Food Restriction and Administration of Immunogens: Impact on Parameters Routinely Monitored in Nonclinical Studies

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1 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 subsets, 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 108) 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 (-7 grams) and sRBC (-10 grams) cohorts, but not in animals administered DI and sRBC or KLH. Lower spleen (absolute, 41.7% and relative 38.5%) and thymus (absolute, 75.5%, and relative, 75%) weights were noted in CPS and sRBC (or KLH) groups but not in DI and sRBC or KLH groups. These changes correlated with lymphoid organ 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.

2 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 (tail vein), followed by once daily oral administration of 5 rats each with de-ionized water (DI) or Cyclophosphamide monohydrate (CPS for 5 consecutive days.

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 *ad libitum*) and 4 (food restricted, offered 16 grams/day/animal) on study days 8 and 15. Animals in Group 3 were placed on a restricted feeding regimen only.

Group	Treatment	Dose Volume (mL/kg)	KLH Dose (µg/animal)	Numer of Female Rats
1	DI Water	5	NA	5
2	DI water + KLH	5	300	5
3	Restricted Feed	NA	NA	5
4	Restricted Feed + KLH	NA	NA	5

Table 1. Administration scheme for cohort 2

Parameters evaluated:

Body weights; food consumption; hematology (differential and leukocyte count); lymphoid organ weight and histology; Immunophenotyping by 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). Blood was gated using pan leukocyte marker.

3 RESULTS

Cumulative body weight changes were lower in feed restricted animals (Figure 1) and CPS + sRBC (Figure 2 cohorts, but not in animals administered DI and sRBC or KLH. The body weight changes correlated with the restricted food regimen and in CSP-treated rats.

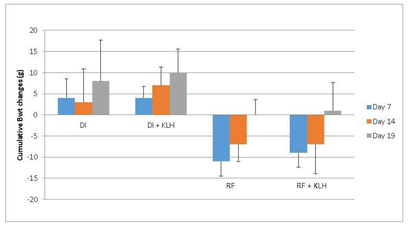

Figure 1. Cumulative body weights changes in the RF and KLH groups

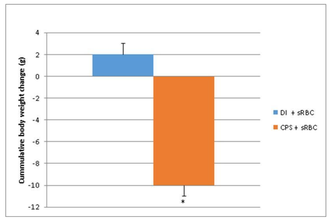

Figure 2. Cumulative body weights changes in CSP + sRBC groups

Feed 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.

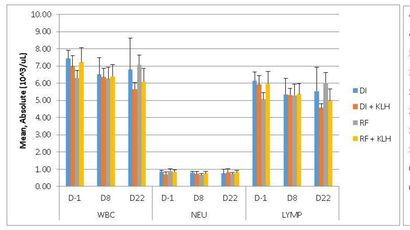

Figure 3. Hematology parameters

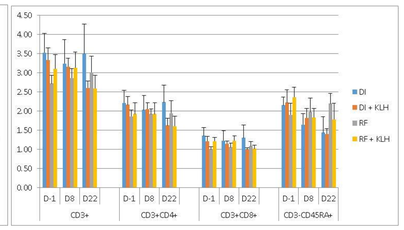

Figure 4. T and B cell subpopulations in peripheral blood

4 RESULTS (Continued)

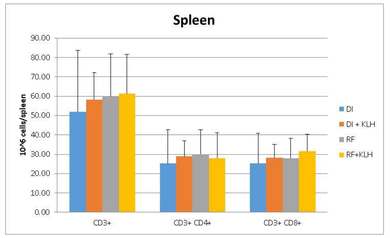

Figure 5. T and B cells in rat spleen

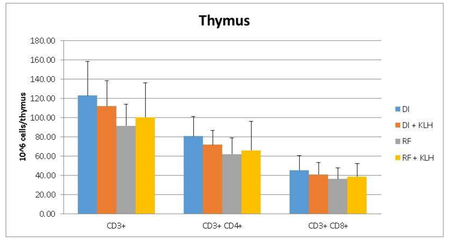

Figure 6. T and B cells in rat thymus

Low splenic and thymus weights (Figure 7) were noted in CPS and sRBC (or KLH) groups but not in DI and sRBC or KLH groups. Histopathology (Table 2): lymphoid depletion in spleen (decreased number of lymphocytes, marginal zone lymphoid follicles), thymus (with atrophy of cortex that lacked a clear separation with the medulla), lymph nodes and bone marrow. Findings correlated with lower organ weights in CPS and sRBC (or KLH) groups.

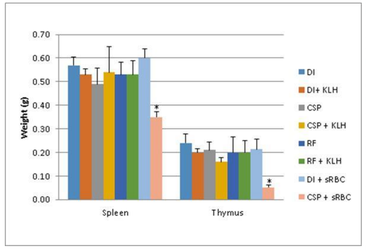

Figure 7. Organ weights changes

	CSP+sRBC	CSP	CSP + KLH
Spleen (N=5)			
Depletion, lymphoid	5	4	5
Mild	3	2	1
Moderate	2	2	4
Thymus (N=5)			
Depletion, lymphoid	5	0	0
Marked	5	0	0
LN, Mesenteric (N=5)			
Depletion, lymphoid	5	0	0
Mild	2	0	0
Moderate	3	0	0
LN, Axillary (N=5)			
Depletion, lymphoid	5	0	0
Mild	1	0	0
Moderate	4	0	0
Bone Marrow, Femur (N=5)			
Hypocellular			
	5	0	0
Mild	2	0	0
Moderate	3	0	0

Table 2. Histopathology findings

5 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 +CSP 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 sRBC or KLH and food restriction is unlikely to cause effects that can interfere with immunotoxicology or other data interpretation in routinely monitored parameters in toxicology studies in rats.