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Analysis of premature death of Sprague-Dawley rats in carcinogenicity studies

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Abstract : To help the interpretation of causes of death, it is critical that the background incidence of factors contributing to death be recorded and archived. Information was gathered from the control groups of 19 rat carcinogenicity studies. All cases of death occurring within the 2-year period were reviewed. Out of 1124 males and 1084 females, 720 male (64.1%) and 689 female (63.6%) decedents were recorded. There was no difference in the probability of survival between two sexes. Analysis of factors contributing to death revealed that 400 males (48.7%) had neoplastic changes, 189 males (23.0%) had non-neoplastic lesions, and 232 males (28.3%) died from unknown causes. In females, these figures were 627 (76.4%), 62 (7.6%) and 132 (16.0%), for neoplastic, non-neoplastic and unknown findings, respectively. It could be suggested that the risk of death by non-neoplastic reasons was higher in the males than in the females, whereas females were more likely to be affected by tumours. In the neoplastic causes of death, pituitary tumours were the most common in both sexes, followed by mammary tumours in females, and haemopoietic tumours in males. In non-neoplastic cause of death, renal diseases were the most common in both sexes, followed by skin diseases and cardiovascular diseases in males, and skin diseases and poditis in males. A relatively large number of animals (28.3% in males and 16.0% in females) were found dead, without any significant clinical or histologically identifiable cause. Most of the animals with pituitary tumours were killed in extremis and the proportion of females (70.1%) being greater than males (46.8%). There were no case which died by accident, and also only minimal incidence which died by bleeding procedures.

Key words : SD rat, control, premature death, type of death

Introduction

The background pathology of laboratory animals is an important confounding factor in the assessment of the toxic effects of chemicals [4]. Death during long-term toxicity studies or other animal experiments are one of the major concerns for toxicologists and pathologists. There were several different categories of premature death, such as accidental death, death during blood sampling, killed for humane reasons, killed in extremis, and animals found dead [13]. As a result of modern animal husbandry methods and strict genetic and microbiological control, the incidence of sporadic death unrelated to treatment has been greatly reduced. However, such death do still occur, and it can cause problems in the interpretation of animal experiments. There is some published information on mortality in laboratory rodents, including causes of death and/or factors contributory to a declining condition or death [2, 4, 11, 13, 14]. However, most of them are focused on factors contributory to death data, rather than being included various type of death, therefore it was considered useful to obtain data on factors contributing to death and death type during long-term carcinogenicity studies.

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Materials and Methods

Animals

Male and female Sprague-Dawley (SD) rats, obtained from Charles River (UK) Limited were maintained as control animals for carcinogenicity studies at Huntingdon Life Sciences UK. At an estimated age of 5 to 6 weeks, 1 to 4 rats were housed at random in suspended solid-bottomed polycarbonate cages with sawdust or wood-chip bedding, or in stainless steel wire cages. Animal room temperature and relative humidity were generally maintained at 18 to 23°C and 38 to 68%, respectively, for the study. Artificial light was set to give 12 hours of continuous light and 12 hours of continuous dark per 24 hours. Filtered air was ducted into the animal room and extracted, to provide approximately 15 air changes per hour. All rats had free access to tap water bottles with sipper tubes, and to ground SDS Rat and Mouse No. 1 modified maintenance diet (SDS Special Diets, Witham). Drinking water and diet were routinely subjected to chemical analysis to monitor possible influences on the study. Food hoppers and water bottles were changed daily or every two days. An acclimatisation period of 14 days was allowed between arrival/allocation to test groups and the start of treatment, during which period a review of animal health was undertaken by a veterinary officer.

Histopathology

All decedent animals underwent complete necropsy, according to GLP-compliant Standard Operating Procedures (SOPs). Samples of all protocol-specified tissues were preserved in 10% neutral buffered formalin (except for eyes, which were preserved in Davidson's fluid, and testes and epididymides, which were initially fixed in Bouin's solution and then transferred to 70% industrial methylated spirits). In addition, samples of any macroscopically abnormal tissue, including all nodules and tissue masses, were routinely preserved, along with samples of adjacent tissues, where appropriate. All tissues were embedded in paraffin wax, and sections cut at 4 to 5 microns were stained with haematoxylin and eosin. For bilateral organs, sections of both the left and right organs were examined. The initial examination was undertaken by the study pathologist, and the results were then subjected to a routine peer review by a second pathologist; the diagnoses reported represented the consensus opinions of both pathologists. All macroscopic and microscopic findings were entered on an automated data collation system.

Study design

This report is based on 19 carcinogenicity studies using SD rat conducted at Huntingdon Life Sciences UK, during the period 1990-2002. Information was collected from control groups (a total of 1124 male and 1084 female rats). Each control group consisted of at least 50 males and 50 females. All cases of death occurring within the 104 weeks of the studies were reviewed, and factors contributing to death, type of death and day of death were recorded.

Statistical analysis

Survival analyses were performed on males and females separately, and the survival curves for the two sexes were compared. Kaplan-Meier estimates were calculated for each sex to show the survival curves [8]. A log-rank test was subsequently performed to compare the survival curves for the two sexes. All statistical analyses were performed in SAS 8.2 [12].

Results

Data on factors contributing to death and the type of death of all decedents up to 2-year were collected, and are presented in Table 1 and Table 2.

Mortality

From a total of 1124 males and 1084 females, 720 male (64.1%) and 689 female (63.6%) decedents were recorded in 2-year carcinogenicity studies. The factors contributory to death were neoplastic in 400 males (48.7%) and 627 females (76.4%), non-neoplastic in 189 males (23.0%) and 62 females (7.6%), and unknown in 232 males (28.3%) and 132 females (16.1%), which did not show any histopathological lesion sufficient to have contributed to their death. There was no significant difference in mortality between two sexes during this period.

Neoplastic findings

The most frequently occurring tumour was adenoma or carcinoma of the pars distalis of the pituitary gland in both sexes, with a higher incidence in females than in males. Premature death types of this tumour were mostly killed in extremis followed by found dead and

Table 1. Type of premature death expressed by cause of death in carcinogenicity studies of male SD rats

Type of death	Accidental death	Died at bleed	Found dead	Humane Kill	Killed in extremis	Total
Causes of death (%)		1 (0.1%)	365 (50.7%)	141 (19.6%)	213 (29.6%)	**720 (100%)**
Neoplastic						
Pituitary tumour			85	24	96	**205**
Haemopoietic tumours			16	13	24	**53**
Skin tumours			6	40	1	**47**
Renal tumours			8		11	**19**
Brain tumours			3	1	5	**9**
Thyroid tumours			4	7	1	**12**
Others			21	20	14	**55**
Sub-total			143 (35.8%)	105 (26.3%)	152 (38.0%)	**400 (48.7%)**
Non-neoplastic						
Renal diseases			32	8	23	**63**
Skin diseases			14	12		**26**
Cardiovascular diseases			20		4	**24**
Poditis			2	17		**19**
Others			22	18	17	**57**
Sub-total			90 (47.6%)	55 (29.1%)	44 (23.3%)	**189 (23.0%)**
Unknown						
Sub-total			164 (70.7%)	60 (25.9%)	8 (3.4%)	**232 (28.3%)**
Total* (%)	–	–	**397 (48.4%)**	**220 (26.8%)**	**204 (24.8%)**	**821 (100%)**

Total 1124 males were used.
*These are total numbers of lesions shown in cases of death.

found humane kill. Mammary tumours in females were the second most frequent, with adenocarcinoma and fibroadenoma. Majority of females with mammary tumour were killed by humane reason and few of them were killed in extremis or found dead. The second most highest incidence tumour in males were haemopoietic tumours, mostly consisted of lymphoma and histiocytic sarcoma. Large number of animals with haemopoietic tumours were killed in extremis and rest of them were found dead or killed by humane reason in both sexes. Various skin tumours were reported, mostly killed by humane reason. In males, renal tumours and brain tumours were identified killed in extreme condition or found dead. Males with thyroid tumours were found dead or killed by humane reason. Other tumours were reported in both sexes but incidences of each tumour were fairly low and there were no any predilection of death type.

Non-neoplastic findings

A spectrum of specific factors contributory to death was identified. Renal diseases, mainly chronic progressive nephropathy, were frequently seen in both sexes, the most frequent death type were found dead and killed in extremis in males and females, respectively. Skin diseases and poditis were also commonly seen

Table 2. Type of premature death expressed by cause of death in carcinogenicity studies of female SD rats

Type of death	Accidental death	Died at bleed	Found dead	Humane Kill	Killed in extremis	Total
Causes of death (%)		1 (0.1%)	145 (21.0%)	219 (31.8%)	324 (47.0%)	**689 (100%)**
Neoplastic						
Pituitary tumour			72	45	274	**391**
Mammary tumours			7	148	9	**164**
Skin tumours				14		**14**
Haemopoietic tumours			8	4	12	**24**
Others			7	10	17	**34**
Sub-total			94 (15.0%)	221 (35.2%)	312 (49.8%)	**627 (76.4%)**
Non-neoplastic						
Renal diseases			5		21	**26**
Skin diseases			4	2		**6**
Poditis				5		**5**
Others			8	10	7	**25**
Sub-total			17 (27.4%)	17 (27.4%)	28 (45.2%)	**62 (7.6%)**
Unknown						
Sub-total			67 (50.8%)	58 (44.0%)	7 (5.3%)	**132 (16.0%)**
Total* (%)	–	–	**178 (21.7%)**	**296 (36.0%)**	**347 (42.3%)**	**821 (100%)**

Total 1084 females were used.
*These are total numbers of lesions shown in cases of death.

contributing factors, with found dead or humane kill in death type in both sexes. Cardiovascular diseases were as a factor contributory to death, mostly found dead were reported in males. Many other non-neoplastic contributing factors to death were recorded in both sexes but without any predominant death type. A relatively large number of animals (28.3% in males and 16.0% in females) were found dead, without any significant clinical or histologically identifiable cause. Premature death type of those animals that died by unknown reasons were mostly found dead, followed by humane kill and killed in extremis. Only single animal in each sex was died at bleed.

Discussion

The variation in the incidence of tumours between studies may be, in part, due to the duration of study and the strain of animal, as well as variations in quality, such as microbiological status and husbandry of the animals, even when obtained from the same breeder [3, 5, 6]. Marked variability in tumour incidence in the same strain of animal kept in the same laboratory has also been observed [9], and has been considered to be partly a reflection of biological variability [1]. Sources of variation could also have been due to differences in the histopathological terminology of different pathologists, as well as variation in diagnoses over time [2]. However, Gopinath [5] indicated that, for a large pool of background control data, with several studies per year, the drift in the incidence rates of tumour types over a period of time is not necessarily so obvious. In this laboratory, most of the variable factors (source of animal supply, laboratory methods, husbandry and feed) are strictly

controlled and standardised, therefore the data in this report, which surveyed studies during an approximately 12-year period, were not thought to be greatly influenced.

Predetermined termination (i.e. of animals killed for humane reasons or in extremis) should be performed, if possible, primarily to avoid undue suffering, and also to avoid or reduce autolysis. Under the experimental conditions of carcinogenicity studies, it is therefore often difficult to identify a precise cause of death for all decedent animals. Hence, by studying data from clinical, necropsy and histopathological findings, pathologists attempt to ascribe a factor or factors contributory to death (although this might sometimes not have been sufficient alone to have been an actual cause of death) [14].

In general, male and female mortality patterns vary slightly from study to study [4]. In this survey, we have confirmed that the probability of survival until 2-year period was similar for males and females. There have been great efforts to improve the microbiological condition of rats, as well as animal husbandry. Because of modern laboratory facilities and systems, there were no cases of animals dying of infectious diseases in this report.

Causes of death in 2-year carcinogenicity studies are mainly due to age-related, well-characterised non-neoplastic and neoplastic lesions [2]. In 2-year SD rat carcinogenicity studies, the major neoplastic causes of death or morbidity were pituitary and skin tumours in males, and pituitary and mammary tumours in females [2, 7, 10]. The tumour incidence profiles were consistent with them, but haemopoietic tumours were also the second and third highest tumours in males and females, respectively in this report. In non-neoplastic cause of death in SD rats, chronic progressive nephropathy and polyarteritis are common age-related findings, with male predominance [2], which was consistent with this report.

Most of the animals with pituitary tumours were killed in extremis and the proportion of females (70.1%) being greater than males (46.8%). The relative number of decedents in which the pathologist failed to determine a cause of death through histopathological examination was slightly greater in males than in females. Due to the good laboratory animal practice, there were no case which died by accident, and also only minimal incidence which died by bleeding procedures. Poditis was thought to be a husbandry-related inflammatory condition, caused by high bodyweight and the use of wire-bottomed cages [4], but occasional death due to poditis were still seen, even some cases in females, in this survey.

In this survey, the risk of death due to neoplastic factors appeared to be greater in females than in males, whereas there were some more incidence of non-neoplastic cause of death in males (23.0%) than in females (7.6%). In most of the animals without tumours, the cause of death was unknown.

To assist in the interpretation of premature death, especially in long-term studies, it is important that the background incidence for factors contributory to death and death type be recorded and archived. This report will give pathologists a useful reference when unexpected death are found in SD rats.

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