Antioxidant protection in mitochondria in chemotherapy-induced neuropathic pain

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Background
Neuropathic pain is a common and dose-limiting adverse effect of several cancer chemotherapeutic agents including paclitaxel. Current treatments for chemotherapy-induced peripheral neuropathy (CIPN) are largely ineffective and the pain can persist long after the cessation of the chemotherapy regimen. Whilst the specific underlying mechanisms are not fully understood, a growing body of research demonstrates the role of oxidative stress and mitochondrial dysfunction in the development of CIPN. There is evidence of mitochondrial dysfunction and induction of oxidative stress in dorsal root ganglion (DRG) cells and sensory axons from rats treated with paclitaxel.1,2 Further evidence of the role of mitochondrial damage in the development of CIPN was the report that paclitaxel-mediated mechanical hypersensitivity was worsened by mitochondrial poisons.3 Antioxidants were also shown to improve mechanical hypersensitivity in a rat model of paclitaxel CIPN.4 However antioxidants which specifically protect mitochondria may be more effective at inhibiting oxidative stress and protecting mitochondrial function than antioxidants which do not specifically act within mitochondria, and may be beneficial as a novel treatment for CIPN.

We therefore undertook studies using two antioxidants which accumulate in mitochondria in comparison with an antioxidant which does not, in both in vitro studies with DRG cells and in vivo using a rat model of paclitaxel-induced CIPN. We also assessed the ability of paclitaxel to kill cancer cells in the presence of the various antioxidants.

Melatonin and MitoVitE are both antioxidants which act within mitochondria. MitoVitE is conjugated to a lipophilic cation which allows it to accumulate in the mitochondria.5 Melatonin possesses both lipophilic and hydrophilic properties and is able to access all parts of the cell and in particular the mitochondria.6,7 Melatonin also promotes endogenous antioxidant activity.6,8 In addition we used Trolox, which is an aqueous analogue of vitamin E and cannot enter mitochondria.

Methods
For the in vitro studies we used the DRG cell line 50B11 which was a kind gift from Professor Ahmet Hoke from Johns Hopkins University, Baltimore, USA. Cells were cultured with a range of concentrations of paclitaxel, with or without the addition of melatonin, MitoVitE or Trolox. Several measures of oxidative stress including free radical production, and glutathione levels, and measures of mitochondrial function, including mitochondrial metabolic rate, membrane potential, mitochondrial pore opening and ATP production were undertaken. For the in vivo studies we used a rat model of paclitaxel-induced CIPN, and assessed the effects of melatonin, MitoVitE and Trolox on behavioural measures of pain.
Results

*In vitro* studies showed that exposure to paclitaxel caused an increase in free radical production and decreased glutathione levels, both indicating induction of oxidative stress. Measures of mitochondrial function including mitochondrial metabolic rate, membrane potential, mitochondrial pore opening and ATP production were decreased with paclitaxel treatments indicating mitochondrial dysfunction/damage. Compared to paclitaxel alone, oxidative stress and mitochondrial dysfunction was less in cells co-treated with melatonin and MitoVitE. The same protective effects were not seen in cells co-treated with Trolox (Table 1, and previous NIAA progress report, available at \(^9\)). The effects of the antioxidant treatments on the cancer cell-killing ability of paclitaxel were also assessed *in vitro*. Cytotoxicity of paclitaxel against breast cancer cells (MCF7) or ovarian cancer cells (A2780) was not affected by any of the antioxidants. *In vivo* studies in rats treated with paclitaxel demonstrated that oral administration of melatonin in drinking water or daily intraperitoneal injection of MitoVitE attenuated paclitaxel-induced mechanical hypersensitivity, whilst oral Trolox did not affect behavioural measures of CIPN (Figure 1).

Diagrams / Figures

**Table 1: Summary of findings from in vitro study**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Paclitaxel alone</th>
<th>+ Melatonin</th>
<th>+ MitoVitE</th>
<th>+ Trolox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free radical production</td>
<td>Increased</td>
<td>Attenuated paclitaxel effect</td>
<td>Attenuated paclitaxel effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Cellular glutathione levels</td>
<td>Decreased</td>
<td>Attenuated paclitaxel effect</td>
<td>Attenuated paclitaxel effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Mitochondrial metabolic rate</td>
<td>Decreased</td>
<td>Attenuated paclitaxel effect</td>
<td>Attenuated paclitaxel effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Mitochondrial membrane potential</td>
<td>Decreased</td>
<td>Attenuated paclitaxel effect</td>
<td>Attenuated paclitaxel effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Mitochondrial pore opening</td>
<td>Increased</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Mitochondrial volume</td>
<td>Decreased</td>
<td>Attenuated paclitaxel effect</td>
<td>No effect</td>
<td>Attenuated paclitaxel effect</td>
</tr>
<tr>
<td>ATP production</td>
<td>Decreased</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
</tbody>
</table>
Mechanical withdrawal thresholds were measured in rats treated with paclitaxel + vehicle control (red), paclitaxel + antioxidant (blue), and Cremaphor (black). Withdrawal thresholds for experiments assessing Trolox, melatonin and MitoVitE are shown in graphs A, C, and E respectively. For analysis, individual area under the curve values were calculated and compared, indicating Trolox did not significantly affect paclitaxel-induced mechanical hypersensitivity (B), whilst melatonin (D) and MitoVitE (F) significantly attenuated paclitaxel-induced mechanical hypersensitivity.

Figure 1: Effects of antioxidant treatments in rat model of CIPN
Conclusion
Paclitaxel treatment of DRG cells in vitro results in evidence of oxidative stress and mitochondrial dysfunction, and was ameliorated by melatonin and MitoVitE which act in mitochondria, but not by Trolox, which does not. The protective effects also extended to improvement of mechanical hypersensitivity in a rat model of paclitaxel induced neuropathy, in that melatonin and MitoVitE were beneficial but Trolox had no effect. Phase I trials have not been undertaken using MitoVitE but melatonin has been safely given to human subjects in several trials and so may represent a future novel therapy for chemotherapy-induced neuropathic pain.

References

Output


Two papers are also currently being prepared for publication and Barry McCormick has submitted his PhD thesis for examination.