

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	No remarkable findings.
Clinical signs	No remarkable findings.
Body weight	Body weights for treated animals were 25 - 35% higher than control values for all treated groups of males and females.
Food consumption	Treated animals from all groups consumed larger quantities of food when compared to controls.
Ophthalmoscopy	No remarkable findings.
Clinical Pathology	The following parameters were significantly different from control: <ul style="list-style-type: none"> • Hemoglobin concentration - Week 7, ↑ Group 3 - 4 ♂; Week 13, ↑ Group 3 ♂ • Hematocrit - Week 7, ↑ Group 3 - 4 ♂; • Mean cell hemoglobin - Week 7, ↑ Group 2 and 3 ♂; Week 13, ↑ Group 2,3 ♂; • Mean cell volume - Week 7, ↑ Groups 2 - 4 ♂; • Glucose - Week 7, ↓ Group 3 - 4 ♂; Week 13, ↓ Groups 3 - 4 ♂.
Gross pathology	
Organ Weights	Heart weights were significantly higher for all treated groups of animals when compared to controls. Kidney and liver weights for high dose males were higher than controls. Thyroid weights were higher than controls for mid dose males. Lung weights were higher for Group 2 females when compared to controls. Adrenal weights were higher for Group 2 and 3 females when compared to controls.
Necropsy	No remarkable findings.
Histopathology	No remarkable findings.

CONCLUSION

The increased body weight and heart weight noted in this study is consistent with that noted in other toxicity studies with this drug. The Sponsor concluded that CGP 25827A was well tolerated up to the highest inhaled dose (0.442 mg/kg/day). Notably, there were no adverse effects on the respiratory system.

10. 6/12 Month Inhalation Study in Rats — Formulation 1/73)**BACKGROUND INFORMATION**

Study Title: 6/12 Month Inhalation Study in Rats — Formulation 1/73)
Sponsor Study No.: 936115
Laboratory Study No.: 653179
Study Dates: June 10, 1993 - August 5, 1994
Report Date: March 8, 1996
Test Facility: _____
GLP Status: Compliant
NDA Volume:Page 39:1

METHODS

Test Article: CGP 25827A (Foradil) Dry Powder Formulation (1/73)
Batch No: 1103/1 and 1173/1
Purity: _____
Control Article: Lactose
Purity: Not stated
Species/Strain: Sprague-Dawley derived albino (Tif:RAIf) rats
Route: Nose Only Pulsed Inhalation
Exposure Conditions: Rotating brush generator with monitored air flow, temperature, and humidity.
Duration of Exposure: 1 year with and 8-week recovery

Group	Dosing Information					Mean % of Particles <3.5 μ m Diameter
	No.	No.	No.	Dose Levels	--	
	Animals/sex: Main Study	Animals/sex: 6-Mo. Interim	Animals/sex: Recovery	(μ g/kg/day)		
1 (Lactose)	20	10	5	0	47.5	
2 (Low)	20	10	5	30	50.4	
3 (Mid)	20	10	5	120	46.7	
4 (High)	20	10	5	400	44.0	

The results of previous toxicity studies and a feasibility study using the exposure conditions described for the current study were considered in dose selection. In a 28-day inhalation study in rats, doses were 15.6, 50.4, and 115.3 μ g/kg/day and findings included significantly increased heart weights at all doses without microscopic correlate. In a 90-day study, doses were 2.5, 8.1, and 26.1 μ g/kg/day and findings included increased heart weights for high dose females only and there was no microscopic correlate.

The low dose was a small multiple of what was thought to be the intended human therapeutic dose (48 μ g/day). The high dose was described by the Sponsor as the "maximum tolerated dose that can be administered to the test model of the 52 week duration of the study without causing unnecessary distress or suffering." The basis for this claim was not stated.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Rectal temperature	Week 25
Ophthalmoscopy	Prior to treatment and at Weeks 6, 12, 25, 39, and 51
Urine for Proof of Absorption	Weeks 26 and 51

Parameter	Frequency of Measurement
Plasma for Proof of Absorption	Weeks 26 and 51 (predose, immediately postdose, and 0.5, 1, 2, 4, and 8 hours postdose)
Clinical pathology	Prior to treatment and at Weeks 7, 13, 36, 39, and 52; and after the 8-week recovery period
Gross pathology (including organ weights)	After 6 months and 12 months of treatment and 8 weeks of recovery
Histopathology	After 6 months and 12 months of treatment and 8 weeks of recovery. Complete tissue list for all control and high dose animals and salivary glands, heart, spleen, lungs, nasal cavity and testes were examined from animals in all groups.

RESULTS

Mortality

No remarkable observations.

Clinical signs

No remarkable observations.

Body weight

Body weight gain was higher for treated males when compared to controls throughout the growth phase of the study (Weeks 1 - 15). Thereafter, body weight gain values for treated males approached that of controls and was less than controls for the latter part of the study. Body weight gain values for females were consistently greater than control values throughout the study.

Food consumption

No remarkable findings.

Rectal temperature

No remarkable findings.

Ophthalmoscopy

No remarkable findings.

Urine for Proof of Absorption

Not included in this report.

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Plasma for Proof of Absorption

Not included in this report.

Clinical Pathology

Glucose levels for all treated groups of animals were consistently decreased compared to control values. Statistically significant differences from control values were reported for various other parameters in hematology and clinical chemistry determinations, however the changes could not be definitively attributed to treatment with CGP 25827A because they did not occur in a dose related response, were inconsistent over time, or were not biologically meaningful.

Gross pathology

Organ Weights

Treatment related changes in organ weights were observed in the heart (all dose groups), lung (all dose groups), liver (mid and high dose groups), and testes (mid and high dose groups). The changes in the liver and testes weights persisted through the recovery period.

Increased heart weights were noted for all groups of treated animals when compared to controls as early as 6 months, persisted through the 12-month observation, but were not present after the 8-week recovery period. Lung weights for all treated groups were higher than control values at the 6 (males and females) and 12 (females) month intervals, but were not noted at the 8-week recovery interval. Thyroid weights were not measured at the 6 month interval but were significantly higher than control for high dose females at the 12 month interval.

Testes weights for the mid and high dose males were lower than control values at the 6- and 12-month intervals and at the 8-week recovery interval. The other organ weight change that persisted through the recover period was lower-than-control liver weights for males at 6 months (high dose) and at the recovery interval (mid and high dose).

Necropsy Observations

An increased incidence of small or flaccid testes was observed in mid and high dose group males at the 6 and 12 month intervals and after recovery when compared to controls.

Histopathology

Remarkable microscopic findings were observed in the testes (Group 3 and 4 males); spleen (Group 3 and 4 females); salivary gland (Group 4 males and females); nasal cavity (Group 4 males and females); lungs (Group 4 females); and heart (Group 4 males). All findings except degeneration of germinal epithelium of the seminiferous tubules resolved during the 8-week recovery period.

The only remarkable finding at the 6 month interval was degeneration of seminiferous tubules. Only high dose and control animals were examined at this interval, thus the finding was only identified in the high dose males. At the 12 month interval, this finding was revealed in mid dose rats as well, and did not resolve for the mid or high dose males after the 8 week recovery period.

The findings in the lung and nasal cavity were confined to mid and/or high dose animals at the 12 month interval and were not present after 8 weeks of recovery. Findings in the lung consisted of an increased severity of "large 'foamy' macrophages" around the terminal bronchioles of the high dose females. In the nasal cavity, an increased incidence and severity of goblet cell proliferation was observed in the anterior region of the cavity in high dose males and females when compared to controls.

Findings in the spleen consisted of increased incidence and/or severity of extramedullary hematopoiesis in mid and high dose animals at the 12 month interval.

In the salivary gland, very mild hypertrophy of serous acini was noted in 1/20, 0/20, 0/18 and 10/20 males in Groups 1 - 4, respectively and in 2/20, 2/20, 2/20 and 9/20 females in Groups 1 - 4, respectively, at the 12 month interval.

The notable finding in the heart was an increased incidence of myocardial fibrosis in high dose males when compared to controls.

CONCLUSION

After one year of treatment with CGP 25827A via dry powder inhalation, rats receiving 120 and 400 $\mu\text{g}/\text{kg}/\text{day}$ had degeneration of seminiferous tubules that did not recover after an 8-week period without treatment. The no observable adverse effect level (NOAEL) for this irreversible finding was 30 $\mu\text{g}/\text{kg}/\text{day}$ in rats, — times the expected human dose daily inhaled dose of — μg .

Findings in the heart (myocardial fibrosis), lung (presence of foam cells) were consistent with those observed in the rat dietary carcinogenicity study with CGP 25827A.

Mice

Three month dose range finding studies were conducted in mice: 1 via dietary administration and 2 via drinking water administration.

After 3 months of dietary administration, mice treated at levels of 40 mg/kg/day and above had increased heart weights without microscopic correlate, and decreased glucose levels and increased urea levels. Red cell parameters were elevated in groups treated at levels of 400 mg/kg/day and above. Kidney weights were higher than control in animals treated at levels of 140 and above, but histopathologic evaluation was not performed on this organ.

After 3 months of treatment with the test material in the drinking water, no remarkable changes were observed in animals dosed as high as 450 mg/kg/day.

A second 3 month study was conducted to supplement information obtained in the carcinogenicity study (reported later in this review). Reviews of this and the other 3 month studies are presented below.

11. 3-Month Range Finding Study in Mice (Administration in Food)**BACKGROUND INFORMATION**

Study Title:	3-Month Range Finding Study in Mice (Administration in Food)
Sponsor Study No.:	906171
Study Dates:	May 22 - August 22, 1990
Report Date:	February 28, 1992
Test Facility:	CIBA-GEIGY Limited Pharmaceuticals Division 4002 Basle Switzerland
GLP Status:	Compliant with GLP Switzerland, Procedures and Principles, March 1986, Section 4, 2.2(e)
NDA Volume:Page	18:1

METHODS

Test Article:	CGP 25827A
Batch No.:	810187
Purity:	_____
Control Article:	Diet
Species/Strain:	Mouse/Tif:MAGf (SPF), hybrids of NIH x MAG
Route:	Oral in the diet
Duration of Exposure:	3 months

Animals were assigned to groups and administered CGP 25827A in the diet. Target doses and actual doses measured in the diet are presented in the following table. In the last column, plasma levels associated with each group are presented; mean values represent a sample size of 2 for each group at the end of the study and ND stands for not detected.

Dosing Information						
Group	No. Animals per sex	Target Dose (mg/kg/day)	Actual Dietary Levels (mg/kg/day)		Mean Plasma Levels after 3 months (pmol/g)	
			Males	Females	Males	Females
1	9	0	-	-	ND	ND
2	9	40	5.73	8.97	ND	ND
3	9	140	22.3	33.5	3.4	2.5
4	9	400	61.8	82.3	7.0	6.9
5	9	1400	246	335	32.9	35.8

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Water consumption	Weekly
Clinical pathology	1 and 3 months
Blood level determinations	1 and 3 months (2/sex/group)
Organ weight	3 months
Gross pathology (tissues were saved)	3 months
Histopathology	3 months (heart only)

RESULTS

In-life Observations

There were no remarkable differences from control in mean body weight values. Treated mice ate and drank more than control mice, but not in a consistent dose-response pattern.

Clinical Pathology

Slightly higher than control values for erythrocyte, hemoglobin, and hematocrit were observed in the females treated at the 400 (Group 4) and 1400 (Group 5) mg/kg/day level. Mean values reported for these parameters are reported in the following table with statistically significant findings noted with an asterisk.

Mean Hematology Values of Parameters Affected by CGP 25827

Group	RBC (T/l)		Hb (mmol/l)		Hct (l)	
	Males	Females	Males	Females	Males	Females
1	11.4	11.1	10.6	10.7	0.503	0.509
2	12.0	11.6	10.7	10.9	0.503	0.523
3	12.1	11.8	11.4	11.2	0.527	0.532
4	12.4	12.5*	11.2	11.5*	0.532	0.551
5	12.1	12.6*	10.9*	11.9*	0.518*	0.568*

Serum chemistry parameters that differed from control included decreased glucose levels (mean values were 7.0, 4.4, 4.6, 4.8, and 4.0 for groups 1 - 5, respectively) and increased urea levels (mean values were 8.3, 11.8, 12.0, 11.2, and 14.3 for groups 1 - 5, respectively) for all treated groups of males. Liver enzyme levels of high dose males were elevated above control.

Gross and Microscopic Pathology

Effects on the heart and kidney were evidenced by changes in organ weight. Mean kidney weights, kidney-to-body and kidney-to-brain weight ratios were higher than control, with statistical significance often achieved in male groups treated at 140, 400, and/or 1400 mg/kg/day levels. Mean heart weight, heart-to-body and heart-to-brain weight ratios were consistently higher than control values for all treated groups of males and females.

There were no remarkable gross findings in tissues examined.

No treatment-related findings were revealed by microscopic evaluation of the heart in animals from any dose group.

CONCLUSION

After 3 months of dosing with CGP 25827A at levels up to 1400 mg/kg/day mice exhibited signs of effects on red cell parameters, heart, kidney, and perhaps liver. Erythrocyte, hemoglobin, and hematocrit values were elevated above control in the 400 and 1400 mg/kg/day female groups. Decreased glucose levels and increased urea levels relative to control were noted for all dose groups. Effects on the liver were evidenced by increases in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in males in the 1400 mg/kg/day dose group. Effects on the heart and kidney were evidenced by increases in weight in groups 2 (heart only), 3, 4, and 5. Only the heart was examined microscopically and no correlate to the increased weight or any other findings were revealed.

12. Thirteen Week Drinking Water Study in Mice**BACKGROUND INFORMATION**

Study Title: Thirteen Week Drinking Water Study in Mice
Sponsor Study No.: — D-8-3
Laboratory Study No.: 2069-102
Study Dates: May 17, 1979 - August 22, 1979
Report Date: March 6, 1980
Test Facility: _____

GLP Status: Compliant
NDA Volume:Page 17:1

METHODS

Test Article: CGP 25827A (BD40A)
Batch No.: L6
Control Article: Acidified water
Species/Strain: Mouse/B6C3F1
Route: Drinking water
Duration of Exposure: 13-weeks

Fifteen mice per sex were randomly assigned to four groups receiving CGP 25827A at concentrations of 0, 0.25, 0.50, and 1.00 mg/ml. Overall mean compound consumption values were 96, 185, and 397 mg/kg/day for males in Groups 2 - 4, respectively, and 101, 194, and 450 mg/kg/day, respectively for females in Groups 2 - 4, respectively. Mice received test material or control water via *ad libitum* exposure in the drinking water. Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Weekly
Body weight	Weekly
Food consumption	Weekly
Water consumption	Twice weekly
Ophthalmoscopic Examinations	Pretest, Weeks 6 and 13
Organ weight data	Week 13
Gross pathology	Week 13
Histopathology	Week 13

RESULTS

No treatment-related findings were noted for any of the parameters evaluated.

CONCLUSION

The no observable effect level (NOEL) for CGP 25827A is 1.0 mg/ml (~ 400 mg/kg/day) in mice treated for 13-weeks via oral administration in the drinking water.

13. 3-Month Oral Toxicity Study in Mice

BACKGROUND INFORMATION

Study Title: 3-Month Oral Toxicity Study in Mice
Sponsor Study No.: 906303
Study Dates: January 28 - May 7, 1991
Report Date: August 6, 1992
Test Facility: CIBA-GEIGY Limited
Pharmaceutical Division
Preclinical Safety
Basel, Switzerland
GLP Status: Compliant with GLP Switzerland, Procedures and Principles,
March 1986, Section 4, 2.2(e)
NDA Volume:Page 17:55

METHODS

Test Article: CGP 25827A
Batch No.: 400190
Control Article: Acidified tap water
Species/Strain: Mouse/Tif:MAGf(SPF)
Route: Drinking water
Duration of Exposure: 3 months

This study was designed to supplement a carcinogenicity study⁵ with information regarding:

- actual water consumption and test article intake
- plasma levels of CGP 25827A
- serum electrolyte levels (to assess adrenal gland function)
- ~~_____~~ analysis of the mitotic index of the non-glandular stomach mucosa and adrenal gland.

Twenty-two mice per sex were randomly assigned to three groups receiving CGP 25827A at concentrations of 0.25, 0.50, and 1.00 mg/ml. Within treatment groups, animals were divided into groups of 6 for clinical chemistry, 6 for hematology, and 10 for satellite

⁵ 104-Week Drinking Water Toxicity Study in Mice, _____
105, September, 16, 1983.

study. A group of 12 control mice per sex received acidified water and was also divided into 2 groups of 6 for clinical chemistry and hematology, respectively. Thus, the total number of animals used for in-life, gross and microscopic evaluations were 12 controls and 22 treated mice/sex/group. Mice received test material or control water via *ad libitum* exposure in the drinking water. Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Daily and then 3 times per week beginning with Week 5
Food consumption	Twice per week
Water consumption	Daily with correction for spillage
Hearing test	Pretest, Weeks 6 and 13
Ophthalmology	Pretest, Weeks 6 and 13
Clinical biochemistry	Pretest, Weeks, 5 and 15
Hematology	Pretest, Weeks 5, 9, and 14
Organ weight	Weeks 13 - 15
Gross pathology	Weeks 13 - 15
Histopathology	Weeks 13 - 15

RESULTS

The Sponsor calculated individual water consumption and test article intake after recovery of spilled water. Water consumption for the high dose males was low and yielded lower than target values for test article intake. The test article intake values for high dose males was similar to that of mid-dose females. Test article intake values for high dose females were about 60% higher than that for high dose males. Using a graph provided the Sponsor, the overall mean test article intake values can be estimated as 65, 100, and 165 mg/kg/day for males and 80, 180, and 270 mg/kg/day for females. Results of plasma analysis were not reported in this study.

There were no remarkable findings in clinical chemistry parameters.

Microscopic evaluation of the adrenal gland revealed minimal focal hypertrophy or hyperplasia in several male mice in the mid and high dose groups. No increase in mitotic activity of adrenal cortical cells was revealed in this study. There was no mention of findings or increases in mitotic indices in the non-glandular stomach.

Other microscopic findings included congestion and increased hematopoiesis in the spleen in all dose groups and hypertrophy of the mandibular salivary gland in females from all dose groups and from males from the high dose group.

CONCLUSION

The Sponsor attempted to replicate the conditions of the referenced carcinogenicity study, with the exception of data collection techniques for water consumption, plasma sampling, and evaluation of mitotic indices in two tissues.

Findings unique to only one of the two studies are summarized in the following table.

Carcinogenicity Study Only	Current Study Only
<ul style="list-style-type: none"> • adrenal spindle cell hyperplasia 	<ul style="list-style-type: none"> • water consumption and thus test article intake calculations were higher for females when compared to males in all dose groups • splenic congestion and hematopoiesis with concomitant changes in red blood cell parameters • hypertrophy of the mandibular salivary gland

While not previously reported for mice, the findings in the mandibular salivary gland had been seen in rats and were considered by the Sponsor to be characteristic of the response to β -agonists.

Dogs

The toxicity of CGP 25827A was tested in dogs via both the oral and inhalation routes of administration for up to 1 year. Effects revealed in these studies are attributed to an exaggeration of pharmacologic effects of beta agonists. Dogs treated for one year by the inhalation route exhibited increased heart rate and force of contraction at daily doses of $\geq 2 \mu\text{g/kg/day}$ ($.002 \text{ mg/kg/day}$) via inhalation and $\geq 0.01 \text{ mg/kg/day}$ via oral administration. Myocardial fibrosis was noted in the dogs treated orally and did not fully resolve during the one-month recovery phase. This finding was not noted in the inhalation study.

There were no signs of overt toxicity in dogs treated with CGP 25827A at a level of $15.16 \mu\text{g/kg/day}$ via inhalation for one year.

Reviews of studies conducted in dogs is presented below.

14. 12 Month Oral Toxicity Study in Beagle Dogs (CGP 25827A)

BACKGROUND INFORMATION

Study Title: 12 Month Oral Toxicity Study in Beagle Dogs (CGP 25827A)
 Sponsor Study No.: 85-751

Laboratory Study No.: Not applicable
Study Dates: March 10, 1986 – April 8, 1987
Report Date: April 18, 1988
Test Facility: CIBA GEIGY Limited
 Experimental Toxicology
 4332 Stein, Switzerland
GLP Status: Compliant
NDA Volume:Page 26:1

METHODS

Test Article: CGP 25827A
Batch No (analytical content of active [mg]): 11/806/1 (1.9); 11/805/1 (0.9); 11/804/1 (0.05); 11/803/1 (0.1); 11/802/1 (0.5); 11/801/1 (1.0)
Control Article: Gelatin capsule with "Placebo EE, batch 09/797/1"
Species/Strain: Beagle dog
Route: Oral capsule
Duration of Exposure: 12 months

Animals were randomly assigned to study groups and the test material was administered in gelatin capsules illustrated in the table below. Doses were selected based on observed toxicity in previous studies with CGP 25827A. The high dose was expected to cause myocardial lesions and was estimated to be the maximum tolerated dose for beagle dogs subjected to a 1-year study. Slight pharmacodynamic effects (increased heart rates) were expected at the low dose. Four dogs/sex/group were treated for 1 year and terminated. An additional 2 dogs/sex/group remained on study for a 1-month recovery period.

Group	No. Animals/Sex	Dose ($\mu\text{g}/\text{kg}/\text{day}$)
1	6	0
2	6	0.01
3	6	0.1
4	6	1.0

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Daily
Electrocardiograph	Prior to treatment and at Weeks 14, 28, and 51, and at the end of the 4-week recovery period. Measurements were taken immediately before the daily dose in all animals and at 1, 3, and 5 hours after dosing in 2 dogs/sex/group.
Ophthalmology	Prior to treatment, during Week 52, and at the end of the 4-week recovery period
Hearing test	Prior to treatment, during Week 52, and at the end of the 4-week recovery period

Parameter	Frequency of Measurement
Hematology	Prior to treatment and during Weeks 13, 27, 52, and at the end of the 4-week recovery period
Serum chemistry	Prior to treatment and during Weeks 13, 27, 52, and at the end of the 4-week recovery period
Urinalysis	Prior to treatment and during Weeks 13, 27, 52, and at the end of the 4-week recovery period
Gross pathology	4 dogs/sex/group after 52 Weeks and 2 dogs/sex/group after the 4-week recovery period
Organ weights	Same schedule as gross pathology. Organs weighed included: adrenals, brain, cervical lymph nodes, epididymides, heart, kidneys, liver, lung, ovaries, parathyroid, pituitary, prostate, spleen, submandibular salivary glands, testes, thymus, thyroid, and uterus.
Histopathology	Same schedule as gross pathology

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Reddening of the skin (ears and abdomen) and mucous membranes (gingival and conjunctiva) were reported for Groups 3 and 4 animals. This observation was first noted shortly following daily dosing and resolved. This observation was not noted during the 4-week recovery period.
Body weight	No remarkable observations.
Food consumption	No remarkable observations.
Ophthalmology	Alterations in the appearance of the fundus were noted in Groups 3 and 4 animals. The incidence of remarkable ophthalmoscopic findings in treated groups is summarized in the following table. Microscopic examination of ocular tissues revealed atrophy of the tapetum lucidum. These findings did not resolve during the 4-week recovery period

Incidence of Treatment-Related Ophthalmoscopic Findings

Finding	0.01 mg/kg/day		0.1 mg/kg/day		1.0 mg/kg/day	
	Males	Females	Males	Females	Males	Females
Expansion of pigmentation in the fundus	0/6	0/6	0/6	1/6	4/6	3/6
Orange spots in the tapetal area	0/6	0/6	1/6	4/6	5/6	4/6
Hyperreflective foci in the tapetal area	0/6	0/6	0/6	0/6	1/6	0/6

Parameter	Remarkable Observations
Hearing test	No remarkable observations.
Electrocardiograph	Bradycardia was noted in all treated groups. Heart rates were slightly increased when compared to controls in all treated groups 1, 3 and 5 hours after dosing and decreased thereafter, reaching markedly lower heart rates than controls by 22 hours after dosing.
Hematology	Decreased hemoglobin in group 3 males, hypochromasia and microcytosis in group 4 animals, increased platelet counts in group 2 males and group 3 females.

Parameter	Remarkable Observations
Serum chemistry	Decreased glucose in all treated groups; decreased cholesterol and HDL in group 4 males; decreased triglycerides in group 3 males and group 4 females; decreased phospholipid in group 4 animals; increased potassium in all treated groups; and increased phosphate in group 4 females.
Urinalysis	No remarkable observations.
Gross pathology	No remarkable observations.
Organ weight	Decreased heart weights in all treated groups of males.
Histopathology	Focal myocardial fibrosis in all groups of treated animals. This effect did not resolve during the one-month treatment-free period.

CONCLUSION

Observations noted in this study were consistent with those reported in previous studies and are attributed to an exaggeration of the pharmacologic activity of this β agonist. All treated groups exhibited pharmacologic signs of exposure to CGP 25827A (Foradil).

Clinical signs (reddening of the skin and mucous membranes) and increased heart rates are likely vasodilatory effects and were not noted during the course of the recovery phase. All signs of toxicity resolved within the 1-month recovery period with the exception of focal fibrosis of the myocardium and atrophy of the ocular tapetum lucidum. Myocardial fibrosis is consistent with findings in other studies with β agonists. The tapetum lucidum atrophy is considered to be of negligible clinical significance since humans are an atepetal species. The NOAEL was not identified in this effect.

15. Preliminary Inhalation Toxicity Study in the Dog

BACKGROUND INFORMATION

Study Title: Preliminary Inhalation Toxicity Study in the Dog
Sponsor Study No.: 926109
Laboratory Study No.: 321906
Study Dates: Not Stated
Report Date: June 8, 1993
Test Facility: _____

GLP Status: Non-compliant
NDA Volume:Page 40:294

METHODS

Test Article: CGP 25827A (Foradil)
Batch No: Not stated
Purity: Not stated
Control Article: None
Purity: Not stated

Species/Strain: Dog (strain not stated)
Route: Inhalation
No. of Animals: 2
Dose levels: 1.5 – 2.4 mg/kg/day
Duration of Exposure: 5 days

Toxicity was assessed by evaluating gross and microscopic pathology.

RESULTS

Myocardial necrosis was observed in both animals and was considered to be a result of treatment with CGP 25827A.

CONCLUSION

This study is of limited value in the overall evaluation of the toxicity of the drug due to several reasons including: the lack of proper controls, low sample size, unspecified conditions of exposure, lack of the full battery of parameters needed to assess toxicity, and the lack of a no observable effect level.

16. Inhalation Feasibility Study in Dogs (CGP 25827A (Foradil) — Formulation

BACKGROUND INFORMATION

Study Title: Inhalation Feasibility Study in Dogs (CGP 25827A (Foradil) —
Formulation
Sponsor Study No.: 93-6077
Laboratory Study No.: 653011
Study Dates: April 2 – 16, 1993
Report Date: June 24, 1994
Test Facility: _____

GLP Status: Compliant
NDA Volume:Page 41:1

METHODS

Test Article: CGP 25827A
Batch No: 1066/1
Purity: _____
Control Article: Not Applicable
Purity: Not Applicable
Species/Strain: Beagle dog
Route: Oral Inhalation

Exposure Conditions: Rotating brush generator with compressed air dispersion.
Duration of Exposure and Dose Levels: Single doses of 20 and 50 $\mu\text{g}/\text{kg}$ were given to one male and one female on separate occasions. The time between doses was not specified in the report. Animals were sacrificed on Day 4.

Particle Size Distribution: _____
 $\mu\text{g}/\text{kg}$ exposures, respectively.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	During and after dosing
Body weight	Weekly
Food consumption	Daily
Electrocardiograph	Predose and 5, 10, 30, 60, 120, and 240 minutes after dosing
Respiratory function	3 times over the course of the study
Blood for proof of absorption	Predose and at 30 minutes after each dose
Urine for proof of absorption	Urine was collected for 24 h immediately after dosing.
Gross pathology of the heart	Day 4
Histopathology of the heart	Day 4

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Increased heart force and reddening of the mouth, gums, and sometimes ventral surface of the abdomen were reported for one or both dogs immediately following exposure.
Body weight	No remarkable observations.
Food consumption	No remarkable observations.
Electrocardiograph	Heart rates were markedly increased with a maximum effect at 30 minutes to 1 hour post dose. Heart rates remained increased for up to 4 hours post dose.
Respiratory function	No remarkable observations.
Gross pathology of the heart	Pale foci were observed in the left ventricular papillary muscle in both animals. Valvular thickening was also observed.
Histopathology of the heart	Acute myocardial degeneration was observed in both animals.

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ON ORIGINAL

CONCLUSION

This study demonstrated that the CGP 25827A dry powder formulation could be administered to dogs in a direct passive inhalation dosing setting.

The heart was affected by treatment with CGP 25827A at levels of 20 and 50 µg. Clinical signs (reddening of the mouth and ventral surface) and increased heart rates are likely vasodilatory effects. The Sponsor reported that the myocardial degeneration was consistent with anoxic damage indirectly associated with vasodilatation.

17. 4-Week Inhalation Toxicity Study in Dogs with CGP 25827A(Foradil)Dry Powder Formulation (1/69) in the Dog**BACKGROUND INFORMATION**

Study Title: 4-Week Inhalation Toxicity Study in Dogs with CGP 25827A(Foradil)Dry Powder Formulation (1/69) in the Dog
Sponsor Study No.: 926074
Laboratory Study No.: 321917
Study Dates: July 9 - august 6, 1992
Report Date: January 22, 1993
Test Facility: _____

GLP Status: Compliant
NDA Volume:Page 41:52

METHODS

Test Article: CGP 25827A (Foradil) Dry Powder Formulation (1/69)
Batch No: 1066/1
Purity: _____
Control Article: Lactose _____ 100 Mesh
Purity: Not stated
Species/Strain: Beagle dog
Route: Oral Inhalation
Exposure Conditions: Rotating brush generator with a _____

Duration of Exposure: 4 Weeks

Animals were randomized into study groups and dosed as follows:

Group	No. Animals/sex	Dosing Information	
		Dose Levels of Lactose or Test Article as Supplied (mg/kg/day)	Active Dose Calculated from Analysis of Filter Samples (µg/kg/day)
1 (Lactose)	3	2	0
2 (Low)	3	0.1	3
3 (Mid)	3	0.5	14
4 (High)	3	2	55

The mean percentage \pm S.D. of particles found at an aerodynamic diameter of 4.6 µm or below was $76.0 \pm 3.1\%$ for CGP 25827A and 48.4 ± 7.6 for lactose.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Weekly
Food consumption	Daily
Ophthalmoscopic exams	Predose and after 4 weeks
Electrocardiograph	Predose Day 1 – 30 minutes postdose (all groups) and 2 hours postdose (Groups 2 and 4) Week 1 – Predose; and at 10 minutes and then 2 hours postdose Week 2 – Predose and 2 hours postdose Week 4 – Predose and 10 minutes and then 2 hours postdose
Respiratory function	Predose, on Day 2 and after 4 weeks
Hematology	Predose and after 4 weeks
Serum Chemistry	Predose and after 4 weeks
Urinalysis	Predose and after 4 weeks
Blood for proof of absorption	After 1 week: Prior to the daily dose and 30 minutes after dosing in Group 4 animals only
Urine for proof of absorption	Urine was collected from all animals for 24 h immediately after dosing at the end of Weeks 1 and 4.
Gross pathology	After 4 weeks.
Organ weights	Weighed organs include: adrenal glands, brain, heart, kidneys, liver, lungs, pituitary gland, prostate gland, spleen, testes, epididymides, thyroid gland with parathyroids.
Histopathology	After 4 weeks.

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RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Erythema of the mucous membranes of the oral cavity and abdominal skin was observed in all dogs treated with CGP 25827A. This observation was noted daily shortly following dose administration and persisted for approximately 1 to 5 hours.
Body weight	No remarkable observations.
Food consumption	No remarkable observations.
Electrocardiograph	Heart rates from all treated groups were increased at 30 minutes and 2 hours post dose on Day 1 when compared to control and pre-test values. This change was accompanied by increased P-wave amplitude and decreased QT-intervals. Frank tachycardia was observed in dogs from all dose groups. These changes were also observed at subsequent intervals.
Respiratory function	No remarkable observations.
Hematology	No remarkable observations.
Serum chemistry	Serum electrolyte levels were slightly off balance in treated animals when compared to controls and pretest values. Potassium levels increased slightly with increasing dose while sodium levels were significantly lower than controls for Groups 3 and 4 females.
Urinalysis	No remarkable observations.
Gross pathology	No remarkable observations.
Organ weights	No remarkable observations.
Histopathology	Myocardial fibrosis was observed in 0/3, 1/3, 0/3, and 2/3 males from Groups 1 - 4, respectively and in 0/3, 0/3, 1/3, and 1/3 females from Groups 1 - 4, respectively. Severity increased with increasing dose. A no effect level for this observation was not identified in this study.

CONCLUSION

The heart was affected by treatment with CGP 25827A at levels of 3, 14, and 55 $\mu\text{g}/\text{kg}/\text{day}$ inhaled over a 4-week period. Clinical signs (erythema of the mucous membranes of the mouth and abdominal surface) and increased heart rates are likely vasodilatory effects associated with the pharmacologic action of the drug. The myocardial fibrosis observed in this study is consistent with that observed in previous studies with this drug.

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18. One Month Inhalation Toxicity Study in Dogs (Test for Toxic Effects of Degradation Products)

BACKGROUND INFORMATION

Study Title: One Month Inhalation Toxicity Study in Dogs (Test for Toxic Effects of Degradation Products)
Sponsor Study No.: 90-6158
Laboratory Study No.: 644190
Study Dates: August 6 - September 6, 1990
Report Date: March 16, 1991
Test Facility: _____
GLP Status: Compliant
NDA Volume:Page 33:1

METHODS

Test Article: CGP 25827A (fresh and expired)
Batch No: 13/617/3 (fresh - expiration date 8/91)
 13/831/3 (outdated - expiration date 11/89)
Purity: Not stated
Control Article: Aerosol fluorocarbon propellants
Purity: Not stated
Species/Strain: Beagle dogs
Route: Oral Inhalation
Exposure Conditions: 100 metered doses of 12 µg/actuation yielding a daily dose of 12 µg
Duration of Exposure: 28 days

The purpose of this study was to compare the effects of fresh and outdated batches of CGP 25827A on beagle dogs, when administered inhaled doses for 28 days. Accordingly, comparisons were made between groups receiving fresh and outdated batches of drug rather than comparisons to controls.

Dosing Information		
Group	No. Animals per sex	Target Dose (µg/day)
1	2	0
2	4	1200 (outdated)
3	4	1200 (fresh)

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Clinical signs	Daily
Body weight	Twice weekly
Food consumption	Daily
Ophthalmoscopic Examinations	Pretest and Week 4
Electrocardiograms	Pretest and Week 4
Respiratory Function	Pretest and Week 4
Clinical Pathology	Pretest and Day 27
Gross pathology (including organ weights)	Study termination
Histopathology	Study termination

RESULTS

Parameter	Findings
Clinical signs	No remarkable findings.
Body weight	No remarkable findings.
Food consumption	No remarkable findings.
Ophthalmoscopic Examinations	No remarkable findings.
Electrocardiograms	No remarkable findings.
Respiratory Function	No remarkable findings.
Clinical Pathology	A slight reduction in hemoglobin and red blood cell counts were noted in dogs treated with CGP 25827A when compared to controls. No differences were noted between groups treated with fresh and outdated CGP 25827A.
Gross pathology (including organ weights)	Spleen weights were lower for dogs treated with CGP 25827A when compared to controls. No differences were noted between groups treated with fresh and outdated CGP 25827A.
	There were no remarkable gross findings.
Histopathology	No remarkable findings.

CONCLUSION

There were no differences between groups treated with fresh CGP 25827A or with CGP 25827A that was outdated.

19. 6/12 Month Inhalation Toxicity Study in Dogs (CGP 25827A (Foradil))

BACKGROUND INFORMATION

Study Title: 6/12 Month Inhalation Toxicity Study in Dogs (CGP 25827A (Foradil))

Sponsor Study No.: 93-6116

Laboratory Study No.: 653184

Study Dates: May 21, 1993 - August 24, 1994

Report Date: March 15, 1996

Test Facility:

GLP Status: Compliant
NDA Volume:Page 42:1

METHODS

Test Article: CGP 25827A (Foradil)
Batch No: 1103/1 (Weeks 1 - 42) and 1173/1 (Weeks 42 - 52)
Purity:
Control Article: Lactose 100 Mesh
Purity: Not stated
Species/Strain: Beagle dog
Route: Oral Inhalation
Exposure Conditions: Rotating brush generator with compressed air dispersion.
Duration of Exposure: 12 months

Animals were randomly assigned to study groups and the test material was administered as follows:

Group	No. Animals/Sex	Target Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Achieved Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Particle Size (% < 6 μm via —)
1	6	0	0	-
2	4	2	2.01	87
3	4	6	5.44	83
4	6	18	15.16	89

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Daily
Electrocardiograph	Predose, immediately following dosing and 1 hour after dosing on Day 1 and during Weeks 6, 12, 25, 38 and 51 and at the end of the 8-week recovery period
Ophthalmology	Prior to treatment and during Weeks 6, 12, 25, 38 and 51 and at the end of the 8-week recovery period
Respiratory function	Prior to treatment and at Weeks 6, 12, 25, 38 and 51 and at the end of the 8-week recovery period
Hematology	Prior to treatment and during Weeks 7, 13, 25 and 52 and at the end of the 8-week recovery period
Serum chemistry	Prior to treatment and during Weeks 7, 13, 25 and 52 and at the end of the 8-week recovery period
Urinalysis	Prior to treatment and during Weeks 7, 13, 25 and 52 and at the end of the 8-week recovery period

Parameter	Frequency of Measurement
Blood for proof of absorption	Serial samples were collected on Day 1 and during Weeks 28 and 52
Urine for proof of absorption	Urine was collected for 24 h on Day 1 and during Weeks 28 and 52
Gross pathology	4 dogs/sex/group after 52 Weeks and 2 dogs/control and high dose groups after an 8 week recovery period
Organ weights	Same schedule as gross pathology. Organs weighed included: adrenals, brain, heart, kidneys, liver, lung, ovaries, pancreas, parathyroid, pituitary, prostate, spleen, testes, thymus, thyroid, and uterus.
Histopathology	Same schedule as gross pathology. Special staining _____ was used to examine mucus-secreting cells of the trachea.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Observations
Mortality	No remarkable observations.
Clinical signs	Increased heart force and reddening of the mouth, gums, ears and ventral surface of the abdomen were reported for all dogs treated with CGP 25827A (Foradil). This observation was not noted during the 8-week recovery period.
Body weight	Body weight values in treated animals were consistently higher than control values during the treatment phase of study. The high dose animals exhibited a gradual body weight loss during the 8-week recovery period.
Food consumption	No remarkable observations.
Ophthalmology	No remarkable observations.
Electrocardiograph	Heart rates were sporadically increased in all groups treated with CGP 25827A (Foradil) when compared to controls, however no consistent patterns were observed during the course of the study.
Respiratory function	No remarkable observations.
Hematology	Red cell parameters were lower in treated animals from all groups when compared to controls throughout the treatment period. This finding was present, but to a lesser degree, at the end of recovery period for high dose animals.
Serum chemistry	Triglyceride levels for treated animals were lower than control values at all serum collection intervals for males and at Weeks 7, 13 and 52 for females. Creatinine levels were consistently higher for treated groups when compared to controls. There were no remarkable observations for animals in the recovery phase of the study.
Urinalysis	No remarkable observations.
Gross pathology	No remarkable observations.
Organ weight	No remarkable observations.
Histopathology	No remarkable observations.

CONCLUSION

Observations noted in this study were consistent with those reported in previous studies and are attributed to an exaggeration of the pharmacologic activity of this β agonist. All

treated groups exhibited pharmacologic signs of exposure to CGP 25827A (Foradil). Results of the proof of absorption studies were not included in this report.

Clinical signs (reddening of the mouth and ventral surface) and increased heart rates are likely vasodilatory effects and were not noted during the course of the recovery phase. The Sponsor attributed the increased body weight effect to the anabolic effect of β agonist compounds. They further speculated that the observed increases in creatinine levels in treated animals could be either the result of an anabolic effect, with a proportional increase in muscle mass, or a consequence of lower glomerular filtration brought on by low blood pressure associated with the pharmacologic action of CGP 25827A (Foradil).

In conclusion, there were no signs of overt toxicity associated with inhalation of CGP 25827A (Foradil) at levels up to 15.16 $\mu\text{g}/\text{kg}/\text{day}$, the NOAEL, for one year in beagle dogs.

Overall Toxicology Summary

The toxicity of Formoterol fumarate was studied in rats, mice, and dogs via oral and inhalation routes of administration. Inhalation toxicology studies in rats and dogs were conducted for up to 1 year. Oral studies in mice and rats were used in selecting the doses of subsequent carcinogenicity studies and were conducted for 3 months. An oral study in dogs was conducted for up to 1 year.

Effects on the heart were consistent across species and routes of administration and were noted in both the 90-day and 1-year studies. Myocardial fibrosis was observed in rats dosed up to 3 months in the drinking water at levels of 10 $\text{mg}/\text{kg}/\text{day}$ and above. Heart weights were increased in rats dosed at levels of 0.34 $\text{mg}/\text{kg}/\text{day}$ via inhalation without microscopic correlate after 3 months. One year of inhalation treatment in rats yielded increased heart weights in groups treated at .03 $\text{mg}/\text{kg}/\text{day}$ and above, with myocardial fibrosis observed in males treated at the .4 $\text{mg}/\text{kg}/\text{day}$ (high dose) level. After 3 months of dietary administration, mice treated at levels of 40 $\text{mg}/\text{kg}/\text{day}$ and above had increased heart weights without microscopic correlate. Clinical signs of effects on the heart were most evident in the dog, which when dosed, turned red (gums, ears, and/or abdomen) and had increased heart rate and force of contraction. These signs were noted at levels of ≥ 2 $\mu\text{g}/\text{kg}/\text{day}$ (.002 $\text{mg}/\text{kg}/\text{day}$) via inhalation and ≥ 0.01 $\text{mg}/\text{kg}/\text{day}$ via oral administration. Myocardial fibrosis was noted in the dogs treated orally (≥ 0.01 $\text{mg}/\text{kg}/\text{day}$) and did not fully resolve during the one-month recovery phase. This finding was not noted in one-year inhalation study up to 15.16 $\mu\text{g}/\text{kg}/\text{day}$.

Body weight and food consumption was higher than controls in all nearly all treated groups across species and routes of administration. The Sponsor attributes this to an anabolic effect of beta agonists.