Unexpected sudden deaths of F344 rats in long-term toxicity studies: relationship between sudden deaths and stomach tube material or feed type

Takumi Ohishi, Masahiro Mochizuki, Tomonori Enami, Kazushi Okazaki and Shuzo Okazaki

Toxicology Division, Gotemba Laboratory, Bozo Research Center Inc., 1284 Kamado, Gotemba-shi, Shizuoka 412-0039, Japan

(Received June 24, 2008; Accepted July 30, 2008)

ABSTRACT — Sudden deaths of F344 rats (F344/Du Crj (Fischer)) have occurred frequently in the late stage of carcinogenicity studies using stomach tubes. To reduce the sudden deaths, the incidence of sudden deaths was compared in the control groups from 104-week carcinogenicity studies using two different stomach tubes (metal and Teflon) and feeds (pellet and powder). The results indicate that replacing metal tubes with Teflon tubes from the first administration or after week 41 of administration was not effective in reducing the sudden deaths. On the other hand, sudden deaths did not occur at all after changing the feed from pellets to powder after week 44 or 79 of administration. In addition, although decreased body weight and retention of feed in the oral, pharyngeal and laryngeal cavities were observed in the animals that died suddenly, there were no abnormalities in histopathological examination. Therefore, it is suggested that changing the feed from pellets to powder should be effective in reducing the sudden deaths of F344 rats in long-term oral gavage studies or carcinogenicity studies.

Key words: F344 rats, Sudden deaths, Stomach tube material, Feed type

INTRODUCTION

Fischer 344 rats have been widely used in long-term toxicity studies, especially carcinogenicity studies, because they are small, easy to handle and maintain high survival rates. Germann et al. (1994, 1995) reported that sudden deaths frequently occurred to F344 rats in long-term carcinogenicity studies with oral gavage techniques using stomach tubes. They pointed out that one of the causes of sudden death could be dysphagia/asphyxia due to physical invasion of the oropharyngeal cavity by metal stomach tubes. In the laboratory, we have experienced many sudden deaths of F344 rats in long-term carcinogenicity studies using metal stomach tubes since 1997. To reduce sudden deaths, the stomach tube material was changed from stainless-steel to flexible Teflon and the feed was changed from pellets to powder. For this study, the control groups in long-term toxicity studies conducted at this facility and the incidences of sudden deaths were compared among 4 test designs with different stomach tube materials and feed types to discuss the relationship between the sudden deaths and the stomach tube material or the feed type.

MATERIALS AND METHODS

Animals

Specific pathogen-free rats of the F344 strain were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) at 4 weeks of age. The animals were quarantined and acclimatized for approximately 2 weeks and used for 104-week carcinogenicity studies at 6 weeks of age. The animals were housed individually in hanging stainless-steel wire mesh cages in an animal room under the following conditions: temperature at 23 ± 3°C, relative humidity at 50 ± 20%, air ventilation at 10 to 15 times per hour and 12-hr illumination (07:00 to 19:00). CRF-1 pelleted or powdered diet (radiation-sterilized, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were supplied ad libitum. In this study, only control groups given vehicle alone were included.

Correspondence: Takumi Ohishi (E-mail: ohishi@bozo.co.jp)
Experimental design and treatments

The group composition and the number of animals examined are shown in Fig. 1. In Group I, the animals were given pelleted feed and administration was done using stainless-steel stomach tubes throughout the administration period. In Group II, the animals were given pelleted feed throughout the administration period and administered by oral gavage using stainless-steel tubes from the beginning of administration to week 44 of administration and thereafter using flexible Teflon tubes. In Group III, the animals were given pelleted feed from the beginning of administration to week 78 of administration and thereafter powdered feed, and the administration was done using flexible Teflon tubes from start to finish of the administration period. In Group IV, the animals were given pelleted feed until week 44 of administration and thereafter powdered feed and dosed using stainless-steel tubes up to week 18 of administration and thereafter using flexible Teflon tubes.

All animals were observed for general condition and weighed during the study period. For the animals that died, the oral, pharyngeal and laryngeal cavities were examined macroscopically and histopathologically.

All procedures in this study were conducted in compliance with “Guidelines for Animal Experimentation” (Japanese Association for Laboratory Animal Science, May 22, 1987) and according to the Protocol approved by the Animal Care and Use Committee at BOZO Research Center Inc. All efforts were made to minimize animal suffering.

RESULTS

In Group I, sudden deaths occurred from week 31 (Fig. 1). The incidence was higher in females than in males (week 1-40: 0% in males and 3.6% in females, week 1-79: 1.8% in males and 9.1% in females, and week 45-104: 1.8% in males and 9.1% in females). No apparent

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**Occurrence of sudden death of F344 rats in oral gavage carcinogenicity studies a)**

<table>
<thead>
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<th>Week of study (W)</th>
<th>1</th>
<th>10</th>
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<th>30</th>
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<th>50</th>
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<th>70</th>
<th>80</th>
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<td>0% 5.5%</td>
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</tbody>
</table>

a): The occurrence of sudden deaths are shown by “x”.

- **stainless steel**
- Teflon
- pellet
- powder

*Fig. 1.* Occurrence of sudden deaths of F344 rats in oral gavage carcinogenicity studies
clinical signs were noted in the in-life observation of the animals that died suddenly. However, their body weights decreased gradually during the study period and the animals had almost the lowest body weight in each group (Fig. 2). Necropsy revealed only retention of food in the oropharyngeal cavity, while histopathological examination revealed no abnormal finding in the oropharyngeal area (Fig. 3). In the other 3 groups, the pathological findings in the animals that died suddenly, the body weight changes and macroscopic and histopathological findings in the oropharyngeal cavity were similar to those in Group I.

Considering the relationship between the sudden deaths and the stomach tube materials (Fig. 1), the incidence of sudden deaths in Group II was 3.6% in males and 0% in females from week 1 to 44 and 1.8% in males and 18.2% in females from week 45 to 104. The incidence of sudden deaths in Group III was 3.3% in males and 8.3% in females from week 1 to 79. The incidence of sudden deaths in Group IV was 0% in males and 0% in females from week 1 to 18 and 0% in males and 5.5% in females from week 19 to 43. The incidence of sudden deaths in these groups was largely comparable to that in Group I. The incidence of sudden deaths was almost the same even if the stomach tube material was changed from stainless-steel to flexible Teflon.

Considering the relationship between the sudden deaths and the feed type (Fig. 1), sudden deaths did not occur...
after the feed type was changed from pellets to powder in Groups III and IV (Group III: from week 80, Group IV: from week 44).

**DISCUSSION**

F344 rats have been widely used in long-term toxicity studies or carcinogenicity studies. But many sudden deaths occurred in long-term/carcinogenicity studies using metal stomach tubes that were conducted in this laboratory after 1997. To explore the causes and to reduce the occurrence of sudden deaths, the incidence of sudden deaths was compared in 104-week carcinogenicity studies conducted in our laboratory after changing the stomach tube materials and feed types.

From the results in Group I in which the animals were given pelleted feed and administration was done using stainless-steel stomach tubes throughout the course of study, the sudden deaths were similar to those reported by Germann *et al.* (1994, 1995) for the following points: 1) the incidence was higher in females than in males, 2) deaths occurred in week 20 of administration or later, with high frequency especially after week 26 of administration, 3) there were no abnormal clinical signs before death, 4) necropsy revealed only retention of food in the oropharyngeal cavity with no histopathological finding, and 5) body weight at the time of death was almost the lowest in each group. These findings were comparable to those in the animals that died suddenly in the other 3 groups.

Germann *et al.* (1994, 1995) suggested partial blockage of the oropharyngeal lumina due to granulomatous inflammation in the sero-mucinous glands as the cause of death. However, the cause of retention of food was unclear because there were no histopathological changes in the

![Fig. 3. Feed packed in the oral, pharyngeal and laryngeal cavities was observed; however no remarkable lesions were found.](image-url)
oral, pharyngeal or laryngeal cavities in our study. In the present examination, the incidence of sudden deaths was almost the same even after the stomach tube material was changed from stainless-steel to flexible Teflon. On the other hand, sudden deaths were prevented completely by changing the pelleted feed for powdered feed which was considered easier to swallow. When the animals were fed pelleted feed, sudden deaths occurred with increased frequency after week 26, suggesting that some animals had difficulty swallowing after repeated oral gavage and retained food might gradually accumulate in the oropharyngeal cavity. Therefore, it was considered that changing feed from pellets to powder could reduce sudden deaths in long-term toxicity or carcinogenicity studies using F344 rats.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Kunio Doi, Emeritus Professor of the University of Tokyo, for reviewing this paper and Mr. Pete Aughton, D.A.B.T., Dip.R.C.Path., ITR Laboratories Canada Inc., for proofreading.

REFERENCES
