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Cytotoxic Effects of Anti-oxidant compounds in Primary Human Peripheral Blood Mononuclear Cells

J.L. Tipper, Liu T-Y, C.L. Bladen

Institute of Medical & Biological Engineering, University of Leeds, Leeds, LS2 9JT, UK

Potential conflict - JL Tipper receives research support from DePuy International

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BACKGROUND

- Sterilisation of UHMWPE joint replacement components by gamma radiation causes the release of free radicals, which if not dealt with by post irradiation processing, can lead to oxidative damage within the polymer [1].
- Oxidation of the UHMWPE components has been shown to lead to altered mechanical properties and increased wear [2].
- The addition of anti-oxidant compounds to UHMWPE, in particular Vitamin E, is a much debated area and UHMWPE containing 1000 ppm Vitamin E (VE) is offered by most orthopaedic manufacturers as an alternative bearing material.
- A number of other anti-oxidant compounds are being added experimentally to UHMWPE such as hindered phenols [3], anthocyanins, lanthanides and nitroxides.
- The emphasis in these studies has been on studying the effect of these compounds on the mechanical properties of the polymer and/or on wear resistance, however the biological consequences have not been investigated.

[1] Oral et al 2004 Biomaterials 25, 5515-22. [2] Muratoglu et al 1999 Biomaterials 20, 1463-70. [3] Narayan et al 2010 Trans 56th ORS p2317.

AIM OF THE STUDY

The aim of this study was to investigate the effects of Vitamin E, hindered phenol, nitroxide and lanthanide anti-oxidant compounds on the cell viability of a human histiocytic cell line and primary monocytes *in vitro*.

MATERIALS

Compound Group	Antioxidant	Supplier
Natural Antioxidant	Vitamin E	Merck
Hindered Phenol – HPAO1	Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate)	Sigma Aldrich Ltd
Nitroxide	TEMPO (2,2,6,6-Tetramethylpiperidine 1-oxyl)	Sigma Aldrich Ltd
Lanthanide	Europium II chloride Europium III chloride	Sigma Aldrich Ltd

Cells

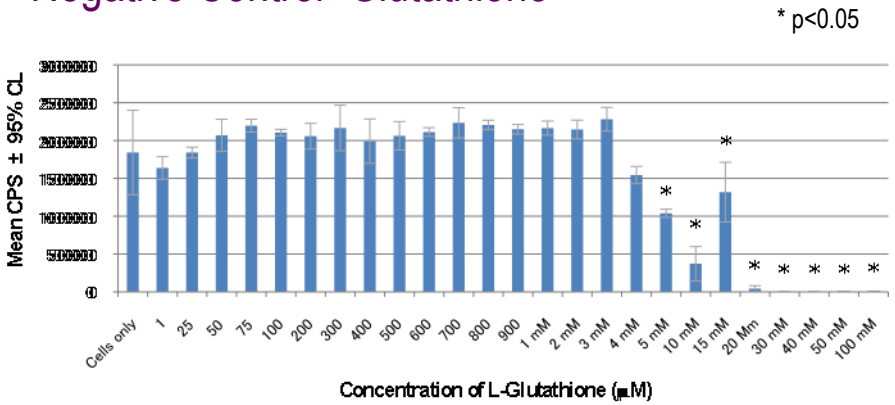
- U937 human histiocytic cell line
- Human peripheral blood mononuclear cells were isolated from 3 healthy volunteer donors (local ethical approval granted)

METHODS

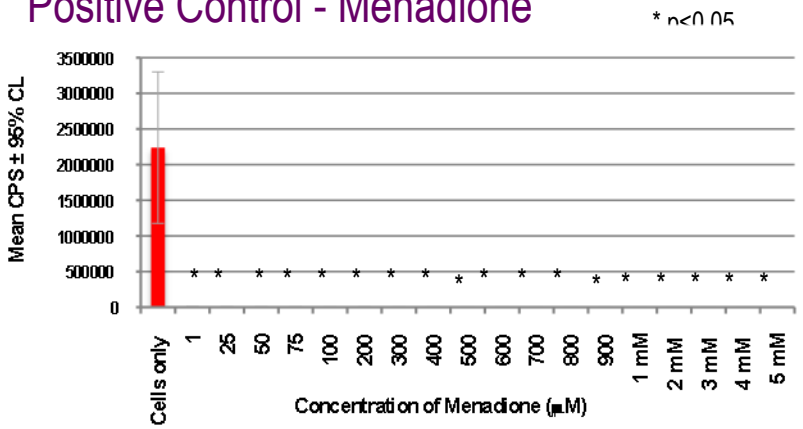
- U937 human histiocytes or peripheral blood mononuclear cells (PBMNCs) were seeded at 2 x 10^4 per well and incubated in RPMI 1640 medium in an atmosphere of 5% (v/v) CO_2 in air (n = 4).
- Antioxidant compounds at concentrations between 1µm and 5 mM were added to the cells and incubated at 37°C for 24h.
- Glutathione (100 µm), a naturally occurring anti-oxidant, and cells only were included as negative controls and 75 µm Menadione, a known inducer of oxidative stress, was included as positive control.
- Cell viability was assessed using the ATP-Lite assay after 24h.
- Results were expressed as mean counts ± 95% confidence limits and values were compared to the negative control using one-way ANOVA ($p<0.05$).

RESULTS U937 CELL LINE

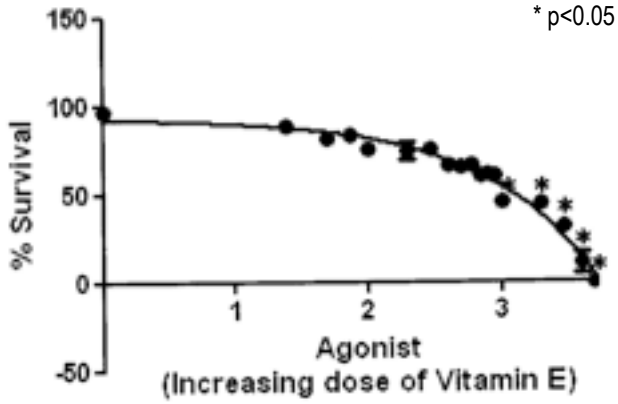
Negative Control- Glutathione



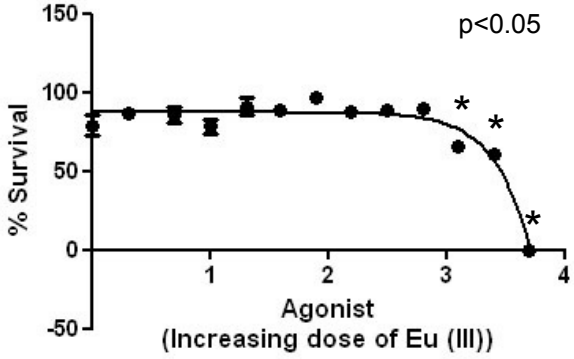
Positive Control - Menadione



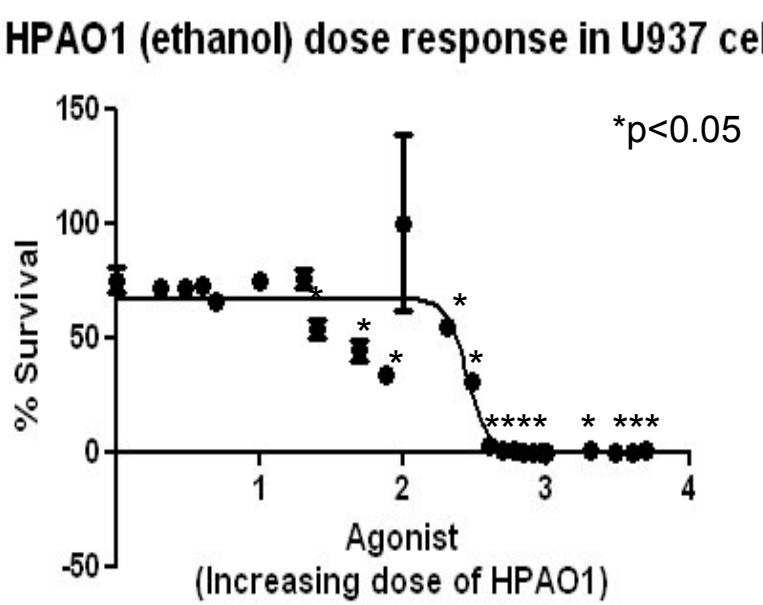
Vitamin E (toxic at 4 mM)



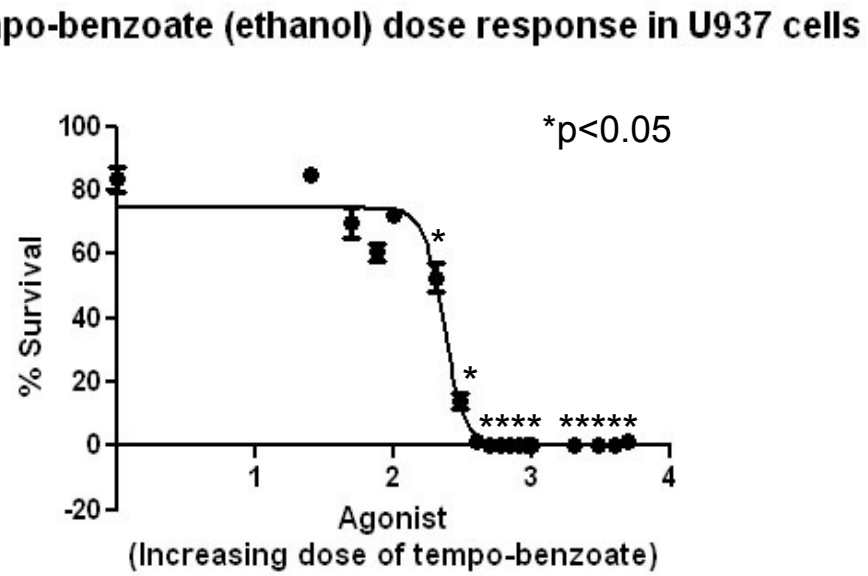
Lanthanides - Europium III (1.25 mM)
Lanthanides - Europium II (125 μM)



U937 - HINDERED PHENOL & TEMPO



HPAO1 toxic at 75 µm

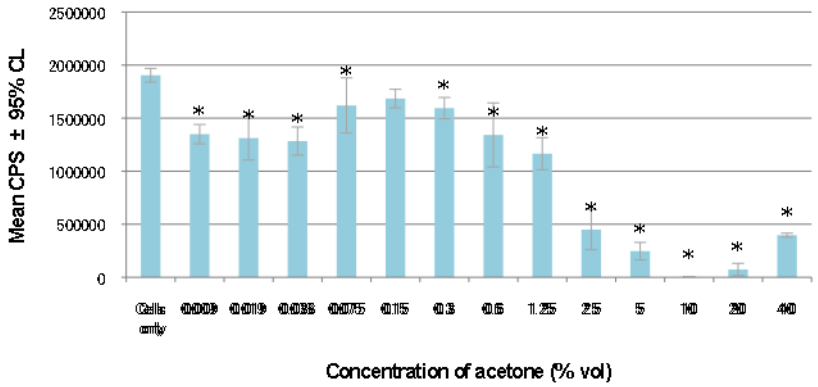


TEMPO toxic at 25 µm

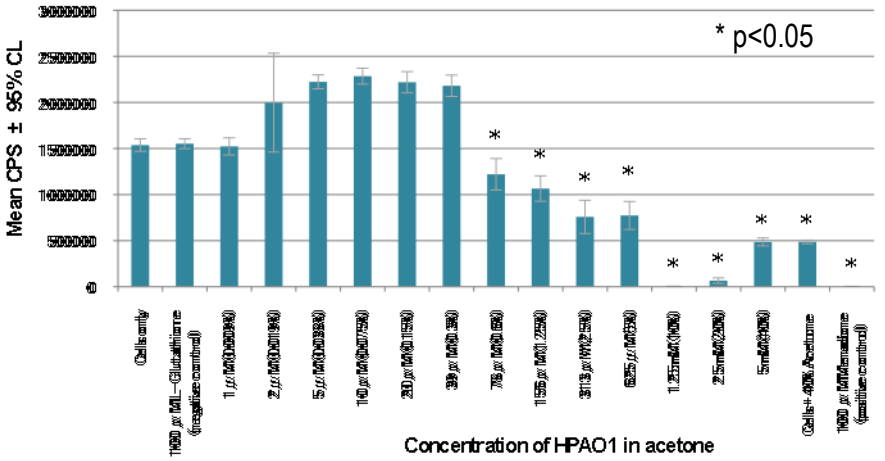
U937 - HINDERED PHENOL & TEMPO

Acetone solvent

* p<0.05

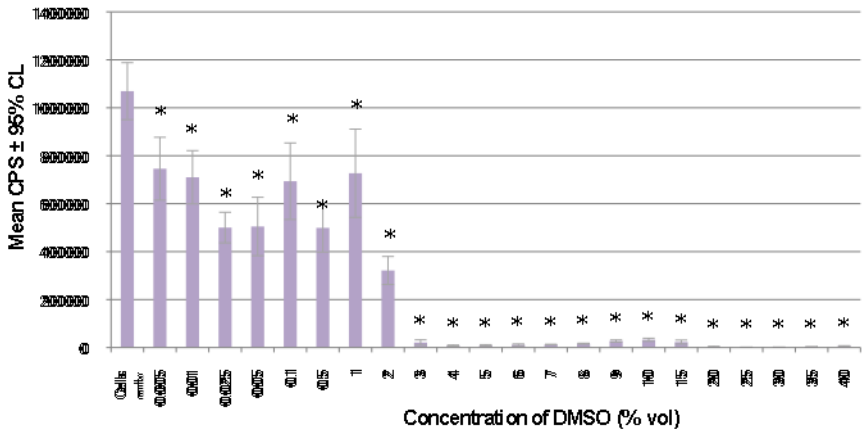


HPAO1

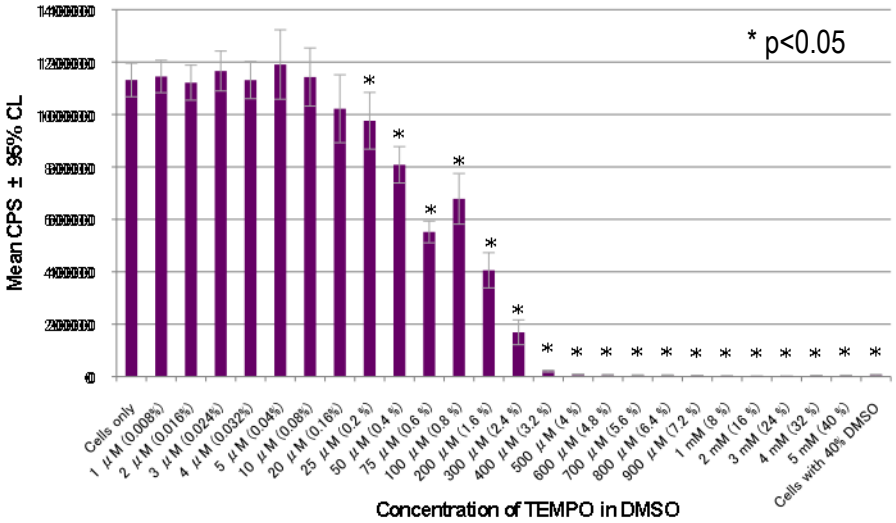


DMSO solvent

* p<0.05

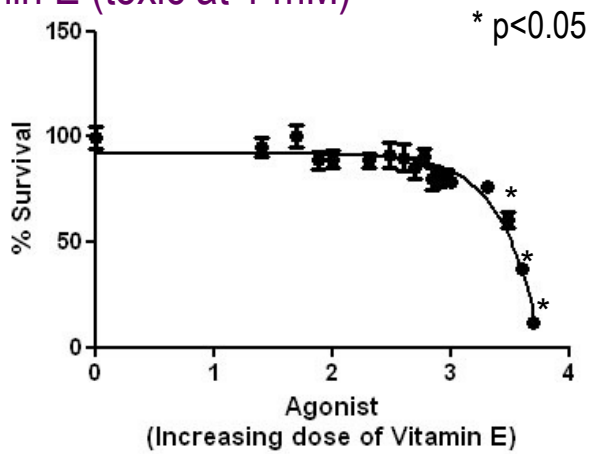


TEMPO

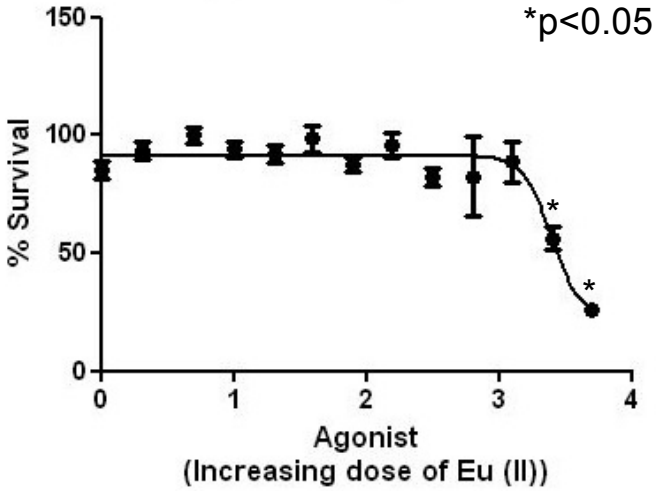


RESULTS PBMNCS

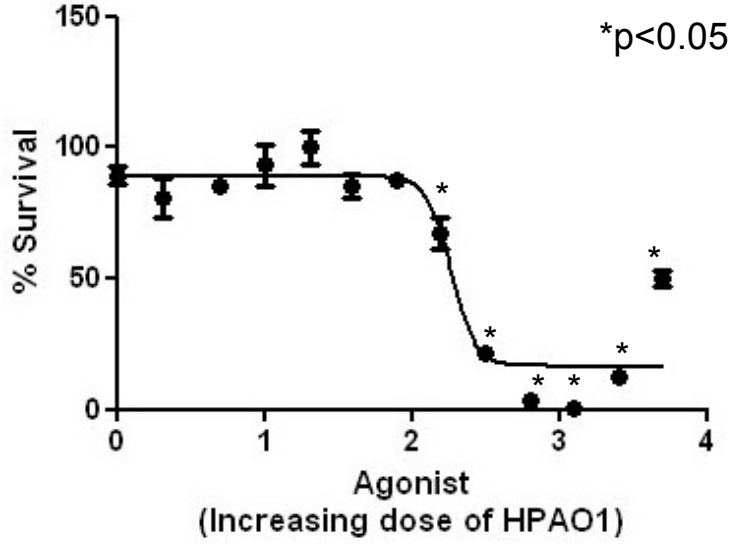
Vitamin E (toxic at 1 mM)



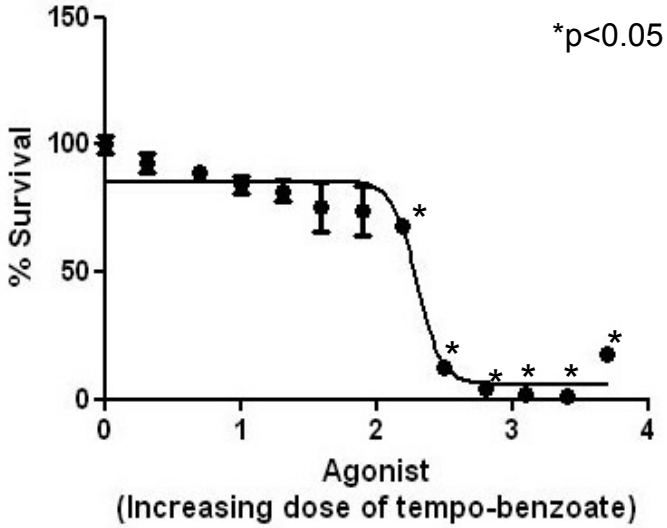
Lanthanides - Europium III(635 µM)



HPAO1 (150 µm)



TEMPO (313 µm)



SUMMARY RESULTS AND DISCUSSION

- Vitamin E and Europium (III) chloride only adversely affected cell viability at high concentrations (mM) in both cell types.
- Whereas HPAO1, TEMPO and Europium (II) chloride adversely affected cells at μm concentrations.
- However, both HPAO1 and TEMPO conferred protection against the toxicity of the solvents (DMSO, acetone and ethanol), an effect shown previously for TEMPO and protection against oxidative damage caused by Cr ions in lymphocytes [4].
- It is not known whether these chemicals would leach from UHMWPE TJR components *in vivo* and therefore pose a cytotoxic risk.
- Previous studies on Vitamin E and HPAO1 have indicated that the compounds are not lost from the bulk material [5, 6]
- However, it is not known whether the compounds will be lost from particulate wear debris which has a comparatively large surface area.

[4] Lewinska *et al*., 2008. Mut Res 649, 7. [5] Oral *et al*., 2006 Biomaterials 27, 2434. [6] King & Sharp Trans ORS 2010 p2286.

CONCLUSION

- Inclusion of antioxidant compounds within UHMWPE for total joint replacement may be beneficial in terms of reducing oxidative damage within the polymer
- The amount of TNF-a released from macrophages exposed to UHMWPE particles containing Vitamin E is significantly reduced compared to virgin UHMWPE (see Bladen et al. tomorrow).
- We are now investigating the biological effects of these AO compounds in terms of cytotoxicity and effect on release of osteolytic cytokines such as TNF-a from macrophages (anti-inflammatory).
- Preliminary results suggest that HPAO1, Europium II and III may exhibit anti-inflammatory similar to Vitamin E in monocytes.

ACKNOWLEDGEMENTS

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