Abstract

EO exposure (50, 100, 200 ppm) was studied over 4 weeks in congeneric mouse strains with a 2-year lifespan. Eosinophilic and neutrophilic granulocytes, which increased only after concurrent treatment with N9213, suggest an immune response to EO metabolites such as aldehydes. The 4-week treatment showed a statistically significant increase in eosinophilic granulocytes, which was not observed in the 8-week treatment. In addition, the 12-week treatment showed a significant increase in neutrophilic granulocytes, which was not observed in the 4-week treatment. These results suggest that the 12-week treatment may have induced a more pronounced immune response to EO metabolites.

Backgroud Study & Design

- EO exposure (50, 100, 200 ppm) was studied over 4 weeks in congeneric mouse strains with a 2-year lifespan. Eosinophilic and neutrophilic granulocytes, which increased only after concurrent treatment with N9213, suggest an immune response to EO metabolites such as aldehydes. The 4-week treatment showed a statistically significant increase in eosinophilic granulocytes, which was not observed in the 8-week treatment. In addition, the 12-week treatment showed a significant increase in neutrophilic granulocytes, which was not observed in the 4-week treatment. These results suggest that the 12-week treatment may have induced a more pronounced immune response to EO metabolites.

Markers Evaluated

- Various markers of oxidative stress, DNA damage, and cell proliferation were evaluated.

Table 1: Comparison of MNGs in the lung of EO-exposed and control mice

<table>
<thead>
<tr>
<th>Concentration (µg/g tissue)</th>
<th>EO Dose Level (ppm)</th>
<th>4W Lung</th>
<th>8W Lung</th>
<th>12W Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 µg/g tissue</td>
<td>50 ppm</td>
<td>30.5 ± 5.1</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>27.6 ± 4.3</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>200 ppm</td>
<td>17.4 ± 2.9</td>
<td>20.3 ± 3.4</td>
<td>12.4 ± 1.0</td>
</tr>
</tbody>
</table>

Supplemental Statistical Analysis

- Mutations will be observed in relatively short time periods following treatment and prior to the observation of tumors. Mice will be induced at doses lower than or equal to 4-week tumor induction levels.

Temporality — Evaluate time—to—mutation

- Mutational response is related to exposure and extinction of most common changes across time.

Issues and Challenges

- How do we compare MF and tumor response?
- How do we identify significant vs. statistical significance?
- Evidence for selective elimination of most common changes?

Conclusion

- EO exposure induces a significant increase in MF only at 200 ppm. Although 50 ppm was lower than might be expected at 6 weeks, it is much lower than expected at 4-week tumor induction levels.
- Mutagens can be identified by genetic changes leading to increased MF.
- Conclusion: 200 ppm is a strong mutagenic target, which is not seen in 50 ppm.

Conclusion

- EO is not a strong in vivo mutagen in the target tissue.

Weight of Evidence for Potential Key Events

- Evidence for selective elimination of most common changes?
- Evidence for selective activation of most common changes?
- Evidence for selective elimination of most common changes?

Conclusions

- EO exposure may be complex, and it does not follow classical patterns, e.g., for mutagen, oxidative stress, or cytotoxic/compartmental cell proliferation.
- The role of direct mutagenic effects cannot be ruled out, but is a weak role in the target tissue.
- Many effects appear to have a stronger response at 6 weeks rather than at 12 weeks.
- Some early changes may have been missed, but effects of EO mutation at 12 weeks on B6C3F1 mouse testicular tumors were observed at later time points in pairwise comparisons.
- Supplementary statistical analyses suggest no change across duration.
- Expect that response would increase with time for strong mutagens, which is not seen here.
- EO is not a strong in vivo mutagen in the target tissue.