Short Paper

Notes on Age-Related Changes in Differential Leucocyte Counts of the Charles River Outbred Albino SD Rat and CD1 Mouse

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Abstract. Comprehensive baseline data from differential leucocyte counts are essential for the evaluation of leucocyte status in long-term carcinogenicity studies. Little information on lifetime variations of leucocyte types in the Charles River outbred albino rat and mouse has been published and this report describes the differential leucocyte count in rats and mice, held in our environment, sampled at 52, 78 and 104 weeks. Data revealed an age-related increase in differential neutrophil count and a corresponding decrease in differential lymphocyte count. Monocyte, eosinophil and basophil counts were unchanged during the lifetime of the animals.

Keywords: Age factors; Leucocyte count; Rats; Mice

Introduction

Carcinogenicity studies are undertaken in-house to detect the development of hyperplastic, preneoplastic and neoplastic lesions, and hence, determine the carcinogenic potential of compounds undergoing final stages of preclinical evaluation. Guidelines recommend that two species of test animals, treated with test compound, be observed for a major portion of their lifespan (OECD 1987). The rat and mouse are the most widely used laboratory animal (Home Office Report 1989) and are especially popular for such research due to their relatively short life span, ready availability and the limited cost of their maintenance in large numbers.

Despite the popularity of these laboratory animals, relatively little baseline data are published on the incidence of spontaneous haematological neoplasia and differential leucocyte counts for such long-term studies. This study assesses the effect of age on differential leucocyte counts, in the outbred Sprague–Dawley rat and Swiss albino mouse, bred by Charles River (UK) Ltd.

It has been demonstrated that under-feeding of an otherwise adequate diet can inhibit tumour induction in rats and mice (Conyeare 1980; Gross and Dreyfuss 1984, 1986) and change differential leucocyte counts in rats (Gaunt et al. 1976). Bodyweights and average daily food intake levels were monitored to ensure that under-feeding, and consequent lack of weight gain, was not a factor in development of neoplasia in our studies. Bodyweights were also measured to provide a comparative reference point for other laboratories.

Materials and Methods

Male and female rats, Crl:VAS CD^® SD BR strain, and mice, Crl:VAS CD–1 (ICR) BR strain, were purchased as weanlings, age approximately 21 days (Charles River (UK) Ltd, Margate, Kent). Rats were housed by sex in groups of four in ‘RC2’ polypropylene cages with stainless-steel suspended grid bottom and tray, mice were housed similarly in ‘MB1’ solid bottom cages (North Kent Plastic Cages Ltd, Erith, Kent). The animals were maintained under specified pathogen free (SPF) conditions, with a temperature of 21 ± 2 °C, in a relative humidity of 55 ± 10%, on a 12h light:12h darkness cycle. Ground SQC rat and mouse mainten-
age diet No. 1 (Special Diet Services Ltd, UK) and mains drinking water were available *ad libitum*.

At 52, 78 and 104 weeks, tail vein blood was obtained (Waynforth 1980) and used to make blood smears (Dacie and Lewis 1984). The smears were then stained with modified Wright’s stain, using an Ames Haematek staining machine (ICN Biomedicals, High Wycombe, Bucks), and 100 cells were examined visually to determine the differential leucocyte count. Anticoagulated blood was not available for the determination of absolute leucocyte counts and other haematological parameters.

Bodyweights and food residues were measured using Sartorius MP8 or U3600 electronic balances (Sartorius Ltd, Epsom, Surrey), and the average food intake (g/animal/day) was calculated for the same time intervals.

The data presented were collected from animals of control groups from studies conducted within Beecham Pharmaceuticals, Research and Development, Stock; eight male and eight female groups of mice, ten male and ten female groups of rats, each group initially consisting of 52 animals. The mean ($\bar{x}$), standard deviation ($s$), and number ($n$) of each control group was used mathematically to calculate the overall mean ($\bar{x}$), standard deviation ($s$), and number ($n$) using the following equations (Armitage and Berry 1987):

$$n = \frac{n_1 + n_2 + \ldots + n_i}{i}$$

$$\bar{x} = \frac{\bar{x}_1 n_1 + \bar{x}_2 n_2 + \ldots + \bar{x}_n n_i}{n_1 + n_2 + \ldots + n_i}$$

$$s = \sqrt{\frac{s_1^2 (n_1 - 1) + s_2^2 (n_2 - 1) + \ldots + s_i^2 (n_i - 1)}{(n_1 + n_2 + \ldots + n_i) - i}}$$

where $i$ = the number of groups

**Results**

There was a progressive increase in the differential neutrophil count, and a progressive decrease in the differential lymphocyte count for both sexes of rat and mouse. Table 1 summarises the results. A doubling in differential neutrophil count in the male rate was the most pronounced change, the least pronounced increase was in the male mouse, 26%, with the increase for the female rat and female mouse being 82% and 58% of the 52 week values respectively.

There were no significant differences seen in the differential monocyte and eosinophil counts of the rat or mouse at the three time points. No basophils were seen in any of the rats or mice.

There were no significant age-related difference in the bodyweights of male or female mice during the two-year period. There was a slight increase in the bodyweights of both the male and female rats between weeks 52 and 78, with the female rats showing a further marginal increase at week 104. There was no significant difference in the food intake of either rat or mouse seen at weeks 52, 78 and 104. Results are summarised in Table 2.

**Discussion**

The results have clearly demonstrated a progressive change in differential neutrophil and lymphocyte counts that cannot be related to either bodyweight or food intake, but appears to be related to the age of the animal.

For most differential leucocyte counts, the standard deviation was fairly large. However, the trend towards an increased differential neutrophil count and decreased differential lymphocyte count is unmistakable, and with such a large sample of animals, the evidence cannot be ignored.

Of the eight control groups used for data collection, one male mouse group showed constant neutrophil and lymphocyte counts for both sexes of rat and mouse. Table 1 summarises the results. A doubling in differential neutrophil count in the male rate was the most pronounced change, the least pronounced increase was in the male mouse, 26%, with the increase for the female rat and female mouse being 82% and 58% of the 52 week values respectively.

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**Table 1. Summary of differential leucocyte counts**

<table>
<thead>
<tr>
<th></th>
<th>Male mouse</th>
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<th>Female mouse</th>
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<th>Male rat</th>
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<th>Female rat</th>
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<tbody>
<tr>
<td></td>
<td>Week</td>
<td>N</td>
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<td>Week</td>
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<tr>
<td></td>
<td>52 (n=370)</td>
<td>35.5</td>
<td>61.6</td>
<td>0.8</td>
<td>2.1</td>
<td>0.0</td>
<td>52 (n=343)</td>
<td>28.9</td>
<td>67.7</td>
<td>1.2</td>
<td>2.1</td>
<td>0.0</td>
<td>52 (n=508)</td>
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<td></td>
<td>Week 78</td>
<td>39.6</td>
<td>56.4</td>
<td>1.8</td>
<td>2.2</td>
<td>0.0</td>
<td>Week 78</td>
<td>35.2</td>
<td>61.3</td>
<td>1.4</td>
<td>2.1</td>
<td>0.0</td>
<td>Week 78</td>
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<td>Week 104</td>
<td>44.8</td>
<td>52.0</td>
<td>0.7</td>
<td>2.4</td>
<td>0.0</td>
<td>Week 104</td>
<td>45.7</td>
<td>51.6</td>
<td>0.8</td>
<td>1.7</td>
<td>0.0</td>
<td>Week 104</td>
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<td>Week 52</td>
<td>28.9</td>
<td>67.7</td>
<td>1.2</td>
<td>2.1</td>
<td>0.0</td>
<td>Week 104</td>
<td>45.7</td>
<td>51.6</td>
<td>0.8</td>
<td>1.7</td>
<td>0.0</td>
<td>Week 104</td>
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<td>Week 78</td>
<td>35.2</td>
<td>61.3</td>
<td>1.4</td>
<td>2.1</td>
<td>0.0</td>
<td>Week 104</td>
<td>47.7</td>
<td>49.6</td>
<td>1.1</td>
<td>1.7</td>
<td>0.0</td>
<td>Week 104</td>
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<td>Week 104</td>
<td>45.7</td>
<td>51.6</td>
<td>0.8</td>
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<td>0.0</td>
<td>Week 104</td>
<td>45.7</td>
<td>51.6</td>
<td>0.8</td>
<td>1.7</td>
<td>0.0</td>
<td>Week 104</td>
</tr>
</tbody>
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Key: N, neutrophil; L, lymphocyte; M, monocyte; E, eosinophil; B, basophil.

Results as mean ± (S)