INTRODUCTION

• Guidelines for the provision of data for genotoxicity in mammalian cells have been re-defined identifying that an ex vivo study undertaken at the same time as in vivo work is preferable to a second in vitro study in mammalian cells.

• As part of a longer term project to incorporate ex vivo end-points into sub-chronic inhalation studies in rodents, we have explored the potential of the Comet assay to monitor DNA damage in isolated rat lung alveolar type II epithelial cells (AEC II).

• AEC II were selected for this study as they are hypothesised to be a target of cigarette smoke exposure in the lung.

METHODS

• Isolation of AEC II
  - Sprague Dawley rats (9 wk, female, 201 ± 225 g) were supplied by Charles River Laboratories.
  - AEC II were isolated from the rat lung using published methods and modifications.

• Cell viability was determined by trypan blue dye exclusion using a Neubauer chamber.

• Statistical analysis
  - <100 cells/slide were assessed at 20X magnification and percentage tail intensity (TI) recorded using Comet assay IV image analysis software.
  - Mean & standard deviation (SD) of TI were calculated.

• Data were analysed by using a published parametric statistical analysis approach.

RESULTS

• Flow cytometry & AP staining results are detailed in Table 2.

• Differences in Leukocyte & AEC II number can be observed at the different stages of the AEC II isolation method.

Table 2: Leukocyte & AEC II at different stages of the isolation method as determined by flow cytometry & AP staining

<table>
<thead>
<tr>
<th>AEC II Isolation Step</th>
<th>Leukocytes</th>
<th>% AEC II</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Filtration - 150 µm</td>
<td>17.6 ± 4.28</td>
<td>62.6% ± 6.84</td>
</tr>
<tr>
<td>5. Filtration - 30 µm</td>
<td>17.6 ± 3.37</td>
<td>60.6% ± 6.26</td>
</tr>
<tr>
<td>6. Percoll® gradient centrifugation</td>
<td>36.6% ± 7.55</td>
<td>59.2% ± 5.64</td>
</tr>
<tr>
<td>7. Culture</td>
<td>22.9% ± 8.55</td>
<td>70.1% ± 8.97</td>
</tr>
</tbody>
</table>

• Basal DNA damage
  - The levels of basal DNA damage, as determined by the Alkaline Comet assay are detailed in Table 3.

Table 3: Levels of basal DNA damage at different stages of the AEC II isolation method

<table>
<thead>
<tr>
<th>AEC II Isolation Step</th>
<th>DNA damage (% DNA tail)</th>
<th>% Cell viability</th>
<th>% AEC II</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Filtration - 150 µm</td>
<td>8.47 ± 17.41</td>
<td>98.2 ± 2.28</td>
<td>49.2 ± 10.73</td>
</tr>
<tr>
<td>5. Filtration - 30 µm</td>
<td>9.42 ± 19.23</td>
<td>99.0 ± 1.35</td>
<td>57.4 ± 20.07</td>
</tr>
<tr>
<td>6. Percoll® gradient centrifugation</td>
<td>15.65 ± 21.93</td>
<td>99.3 ± 0.85</td>
<td>63.5 ± 10.61</td>
</tr>
<tr>
<td>7. Culture</td>
<td>27.36 ± 31.27</td>
<td>99.8 ± 0.51</td>
<td>73.2 ± 12.33</td>
</tr>
</tbody>
</table>

• 1 week inhalation study
  - DNA damage, as determined by the Alkaline Comet assay, was 5.8 ± 11.22% compared to 8.31 ± 14.94% for sham air & CS exposed animals respectively. Figure 2A & B.
  - The level of DNA damage, as determined by the Modified Alkaline Comet assay was significantly different for sham air exposed animals when compared to CS, 21.81 ± 21.55% vs. 49.60 ± 23.02%, p<0.01 Figure 2C & D.

CONCLUSIONS

• We have developed methods for AEC II isolation & the ex vivo Comet assay.

• These methods may have potential use to determine DNA damage resulting from CS exposure.

REFERENCES

The suitability of the ex vivo Comet assay to determine DNA damage induced by cigarette smoke in isolated rat lung cells

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