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Bone marrow spontaneous lesions in rodents from nonclinical 104-week carcinogenicity studies

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HIGHLIGHTS

- The incidence of spontaneous lesions in the bone marrow was higher in mice than in rats.
- In both species non-neoplastic lesions were more common than neoplastic lesions.
- In mice, there were occasional sex and site differences (sternum marrow vs femur marrow) in the incidence of a few types of bone marrow lesions.
- In rats, no sex predilection in the incidence of bone marrow lesions was apparent.

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ABSTRACT

The authors performed a retrospective study to determine the incidences and range of spontaneous lesions in the bone marrow (sternum and femur) of control mice and rats. Data was collected from 2186 mice (Crl:CD-1(ICR)BR), and 2347 rats (Han Wistar and CD(SD) rats) from the control dose groups of 104-week carcinogenicity studies carried out between 2005 and 2014. The incidence of spontaneous lesions in the bone marrow was higher in mice than in rats, and in both species non-neoplastic lesions were more common than neoplastic lesions. In mice, the most common non-neoplastic lesions in the bone marrow were increased cellularity, pigmented macrophages, and decreased cellularity, and the most common neoplastic lesions were malignant lymphoma, granulocytic leukemia and histiocytic sarcoma. There were occasional sex and site differences (sternum marrow vs femur marrow) in the incidence of a few bone marrow lesions in mice. In rats, the most common non-neoplastic lesions were increased cellularity and stromal fibrosis, and the most common neoplastic lesion was malignant lymphoma. In rats, no sex predilection in the incidence of bone marrow lesions was apparent, and there were no significant site differences in the incidence of lesions. To the best knowledge of the authors, there are no recent reports on spontaneous pathological findings in bone marrow of rodents, and we believe that these results will facilitate the interpretation of background findings and/or their increased incidence in carcinogenicity studies.

1. Introduction

The bone marrow is one of the tissues considered important in the assessment of human populations exposed to potential toxicants (Bloom et al., 2013). This may be explained by various factors. The bone marrow, like the intestinal mucosa and gonads, is highly sensitive to cytoreductive/antimitotic agents (e.g. anticancer drugs, immunomodulatory compounds and radiotherapy) (Bloom et al., 2013; Greaves, 2012). The bone marrow, and consequently the peripheral blood, can be susceptible to toxic compounds affecting directly the hematopoietic system (primary hematoxicity such as that induced by clozapine and zidovudine), but can also be secondary to the effects of xenobiotics that affect other tissues (e.g. kidney and intestine), or the supply and/or the metabolism nutrients (e.g. iron and vitamin B12) (Bloom et al., 2013; Greaves, 2012).

It is generally considered that a good concordance exists between the directly mediated effects of drugs on the hematopoietic system in laboratory animals and humans. However, prediction from preclinical studies may be imprecise and this can

Abbreviations: GLP, good laboratory practice; M:E ratio, myeloid:erythroid ratio; NOS, no otherwise specified.

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generate some degree of over- and under-prediction of findings in laboratory animals (Greaves, 2012; Greaves et al., 2004).

In preclinical toxicology setting, bone marrow examination can include histological examination, cytological examination, and flow cytometry evaluation (Ramaiah et al., 2013; Reagan et al., 2011). Cytological examination and flow cytometry evaluation may be needed on case-by-case basis and most often in a second instance based on histological findings and/or haematology results during a preclinical toxicology study (Reagan et al., 2011). However, in carcinogenicity studies, histological bone marrow evaluation still remains the main procedure (Cline and Maronpot, 1985; Reagan et al., 2011). Electron microscopy and clonogenic assays for mechanistic information on test item related effects on the hematopoietic system may also be required (Reagan et al., 2011). Histological evaluation can provide information regarding the overall cellularity and distribution of a lesion. It can also provide a general estimation of the proportion and maturation of hematopoietic cell lines as well as an estimation of iron stores. It also offers an opportunity to evaluate changes in the structural organization of the hematopoietic environment including associated tissues (i.e. endosteum, bone, interstitium, and adipose tissue) (Reagan et al., 2011; Travlos, 2006). Therefore, the reporting and regular updating of background pathological findings from control animals used in non-clinical toxicology studies are required in order to properly and fully interpret drug-induced lesions.

The main aim of the current study was to present the range and incidences of bone marrow spontaneous lesions of control rats and mice and discuss summarized results from studies carried out at Charles River, Edinburgh. This paper may help in providing the up to date basis for further investigation on bone marrow pathology in rodents.

2. Materials and methods

2.1. Animals

Bone marrow (femur and sternum) samples from a total of 3910 mice (1961 males and 1949 females) and 4172 rats (2086 males and 2086 females) were obtained from fifty-six 104-week carcinogenesis studies (22 mice studies, 34 rat studies) evaluated over a period of 15 years (2000 and 2014) at Charles River, Edinburgh (Table 1). Data was collected for one mice strain (CD-1 mice) and two different rat strains (Han Wistar and CD(SD) rats). The animals were purpose-bred for laboratory use and came from Charles River UK Ltd. (Margate, Kent, UK). All control animals were obtained from groups of animals that were not dosed, or animals that were dosed with commonly used vehicles. Studies for which control animals were dosed with a novel vehicle were excluded.

For rats, males or females were housed up to 4 per cage by sex, and for mice, males were housed individually and females were housed 2 or 3 per cage in appropriately sized standard suspended polycarbonate/polypropylene cages with stainless steel grid tops and solid bottoms. The temperature and humidity were automatically controlled at 19–23 °C and 40–70%, respectively, with a minimum of 15 air changes per hour. An automatic 12-h light cycle of 07:00–19:00 was maintained. Animals were fed an ad libitum commercial rodent diet (Rat and Mouse modified No. 1 Diet SQC Expanded, Special Diet Service Ltd., 1 Stepfield, Witham, Essex, UK). Wooden chew-sticks and play tunnels were also offered to all animals for environmental enrichment.

All studies were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986, which conforms to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, Council of Europe).

2.2. Histopathological evaluation

Animals were humanely euthanized by a rising concentration of carbon dioxide and exsanguinated via femoral veins. A detailed necropsy was performed under the supervision of a veterinary pathologist. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin wax, sectioned to a 4–5 m thickness, and stained with hematoxylin and eosin. They were examined histologically, and the findings were entered directly into a computerized database. Non-neoplastic lesions were graded with scores ranging from minimal to severe. Each study was subjected to an internal peer review and all data reviewed by the Quality Assurance Department at Charles River's Edinburgh facility prior to release of the final pathologist's report.

2.3. Study design

Data were collected retrospectively from control groups of rat and mouse studies evaluated over a period of 15 years from 2000 to 2014 (Table 1). From this pool of information, studies incorporated into the present investigation were selected on the following criteria: 1) At least one control or untreated group (controlled studies); 2) GLP-compliant toxicological studies, and 3) Evaluation of bone marrow sections (sternum and/or femur). Study material including histological incidence tables and individual animal data listings were analyzed for pathology findings under each body and organ system. A few selected glass slides were retrieved from the archives for imaging purposes and to permit more detailed description of lesions. A limited number of slides with lesions of interest were re-evaluated by qualified veterinary pathologist.

Statistical analysis of lesions incidence was performed at 1% significance level ($p < 0.01$) using Fischer's exact test comparing males vs females. Fischer's exact test was also used to evaluate differences in the incidence of each type lesion in the different anatomic location (sternum vs femur) within the same sex.

3. Results

Tables 2 and 3 present the spontaneous histopathological findings recorded in scheduled euthanized mice and rats from 104-week carcinogenicity studies at our facility in 15-year time period

Table 1
Details of data sources: 104-Week carcinogenicity studies in rats and CD-1 mice (2000–2014).

Period of study	Total number of studies	Route of administration: number of studies				Total numbers of animals		
		Oral gavage	Dietary	Subcutaneous injection	Inhalation	Males	Females	Total
Rats all strains	17	7	4	2	4	1173	1174	2347
Sprague–Dawley Rat	8	3	3	1	1	334	332	666
Hanover Wistar rat	9	4	1	1	3	839	842	1681
CD-1 mice	11	6	1	2	2	1093	1093	2186