The Effects of Vitamin E Analogues α-Tocopherol and γ-Tocotrienol on the Human Osteocyte Response to Ultra-high Molecular Weight Polyethylene Wear Particles

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Abstract

Background

Polyethylene (PE) liners are a common bearing surface of orthopaedic prostheses. In some products, vitamin E is added to PE as a preventive measure against oxidative degradation. Ultra-high molecular weight PE (UHMWPE) wear particles contribute to periprosthetic osteolysis, a major cause of aseptic loosening. Osteocytes are an important cell type for controlling both bone mineralisation and bone resorption, and respond to UHMWPE particles by inducing both osteocytic osteolysis and support of osteoclastogenesis. Here, we examined the effects of the vitamin E analogues α-tocopherol and γ-tocotrienol alone or in the context of wear particles on human osteocyte gene expression and mineralisation behaviour.

Methods

Human primary osteoblasts isolated from patients undergoing hip arthroplasty were differentiated for 28d to an osteocyte-like stage. Cultures were exposed to UHMWPE wear particles in the presence or absence of either α-Tocopherol or γ-Tocotrienol. Gene expression related to the oxidative response, osteocytic osteolysis and osteoclastogenesis was measured by real-time RT-PCR. The effect on calcium release from mineralised osteocyte-like cultures was also measured.

Results

Both α-Tocopherol and γ-Tocotrienol upregulated expression of several antioxidant-related genes. UHMWPE particles also upregulated antioxidant gene expression, suggesting the involvement of oxidative stress pathways. UHMWPE particles upregulated the pro-osteoclastogenic *RANKL*:*OPG* mRNA ratio, however, this was reduced in the presence of vitamin E analogues. UHMWPE particles also upregulated mRNA expression of osteocytic-osteolysis related genes. However, treatment with either analogue alone and in combination

with UHMWPE particles also resulted in significant upregulation of these genes. Consistent with this, both vitamin E analogues promoted calcium release from mineralised cultures of osteocyte-like cells.

Discussion

Our findings suggest that vitamin E analogues may suppress osteocyte support of osteoclastogenesis in response to UHMWPE particle exposure. Vitamin E analogues exert an antioxidant effect on osteocytes, however, this appears to involve the induction of osteocytic osteolysis, which could promote periprosthetic osteolysis. It will be important to further elucidate the effects of vitamin E on the cells of the prosthetic joint in order to understand the potential effects of their inclusion in prosthetic materials.

Introduction

Implant loosening is a major problem associated with total hip replacement (THR). A main cause of aseptic loosening is the development of peri-prosthetic osteolytic lesions. Wear particles, in particular ultra-high molecular weight polyethylene (UHMWPE) wear particles, are directly associated with the production of osteolytic lesions, where high volumetric wear rates correlate with the greatest progression of lesion volume (1). UHMWPE wear particles are produced from the mechanical friction of the liner against the metal femoral head, exacerbated by the oxidative degradation of the polyethylene (PE). UHMWPE wear particles have been shown to stimulate a variety of cellular pathways, including promoting bone resorption, inducing inflammation, and inhibiting bone formation (2), as well as inducing osteocyte peri-lacunocanalicular remodelling or osteocytic osteolysis (3).

The addition of Vitamin E analogues is a recent approach to improving the endurance of UHMWPE liners by preventing oxidation. The aim of imbuing PE liners with Vitamin E is to reduce the formation of free radicals within the PE, as well as improving fatigue properties that would normally occur after post-irradiation melting, without sacrificing fatigue strength (4). Vitamin E, specifically α-tocopherol, can be blended with UHMWPE powder prior to radiation, which protects against oxidation within the PE chain but reduces the crosslinking efficiency (5-7). Alternatively, UHMWPE is diffused with vitamin E after radiation, circumventing possible changes to the crosslinking efficiency (8). However, diffusion of vitamin E would result in relatively less vitamin E covalently bound within the PE chain compared to addition prior to radiation, potentially allowing unbound vitamin E to leach into the synovial fluid and surrounding tissues, including bone, *in vivo* (9).

Both Tocopherols and Tocotrienols play potentially important roles in regulating bone resorption (10, 11). α-Tocopherol has been shown to have varying effects on both bone formation and resorption, dependent on the dose and model system investigated (10). For

example, specific knockdown of the α-tocopherol transfer protein in mice, where vitamin E absorption is blocked, showed a significantly higher bone mass due to lower bone resorption and reduced osteoclast surface (12). Tocotrienol is able to reduce oxidative damage in osteoblasts, promoting osteoblast differentiation and survival (13). Human epidemiological studies have also investigated the relationship between vitamin E and bone metabolism, however, with mixed results. One study, in a US population aged 50 and older, described a negative correlation between the high serum concentration of tocopherol and the femoral neck BMD (14). Similarly, a study by Wolf *et al.* showed a negative correlation between total vitamin E intake and femoral neck BMD in an age-adjusted regression analysis of women in the USA (15). However, a longitudinal study on Swedish subjects over the age of 50 and monitored for 19 years, has shown an association between low intake and serum concentration of α-tocopherol, and an increased rate of fracture (16).

Osteocytes are the most abundant cell type in bone and are critical in controlling bone remodelling and maintaining skeletal integrity (17). Osteocytes are capable of resorbing their perilacunar matrix by producing bone degrading enzymes, including matrix metallopeptidase 13 (*MMP13*) (18), Cathepsin K (*CTSK*) (19) and carbonic anhydrase II (*CA2*) (20). It was previously shown that human osteocytes exposed to UHMWPE particles *in vitro* display both osteocytic osteolytic and pro-osteoclastogenic responses (3, 21). Examination of human bone biopsies from implant patients with aseptic loosening and with confirmed periprosthetic osteolysis, also showed significantly increased osteocyte lacunar area compared to those in primary THR biopsies (3, 21). Little is known regarding the effects of UHMWPE particles on bone cells such as osteocytes in the added presence of vitamin E, in terms of the catabolic bone remodelling response. In this study, we investigated the biological effects of two major

analogues of vitamin E, α-Tocopherol and γ-Tocotrienol, in the presence or absence of UHMWPE particles, on human primary osteocyte-like cells *in vitro*.

Materials and Methods

Ethics statement

All patient-derived samples were obtained with written, informed consent and human research ethics approval (Royal Adelaide Hospital Human Research Ethics Committee approval no. 090101).

Human osteocyte-like cells

Human primary osteoblasts were derived from proximal femoral cancellous bone biopsies, taken from patients undergoing total hip replacement surgery, and cultured *ex vivo*, as described previously (22). Human primary osteoblasts were cultured for 28 days until the cells reached a mature osteocyte-like stage, as described previously (3). The cells were then treated for a further 7d with either α-Tocopherol or γ-Tocotrienol (Sigma Aldrich, St Louis, MO, USA) at concentrations of 100 nM, 300 nM, 1 µM or untreated control.

Similarly, osteocyte-like cells after 28d of culture were overlaid with a collagen gel (Cellmatrix Type 1-A, Nitta, Tokyo, Japan) (3), containing UHMWPE particles (Ceridust 3615, Clariant Company) (100µg/ml), with the addition of either α-Tocopherol or γ-Tocotrienol (Sigma Aldrich, St Louis, MO, USA) (1 µM), or particle free and vehicle controls, and cultured for a further 21d. UHMWPE particles were confirmed to be endotoxin free and were sized from 0.3-10 µM. Either α-Tocopherol or γ-Tocotrienol, 1 µM was added to the culture medium every 3 days.

Gene Expression

Total RNA was extracted using TRIzol (Life Technologies, NY, USA) and complementary DNA was synthesised using iScript™ RT kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. Real time RT-PCR was performed using SYBR Green Fluor qPCR Mastermix (Qiagen, Limburg, The Netherlands) on a CFX Connect (Bio-Rad, Hercules, CA, USA). Gene expression was normalised to that of the housekeeping gene, *HPRT1*. Oligonucleotide sequences for the mRNA-specific amplification of *MMP13*, *CA2*, *CTSK*, *RANKL*, *OPG* and *CSF1* are published (3). Oligonucleotide sequences for *HPRT1*, *SOD1*, *SOD2* and *CAT* are listed in **Table 1**. All primers were designed in-house to be mRNA specific by at least one per pair flanking an intron-exon boundary, and all were purchased from Sigma Aldrich (St. Louis, MO, USA).

Gene	Sequence (5′ – 3′)	Direction	Amplicon size
HPRT1	TGACCTTGATTTATTTTGCATACC CGAGCAAGACGTTCAGTCCT	Forward Reverse	102bp
SOD1	TGAAGAGAGGCATGTTGGAGAC TGGGCGATCCCAATTACACC	Forward Reverse	231bp
SOD2	GGCCTACGTGAACAACCTGA GCAACTCCCCTTTGGGTTCT	Forward Reverse	185bp
CAT	CCTGTGAACTGTCCCTACCG AATGCCCGCACCTGAGTAAC	Forward Reverse	218bp

Table 1: Sequences of sense (S) and anti-sense (AS)-specific oligonucleotide primers and predicted PCR product sizes. Primers were designed to be mRNA-specific.

In vitro mineralisation

Calcium deposition was visualised using the Alizarin Red stain, as described previously (23). Briefly, quadruplicate wells per treatment were washed with PBS and then fixed with 10% neutral buffered formalin for 1 hour at RT and then washed with PBS (3x). Each well was stained with aqueous 2% (w/v) Alizarin red solution (Sigma Aldrich) at pH 4.2, for 5 minutes at room temperature and then washed with distilled water to remove any unbound stain. Each well was imaged (Olympus SZ2-ILST, Tokyo, Japan) and then the bound stain was solubilised with 100 µl of 10% Cetylpyrridium chloride (CPC) (Sigma Aldrich) in 10mM Sodium phosphate monobasic monohydrate (pH 7.0) added to each well for 15 minutes at RT. The optical density was determined at 560nm by spectrophotometry (Thermo Multiskan Ascent).

Results and Discussion

Effects of UHMWPE and Vitamin E analogues on the oxidative stress response

In order to investigate the effects of vitamin E analogues and UHMWPE particles, we used a clinically relevant model where human primary osteoblasts isolated from THR implant recipients, the population at risk from developing periprosthetic osteolysis, were cultured for 28 days under pro-osteogenic conditions to generate osteocyte-like cultures (3). Once established, the cells were overlaid with a type I collagen gel containing UHMWPE particles, with the addition of either α-Tocopherol or γ-Tocotrienol and cultured for a further 21 days.

Vitamin E is an attractive bone supplement due to its anti-inflammatory and antioxidant activities. The induction of antioxidant gene expression can be both a marker of oxidative stress and an indication of a physiological response to the causative reactive oxygen species (ROS). Therefore, we investigated the expression of key antioxidant genes, superoxide dismutases 1 and 2 (*SOD1* and *SOD2*), which are produced in response to ROS and act by binding to superoxide radicals, metabolising them to oxygen and hydrogen peroxide (24). The expression

of catalase (*CAT*) was also examined, which converts hydrogen peroxide into water and oxygen (24). Osteocyte-like cultures treated with either α-Tocopherol or γ-Tocotrienol significantly upregulated the expression of all three antioxidant genes (**Fig. 1A-C**). Moderate upregulation of these genes occurred with exposure to UHMWPE particles alone, suggesting induction of oxidative stress. Importantly, both Vitamin E analogues were able to further increase the expression of these genes in the presence of UHMWPE particles. Previous studies have shown that PE liners and the resulting particles undergo oxidative degradation and can generate ROS (25, 26). Therefore, upregulation of antioxidant genes could be a protective mechanism by the osteocyte in response to ROS produced by the UHMWPE particles, preventing DNA damage and potential cell death. Consistent with this protective mechanism, we have previously reported that UHMWPE particles do not induce apoptosis in human osteocyte-like cells (3).

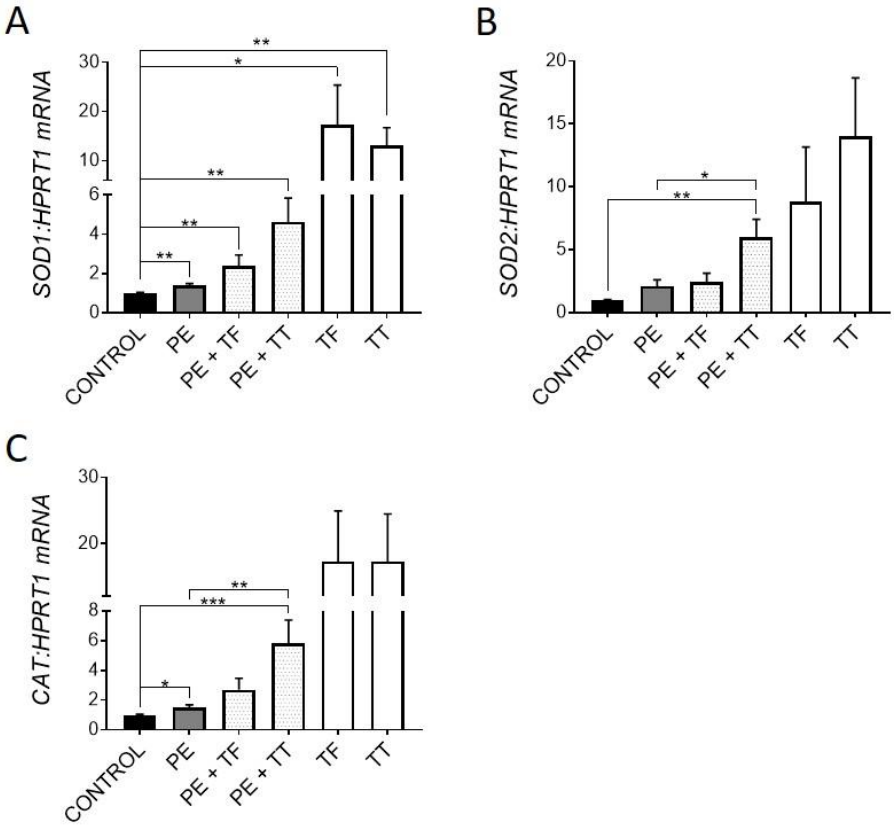

Figure 1: Effect of vitamin E and UHMWPE particles on antioxidant gene expression. Human osteocyte-like cultures were overlaid with collagen gel containing UHMWPE particles and either α-Tocopherol (TF) or γ-Tocotrienol (TT), and cultured for a further 21d. Relative gene expression was measured for antioxidant genes *SOD1*, *SOD2* and Catalase (A-C). Data shown are means ± standard error of the mean (SEM) of quadruplicate experiments analysed in duplicate. Significant differences are denoted by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Effects of UHMWPE and Vitamin E on the pro-osteoclastogenic response

We next investigated the expression of genes associated with osteoclastogenesis. It was previously shown that osteocytes respond to UHMWPE wear particles by upregulating the pro-osteoclastogenic *RANKL*:*OPG* mRNA ratio, as well as upregulating key osteoclastic regulatory genes, *CSF1* and *IL8* (3, 27). Here, osteocytes responded to treatment with either α-Tocopherol and γ-Tocotrienol by downregulating the pro-osteoclastogenic *RANKL*:*OPG* mRNA ratio. In particular, treatment with γ-Tocotrienol suppressed *RANKL* and increased *OPG* mRNA expression in the primary human osteocytes, (**Fig. 2A-B**), whereas α-Tocopherol, whilst having

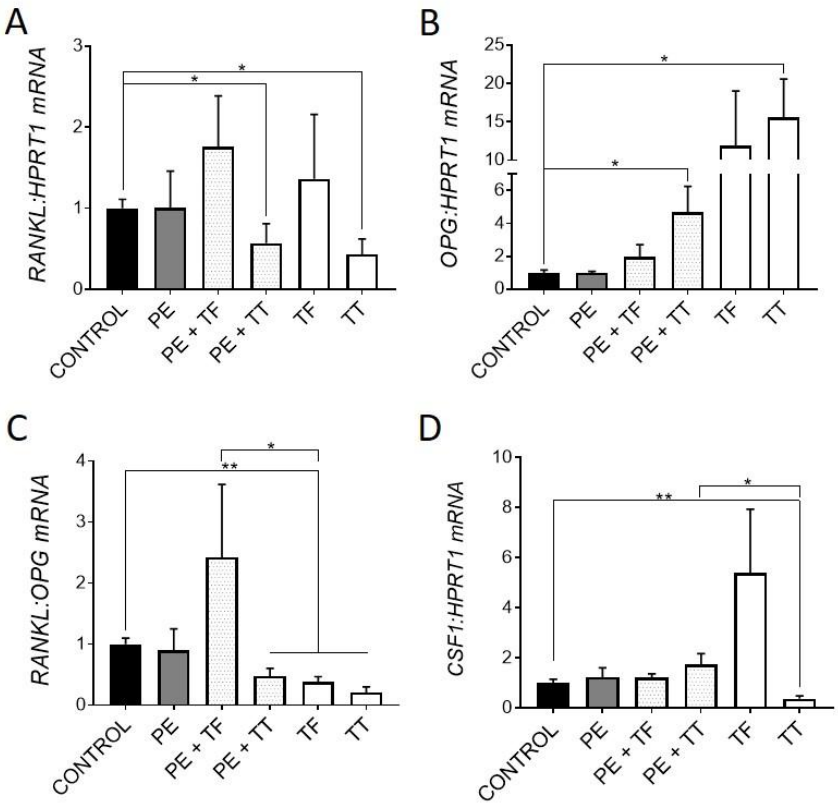

Figure 2: Effect of vitamin E and UHMWPE particles on osteoclastogenic gene expression. Relative gene expression was measured for genes associated with osteoclastogenesis: *RANKL*, *OPG*, the pro-osteoclastogenic *RANKL*:*OPG* mRNA ratio and *CSF1* mRNA expression (A-D). Data shown are means ± standard error of the mean (SEM) of quadruplicate experiments analysed in duplicate. Significant differences are denoted by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

non-significant effects on either gene alone, also resulted in reduced *RANKL*:*OPG* expression overall. The addition of α-Tocopherol had no effect on the UHMWPE response in terms of this ratio. However, γ-Tocotrienol significantly reduced the *RANKL*:*OPG* mRNA ratio in the presence of particles. UHMWPE particles also strongly upregulated *CSF1* mRNA expression, which was effectively suppressed by either vitamin E analogue (Fig. 2D). Vitamin E analogues alone significantly downregulated *CSF1* expression. Overall, these findings suggest that both α-Tocopherol and γ-Tocotrienol exert an anti-osteoclastogenic effect on human osteocyte-like

cells, in the presence or absence of UHMWPE particles. Previous studies have shown that mice treated with vitamin E, either α-Tocopherol or γ-Tocotrienol, have decreased bone resorption (11, 28). Consistent with these results, a study by Bichara *et al*. (29) showed Vitamin E bound UHMWPE wear particles significantly decreased the amount of bone resorption in a mouse calvarial model of osteolysis when compared to conventional UHMWPE particles.

Effects of UHMWPE and Vitamin E on the osteocytic osteolysis response

Consistent with a previous study (3), UHMWPE particles induced expression of genes associated with osteocytic osteolysis, including *MMP13*, *CA2* and *CTSK* (**Fig. 3A-C**).

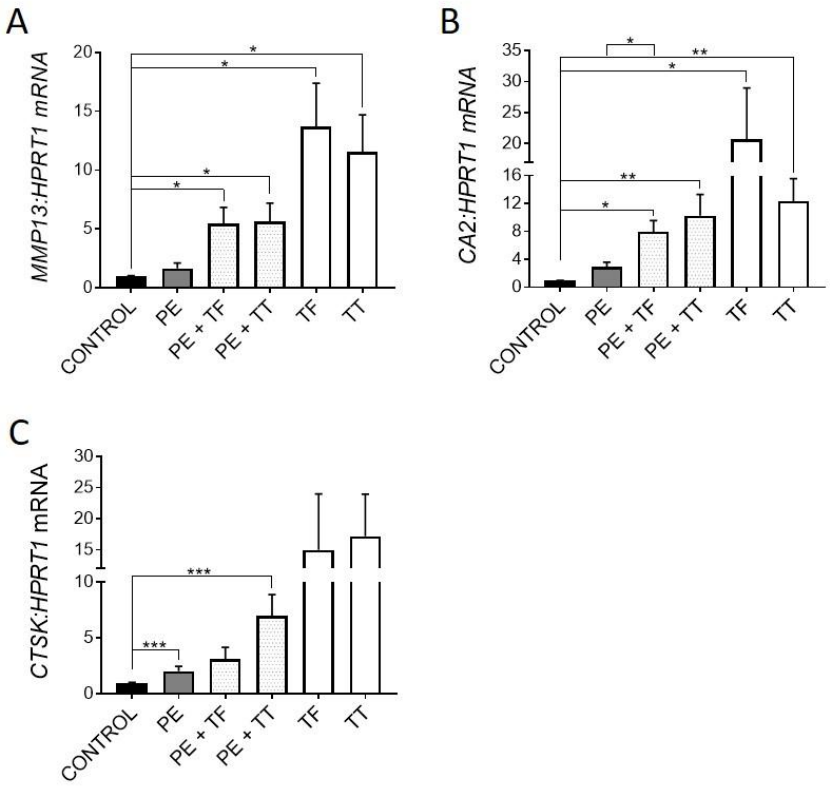

Figure 3: Effect of vitamin E and UHMWPE particles on expression of genes associated with osteocytic osteolysis. A. *MMP13* mRNA expression, B. *CA2* mRNA expression, C. *CTSK* mRNA expression. Data shown are means ± standard error of the mean (SEM) of quadruplicate

experiments analysed in duplicate. Significant differences are denoted by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Similarly, UHMWPE particles increased the expression of *CA2* and *CTSK*, concurrent with the increase in antioxidant genes as described in Figure 1. Treatment with either Vitamin E analogue significantly upregulated the expression of all three genes, suggesting that Vitamin E could promote osteocytic osteolysis. Both α-Tocopherol and γ-Tocotrienol were able to further increase the expression of these genes in the presence of PE particles. As mentioned previously, UHMWPE particles are capable of producing ROS, which play a key role in osteoclastic differentiation and activity (30). Therefore, the osteocytes in this study could be responding to ROS through one of two pathways. Firstly, the inhibition of ROS in human osteocytes by treatment with Vitamin E analogues alone and in the presence of UHMWPE particles may have caused the increase in expression of genes associated with osteocytic osteolysis, *MMP13*, *CTSK* and *CA2*. Secondly, UHMWPE particles may stimulate the production of ROS by the osteocyte, inducing oxidative stress, triggering a perilacunar remodelling response. This effect would explain why there is a significant upregulation of antioxidant genes by the osteocyte in response to UHMWPE. Therefore, removal of perilacunar bone could be one of the mechanisms used by osteocytes to relieve oxidative stress. Further investigation is required to determine if ROS produced by the osteocyte plays a role in regulating osteocytic osteolysis.

Effects of Vitamin E analogues on osteocytic regulation of mineralisation

To investigate the effects of α-Tocopherol and γ-Tocotrienol on osteocytic regulation of mineralisation, osteocyte-like cultures were exposed to either analogue (0, 100 nM, 300 nM and 1 µM) in the absence of UHMWPE particles. Calcium deposition was measured using the Alizarin Red stain, as described previously (23). Consistent with the observed regulation of osteocytic osteolysis related genes, treatment with either Vitamin E analogue resulted in decreased mineralisation (**Fig. 4**). This is consistent with the upregulation by Vitamin E

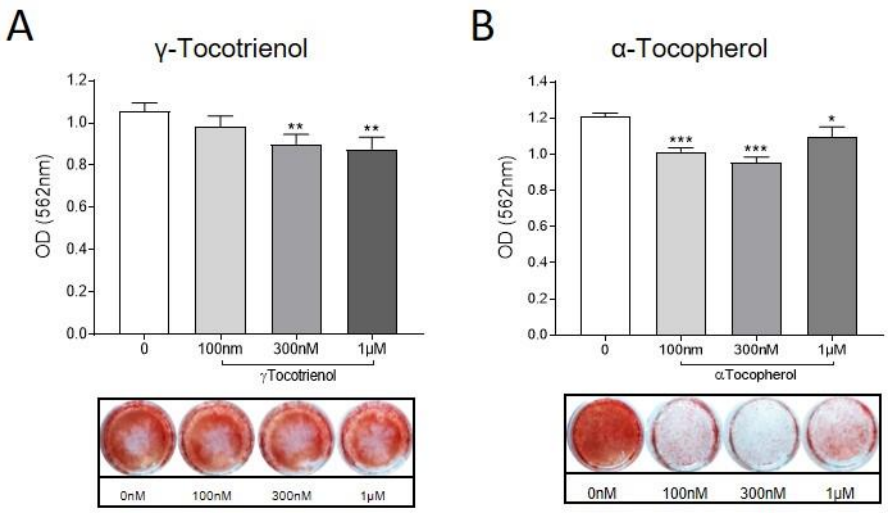

Figure 4: Effect of vitamin E analogues on osteocyte mineral handling. Human osteocyte-like cultures were treated with either α-Tocopherol (TF) or γ-Tocotrienol (100nM, 300nM, 1µM) or untreated (0) for a further 6d. Cells were stained with Alizarin Red and then quantified using Cetylpyrridium chloride to elute the bound stain. Absorbance at 560nM was measured by spectrophotometry. Data shown are means ± standard deviation of quadruplicate wells. Significant differences are denoted by $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

treatment of genes associated with perilacunar remodelling, including *CA2*, which has been shown to be important during calcium release through osteocytic acidification of the bone matrix (20). This suggests that vitamin E could contribute to the loss of bone that occurs during

periprosthetic osteolysis specifically through promoting osteocytic removal of perilacunar bone.

Conclusion

This study demonstrates the potential effects of α-Tocopherol and γ-Tocotrienol on the regulation of bone resorption by the human osteocyte. Our data suggest that UHMWPE particles induce oxidative stress in osteocytes, which could be a potential trigger of perilacunar remodelling. Two distinct actions of the vitamin E analogues were observed: suppression of the pro-osteoclastic response, and stimulation of the osteocytic osteolysis response. Thus, while our findings are consistent with vitamin E suppressing the osteoclastic response to UHMWPE particles, it could exacerbate an osteocytic osteolysis response, which could contribute to periprosthetic osteolysis. In conclusion, the effects of vitamin E analogues on osteocytes requires further investigation to determine if their lytic effects have potential consequences in the development of periprosthetic osteolysis and contribute to aseptic loosening.

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References

1. Howie DW, Neale SD, Stamenkov R, McGee MA, Taylor DJ, Findlay DM. Progression of acetabular periprosthetic osteolytic lesions measured with computed tomography. J Bone Joint Surg Am. 2007;89(8):1818-25. PubMed PMID: 17671023.

2. Goodman S, Aspenberg P, Song Y, Regula D, Lidgren L. Polyethylene and titanium alloy particles reduce bone formation. Dose-dependence in bone harvest chamber experiments in rabbits. Acta orthopaedica Scandinavica. 1996;67(6):599-605. Epub 1996/12/01. PubMed PMID: 9065075.

3. Ormsby RT, Cantley M, Kogawa M, Solomon LB, Haynes DR, Findlay DM, et al. Evidence that osteocyte perilacunar remodelling contributes to polyethylene wear particle induced osteolysis. Acta Biomater. 2016;33:242-51. Epub 2016/01/23. doi: 10.1016/j.actbio.2016.01.016. PubMed PMID: 26796208.

4. Costa L, Luda MP, Trossarelli L, Brach del Prever EM, Crova M, Gallinaro P. Oxidation in orthopaedic UHMWPE sterilized by gamma-radiation and ethylene oxide. Biomaterials. 1998;19(7-9):659-68. PubMed PMID: 9663738.

5. Oral E, Beckos CG, Malhi AS, Muratoglu OK. THE EFFECTS OF HIGH DOSE IRRADIATION ON THE CROSS-LINKING OF VITAMIN E-BLENDED ULTRAHIGH MOLECULAR WEIGHT POLYETHYLENE. Biomaterials. 2008;29(26):3557-60. doi: 10.1016/j.biomaterials.2008.05.004. PubMed PMID: PMC2516939.

6. Oral E, Greenbaum ES, Malhi AS, Harris WH, Muratoglu OK. Characterization of irradiated blends of alpha-tocopherol and UHMWPE. Biomaterials. 2005;26(33):6657-63. Epub 2005/07/05. doi: 10.1016/j.biomaterials.2005.04.026. PubMed PMID: 15993487; PubMed Central PMCID: PMCPMC1463566.

7. Parth M, Aust N, Lederer K. Studies on the effect of electron beam radiation on the molecular structure of ultra-high molecular weight polyethylene under the influence of alpha-

tocopherol with respect to its application in medical implants. Journal of materials science Materials in medicine. 2002;13(10):917-21. Epub 2004/09/07. PubMed PMID: 15348184.

8. Oral E, Wannomae KK, Hawkins N, Harris WH, Muratoglu OK. Alpha-tocopherol-doped irradiated UHMWPE for high fatigue resistance and low wear. Biomaterials. 2004;25(24):5515-22. doi: 10.1016/j.biomaterials.2003.12.048. PubMed PMID: 15142733.

9. Jarrett BT, Cofske J, Rosenberg AE, Oral E, Muratoglu O, Malchau H. In vivo biological response to vitamin E and vitamin-E-doped polyethylene. The Journal of bone and joint surgery American volume. 2010;92(16):2672-81. doi: 10.2106/JBJS.I.00068. PubMed PMID: 21084577.

10. Chin KY, Ima-Nirwana S. The effects of alpha-tocopherol on bone: a double-edged sword? Nutrients. 2014;6(4):1424-41. Epub 2014/04/15. doi: 10.3390/nu6041424. PubMed PMID: 24727433; PubMed Central PMCID: PMC4011043.

11. Chin KY, Ima-Nirwana S. The biological effects of tocotrienol on bone: a review on evidence from rodent models. Drug Des Devel Ther. 2015;9:2049-61. Epub 2015/04/22. doi: 10.2147/dddt.s79660. PubMed PMID: 25897211; PubMed Central PMCID: PMCPMC4396581.

12. Fujita K, Iwasaki M, Ochi H, Fukuda T, Ma C, Miyamoto T, et al. Vitamin E decreases bone mass by stimulating osteoclast fusion. Nat Med. 2012;18(4):589-94. Epub 2012/03/06. doi: 10.1038/nm.2659. PubMed PMID: 22388090.

13. Fatokun AA, Stone TW, Smith RA. Responses of differentiated MC3T3-E1 osteoblast-like cells to reactive oxygen species. European journal of pharmacology. 2008;587(1-3):35-41. doi: 10.1016/j.ejphar.2008.03.024. PubMed PMID: 18448093.

14. Zhang J, Hu X, Zhang J. Associations between serum vitamin E concentration and bone mineral density in the US elderly population. Osteoporosis Int. 2016:1-9. doi: 10.1007/s00198-016-3855-5.

15. Wolf RL, Cauley JA, Pettinger M, Jackson R, Lacroix A, Leboff MS, et al. Lack of a relation between vitamin and mineral antioxidants and bone mineral density: results from the Women's Health Initiative. Am J Clin Nutr. 2005;82(3):581-8. PubMed PMID: WOS:000231809900015.

16. Michaëlsson K, Wolk A, Byberg L, Ärnlöv J, Melhus H. Intake and serum concentrations of α-tocopherol in relation to fractures in elderly women and men: 2 cohort studies. The American Journal of Clinical Nutrition. 2014;99(1):107-14. doi: 10.3945/ajcn.113.064691. PubMed PMID: PMC3862449.

17. Prideaux M, Findlay DM, Atkins GJ. Osteocytes: The master cells in bone remodelling. Curr Opin Pharmacol. 2016;28:24-30. doi: 10.1016/j.coph.2016.02.003. PubMed PMID: 26927500.

18. Tang SY, Herber RP, Ho SP, Alliston T. Matrix metalloproteinase-13 is required for osteocytic perilacunar remodeling and maintains bone fracture resistance. J Bone Miner Res. 2012;27(9):1936-50. Epub 2012/05/03. doi: 10.1002/jbmr.1646 [doi]. PubMed PMID: 22549931; PubMed Central PMCID: PMC3415585.

19. Qing H, Ardeshirpour L, Pajevic PD, Dusevich V, Jahn K, Kato S, et al. Demonstration of osteocytic perilacunar/canalicular remodeling in mice during lactation. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2012;In Press. Epub 2012/02/07. doi: 10.1002/jbmr.1567 [doi]. PubMed PMID: 22308018.

20. Kogawa M, Wijenayaka AR, Ormsby RT, Thomas GP, Anderson PH, Bonewald LF, et al. Sclerostin Regulates Release of Bone Mineral by Osteocytes by Induction of Carbonic Anhydrase 2. J Bone Miner Res. 2013;28(12):2436-48. Epub 2013/06/06. doi: 10.1002/jbmr.2003. PubMed PMID: 23737439.

21. Ormsby RT, Solomon LB, Crotti TN, Haynes DR, Findlay DM, G.J. A. Osteocytes respond to clinically relevant UHMWPE and metal wear particles by up-regulation of resorptive and inflammatory pathways. 2018.

22. Atkins GJ, Welldon KJ, Halbout P, Findlay DM. Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2008. PubMed PMID: 18763010.

23. Kumarasinghe DD, Sullivan T, Kuliwaba JS, Fazzalari NL, Atkins GJ. Evidence for the dysregulated expression of TWIST1, TGFbeta1 and SMAD3 in differentiating osteoblasts from primary hip osteoarthritis patients. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2012;20(11):1357-66. doi: 10.1016/j.joca.2012.07.005. PubMed PMID: 22820497.

24. Fattman CL, Schaefer LM, Oury TD. Extracellular superoxide dismutase in biology and medicine. Free Radic Biol Med. 2003;35(3):236-56. PubMed PMID: 12885586.

25. Premnath V, Harris WH, Jasty M, Merrill EW. Gamma sterilization of UHMWPE articular implants: an analysis of the oxidation problem. Ultra High Molecular Weight Poly Ethylene. Biomaterials. 1996;17(18):1741-53. PubMed PMID: 8879511.

26. Neuerburg C, Loer T, Mittlmeier L, Polan C, Farkas Z, Holdt LM, et al. Impact of vitamin E-blended UHMWPE wear particles on the osseous microenvironment in polyethylene particle-induced osteolysis. Int J Mol Med. 2016;38(6):1652-60. Epub 2016/10/26. doi: 10.3892/ijmm.2016.2780. PubMed PMID: 27779642; PubMed Central PMCID: PMCPMC5117748.

27. Atkins GJ, Welldon KJ, Holding CA, Haynes DR, Howie DW, Findlay DM. The induction of a catabolic phenotype in human primary osteoblasts and osteocytes by

polyethylene particles. Biomaterials. 2009;30(22):3672-81. Epub 2009/04/08. doi: 10.1016/j.biomaterials.2009.03.035. PubMed PMID: 19349075.

28. Abdul-Majeed S, Mohamed N, Soelaiman IN. Effects of tocotrienol and lovastatin combination on osteoblast and osteoclast activity in estrogen-deficient osteoporosis. Evid Based Complement Alternat Med. 2012;2012:960742. doi: 10.1155/2012/960742. PubMed PMID: 22927884; PubMed Central PMCID: PMC3425381.

29. Bichara DA, Malchau E, Sillesen NH, Cakmak S, Nielsen GP, Muratoglu OK. Vitamin E-diffused highly cross-linked UHMWPE particles induce less osteolysis compared to highly cross-linked virgin UHMWPE particles in vivo. J Arthroplasty. 2014;29(9 Suppl):232-7. Epub 2014/07/08. doi: 10.1016/j.arth.2014.03.044. PubMed PMID: 24998319.

30. Callaway DA, Jiang JX. Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. J Bone Miner Metab. 2015;33(4):359-70. Epub 2015/03/26. doi: 10.1007/s00774-015-0656-4. PubMed PMID: 25804315.