Five-week repeated dose oral toxicity study of Cetirizine in juvenile dogs

Study Number :  FLS 91-4534

Date of submission : June 1, 1993

Sponsor : 2-8, Doshomachi 2-chome, Chuo-ku, Osaka
541 JAPAN

Sumitomo Pharmaceuticals Co., Ltd.

10221, Kobuchisawa-cho, Kitakoma-gun, Yamanashi-pref.
408 JAPAN

Fuji Life Science Incorporated

Study director : Yasuki Akio
SUMMARY

Five-week repeated dose oral toxicity study of Cetirizine was carried out in juvenile beagles. Three or four week-old beagles were treated orally with Cetirizine at a daily dose of 4, 20, 50 or 100mg/kg for 5 weeks. Five groups including a control were provided, and each group consisted of three males and three females. During the administration period, the animals were observed for general signs, body weight changes and food consumption. Hematological examination, blood chemistry examination, urinalysis, physiological examination, ophthalmological examination and pathological examination were also performed. The results are as follows:

1) All males and two females in 100mg/kg group were killed in a moribund state on the 10th - 17th day of administration.

2) In the clinical observation, salivation, depression of spontaneous movement and noisy breath sounds were found in all moribund animals before the end of administration, and they eventually became moribund. In remaining animals, incidence of vomiting was slightly higher in 50 and 100mg/kg groups, and that within one hour after administration was markedly high in these groups.

3) In the body weight measurement, a weight reduction was observed in all moribund animals before sacrifice. In remaining animals, any body weight changes which appeared attributable to the test article were not observed.

4) With respect to food consumption, all moribund animals could not ingest food before sacrifice. In remaining animals, no changes attributable to the test article were found in the uptake of paste food or pellet food.

5) In hematological examination, blood chemistry examination, urinalysis, electrocardiography, body temperature, ophthalmological examination, organ weight and histological examination, changes which appeared attributable mainly to aggravation of general conditions were observed in a few items in moribund animals. However, no changes attributable to the test article were detected in any animals including remaining animals.
Based upon these results, it was concluded that the no-effect level of Cetirizine was 20mg/kg in both sexes, because an increased incidence of vomiting was observed at a dose of 50mg/kg and above.
MATERIALS AND METHODS

1. Test animals

Beagle dogs (15 males and 15 females aged 3 weeks, respectively) were obtained on August 16, 1991, transferred into an animal room and used for the study. These animals had been fed with formula and weaning food from the age of 2 weeks and weaned completely in about 1 week by the breeder (CSK Research Park Inc.). They were regarded as healthy from clinical observation, fecal examination (for parasites) and hematological examination carried out by the breeder. However, due to an accident in fixing organs and tissues, data from three males and two females could not be obtained. Therefore, additional seven males and six females (3 males and 3 females for each of 50mg/kg group, and 1 male and 1 female for reserves) were obtained on October 25. But, all of these additional animals were killed, because increased leukocytes were sporadically observed among these supplemental animals during the acclimation period and another seven males and six females that were raised similarly to those described above were obtained on November 15.
3. Grouping and dose groups

Each dog was allocated to one of the treatment group the day before the commencement of administration. Body weight and litter of origin were two factors taken into account during this procedure.

4. Administration

1) Route and procedure

The test article was inserted into gelatine capsules (Japanese Pharmacopoeia Grade) and the animals dosed orally in accordance with the intended clinical route. The control animals received the same number of empty capsules as that for the high dose group animals.

2) Dosage levels and groups

The identification was as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control (Empty capsules)</td>
<td>0</td>
<td>3 Males 3 Females</td>
</tr>
<tr>
<td>2 Cetirizine, low dose</td>
<td>4</td>
<td>3 Males 3 Females</td>
</tr>
<tr>
<td>3 Cetirizine, medium dose 1</td>
<td>20</td>
<td>3 Males 3 Females</td>
</tr>
<tr>
<td>4 Cetirizine, medium dose 2</td>
<td>50</td>
<td>3 Males 3 Females</td>
</tr>
<tr>
<td>5 Cetirizine, high dose</td>
<td>100</td>
<td>3 Males 3 Females</td>
</tr>
</tbody>
</table>

The data of two animals in control group, one animal in low dose group and two animals in medium dose 1 group (totally 5 animals) were excluded from this report due to an accident during fixation of organs and tissues.

3) Justification of the dose levels

As safety evaluation of Cetirizine in juvenile beagles, an oral toxicity study with a single administration has already been carried out.
In that study, vomiting and decreased locomotive activity were observed in dose groups of 100mg/kg or above, and salivation was seen in 300mg/kg group (high dose group). No changes were observed in 30mg/kg group (low dose group). In one month oral toxicity study in adult beagles, tremor, salivation, ataxy and increased vomiting were observed in 135mg/kg/day group (high dose group), but no abnormalities were detected in 45mg/kg/day group (medium dose) or lower dose groups.

Based on the above results, 100mg/kg was selected as the high dose level for this study, the remaining levels of 4 and 20 were about 3-fold decreases over the high dose.

However, five animals out of six in high dose group (100mg/kg/day) were killed during the administration period. Since no obvious changes were detected in medium group 1 (20mg/kg/day), an additional administration group between these two dose levels appeared to be necessary for toxicity evaluation of the test article in this study. In the latest one month study in adult dogs, no noteworthy changes were found at 45mg/kg/day (intermediate dose), and in a 6 month study, increased vomiting and hypersensitivity were observed in 75mg/kg/day group (high dose). Therefore, an additional 50mg/kg/day group was set as medium group 2 in consideration of the use of juvenile animals.

4) Administration period
The animals dosed once a day in the morning for 5 weeks.

5. Observation and measurement
1) General signs
Every animal was observed once a day during the acclimation period and three times a day (before administration, about 1 hour after administration and in the evening) during the administration period. In addition, animals were also observed 0.5 hour after administration till day 5.

2) Body weight
Each animal was weighed on the arrival, grouping, 1st administration and necropsy days, and twice a week during the administration period. Moribund animals were weighed before necropsy.
3) Food consumption

Daily food intake by each animal was measured once in the acclimation period, and twice a week during the administration period.

6. Clinical pathology

Clinical pathology were performed once before the administration commenced and then during dose weeks 2 and 5. Blood sample was taken from the external jugular vein without using any anesthetic. Urine was collected with a tray. Blood sample was collected in the morning during the acclimation period, and before daily feeding and administration during the administration period. Fresh urine was collected before daily administration.
8. Pathological examination

1) Necropsy

After the final administration, all animals were fasted overnight. The animals were then anesthetized by injection of a sodium pentobarbital solution into the cephalic vein of the forelimb, killed by exanguination from the bilateral axillary arteries, and necropsied.

2) Organ weights

After necropsy, the following organs from all animals were weighed:
- Brain, heart, thymus, spleen, lungs, submaxillary glands.
- Liver, kidneys, testes, prostate, ovaries, uterus, pituitary, thyroid, adrenals.
Relative organ weight (organ weight/body weight) was calculated from the body weight of the necropsy day.

3) Histological examination

Samples of the following organs/tissues were removed and fixed in 10% neutral-buffered formalin, except eyes which were preserved in Davidson's solution.
- Cerebrum, cerebellum, medulla oblongata, spinal cord (thoracic region), sciatic nerve, heart, thoracic aorta, thymus, spleen, mesenteric lymph nodes, sternum (with bone marrow), trachea, bronchus, lung, submaxillary glands, liver, gallbladder, pancreas, tongue, esophagus, stomach, duodenum, jejunum, ileum, colon, kidneys, urinary bladder, testes, epididymides, prostate; ovaries, uterus, vagina, pituitary, thyroid, parathyroids, adrenals, skin (posterior dorsal portion), femoral muscle.
These samples of the fixed tissue were processed, by standard histological techniques, embedded in paraffin wax, sectioned, and stained with hematoxylin-eosin (H.E.), and then examined microscopically. Also, sections of the liver of every animal were stained by the PAS reaction, and sections of the heart of one male in 4mg/kg group, one female in 20mg/kg group and two males in 100mg/kg group were subjected to the Azan stain and Elastica van Gieson's stain, and examined microscopically. Frozen sections of the liver and kidneys of one male and one female of control and 100mg/kg groups, and one female of
50mg/kg group were prepared, stained with Oil Red O and examined microscopically. In addition, the larynx, hilus lymph nodes and submaxillary lymph nodes were fixed in 10% neutral-buffered formalin, and the hilus lymph nodes of two males and two females in 100mg/kg group were sectioned, H.E. stained and examined microscopically.

9. Statistical analysis

Quantitative data was analyzed by the F test for homogeneity of variance. When the F test indicated homogeneous variance, Student's t test was used, and when non-homogeneous, Aspin-Welch's t test was used for comparison of control and administration groups.
RESULTS

1. Mortalities

All males and two females in 100mg/kg group were killed in a moribund state from the 10th to the 17th day of administration. In these animals, salivation, depression of spontaneous movement and noisy breath sounds observed from the 9th - 13th day of administration, and then, decumbence was seen. All males and one female in 100mg/kg group were killed the day after the first appearance of abnormal signs, and one female was killed on 5 days after the first appearance.

2. General signs

In moribund animals, abnormal signs observed from the first appearance on were; depression of spontaneous movement, salivation and decumbence in all animals, paleness of visible mucosa in all males, limping, noisy breath sounds and mucus or food residue in the oral cavity in one male and two females, erosion of the tongue in one male, nasal discharge, emaciation and blood in foamy mucous vomit in one female.

In remaining animals, frequency of vomiting was slightly higher in one female in control, two males and one female in 50mg/kg group, and one female in 100mg/kg group throughout the administration period. Vomiting was marked within one hour after daily administration in two animals in 50mg/kg group and one animal in 100mg/kg group. On the 23rd day of administration, a test article-like material was found in vomit of one male in 50mg/kg group. However, the frequency of vomiting in one female in control group and one male in 50mg/kg group were similar before dosing commenced. With respect to stool, high incidence of soft stool or mucous stool was observed in two males in 20mg/kg group, one male in 50mg/kg group and one male in 100mg/kg group during the early period of administration. On the 22nd day of administration, muddy stool was found in one female of 20mg/kg group. However, similar changes of stool were observed in these animals before dosing commenced and in control animals during the administration period. Since the occurrence of abnormal stool decreased with the decrease in water content in the food due to the change from paste to pellets, the changes of fecal properties were not regarded to be attributable to the test article.
3. Body weight

All moribund animals reduced body weight from 1 to 4 days before sacrifice. Individual loss was 60 - 220g.

In remaining animals, decreased body weight due to decreased food intake was observed in one female in 50mg/kg group from the 12th until 22nd day of administration. However, this animal showed normal body weight gain thereafter. All the other animals normally gained weight.

4. Food consumption

In moribund animals, two males and one female became unable to take food before sacrifice.

In remaining animals, one female in 50mg/kg group scarcely ingested pellet food until the 23rd day of administration, but became normal thereafter. Since this animal ingested paste food well, decreased ingestion of pellet food appeared attributable to its preference. No clear differences were observed in consumption of paste food between control group and administration groups.

5. Hematology

In moribund animals, increased Ht in all animals, increased RBC in two males and two females, increased WBC in one male and one female, increased Pl. in one female, prolonged APTT in one male and one female, high percentage of Stab. and low percentage of Lympho. in one male, high percentage of Seg. and low percentage of Lympho. in one female, and decreased WBC in one male were observed in the examination performed shortly before sacrifice.

In remaining animals, MCHC, Pl. and Reti. values were significantly higher than control values during weeks 2 or 5, but, these high values were similar than the values before the commenced of administration.

6. Blood chemistry

In moribund animals, decreased K in one female and two males, increased TG in two males and one female, increased Glu. in one male, and increased T.cho., BUN and Na in one female were observed in the examination performed shortly before sacrifice. In addition, decreased GOT and GPT in two males and one female, and decreased T.bil. in one female were found, but these low values were regarded to be clinically insignificant.
In remaining animals, significantly different values of GTP, A/G, T.bil., BUN and Cl values in males, and GOT, TP, Na and Ca values in females from those of control were sporadically observed during weeks 2 or 5 of administration. However, these changes were not dose-dependent, or within a range of normal deviation. One female in 4mg/kg group showed high GTP level in the 5th week of administration.

7. Urinalysis

In moribund animals, casts were observed in one male of 100mg/kg group in the 2nd week of administration. In the examination of urine collected from the urinary bladder at necropsy, positive urinary protein was observed in two males and two females, casts were found in two males and epithelial cells were detected in one males.

In remaining animals, increased urine volume and total amount of Na, K and Cl excretion were observed in females of 20mg/kg group and higher groups in the analysis of 24-hour urine in the 5th week of administration. Among these values, the amount of K excretion in 50mg/kg group was significantly higher than that of control. However, these changes were within a normal range, and no such a tendency was observed in males.

8. Electrocardiography

In all moribund animals, decreased heart rate and elongation of QT interval were observed.

In remaining animals, significant differences were observed in QRS axis in females of 20mg/kg group and in heart rate in females of 50mg/kg group in the 5th week of administration, when compared with control. However, these changes were within a range of physiological deviation.

9. Body temperature

No abnormalities were detected at any point of examination.

10. Ophthalmological examination

In the examination before dosing commenced, hemorrhage in the vitreous body was observed in one male in 20mg/kg group and one female in 50mg/kg group. However, in the 5th week of administration, hemorrhage in the former animal disappeared and that in the latter was on the mend. In addition, slight dysplasia of the retina was observed in one male each in control and 20mg/kg groups. However, this dysplasia remained unchanged
in the 5th week of administration.

In juvenile beagles, hemorrhage in the vitreous body, together with remnants of the arteries in the vitreous body, is frequently observable. Its incidence is 1.7 - 6.7%, and this type of hemorrhage is known to be absorbed with growth and disappear. Dysplasia of the retina is a non‐progressive congenital change. During the experimental period of this study, hemorrhage in the vitreous body disappeared mostly and dysplasia of the retina remained unchanged. Therefore, these changes did not affect the quality of this study.
2) Organ weight

In moribund animals, low spleen and thymus weight, and high adrenals weight both in relative and absolute weights were observed in one female.

In remaining animals, absolute brain weight was significantly lower and relative heart weight was significantly higher in males of 50mg/kg group than those of control group. However, the differences were slight, and the values were within a range of normal deviation. Also,
submaxillary glands absolute weight was significantly lower in females of 4mg/kg group than that of control group, but the change was slight and no dose dependency was observed.

3) Histological examination
Moribund animals (3 males and 2 females in 100mg/kg group):
In liver, increased fat-droplets in the peripheral region of the lobules and decreased PAS-positive glycogen granules in the hepatocytes were observed in all animals. In the case of markedly increased in fat-droplets, fat-droplets were observed diffusively. Also, slight or moderate dilatation of the sinusoid, activation of Kupffer cells and focal aggregation of activated Kupffer cells were observed in all males and one female. In addition, focal necrosis consisted of focal karyopyknosis, increased cytoplasmic eosinophilia of hepatocytes, and activated Kupffer cells were found in two males. Other observed changes were scattered small foci of mineralization, and infiltration of neutrophils or mononuclear cells in the portal area and lobules.

In kidneys, slight or moderate increased in fat-droplets mainly in the epithelium of the proximal straight tubules was observed in all animals. Slight dilatation of the distal tubules and congestion were found in one female.

In lymphatic and hematopoietic system, hypocellularity in the sternal bone marrow was observed slightly in all males and moderately in one female. In spleen, moderate extramedullary hematopoiesis was observed in all males and one female. However, in the other female, no extramedullary hematopoiesis was found, but atrophy of the systemic lymphatic tissues such as spleen, thymus, mesenteric lymph nodes, hilus lymph nodes and Peyer’s patches in ileum were observed.

In respiratory system, slight to severe trachitis, bronchitis, focal bronchopneumonia accompanied by infiltration of neutrophils in the hilus lymph nodes were observed in two males and one female.
Congestion of the hilus lymph nodes was also observed in these two males. In addition, slight infiltration of neutrophils and slight congestion was found in the hilus lymph nodes of one female which did not show pneumonia.

In other organs, ulcer in the tongue and the upper lip, and erosion and neutrophil infiltration in palatine tonsil were found in one male, and erosion of mucosa of the gastric fundus and increased vacuoles in
the adrenocortical cells were seen in one female. Also, infiltration of neutrophils and mononuclear cells in mucosa of the esophagus was observed in two males. In addition, infiltration of neutrophils or mononuclear cells in the coronary adipose tissue of heart, infiltration which is sometimes observable in juvenile beagles, was found in two males. Hemorrhage under the epicardium and endocardium, and intimal fibrous thickening of the right coronary artery was observed in one male, and slight infiltration of neutrophils or mononuclear cells in the mucosa of the urinary bladder was seen in all animals except one male. However, these changes were similar to those observed frequently in adult beagles maintained under routine conditions.

Moderate infiltration of neutrophils in the mucosa of the gallbladder was seen only in one female.

Remaining animals (terminal sacrifice):

In liver, similarly to moribund animals, slight increased in fat-droplets was observed in one female of 50mg/kg group, and slight decreased in PAS-positive glycogen granules was observed in one male and two females of 50mg/kg group. However, these changes were not detected in a remaining female in 100mg/kg group or in animals of 20 and 4mg/kg groups, including one female of 4mg/kg group which showed a high GTP activity in blood chemistry examination. Other changes observed were increased single cell vacuolation or ballooning, and infiltration of mononuclear cells in the portal area and lobules in 50mg/kg group.

In kidneys, similarly to moribund animals, slight increased in fat-droplets was observed in one male and one female of 50mg/kg group. Acute pyelitis that was not dose-dependent and appeared to be attributed to spontaneous occurrence was observed in one of the two.

In other organs, a slight erosion and infiltration of neutrophils and mononuclear cells in the mucosa of the esophagus was observed in one female of 100mg/kg group which showed frequent vomiting. However, no changes in the esophageal mucosa were found in one female of 50mg/kg group which also showed vomiting at a similar frequency.

Extramedullary hematopoiesis in the spleen was observed in all groups including control. Although the degree of this change was slight in males and slight or moderate in females, this change has no difference among groups, and was within a normal range. Other dose-independent
changes that were also observed in control group were slight or moderate trachitis and bronchitis, and slight focal aggregation of neutrophils or mononuclear cells in the interstitium of the lung. Changes of single occurrence or dose-independent changes were a slight periarteritis of the right coronary artery and moderate hemorrhage in the mucosa of the urinary bladder. As changes similar to those frequently observable in adult beagles maintained under routine conditions, focal intimal fibrous thickening of the right coronary artery or slight infiltration of neutrophils or mononuclear cells in the mucosa of urinary bladder was observed in one animal. No abnormalities were observed in the eyes of one male in 20mg/kg group and one female in 50mg/kg group in which eyes hemorrhage in the vitreous body was found in ophthalmological examination. In the eyes of one male each in control and 20mg/kg groups in which eyes dysplasia of the retina was observed, focal retinal projection into the vitreous body of every layer except the pigmented epithelial membrane of the retina was found. However, no abnormal cells in each layer were observed, and this change was attributed to abnormal development.
DISCUSSION AND CONCLUSION

The toxicity of Cetirizine was investigated by repeated orally administration to juvenile beagles at a daily dose of 4, 20, 50 or 100mg/kg for 5 weeks.

All males and two females in 100mg/kg group started to show salivation, depression of spontaneous movement and noisy breath sounds on the 9th - 13th day of administration. Since the animals became moribund 1 to 5 days after the appearance of these signs, they were killed.

All changes observed in clinical examination before sacrifice of moribund animals, that is, high values of RBC, Ht., Glu., Na, T.cho., BUN and TG, low value of K, protein-positive urine, and decreased heart rate and prolonged QT interval in electrocardiography were regarded to be secondary changes due to primary aggravation of general conditions.

In pathological examination, activation of Kupffer cells and focal aggregation of these cells were observed in the liver of all males and one female in moribund animals. In two males of them, scattered necrotic foci which were observed in the liver of untreated animals of a similar age were found. However, no changes indicating liver failure were obtained from blood chemistry examination of these animals and no changes in Kupffer cells were observed in one female in moribund animal and all remaining animals, the changes of Kupffer cells appeared to be related to aggravation of general conditions. On the other hand, as common histological findings increased fat-droplets in the peripheral region of the hepatic lobules, decreased PAS-positive glycogen granules in hepatocytes, and increased fat-droplets in the epithelial cells of the renal proximal straight tubules were observed in all moribund animals. However, these changes were slight or moderate, and not serious. Therefore, these changes could not be regarded as the cause of the moribund state of the animals. In remaining animals, similar changes were sporadically observed in 50mg/kg group but not in 100mg/kg group. Since increased in fat-droplets in liver and kidneys is also spontaneously observed, these were not regarded to be attributed to the test article. Moderate or severe bronchitis or erosion in the tongue was observed in moribund animals. These changes appeared to be related to noisy breath sounds observed during clinical observation, and may have intensified aggravation of general
conditions or inability of food ingestion. However, these changes were not common to moribund animals, and were not regarded to be the direct effect of the test article. Decreased cell density in the sternal bone marrow was observed in moribund animals. However, this change was not seen in one remaining animal in 100mg/kg group, or no results which indicated the effects on bone marrow were obtained in hematological examination. Therefore, this change appeared attributable to the aggravated general conditions. In one moribund female animal after a long period from the first appearance of abnormal sign, emaciation was marked, and stomach ulcer, atrophy of systemic lymphatic tissues and enlargement of the adrenals accompanied by increased vacuolation in adrenocortical cells were observed. These changes are common to weakened animals.

Increased WBC in hematological examination appeared attributable to bronchitis or aggravated general conditions as was described above. The cause for low WBC, high Pt. and prolonged APTT were not clear.

During the clinical observation, slight increased in vomiting was seen in 50mg/kg and 100mg/kg groups, and this condition was marked within 1 hour after administration. In a 4 week oral toxicity study in adult dogs, increased vomiting was also observed. Therefore, this was regarded to be attributed to the test article. In histological examination, erosion was found in the esophageal mucosa of one animal that showed increased vomiting in 100mg/kg group.

In blood chemistry examination, high GPT level was observed in one female of 4mg/kg group in the 5th week of administration. However, this change was not dose-dependent, and no other abnormalities were found in blood chemistry examination or no changes in liver were detected in pathological examination in this animal. Although, the cause of this change was not clear, and this change appeared to be independent of the test article. No abnormalities were observed in hematological examination, urinalysis, ophthalmological examination, electrocardiography or body temperature.

Based upon these results, it was concluded that the no-effect level of Cetirizine was 20mg/kg in both sexes, because increased incidence of vomiting was observed at a dose of 50mg/kg and above.