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Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies

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Abstract

The purpose of this paper is to provide historical data pertaining to clinical chemistry and haematology parameters, obtained from control Sprague-Dawley rats, used in pre-clinical toxicity studies. Mean, standard deviation, minimum and maximum values for haematological and coagulative profiles, haemato-biochemistry and urine analysis data, and the differences per sex and study duration, 4 versus 13 weeks, are presented. The studies were conducted in agreement with the GLP (Good Laboratory Practice) regulations. Statistically significant differences, at the confidence level of 99%, for the red blood cell (RBC) parameters, the white blood cell (WBC) series parameters, plasmatic albumin/globulin (A/G), alanine amino-transferase (ALT), alkaline phosphatase (ALP), creatinine, globulin, glucose, sodium, total protein, tryglycerides, urea and urine volume were observed in males, when 4-week study values were compared with those obtained from 13-week studies. Female rats showed statistically significant variations, at the confidence level of 99% for RBC number and mean corpuscular haemoglobin (MCH), mean red blood cell volume (MCV), WBCs count and lymphocytes percentage, A/G, albumin, ALT, AST, ALP, creatinine, globulin, and sodium, when 4-week study values were compared to 13-week studies. Similar differences were observed comparing the female with male haematological and biochemical data for the two different times of the sample collection. These data could be useful as a reference for evaluation of background pathology in Sprague-Dawley rats, when used in studies performed to evaluate the toxicological profile of a new chemical entity (NCE) in agreement with requirements from international regulatory agencies.

Keywords: Clinical pathology; Haematology; Historical data; Reference range value; Sprague-Dawley rats

Introduction

The demands of international harmonisation concerning pre-clinical toxicity and safety studies require the standardisation of animal housing and laboratory procedures. Over the last decade, as a result of toxicology and toxicological and clinical pathology rapid expansion, pre-clinical toxicity and safety studies became of increased importance. The toxicology and toxicological and clinical pathology fields are of fundamental importance because many countries in the world have different regulatory authorities, whose duty is to evaluate toxicological data from pre-clinical toxicity and safety studies for a new chemical entity (NCE). A license must be issued before marketing any new drugs, additives, pesticides, or new chemical products. The regulatory authorities, for example, European Medicine Evaluation Agency (EMEA), US Food and Drug

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Administration (FDA), US Environmental Protection Agency (EPA), Japanese Ministry of Health, Labor and Welfare (MHLW) and national government agencies issue guidelines concerning toxicology and toxicological and clinical pathology. The harmonisation is also important, due to different guidelines in the various countries. For example, the JMHLW require more detailed clinical pathology parameters than guidelines issued from other authorities (Matsuzawa et al., 1995).

Abnormal treatment-related values could represent changes pertaining to pharmacological and/or toxicological effects. These changes could be regarding tissue morphology, detected by histopathological evaluation, and/or alterations in a series of in vivo analysed parameters. Dose-related changes are also of crucial importance (Matsuzawa et al., 1995). Among these parameters, clinical chemistry and haematology data are of great importance for determining effects induced by treatment. Good reference range values must be obtained before defining any alterations. These data can be obtained from control groups during each study. Another excellent source is historical data from a number of control animal groups. This can provide information on the normal value in standard conditions for multiple points (animal housing, nutrition, beverage, hours of light per day, sex and age of animals, etc.). These background data, obtained in any research centre (company, university or contract laboratory for toxicological evaluation), are essential for a good evaluation of the clinical and haematological pathology data, obtained from experimental studies (pre-clinical toxicity, safety and others). Furthermore, historical data can also be useful to evaluate background pathology in animal populations, used in each laboratory and to discern the non-NCE-related variations, possibly detected during the experimental phase of a study. Finally, these data, in combination with data from other laboratories, could add new information with new analytical methods with respect to the past.

The aim of this paper is to provide historical data pertaining to clinical chemistry and haematology parameters used in pre-clinical toxicity studies. In particular, we evaluated the normal range value in Sprague-Dawley rats, the differences between male and female animals for all parameters, and the differences between the two major intra-sex groups at 4 and 13 weeks of the study.

Materials and methods

Animals

Male and female Sprague-Dawley rats came from the same breeder (Harlan, Italy S.r.l., S. Pietro al Natisone, Udine, Italy), 5–6 weeks old and weighing approximately 170 g, at commencement of treatment, from control groups of 4- and 13-week pre-clinical toxicity studies, were used in this review. The animals were housed in a limited access rodent facility, up to 5 per sex to a cage, in clear polycarbonate cages with a stainless steel mesh lid and floor. Each cage tray held absorbent paper. Animal room controls were set to maintain temperature and relative humidity at $22 \pm 2\,^{\circ}C$ and $55 \pm 10\%$, respectively. There were approximately 15–20 air changes per hour and the rooms were lit by artificial light for 12 h each day. Drinking water was supplied ad libitum and animals were fed an Altromin MT pellet diet (A. Rieper, Bolzano, Italy).

The studies were conducted in compliance with the GLP regulations of: US FDA [21 CFR part 58, 22 December 1978] and subsequent revisions; Commission Directive 1999/11/EC of 8 March 1999 (adoption of the "OECD principles on Good Laboratory Practice – as revised in 1997") and subsequent revisions; Decreto Legislativo no. 120 of 27 January 1992 and subsequent revisions. Procedures and facilities complied with the requirements of Commission Directive 86/609/EEC (The Council Directive of the European Communities) concerning the protection of animals used for experimental and other scientific purposes. National legislation, harmonising with this Directive, is defined in Decreto Legislativo no. 116 of 27 January 1992.

Haematology and clinical chemistry

Animals were fasted for approximately 16 h and blood samples were withdrawn. Samples of blood for haematological and clinical chemistry examinations were withdrawn under light ether anaesthesia from the retro-orbital sinus of the last 10 surviving male and female animals from each main group, under conditions of food and water deprivation. Blood aliquots were put in different anticoagulants, according to the type of investigations. For the evaluation of haematological parameters, an aliquot of blood per animal was placed in ethylen-diamino-tetracetic-acid (K_3-EDTA). The measurements were performed by Bayer H1 instrument, using Bayer reagents (Bayer Diagnostics S.r.l., Milan, Italy).

For the evaluation of the blood coagulation profile, one blood aliquot per animal was placed in a tube containing sodium citrate to a final blood:sodium citrate ratio of 10:1, in order to obtain an adequate amount of plasma to measure prothrombin time (PT). PT was evaluated by the coagulometer ACL 3000 Plus IL (IL Instrumentation Laboratory S.p.A., Milan, Italy) and PT kit (IL test PT-Fibrinogen HS, IL Instrumentation Laboratory S.p.A., Milan, Italy).

For the evaluation of biochemical parameters, one aliquot of blood per animal was placed in a tube