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(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF BRISTOL** [GB/GB]; Senate House, Tyndall Avenue, Bristol BS8 1TH (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MANSEL, Jason** [GB/GB]; Department of Oral and Dental Science, University of Bristol Dental School, Lower Maudlin St, Bristol BS10 5NB (GB). **NOWGHANI, Maryam** [GB/GB]; Department of Oral and Dental Science, University of Bristol Dental School, Lower Maudlin St, Bristol BS10 5NB (GB).

(74) Agent: **TURNER, Rhiannon**; Greaves Brewster LLP, Indigo House, Cheddar Business Park, Wedmore Road, Cheddar Somerset BS27 3EB (GB).

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(54) Title: LIGANDS OF VITAMIN D NUCLEAR RECEPTORS WITH CELL MATURATION PROMOTION FACTORS

(57) Abstract: This invention relates to the use of non-calcaemic ligands of vitamin D receptors (VDRs) in medical and/or surgical applications, particularly orthopaedic applications, particularly where osteoblast maturation for enhancement of bone repair and regeneration is desirable.

LIGANDS OF VITAMIN D NUCLEAR RECEPTORS WITH CELL MATURATION PROMOTION FACTORS

Field of the invention

This invention relates to the use of non-calcaemic ligands of vitamin D receptors (VDRs) in medical and/or surgical applications, particularly orthopaedic applications, particularly where osteoblast maturation for enhancement of bone repair and regeneration is desirable.

Background to the invention

Mature osteoblasts are responsible for providing a mechanically competent mineralised collagenous matrix. A key molecule to ensuring that bone is effectively calcified is 1α,25-dihydroxy vitamin D3 (D3) (van Driel *et al.* (2004) Current Pharmaceutical Design **10** 2535-2555; van Driel *et al.* (2006) J. Cell. Biochem. **99** 922-935). D3 deficiency in childhood leads to rickets and an inadequate D3 status in adults results in osteomalacia (Berry *et al.* (2002) Semin. Musculoskelet. Radiol. **6** 173-182).

Vitamin D exerts its effects by binding to the vitamin D receptor (VDR), which is a member of the nuclear receptor family of transcription factors. In human beings, the VDR is encoded by the gene having GenBank accession no. NG_008731 (mRNA sequence having GenBank accession no. J03258; Baker *et al.* (1988) Proc. Natl. Acad. Sci. U.S.A. **85** 3294-3298). Alternative splicing results in multiple transcript variants encoding the same protein, having the amino acid sequence of NCBI accession no. NP_000367.

Lithocholic acid (LCA), also referred to as 5β-cholanic acid, is a secondary bile acid that is also a ligand of the vitamin D receptor (Makashima *et al.* (2002) Science **296** 1313-1316). LCA and its derivative LCA acetate (also referred to as 5β-cholanic acid-3α-ol-acetate) have been shown to inhibit the proliferation and promote differentiation of human leukaemia THP-1 cells (Adachi *et al.* (2005) J. Lipid Res. **46** 46-57). Cooperation between LCA acetate and cotylenin A, a botanical fusicoccane diterpene glycoside, has shown promising pro-differentiating effects upon primary human myeloid leukaemia cells in vitro (Horie *et al.* (2008) Leuk. Res. **32** 1112-1123). In addition LCA effectively suppresses inflammatory signals in human colon epithelial Caco-2 cells, via binding to the VDR (Sun *et al.* (2008) J. Steroid. Biochem. Mol.

Biol. **111** 37-40). Collectively, these studies support LCA and its derivatives as acting as D3 surrogates.

Summary of the invention

According to a first aspect of the invention there is provided a composition comprising a non-calcaemic ligand of a VDR and a cell maturation promotion factor.

The term "cell maturation promotion factor", as used throughout this specification, indicates a compound which is capable of promoting cell maturation, i.e., which can advance maturation of cells by, for example, accelerating the life cycle of a cell and/or promoting progression of a cell to a terminally differentiated state. The cell may be an osteoblast and the term "osteoblast maturation promotion factor" has an equivalent meaning in the specific context of osteoblast cells.

The inventors have identified that non-calcaemic ligands of the VDR can act in concert with the intercellular lipid mediator lysophosphatidic acid (LPA or 1-acyl-*sn*-glycerol-3-phosphate) to promote cell maturation. In particular, it has been found that non-calcaemic ligands of the VDR expressed by osteoblasts can act in concert with LPA to promote osteoblast maturation. Therefore, the cell maturation promotion factor may be, for example, LPA.

The inventors have surprisingly identified the suitability of lithocholic acid (LCA) and its derivatives for use to act as a surrogate molecule for D3 in supporting osteoblast maturation. This is particularly advantageous because LCA and its derivatives are capable of replicating a D3 response in a variety of cell types without being associated with the undesirable side effect of eliciting a hypercalcaemic response (Nehring *et al.* (2007) Proc. Natl. Acad. Sci. U.S.A. **104** 10006-10009; Ishizawa *et al.* (2008) J. Lipid. Res. **49** 763-772). This particular property makes LCA and its derivatives very attractive in a bone tissue engineering context. Furthermore, the cost of LCA and LCA derivatives are markedly less than that of D3, making it a potentially economic surrogate steroid for bone regenerative applications.

In particular embodiments of the invention the VDR may be a receptor which is expressed by osteoblasts (for example, a receptor encoded by the gene having the nucleotide sequence of GenBank accession no. NG_008731 and/or a receptor encoded by the mRNA having the nucleotide sequence of GenBank accession no. J03258

and/or a receptor having the amino acid sequence having NCBI accession no. NP_000367; the skilled person is readily able to determine non-human equivalents of this gene and protein, such as are known in mice and rats, as well as other species). The inventors have surprisingly demonstrated that a pairing of LCA or derivatives thereof with LPA evokes a synergistic increase in the maturation of osteoblasts.

Therefore, in specific embodiments of the invention, the non-calcaemic ligand of VDR is LCA or a derivative thereof. Examples of suitable LCA derivatives are, but not limited to, LCA acetate; LCA acetate methyl ester (also referred to as methyl-3α-acetoxychonate and methylacetoxylithocholate) and LCA propionate.

The LPA, LCA or derivatives thereof mentioned throughout can be naturally-derived or synthetic analogues. The term "naturally-derived" indicates that the compound can be found occurring naturally in a cell and may, for example, be obtained by purification from a cell. The compound may also be obtained by use of recombinant technology or by synthetic chemistry methods.

In one embodiment of the invention there is provided a composition comprising LCA or a derivative thereof in combination with a cell maturation promotion factor (i.e., a second agent capable of promoting cell maturation).

In embodiments of the invention the cell is an osteoblast.

In embodiments of the invention the composition is capable of delivering an optimised therapeutic concentration (i.e., an amount shown to be therapeutically effective, for example, by routine trials) of LCA or derivative(s) thereof, wherein the amount of LCA or derivative(s) thereof administered is in the range of between about 0.5μm to 50μm, or between about 0.5μm to 30μm, or between about 0.5μm to 20μm, or between about 0.5μm to 10μm, or between about 0.5μm to 5μm.

In embodiments of the invention the composition is capable of delivering an optimised therapeutic concentration of LCA or derivative(s) thereof, wherein the amount of LCA or derivative(s) thereof administered is about 0.5μm, about 1.0μm, about 1.5μm, about 2.0μm, about 2.5μm, about 3.0μm, about 3.5μm, about 4.0μm, about 4.5μm, about 5.0μm, about 5.5μm, about 6.0μm, about 6.5μm, about 7.0μm, about 7.5μm, about 8.0μm, about 8.5μm, about 9.0μm, about 9.5μm or about 10.0μm.

In embodiments of the invention the cell maturation promotion factor (i.e., the second agent capable of promoting osteoblast maturation) is a growth factor, for example transforming factor beta (TGF-β), epidermal growth factor (EGF), a bone morphogenetic protein (BMP, for example, BMP-1, BMP-2, BMP-3, etc.), or LPA.

In embodiments of the invention the composition is capable of delivering an optimised therapeutic concentration of LPA, wherein the amount of LPA administered is in the range of between about 0.5μm to 50μm, or between about 0.5μm to 30μm, or between about 0.5μm to 20μm, or between about 0.5μm to 10μm, or between about 0.5μm to 5μm.

In a specific embodiment of the invention, the amount of LCA or derivatives thereof in the composition is sufficient to provide a therapeutic amount (e.g., concentration) of between 0.5μm to 5μm and the amount of LPA in the composition is sufficient to provide a therapeutic amount of about 20μm.

In a specific embodiment of the invention the composition comprises LCA and one or more of TGF-β, EGF, BMP and/or LPA. Alternatively, the composition comprises LCA acetate and one or more of TGF-β, EGF, BMP and/or LPA. In a further alternative, the composition comprises LCA acetate methyl ester and one or more of TGF-β, EGF, BMP and/or LPA. In a yet further alternative, the composition comprises LCA propionate and one or more of TGF-β, EGF, BMP and/or LPA. In an additional alternative, the composition may comprise one or more of LCA, LCA acetate, LCA acetate methyl ester and/or LCA propionate in combination with one or more of TGF-β, EGF, BMP and/or LPA.

The compositions according to the first aspect of the invention may be administered as a single agent or in combination with an adjunct therapy, excipient or implant.

The profile of release of the components of the compositions may optionally be time release, delayed release, sustained release, pulsed release or bulk release. The preparation of compositions having such characteristics is within the routine abilities of the skilled person.

In particular embodiments of the invention the compositions according to the invention are injectable.

In particular embodiments of the invention the compositions are associated with an orthopaedic implant, for example (but not limited to) a nail, a screw, a plate, a scaffold, a fracture putty or a joint prosthesis. The term "associated with" may indicate, for example, a coating of the composition applied to a surface of such an implant, either in the form of the composition alone or in combination with another coating composition such as (but not limited to) a varnish, a solid coating, a coating of a polymeric material, a nanocoating, a cream or a gel. Alternatively or additionally, the implant may be formed in such a way that the composition forms part of the structure of the implant. In some embodiments, the implant may be designed to degrade or partially degrade over time, to be replaced by bone formation promoted by the presence of the composition according to the invention.

The composition according to the first aspect of the invention may be for use in therapy, for example to promote osteoblast maturation and/or for use in the treatment of a bone pathology in which the promotion of osteoblast maturation is therapeutically advantageous. Such bone pathology may include, by way of non-limiting example, broken bones resulting from an accident and/or bone degradation as the result of the progression of a disease such as a cancer, osteoporosis, Paget's Disease or arthritis.

According to a second aspect of the invention there is provided a method of promoting osteoblast maturation, wherein the method comprises the step of exposing an osteoblast (i.e., one or more osteoblasts) to a composition according to the first aspect of the invention.

This method can be used for the *in-vitro* or *ex-vivo* maturation of osteoblasts.

Additionally or alternatively, this method can be used for the *in-vivo* maturation of osteoblasts in a subject in need thereof. The subject can be a human or non-human animal.

Maturation of an osteoblast can be determined, for example, by observation of an increase in alkaline phosphatase activity, as described herein.

According to a third aspect of the invention there is provided a method of promoting bone repair and regeneration in a subject in need thereof, wherein the method

comprises the steps of administering to the subject a composition according to the first aspect of the invention. The subject can be a human or non-human animal.

According to a fourth aspect of the invention there is provided a method of promoting osteoblast maturation wherein the method comprises the steps of;

i) exposing an osteoblast to a first composition comprising a non-calcaemic ligand of a VDR; and

ii) exposing said osteoblast to a second composition comprising an osteoblast maturation promotion factor.

For example, the osteoblast maturation promotion factor may be LPA. The non-calcaemic ligand of a VDR may be LCA or a derivative thereof.

Steps i) and ii) of the fourth aspect of the invention can be performed in combination or separately (for example sequentially), as long as the first and second compositions are both present at the nuclear receptor for a sufficient period of time in order that they can interact and produce a synergistic response. The first and second compositions may be combined into a single composition.

Suitable components of the first and second compositions used according to the fourth aspect of the invention are described in relation to the first aspect of the invention.

According to a fifth aspect of the invention there is provided the use of a non-calcaemic ligand of a VDR in combination with an osteoblast maturation promotion factor, in the treatment of a bone pathology in which the promotion of osteoblast maturation is therapeutically advantageous. Such bone pathology may include, by way of non-limiting example, broken bones resulting from an accident and/or bone degradation as the result of the progression of a disease such as a cancer, osteoporosis, Paget's Disease or arthritis.

The non-calcaemic ligand of a VDR may be, for example, LCA or derivatives thereof, such as LCA acetate, LCA acetate methyl ester and LCA propionate. The osteoblast maturation factor may be, for example, LPA, TGF-β, EGF or BMP.

Suitable concentrations of said ligands and LPA are discussed above in relation to the first aspect of the invention.

According to a sixth aspect of the invention there is provided the use of a non-calcaemic ligand of a VDR and an osteoblast maturation promotion factor in the manufacture of a medicament for use in the treatment of a bone pathology in which the promotion of osteoblast maturation is therapeutically advantageous. The non-calcaemic ligand of a VDR may be, for example, LCA or derivatives thereof, such as LCA acetate, LCA acetate methyl ester and LCA propionate. The osteoblast maturation factor may be, for example, LPA, TGF-β, EGF or BMP.

According to a seventh aspect of the invention there is provided an orthopaedic device comprising a composition according to the first aspect of the invention. According to an eighth aspect of the invention, there is provided a kit, for example a surgical kit, comprising an orthopaedic device and a composition according to the first aspect of the invention. An orthopaedic device may include any which is intended to engage with and/or replace a naturally occurring bone in a body (for example, a human body), such as (but not limited to) a nail, a screw, a plate, a scaffold, a fracture putty or a joint prosthesis.

According to a ninth aspect of the invention there is provided a composition, method, use or orthopaedic device as substantially herein described with reference to the accompanying Examples and Figures.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to" and do not exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking, the invention extends to any novel one, or any novel

combination, of the features disclosed in this specification (including the accompanying claims and drawings). Thus, features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

Moreover, unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

Brief Description of the Figures

Embodiments of the invention will now be described, by way of example only, with reference to the following Figures 1-5 in which:

Figure 1A shows that, within 72 hours of treatment with LPA in conjunction with LCA generates an increase in alkaline phosphatase (ALP) activity is generated, a reliable marker of osteoblast maturation;

Figure 1B shows that the data obtained for ALP activity is not attributed to increased cell numbers;

Figure 2A shows that LCA Ac cooperates synergistically with LPA in eliciting a maturation response in MG63 cells, with Figure 2B showing that cell numbers reached a maximum within 48 hours;

Figure 3A shows that LCA Ac MeO cooperated synergistically with LPA in eliciting a maturation response in MG63 cells, with Figure 3B showing that cell numbers reached a maximum within 48 hours;

Figure 4 shows that co-stimulation of osteoblasts with LCA acetate and LPA generates a demonstrable, synergistic increase in p-NP and therefore alkaline phosphatase (ALP) activity compared with all other treatment groups; and

Figure 5 shows that LCA Ac treatment results in a significant increase in AP-1 activity over the vehicle control group.

Examples

Materials and Methods

Tissue culture reagents

Tissue culture medium and fetal calf serum were obtained from Gibco (Paisley, Scotland) and essentially fatty acid free human serum albumin (FAFA) from Sigma (Poole, UK). Stocks of D3, LCA (Sigma, Poole UK), LCA acetate, LCA acetate methyl ester, LCA hemisuccinate (Makaira, London) and ZK159222 (Bayer Schering Pharma, Berlin) were prepared in ethanol and stored at -20°C. LPA (Biomol) was prepared in 1:1 ethanol:water (10mM) and stored at -20°C. Orthopaedic grade titanium (Ti) discs (12.7mm diameter, depth 2.5mm) were a generous gift from Lars Senneby, Gothenburg, Sweden. Hydroxyapatite (HA) discs (11.1mm diameter) were obtained from HiMed Inc. Bethpage, New York.

MG63 cell culture

Human osteoblast-like cells (MG63) were cultured in conventional tissue culture flasks (250 ml, Greiner) in a humidified atmosphere at 37°C and 5% CO2. Cells were grown to confluence in DMEM/F12 nutrient mix supplemented with sodium pyruvate (1 mM final concentration), L-glutamine (4 mM), streptomycin (100 ng/ml), penicillin (0.1 units/ml) and 10% v/v fetal calf serum. The growth media (500ml final volume) was also supplemented with 5ml of a 100 X stock of non-essential amino acids. Cells were grown to confluence and subsequently dispensed into blank 24-well plates (Greiner, Frickenhausen, Germany) or plates containing titanium or hydroxyapatite discs. In each case wells were seeded with 1ml of a $2x10^4$ cells/ml suspension (as assessed by haemocytometry). Cells were then cultured for 65 hours, the media removed and the cells treated with the same medium but lacking serum for 6-24 hours. In addition to the 24-well plate experiments cells were also seeded into 12-well plates for an activator protein-1 (AP-1) reporter assay.

Co-treating MG63 cells with D3/LCA/LCA derivatives and LPA. 24 –well plate experiments

The establishment of MG63 cells for stimulations within 24 well plates was identical to that described above. These osteoblast-like cells were treated with either LCA, LCA acetate, LCA acetate methyl ester or LCA hemisuccinate (0.5-30mM) in the presence or absence of 20μM LPA. As a positive control for MG63 maturation, cells

were also co-treated with LPA and 100nM D3. To substantiate that the results obtained for LCA/LCA derivatives were via the VDR, co-treated cells were exposed to ZK159222 (10μM). Cultures were left for a maximum of 72 hours prior to an assessment of cellularity and alkaline phosphatase activity (Yarram *et al.* (2004) Mol. Coll. Endocrinol. **220** 9-20; Gidley *et al.* (2006)Prost. Lipid. Med. **80** 46-61)

Assessment of cell number

An assessment of cell number was performed using a combination of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfo phenyl)-2H-tetrazolium, inner salt (MTS, Promega, UK) and the electron coupling reagent phenazine methosulphate (PMS). Each compound was prepared separately in pre-warmed (37°C) phenol red free DMEM/F12, allowed to dissolve and then combined so that 1ml of a 1mg/ml solution of PMS was combined to 19ml of a 2mg/ml solution of MTS. Immediately prior to treating cells with the MTS/PMS reagent, medium was aspirated from each of the wells and replaced with 0.5ml prewarmed (37°C) phenol red free DMEM/F12. Each well was subsequently treated with 0.1ml of the MTS/PMS reagent mixture and the plates returned to the incubator for one hour. A blank consisted of media alone (0.5 ml) plus 0.1ml of the MTS/PMS reagent mixture. Once incubated, samples (0.1ml) from each well were dispensed onto a 96 well microtitre plate and the absorbances at 492 nm read using a multiplate reader. Plates were staggered to ensure that all samples were recovered for 96-well plating within 5 minutes to minimise any error introduced during the formation of further formazan product by the cell monolayer.

Measurement of alkaline phosphatase (ALP) activity

An assessment of ALP activity is reliably measured by the generation of p-nitrophenol (p-NP) from p-nitrophenylphosphate (p-NPP) under alkaline conditions. The treatment of cells to quantify ALP activity was similar to that described previously (Yarram *et al.* 2004, Gidley *et al.* 2006). Briefly, the remaining MTS/PMS reagent was removed and the monolayers rinsed with 1ml of phenol red free DMEM/F12 which was subsequently removed and the monolayers lysed with 0.1 ml of 25 mM sodium carbonate (pH 10.3), 0.1% (v/v) Triton X-100. After 2 min each well was treated with 0.2 ml of 15 mM p-NPP (di-tris salt, Sigma, UK) in 250 mM sodium carbonate (pH 10.3), 1.5 mM $MgCl_2$. Lysates were then left under

conventional cell culturing conditions for 1 hour. After the incubation period, 0.1ml aliquots were transferred to a 96 well microtitre plate and the absorbance read at 405nm. An ascending series of p-NP (25-400 mM) prepared in the incubation buffer enabled quantification of product formation.

Activator protein-1 (AP-1) reporter assay

MG63 osteoblasts were seeded into 12-well plates such that 2ml of a $2x10^4$ cells/ml was dispensed per well. Once the cells had reached approximately 60-70% confluence (typically within 24hr) the media was removed and replaced with serum free culture medium and the cells transiently transfected with 0.5mg/well each of a Renilla control vector, pRL-TK (Promega), and 7AP-1-luc using Fugene6 transfection reagent (Roche). After 6 hours the cells were then treated with test reagents in serum free culture media and left for 18 hours prior to processing for luminometry using the Dual luciferase reagents as instructed (Promega). Luminescence was measured and the luciferase values normalised to control for transfection efficiency using the data obtained for the co-transfected control as previously described (Griffiths *et al.* (1998) Biochem. J. **335** 19-26).

Statistical analysis

Unless stated otherwise, all experiments described above were performed at least twice and all data were subject to a one-way analysis of variance (ANOVA) to test for statistical significant. When a p value of <0.05 was found, a Tukey multiple comparisons post-test was performed between all groups.

Results and discussion

Within 72 hours of treatment, LPA (20μM LPA) in conjunction with either 2.5 or 5μM LCA generated a modest, yet statistically significant (*$p<0.001$) increase in alkaline phosphatase (ALP) activity (Figure 1A), providing a reliable marker of osteoblast maturation. The data obtained for ALP activity was not attributed to increased cell numbers (Figure 1B); indeed, with the exception of cells treated with LPA alone, cells co-treated with LCA and LPA appeared to have maximal numbers at 48 hours.

At all concentrations used, LCA Ac (Figure 2A) and LCA Ac MeO (Figure 3A) cooperated synergistically with LPA (*$p<0.001$ in both cases) in eliciting a maturation

response in MG63 cells. The change in p-NP generation (and, therefore, alkaline phosphatase (ALP) activity) was most noticeable from 48 hours of treatment. As shown in Figures 2B and 3B, with the exception of LPA alone, cell numbers reached a maximum within 48 hours. After this time cell numbers appeared to decline modestly, although these data did not reach statistical significance.

The co-stimulation of osteoblasts with LCA acetate (5μM) and LPA (20μM) generated a demonstrable, synergistic increase in p-NP and therefore alkaline phosphatase (ALP) activity compared with all other treatment groups (Figure 4). The marked increase in ALP activity (and, therefore, MG63 maturation) occurred for cells grown upon both hydroxyapatite (*$p<0.001$) and commercially pure titanium discs (**$p<0.001$).

Finally, osteoblasts were seeded into wells of 12 well-plates and allowed to reach approximately 50-60% confluency prior to their transfection with a Renilla control vector, pRL-TK, and 7AP-1-luc using Fugene6 transfection reagent. Cells were subsequently treated with either vehicle control, 50ng/ml phorbol myristate acetate or 30μM LCA Ac for approximately 18 hours. Following the stimulation period, the cells were processed for luminometry to assess luciferase activity and therefore AP-1 activity relative to the vehicle control group. As shown in Figure 5, the data clearly indicate that LCA Ac treatment resulted in a significant increase (*$p=0.01$) in AP-1 activity over the control group.

A possible explanation for the synergy observed for alkaline phosphate activity in response to LCA and LPA is MEK dependent stimulation of activator protein-1 (AP-1). It is known that the AP-1 family of transcription factors plays an important role in the development and maturation of osteoblasts (Wagner (2002) Ann. Rhem. Dis. **61** 40-42; Marie (2008) Arch. Biochem. Biophys. **473** 98-105). Furthermore, the inventors have already established that the maturation incurred by co-treating osteoblasts with LPA and D3 is MEK dependent (Gidley *et al.* 2006). It has been found that a short stimulation period of 18 hours with 30mM LCA acetate results in a demonstrable increase in the activation of the AP-1 transcription complex. Thus, without wishing to be bound by theory, LCA/LCA derivatives may aid human osteoblast differentiation by influencing the transcriptional activity of this factor. It is proposed that the synergy incurred to ALP activity in response to LPA and LCA

might be a consequence of two transcriptional factors, i.e., AP-1 and the LCA-VDR complex, acting at different loci within the ALP promoter.

Claims

1. A composition comprising a non-calcaemic ligand of a VDR, and a cell maturation promotion factor.

2. A composition according to claim 1, wherein the VDR is a receptor which is expressed by an osteoblast.

3. A composition according to claim 1 or 2, wherein the non-calcaemic ligand of a VDR is LCA or a derivative thereof.

4. A composition according to claim 3, wherein the derivative is LCA acetate, LCA acetate methyl ester or LCA propionate.

5. A composition according to any of claims 2-4, wherein the cell is an osteoblast.

6. A composition according to any preceding claim, wherein the cell maturation promotion factor is a growth factor.

7. A composition according to any preceding claim, wherein the cell maturation promotion factor is transforming factor beta (TGF-β), epidermal growth factor (EGF), or LPA.

8. A composition according to claim 5 wherein the cell maturation promotion factor is a bone morphogenetic protein (BMP).

9. A composition according to any preceding claim for use in therapy.

10. A composition according to claim 9 for use in promoting osteoblast maturation.

11. A composition according to claim 9 or 10 for use in the treatment of a bone pathology in which the promotion of osteoblast maturation is therapeutically advantageous.

12. A method of promoting osteoblast maturation, wherein the method comprises the steps of exposing an osteoblast to a composition according to any of claims 1-8.

13. A method of promoting bone repair and regeneration in a subject in need thereof, wherein the method comprises the step of administering a composition according to any of claims 1-8.

14. A method of promoting osteoblast maturation, wherein the method comprises the steps of;
    i) exposing an osteoblast to a first composition comprising a non-calcaemic ligand of a VDR; and
    ii) exposing the osteoblast to a second composition comprising an osteoblast maturation promotion factor.

15. A method according to claim 14 wherein the non-calcaemic ligand of a VDR is LCA or a derivative thereof.

16. A method according to claim 15 wherein the osteoblast maturation promotion factor is LPA.

17. Use of a non-calcaemic ligand of a VDR and an osteoblast maturation promotion factor in the manufacture of a medicament for use in the treatment of a bone pathology in which the promotion of osteoblast maturation is therapeutically advantageous.

18. Use according to claim 17 wherein the non-calcaemic ligand of a VDR is LCA or a derivative thereof.

19. An orthopaedic device comprising a composition according to any of claims 1-8.

20. A kit comprising an orthopaedic device and a composition according to any of claims 1-8.

Fig. 1A

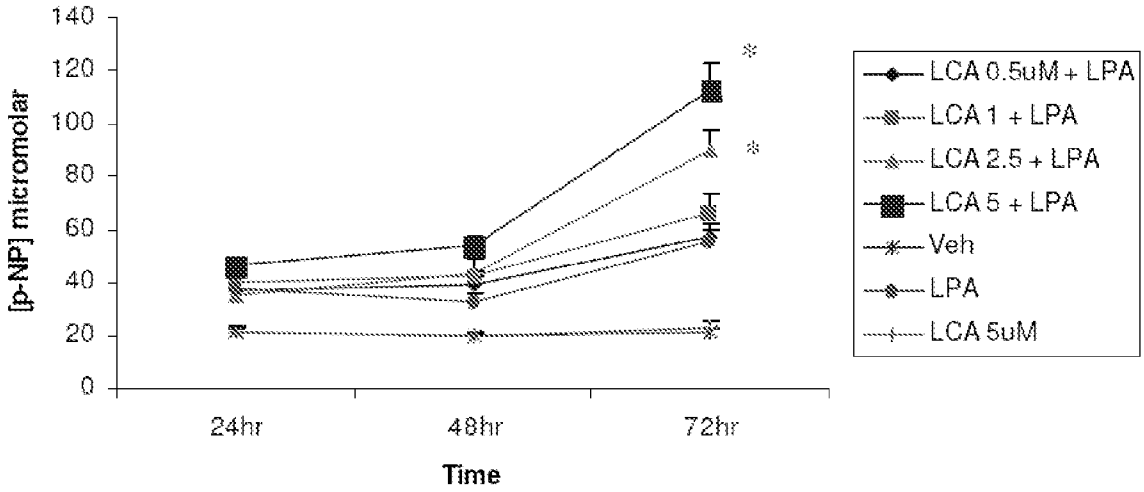

Fig. 1B

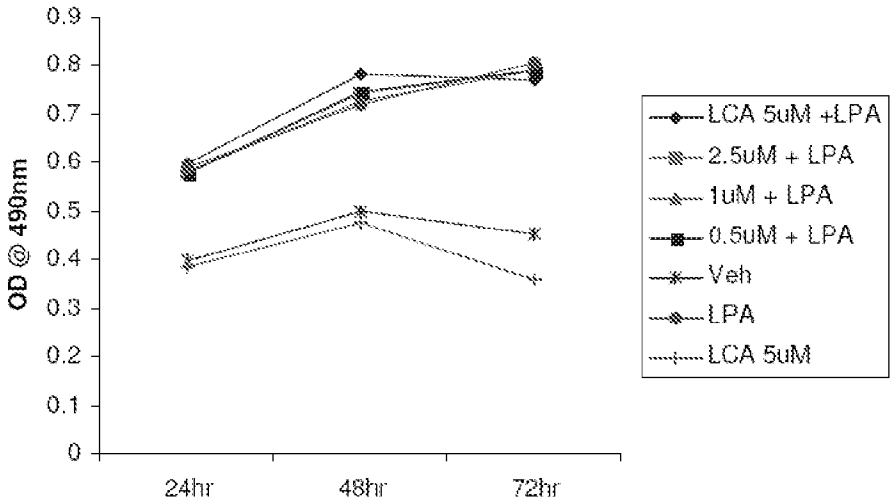

Figure 1

Fig 2A

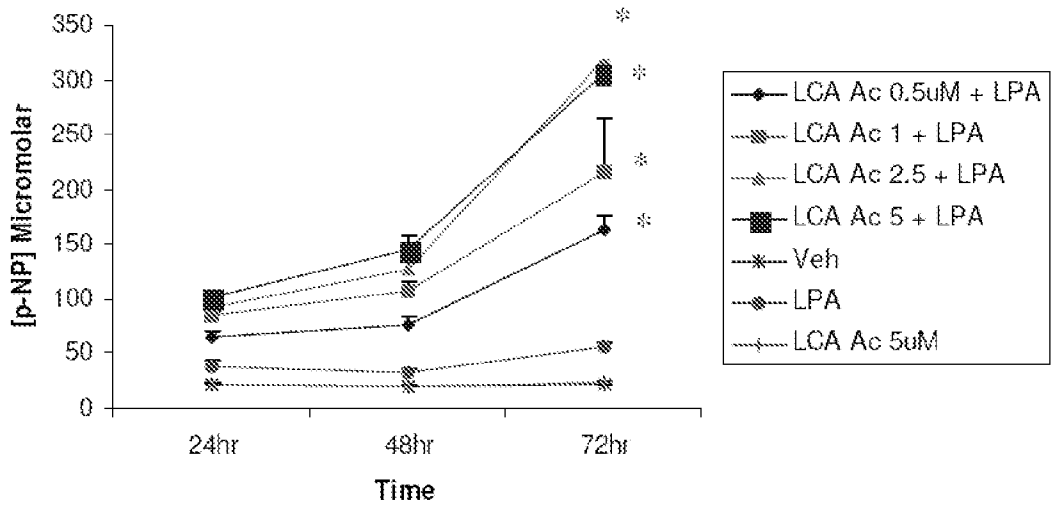

Fig. 2B

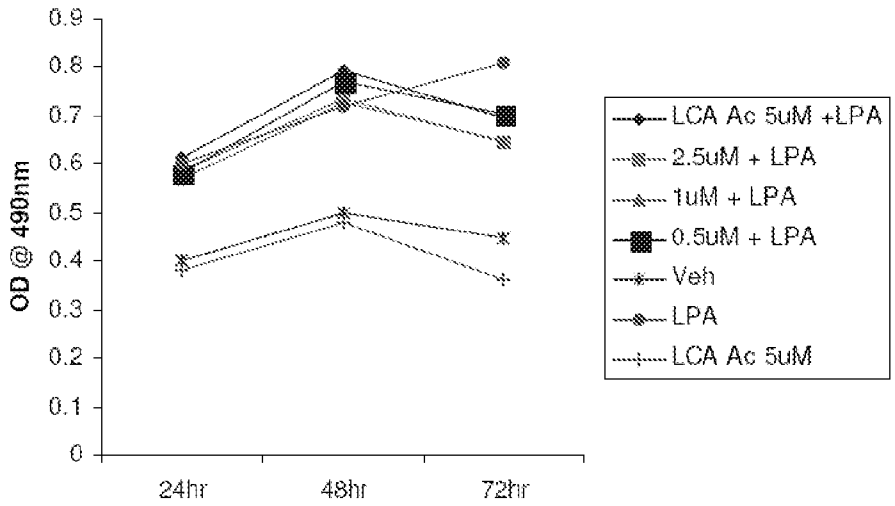

Figure 2

Fig 3A

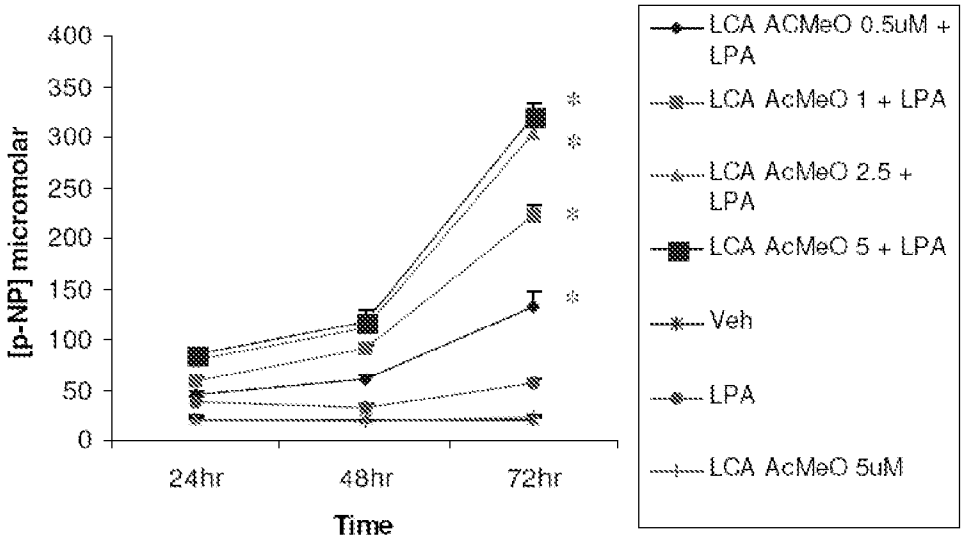

Fig 3B

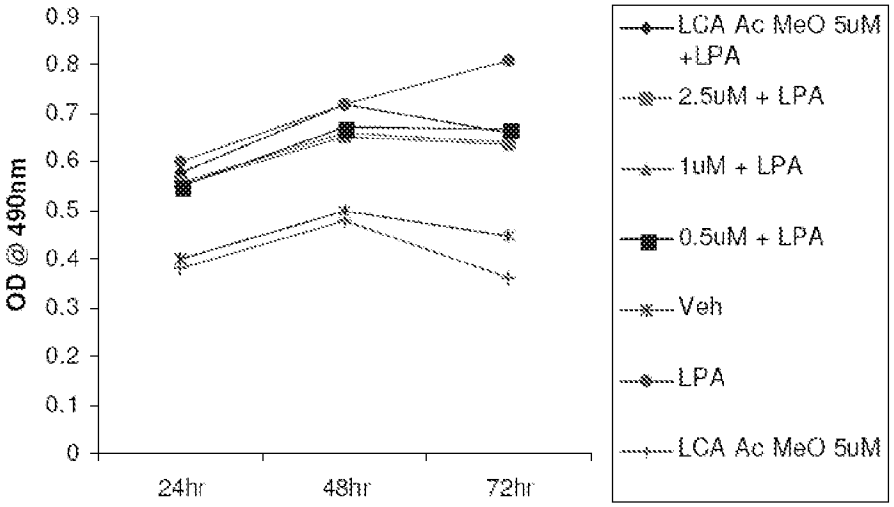

Figure 3

Fig. 4.

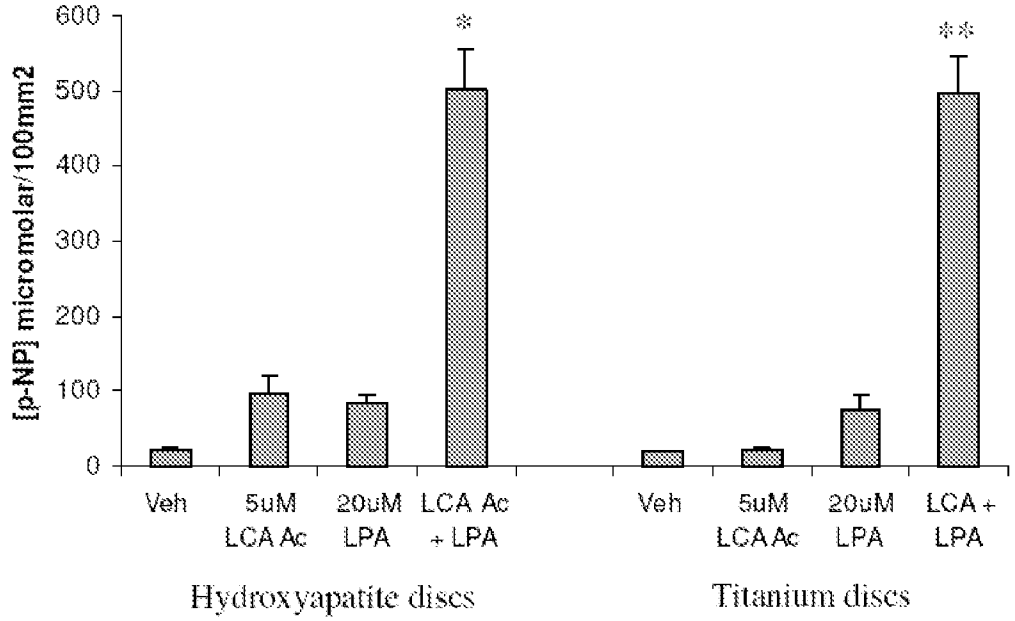

Figure 4

Fig. 5.

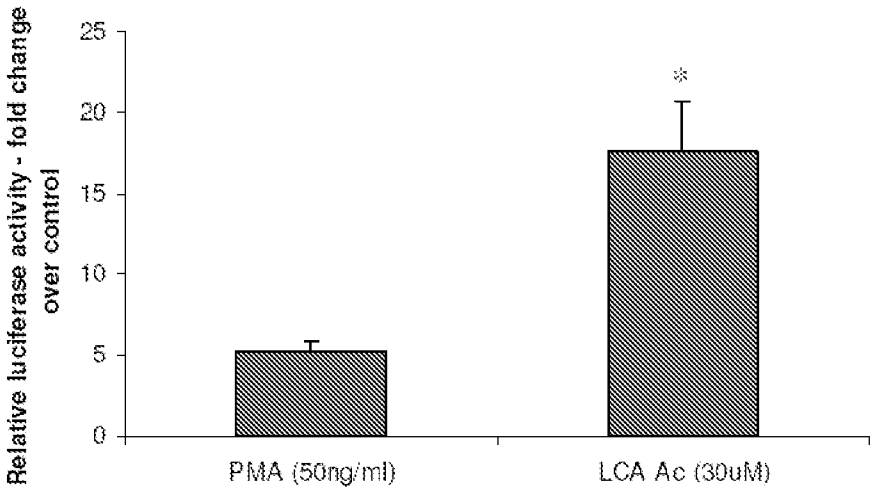

Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/051505

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/575 A61K31/661 A61K38/18 A61K45/06 A61P19/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/110465 A2 (PLIVA ISTRAZIVACKI INST D O O [HR]; VUKICEVIC SLOBODAN [HR]; SIMIC PET) 24 November 2005 (2005-11-24) claims 1,19	1-3,5,8
X	WO 2005/027926 A1 (PFIZER PROD INC [US]; CAMPAGNARI JUDITH LEE [US]) 31 March 2005 (2005-03-31) claims	1-2,5, 8-9,11, 17

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2010	08/03/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pacreu Largo, Marta

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GIDLEY J ET AL: "Lysophosphatidic acid cooperates with 1alpha,25(OH)2D3 in stimulating human MG63 osteoblast maturation" PROSTAGLANDINS AND OTHER LIPID MEDIATORS, ELSEVIER, US, vol. 80, no. 1-2, 1 July 2006 (2006-07-01) , pages 46-61, XP025172697 ISSN: 1098-8823 [retrieved on 2006-07-01] abstract	1-20
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A	ISHIZAWA MICHIYASU ET AL: "Lithocholic acid derivatives act as selective vitamin D receptor modulators without inducing hypercalcemia" JOURNAL OF LIPID RESEARCH, vol. 49, no. 4, April 2008 (2008-04), pages 763-772, XP002569346 ISSN: 0022-2275 cited in the application	1-4, 10-20
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	MANSELL J P ET AL: "Lithocholate-A promising non-calcaemic calcitriol surrogate for promoting human osteoblast maturation upon biomaterials" STEROIDS, ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY, US, vol. 74, no. 12, 4 November 2009 (2009-11-04), pages 963-970, XP026587184 ISSN: 0039-128X [retrieved on 2009-07-29] the whole document	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2009/051505

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CN 101001641 A	18-07-2007
		EP 1750743 A2	14-02-2007
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