ABSTRACT

It is established that decreased feed consumption in toxicology studies correlates with decreased body weights. There is however limited information relating reduced food consumption to hematology, peripheral blood and lymphoid tissue T and B cells and subclues, organ weights and histopathology. In this study parameters were evaluated in food restricted female rats. Five rats each were assigned to four cohorts: water (DI) alone; DI and Keyhole Limpet Hemocyanin (KLH); restricted food, restricted food and KLH. Another cohort of 10 rats were administered sheep red blood cells (sRBC, 1 x 10^8) and one group of 5 administered DI and the second group cyclophosphamide (CPS) once at 15 mg/kg/day. Results showed no changes in hematology or in blood and tissue lymphocytes measured by flow cytometry in the food restriction group and in the food restriction with sRBC or KLH groups. Cumulative body weights were lower in CPS (-17 grams) and sRBC (-10 grams) cohorts, but not in animals administered DI or sRBC or KLH. Lower spleen (absolute, 41.7% and relative 38.5%) and thymus (absolute, 75.5%, and relative, 70%) weights were noted in CPS and sRBC on KLH groups but not in DI and sRBC or KLH groups. These changes correlated with lymphoid cell depletion. Since none of these immunotoxicology effects were seen in food restricted animals, including those administered KLH we conclude that sRBC, KLH or food restriction is unlikely to cause effects that can interfere with data interpretation in some of the routinely monitored parameters in toxicology studies in rats.

MATERIALS AND METHODS

Forty female Sprague-Dawley rats (Crl: CD), 13-15 weeks of age, 290 grams average weight were used on this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee. Animals were divided into 2 study cohorts:

Cohort 1 (sRBC immunogen):
- A total of ten (10) rats were immunized once with 1x10^8 sRBC in 0.2 mL of 0.9% sodium chloride by intravenous injection. Blood was gated using pan leukocyte marker (CD3) and subsets (CD4, CD8) and B cells (CD45RA). Tissues were gated using live, dead and pan leukocyte marker (CD45).

Cohort 2 (KLH immunogen):
- Table 1 shows the experimental design. KLH (Thermo Fisher Scientific, Pierce Biotechnology, Product No. NCI77600B, reconstituted using sterile water for injection and diluted with 0.9% Sodium Chloride for Injection) was administered at 5 mg/mL via intramuscular injection to animals in Groups 2 (food restricted - KLH) and 4 (restricted feed, added KLH). Cyclophosphamide (CPS, 75.5 mg/kg/day) was administered by intraperitoneal injection over 5 consecutive days.

RESULTS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>DI</th>
<th>KLH</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DI Water</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>DI Water + KLH</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Restricted Feed</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Restricted Feed + KLH</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1: Administration scheme for cohort 2

Parameters evaluated:
- Body weights, food consumption, hematology (differential and leukocyte count), lymphoid organ weight and histology.
- Immunotoxicology (flow cytometry, peripheral blood, lymphoid tissues, bone marrow, spleen, and thymus for T lymphocytes (CD3) and subsets (CD4, CD8), and B cells (CD45RA). Tissues were gated using live, dead and pan leukocyte marker (CD45).

Food restriction with or without sRBC or KLH administration in rats did not cause changes in hematology parameters (Figure 3), T and B cells in blood (Figure 4), lymphoid tissues (Figures 5 and 6) or bone marrow.

CONCLUSIONS

KLH and food restriction did not have an effect on hematological or lymphocyte parameters. Rats treated with Cyclophosphamide and sRBCs had lymphoid depletion in the lymphoid organs examined, while KLH + CPS caused lymphoid depletion in the spleen. This finding may have implications for the choice of immunogen when designing studies with a T-cell dependent antigen response arm in rats. Overall, immunization with KLH or sRBC and food restriction is unlikely to cause effects that can interfere with histopathology or other data interpretation in routinely monitored parameters in toxicology studies in rats.