SIMs reduce bone loss, preventing osteoclast activity and osteoclastogenesis by decreasing the differentiation of macrophages or monocytes into osteoclast (38,39). Studies from this systematic review reinforce this information, since all manuscripts suggested that SIM associated with calcium phosphate enhanced bone regeneration, with seven out of eight studies producing statistically significant results (11,27-32). The positive effect of SIM was acknowledged when new bone formation area or volume significantly greater in the group that used SIM with calcium phosphate than the group of calcium phosphate alone. Therefore, this parameter was considered as a well succeed result.

It is a common sense that no animal model is capable of completely mimics human bone regarding macroscopy, microscopy and remodeling (40). However, there are evidence that almost 50% of all animal models used to analyze bone repair are rats and rabbits (41). Two different animal models were considered in this study, rat and rabbit. Nevertheless, the use of both models resulted in the same conclusion: calcium phosphate as a simvastatin carrier promoted significantly greater bone formation than the calcium phosphate alone. Indeed, the analysis of distinguished animal models reaching the same outcome reinforce the conclusion of this study. There are limitations in extrapolating results from animals to humans, but positive results in two distinct animal models can indicate more predictability of clinical outcomes. Several clinical studies already confirmed previous in vivo studies, showing a good agreement between pre-clinical and clinical studies regarding bone graft (42,43).

Furthermore, in the included studies, the bone grafts were evaluated into two different types of bones: femur and calvaria, formed by distinct ossification process, endochondral and intramembranous, respectively. However, SIM significantly enhanced bone repair, despite the bone origin, reinforcing its positive effect.

Also, regarding the biomaterial preparation, it was observed that in most of the included studies, the incorporation of SIM to calcium phosphate was similar. First, SIM was dissolved in ethanol and then incorporated to calcium phosphate by dropping under sterile conditions or soaking. Only one study (32) mixed SIM with the solid portion of the calcium phosphate cement, and then the solid and liquid phase were mixed. Independently of the biomaterial-SIM association process, the SIM group showed statistically higher new bone formation.

Another point to be analyzed is that one of the most important characteristics of biomaterial is biodegradability. In Shahrezaie's study, the nHA, with or without SIM, degraded within 30 days after implantation and was replaced by woven bone (31). Furthermore, after 60 days

they were replaced by corticomedullary compact bone. However, another study demonstrated that HA was the least degradable (30), showing that the nanostructure of the previous study may have contributed to its biodegradability by increasing the surface-to-volume ratio, which could, in turn, lead to a higher resorption rate (44). Moreover, α-TCP and β-TCP are less stable than HA and are thus more soluble in aqueous environments (45), so β-TCP ceramics can be degraded more rapidly than HA ceramics in the body (46,47). Furthermore, α-TCP was shown to have the most bioresorption, followed by β-TCP and HA (30). In this same study, α-TCP and β-TCP carrying simvastatin exhibited more resorption, which can be explained by the degradation possibly being affected by the bone remodeling around the biomaterial (30).

Calcium phosphate biomaterials have been shown to be osteoconductive and biocompatible (3,48,49). Degradation of calcium phosphate may contribute to calcification due to its release of calcium and phosphate ions into the microenvironment, which could enhance bone formation (50). It could also work as a space maintainer, being highly advantageous in bone regeneration (29). Calcium phosphate has been shown to be an ideal carrier for SIM because the association of SIM with biphasic calcium phosphate induced greater bone formation than SIM with collagen sponge (11). In addition, the association of nHA with SIM showed a synergistic effect, inducing a greater bone volume compared with SIM or nHA alone (31). Furthermore, Stein et al. (35). Nevertheless, Nyan et al. used the same dosage of SIM carried by α-TCP and demonstrated a significantly higher bone formation when compared with the control group (29). The difference may therefore be attributed to the simvastatin carrier. However, Rojbani et al. (30) demonstrated that the percentage of bone formation when SIM was carried by HA was not significant, suggesting that it could be associated with the slow biodegradability of HA.

In conclusion, the analyzed literature sustains that locally applied calcium phosphate as a SIM carrier is biocompatible and induces greater bone formation, which is statistically significant, regenerating from 18.3 to 107.97% more bone than calcium phosphate alone. The results so far reveal that calcium phosphate in association with SIM increases bone repair, being relevant for clinical regeneration of bone defects. Nevertheless, more standardized research is recommended since no optimum dose of the biomaterial has yet been defined because of the lack of a pattern in SIM dose and type of calcium phosphate.

Thus, the analysis of pertinent pre-clinical studies evidenced the calcium phosphate biocompatibility and its effectiveness in delivering SIM to improve the repair of bone defects. So, clinical trials are encouraged to investigate the impact of SIM associated with calcium phosphate bone graft in repairing bone defect in humans.

Resumo

Muitos estudos objetivaram desenvolver biomateriais terapêuticos alternativos para o reparo ósseo. O objetivo desta revisão sistemática foi avaliar o efeito da estatina carreada por fosfato de cálcio na formação e regeneração de tecido ósseo em modelos animais quando comparado com outros biomateriais ou coágulo. Esta revisão sistemática seguiu as recomendações do Cochrane Handbook for Systematic Reviews of Interventions, PRISMA guidelines, e o Preclinical Systematic Review & Meta-analysis Facility (SyRF). O protocolo desta revisão sistemática foi registrado no PROSPERO (CRD42018091112) e no CAMARADES. Além disso, o guia ARRIVE foi utilizado com o objetivo de aumentar a qualidade e transparência do estudo. Uma pesquisa eletrônica foi realizada através do MEDLINE/PubMed, Scopus, SciELO, e biblioteca do PROSPERO. Os autores utilizaram uma estratégia de busca específica para cada base de dados, e uma busca foi conduzida na literatura cinza e nas referências dos artigos selecionados. Os critérios de elegibilidade foram estudos em animais, os quais avaliaram o repara do ósseo tratado com fosfato de cálcio como carreador de estatina. O processo de seleção obteve 8 estudos dos 657 encontrados. Todos os estudos concluíram que a aplicação local da sinvastatina carreada pelo fosfato de cálcio é biocompatível, melhora o reparo ósseo e induz uma formação óssea significantemente maior que coágulo ou fosfato de cálcio sozinho. Em conclusão, os estudos pré-clínicos pertinentes evidenciaram a biocompatibilidade do fosfato de cálcio e sua eficácia na entrega do SIM para melhorar o reparo de defeitos ósseos. Assim, estudos clínicos são encorajados a investigar o impacto do SIM associado ao enxerto ósseo de fosfato de cálcio na reparação de defeito ósseo em humanos.

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