Spontaneous convulsions in Sprague-Dawley rats in carcinogenicity studies

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ABSTRACT — Spontaneous clonic convulsions in Sprague-Dawley (SD) rats are occasionally observed in chronic toxicity studies and carcinogenicity studies. To estimate the incidence and features of the spontaneous convulsions, data from 11 oral gavage carcinogenicity studies using Charles River SD rats, which were conducted at our laboratory between 2003 and 2010, were collected (N = 2990 for each sex). The total incidence of animals which showed spontaneous clonic convulsions at least once during the 2-year study period was 2.4% (2.9% in males and 1.9% in females). In those carcinogenicity studies, the earliest observation of convulsions was 25 weeks of age in males and 20 weeks of age in females, and the average age at the first occurrence of convulsions in all animals was approximately 66 weeks of age. Some animals showed convulsions only once, but others on several or numerous occasions. Most convulsions were observed during the oral gavage procedure, especially when holding the animals. Approximately 0.3% of animals died immediately after the seizure. No related histopathological abnormalities in the brain have been recorded for such dead animals following routine examination.

Key words: Spontaneous convulsions, Rat, Carcinogenicity study

INTRODUCTION

The rat is one of the requisite species for animal studies to evaluate the toxicity of chemicals and pharmaceuticals. In carcinogenicity/toxicity studies, we have often experienced spontaneous clonic convulsions in Sprague-Dawley (SD) rats; however, the precise incidence was not clear up to now. Although there is one report of the incidence of spontaneous convulsions in Wistar rats (Nunn and Macpherson, 1995), none could be found for SD rats. Because some kinds of chemicals induce convulsions, e.g. cholinesterase inhibitors (Raffaele et al., 1987), dopamine agonists (Shiosaki et al., 1996), aminophylline (Walker, 1981), quinolone antibacterial agents (Kato et al., 1992) and gamma-aminobutyric acid (GABA) agonist withdrawal (Essig, 1966; Suzuki et al., 1992) in addition to typical convulsants (De Deyn et al., 1992), knowing the incidence and features of spontaneous convulsions is important for proper evaluation of carcinogenicity/toxicity studies. The present report is intended to document the incidence of spontaneous clonic convulsions in Charles River SD rats from 11 carcinogenicity studies conducted at our laboratory.

MATERIALS AND METHODS

Animals

Male and female specific pathogen-free rats of the Sprague-Dawley strain, Crl:CD(SD), were obtained from Atsugi or Tsukuba Breeding Center, Charles River Laboratories Japan, Inc., between 12 May 2003 and 18 January 2008. The animals were 4 or 5 weeks old on arrival and used for each carcinogenicity study at 6 weeks of age after 1 or 2 weeks of quarantine/acclimatization. All carcinogenicity studies were conducted in compliance with the Good Laboratory Practice and Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006).

Husbandry

All animals were housed in animal rooms in an SPF Rodent Barrier Facility which has been designed to ensure that studies proceed without interference from infection and environmental variability. Animal rooms were maintained at temperature of 23 ± 3°C with the relative humidity at 50 ± 20%, the air ventilation at 12 to 17 times
per hour, and 12 hr illumination (07:00 to 19:00). Male
and female rats were housed individually in bracket-type
stainless steel wire mesh cage (W 254 × D 350 × H 170
mm: Lead Engineering Co., Ltd., Tokyo, Japan). Cages
were exchanged at least once every 2 weeks, and rooms
were cleaned every day. Pelleted or powdered diet (radi-
ation sterilized CR-LPF, Oriental Yeast Co. Ltd., Tokyo,
Japan) and drinking water (via automatic water-supply
system) were provided ad libitum.

Experimental procedures
The animals were used for 2-year carcinogenicity stud-
ies during the safety evaluation of a variety of novel phar-
macueticals. The number of animals used for the present
research was 2,990 of each sex from 11 oral gavage carci-
nogenicity studies and 240 of each sex from 1 dietary car-
nogenicity study. These studies generally consisted of
a control group and 3 to 4 dose groups administered the
test compound. In these studies, all animals were inspect-
ed at least twice daily for gross abnormalities or mortali-
ty and given a detailed physical examination weekly. The
animals were also handled at the daily oral gavage admin-
istration, and when body weights were being recorded
(usually weekly up to 14 weeks of study and every
2 weeks thereafter). On completion of the study periods,
surviving animals were killed by exsanguination under
diethyl ether or isoflurane anesthesia and given a full
necropsy. Animals that died or were killed as moribund
were similarly treated. Tissues from all major organs were
taken for routine histological examinations. For the brain,
3 thin transverse HE sections were prepared and exam-
ined by light microscopy (2 sections for the cerebrum;
1 section for the cerebellum).

RESULTS AND DISCUSSION
From oral gavage carcinogenicity studies conducted at
our laboratory between May 2003 and January 2010, the
overall incidence of animals which showed spontaneous
clonic convulsions at least once during the 2-year study
period was 2.4% (2.9% in males and 1.9% in females,
see Table 1). A total of 5,980 rats (2,990 males and 2,990
females) were used for those carcinogenicity studies. The incidence of convulsions in the control group was 2.2%
(2.7% in males and 1.7% in females) and it was quite
similar to the above overall incidence. Because the treat-
ment with test compounds appeared not to be a cause of
convulsions in these carcinogenicity studies, the overall
incidence was considered to be reliable as historical con-
trol data. Animals of some studies were apparently more
susceptible than others; the incidence of convulsions was
4.9% in Study D but only 0.8% in Study A. The suscep-
tibility might be different among the production lots (the
period of purchase) of animals.

The most common type of convulsion was clonic state
with rhythmic muscle contractions, but tonic convulsion
was frequently observed with opisthotonus (i.e. stretch-
ing of the limbs or elevation of the tail) and/or then fol-
lowed by clonic phase. A few animals screamed briefly
just before the seizure. The convulsions stopped several
minutes later, but animals died infrequently as described
later. No typical clinical abnormalities which prelude con-
vulsions were observed among animals with seizure.

The earliest observation of these convulsions has been
recorded at 25 weeks of age in males and 20 weeks of age
in females. Thereafter, animals which showed convul-
sions for the first time appeared at a constant frequency,
and there were no age-related increases in the incidence
or severity of convulsions. The average age at the first
observation of convulsions, calculated from all of ani-
mals which showed convulsions more than one time, was
approximately 66 weeks of age (69 weeks of age in males
and 62 weeks in females).

Some animals exhibited convulsions only once, but
others on several or numerous occasions throughout the
study. The average frequency of convulsions through-
out the study period was calculated from 38 males and
27 females that survived until the end of study, and it
was 4.4 times during 2 years (3.6 times in males and 5.6
times in females). Convulsions were mostly observed at
the time of oral gavage procedure (over 90%) and were
not observed in a dietary carcinogenicity study (N = 240
for each sex) in which the frequency of animal handling
was lower than in oral gavage study. From those results,
it was suggested that the most convulsions were triggered
by the handling of the animals.

Approximately 0.3% of animals (15 out of 5,980 ani-
mals) died immediately after seizure (see Table 1). The
incidence of death was not correlated well with the sever-
ity of convulsions because some animals died in spite of
mild convulsions. Although routine histological exam-
inations were conducted for all animals, no histopatho-
logical abnormalities which cause convulsions were
found in the brain of any animal. In this strain of rat, it
is well known that pituitary tumor is the most common
(McMartin et al., 1992), and the grown pituitary tumor
often presses into the bottom of the brain and creates dys-
function of the brain stem. However, there was no rela-
tionship between the pituitary tumor and spontaneous
convulsions because pituitary tumor was found only in
5 out of 15 animals that died with seizure. We have seen
convulsions caused by chemical substances which affect
the central nervous system in toxicity studies of rodents, and biting of the tongue was often seen in these cases. However, biting of the tongue was not observed at necropsy in the 15 animals that died with seizure in this research.

Generally, convulsions which are induced by test compounds in toxicity studies are observed dose-dependently, in several animals in a group and around the time of maximum drug concentration. In the situation of convulsions, animals often show clinical abnormalities which suggest effects of the test compound on the nervous system, such as change in locomotor activity, piloerection, twitching and tremor, in addition or prior to convulsions. However, spontaneous convulsions in the present research were different from such test compound-induced convulsion in many aspects: spontaneous convulsions in SD rats are observed sporadically, by external stimuli (especially animal handling), at several months after the start of administration (20-25 weeks of age or later), and without any signs of abnormality. To determine whether the convulsion which is observed in a toxicity study is test compound-related or not, recording the details of relationship between the timing of onset of seizure and experimental procedure is especially important.

The present study revealed that clonic convulsions occurred spontaneously in SD rats with a certain probability and some animals died immediately after the seizure. Therefore, it is important to know these phenomena for toxicity evaluation of compounds in rats, especially in long term toxicity studies.

### Table 1. Incidence of spontaneous convulsions in oral gavage carcinogenicity studies in Crl:CD(SD) rats

<table>
<thead>
<tr>
<th>Study</th>
<th>Incidence of animals showing spontaneous convulsions</th>
<th>Age of animal exhibiting first convulsions (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td></td>
<td>Control Low Mid-Low Mid-High High</td>
<td>Control Low Mid-Low Mid-High High</td>
</tr>
<tr>
<td>A</td>
<td>0/60</td>
<td>0/60</td>
</tr>
<tr>
<td>B</td>
<td>0/60</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>0/60</td>
<td>0/60</td>
</tr>
<tr>
<td>E</td>
<td>2/55</td>
<td>1/55 (1)</td>
</tr>
<tr>
<td>G</td>
<td>2/60</td>
<td>2/60</td>
</tr>
<tr>
<td>H</td>
<td>5/120 (1)</td>
<td>4/60</td>
</tr>
<tr>
<td>I</td>
<td>2/60</td>
<td>2/60</td>
</tr>
<tr>
<td>J</td>
<td>1/60</td>
<td>1/60</td>
</tr>
<tr>
<td>K</td>
<td>0/60</td>
<td>1/60 (1)</td>
</tr>
<tr>
<td>Control total</td>
<td>22/815 = 2.7%</td>
<td>14/815 = 1.7%</td>
</tr>
<tr>
<td>Single sex total</td>
<td>86/2990 = 2.9% (11/2990 = 0.4%)</td>
<td>58/2990 = 1.9% (4/2990 = 0.1%)</td>
</tr>
<tr>
<td>Overall total</td>
<td>144/5980 = 2.4% (15/5980 = 0.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses indicates a number of animals which died immediately after seizure.

### Reference


