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

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Potential conflict - JL Tipper receives research support from DePuy International
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], ANTHOXYANINS, 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.

The aim of this study was to investigate the effects of Vitamin E, hindered phenol, nitrooxide and lanthanide anti-oxidant compounds on the cell viability of a human histiocytic cell line and primary monocytes \textit{in vitro}.
### MATERIALS

<table>
<thead>
<tr>
<th>Compound Group</th>
<th>Antioxidant</th>
<th>Supplier</th>
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<tbody>
<tr>
<td>Natural Antioxidant</td>
<td>Vitamin E</td>
<td>Merck</td>
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<tr>
<td>Hindered Phenol – HPAO1</td>
<td>Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate)</td>
<td>Sigma Aldrich Ltd</td>
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<tr>
<td>Nitrooxide</td>
<td>TEMPO (2,2,6,6-Tetramethylpiperidine 1-oxyl)</td>
<td>Sigma Aldrich Ltd</td>
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<tr>
<td>Lanthanide</td>
<td>Europium II chloride</td>
<td>Sigma Aldrich Ltd</td>
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<td></td>
<td>Europium III chloride</td>
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</tbody>
</table>

**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) CO₂ 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 (ethanol) dose response in U937 cells**

- Agonist (Increasing dose of HPAO1)
- % Survival

- HPAO1 toxic at 75 μm

**Tempo-benzoate (ethanol) dose response in U937 cells**

- Agonist (Increasing dose of tempo-benzoate)
- % Survival

- TEMPO toxic at 25 μm

*p<0.05*
U937 - HINDERED PHENOL & TEMPO

Acetone solvent

* p<0.05

HPAO1

* p<0.05

DMSO solvent

* p<0.05

TEMPO

* p<0.05
RESULTS PBMNCS

Vitamin E (toxic at 1 mM)  
Lanthanides - Europium III (675 mM)

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.

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.
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