COMPARATIVE TOXICOLOGICAL ASSESSMENT OF A WATER-SOLUBLE AZOLE ADMINISTERED BOTH ORALLY AND BY INTRAVENOUS CONTINUOUS INFUSION IN CYNOMOLGUS MONKEYS

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ABSTRACT — The water-soluble azole, 4-[1-[(3-methylaminoacetoxymethyl-pyridin-2-yl)-methyl-carbamoyloxy]-ethyl]-1-[(2R,3R)-3-[4-(4-cyano-phenyl)-thiazol-2-yl]-2-(2,5-difluoro-phenyl)-2-hydroxy-butyl]-1H-[1,2,4]triazol-4-ium chloride hydrochloride (hereinafter referred to as WSA), is a new water-soluble triazole anti-fungal compound. WSA is rapidly converted into its active metabolite after administration by either the intravenous or oral route. The clinical effects of WSA are expected to be consistent whether it is administered by injection (intravenous) or orally.

To evaluate and compare the toxicities of WSA via oral and intravenous administration, repeated toxicity studies of WSA were conducted in cynomolgus monkeys (oral dosage levels: 0, 10, 30 and 90 mg/kg/day over a 4-week period; intravenous infusion dosage levels: 0, 10, 30 and 60 mg/kg/day over a 2-week period). In addition, a 2-week oral toxicity study of the active metabolite of WSA was conducted with similar dosages (0, 10, 30 and 90 mg/kg/day).

From the results obtained from the evaluation, hepatotoxicity (liver) and endocrinological toxicity (adrenals) were the major toxicities, a finding which is comparable to other previously tested azole compounds.

In the 4-week oral toxicity study, the lethal dose of WSA was determined to be 90 mg/kg. However, no animal mortality was observed during the 2-week intravenous continuous infusion study.

The toxicokinetics (TK) profile of WSA at 10 and 30 mg/kg dosages illustrated a rapid, dose-dependent conversion of WSA to its active metabolite, and the plasma levels of $C_{\max}$ and AUC were well correlated to their toxicities. However, at high dosages (60 and 90 mg/kg), WSA demonstrated high exposure beyond the dose-proportional manner. Overall, the results obtained indicate that maintaining an effective concentration and a non-toxic dosage level would be manageable by utilizing an initial intravenous continuous infusion followed by oral administration.

KEY WORDS: Antifungal, Triazole, Water-soluble azole, Intravenous infusion

INTRODUCTION

Recently, the number of patients who are suffering from deep-seated mycosis is increasing, and the necessity for development of systemic antifungal therapy is increasing. However, current drugs for the treatment of deep-seated mycosis are usually administered via an oral route, because of their high lipophilicity (low solubility). These drugs also have a narrow antifungal spectrum with several toxicities in the liver and endocrinological organs (Van Cauteren *et al.*, 1989).

Furthermore, drug-resistant strains of fungus have been encountered after treatment with the existing azole antifungal drugs (Yamaguchi, 2000; Niki *et al.*, 1999). To improve these critical issues, a water-soluble azole drug has been sought for the treatment of severe and deep seated mycoses, especially for hospitalized patients, which can be applicable orally and via intravenous administration.

WSA is a new water-soluble azole derivative with a broad antifungal spectrum of efficacy, and is suitable for both injectable and oral treatment. WSA is rapidly

converted into its active form by esterase, and the active compound is highly inhibitory against ergosterol synthesis. Its active compound was identified as having a broad antifungal spectrum and a good safety profile including a low drug-drug interaction. However, water solubility was too low for intravenous injection, so a prodrug (WSA) was designed for improving water solubility.

This paper examines the toxicological profile of WSA via oral administration and intravenous continuous infusion in monkeys, as well as its safety assessment for this compound.

MATERIALS AND METHODS

The study described in this paper was carried out in accordance with the guidelines of the Nippon Roche Research Center Committee for Experimental Animals (based on the Guide for the Care and Use of Laboratory Animals, NIH OACU) on the use of laboratory animals in research.

Test article

The test article, a water-soluble azole (WSA), is a white powder, and has the chemical name of 4-[1-[(3-methylaminoacetoxymethyl-pyridin-2-yl)-methyl-carbamoyloxy]-ethyl]-1-[(2R,3R)-3-[4-(4-cyano-phenyl)-thiazol-2-yl]-2-(2,5-difluoro-phenyl)-2-hydroxy-butyl]-1H-[1,2,4]triazol-4-ium chloride hydrochloride.

The active metabolite is also a white powder and has the chemical name of (1R,2R)-4-[2-[2-(2,5-difluoro-phenyl)-2-hydroxy-1-methyl-3-[1,2,4]triazol-1-yl-propyl]-thiazol-4-yl]-benzonitrile. The WSA and the active metabolite utilized in this study were produced at Nippon Roche Research Center (NRRC). The chem-

ical structures of WSA and the active metabolite are shown in Fig. 1.

Animals and housing conditions

Healthy cynomolgus monkeys (*Macaca fascicularis*, purpose-bred; 3 to 6 years old), purchased from SNBL Service (Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan) were used in this study following an appropriate quarantine and acclimation period.

The body weights of these monkeys at the initiation of the treatment period range from 3.7 to 5.0 kg for the males and from 2.6 to 3.8 kg for the females. The animals were housed in an air-conditioned room (room temperature $25 \pm 2^\circ\text{C}$; relative humidity $55 \pm 10\%$) and kept individually in metal cages, with 12-hr periods of light and dark. They were provided approximately 100 g/day of food to eat (Primate diet #5048, PMI Feeds Inc., USA) and tap water (*ad libitum*).

For the infusion study, the catheter was implanted into the vena cava via the femoral vein under anesthesia, approximately two weeks prior to the initiation of the treatment period (Mendenhall, 2000; Walker, 2000).

Study types, dosages and drug preparation/administration methods

Study types for the comparative safety assessment of oral and infusion administration consisted of a 4-week oral administration of WSA with 2-week interim tests, a 2-week infusion administration of WSA, and a 2-week oral administration of the active metabolite.

The dosages, drug preparation and drug administration methods employed in this study are summa-

WSA

Molecular Weight: 789.7

Water solubility: > 1000 mg/ml

Purity: 98.35%

Active metabolite of WSA

Molecular weight: 437.5

Purity: 99.71%

Fig. 1. Chemical structure of WSA and the active metabolite of WSA.

rized in Table 1.

Observations and examination designs

1. General conditions

All of the monkeys were observed daily during both the treatment and recovery periods, and mortality and clinical signs were recorded.

The body weight measurements for each monkey were carried out and recorded, once a week for the 4-week study and twice a week for the 2-week studies. Daily food consumption was also monitored and recorded for each monkey.

2. Clinical testing

1) Hematological examinations

Blood samples were collected from the femoral or limb veins of each monkey. EDTA-2K was used as the anticoagulant. Blood samples were obtained before the initial WSA or active metabolite treatment (Day 0) and on the final drug administration day (Day 13 for the 2-week studies, Day 27 for the 4-week study), at the midpoint of the treatment period for the 4-week study (Day 13) and at the end of the recovery period.

The following values were determined or calculated from assay data measured by a multipurpose automatic cell counter (Technicon auto-analyzer H*1, Technicon Co., Ltd., USA): red blood cell count, white blood cell count, hemoglobin concentration, hematocrit, platelet count, mean corpuscular volume, mean

corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Hemogram (differential cell count: neutrophil, eosinophil, basophil, monocyte and lymphocyte) was indicated as a ratio or percentage. The reticulocytes were counted by the Brecher method using Sysmex F-300 (TOA Medical Electronics, Japan).

2) Blood chemical analyses

Blood samples for serum analyses were collected utilizing the same procedure as was previously described above for hematology, excluding the anticoagulant. The following clinical assays were performed on an autoanalyzer, the H7150 (Hitachi Ltd., Tokyo, Japan): GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase), ALP (alkaline phosphatase), total protein, albumin, glucose, TG (triglyceride), T-CHO (total cholesterol), BUN (blood urea nitrogen), creatinine, total bilirubin, calcium, inorganic phosphorus, sodium, potassium and chloride, and an A/G (albumin/globulin ratio) was calculated.

3. Pathological examination

1) Necropsy findings

The monkeys that expired during the treatment period were necropsied as soon as possible after the confirmation of their death. All surviving monkeys were euthanized either at the end of the treatment period or at the end of the recovery period. These mon-

Table 1. Study types, dosages, drug preparation and drug administration methods.

Test article	WSA	WSA	Active metabolite of WSA
Administration route	p.o.	i.v. continuous infusion	p.o.
Administration period	4 weeks	2 weeks	2 weeks
Recovery period	4 weeks	4 weeks	—
Dose levels	0, 10, 30, 90 mg/kg	0, 10, 30, 60 mg/kg	0, 10, 30, 90 mg/kg
Carrier	water (pH 3.5 $\pm$ 0.1)	saline (pH 3.5 $\pm$ 0.1)	0.5%CMC-Na solution
Concentration	5, 15, 45 mg/ml	0.8, 2.4, 4.8 mg/ml	5, 15, 45 mg/ml
Dosage volume	2 ml/kg	6.25 ml/kg/hr, 2 hr/day	2 ml/kg
Dosage speed	—	12.5 ml/kg/2 hr/day	—
Administration method	naso-gastric tube	syringe pump: KDS220	naso-gastric tube
Number of animals used	4/sex/dose [#]	4/sex/dose [#]	1/sex/dose

[#] Three animals/sex/dose were euthanized at the end of the treatment period and one animal/sex/dose was euthanized at the end of the recovery period.

keys were terminated by exsanguination using the axillary artery/vein under anesthesia and their necropsy findings were recorded.

2) Organ weights (absolute and relative)

The following organs were weighed and the relative organ weights (organ weight per kg of body weight) were calculated based on the final body weight measurement: brain (including cerebellum and brain stem), pituitary, thyroids (including parathyroid), salivary glands (submandibular and sublingual glands), thymus, lung (including trachea and bronchus), heart, liver, pancreas, spleen, adrenals, kidneys, testes, epididymides, seminal vesicles, prostate, ovaries, and uterus (including vagina).

3) Histopathological examination

Histopathological specimens for all of the monkeys were sampled and prepared from the following organs and tissues: brain (cerebrum, cerebellum and medulla oblongata), pituitary, thyroids, parathyroid, salivary glands (submandibular and sublingual glands), thymus, lung, bronchus, trachea, heart, liver, gallbladder, spleen, adrenals, kidneys, testes, epididymides, seminal vesicles, prostate, ovaries, uterus, vagina, pancreas, stomach, duodenum, jejunum, ileum, colon, rectum, spinal cord, aorta, tongue, esophagus, skin, eyeballs, mammary gland, skeletal muscle, urinary bladder, mesenteric lymph node, bone marrow (femur), sternum, sciatic nerve, lachrymal gland, injection site (tissue around the vein where the tip of the catheter was located) and any other gross lesions that were uncovered.

The organs above, excluding the eyeballs, testes and epididymides, were fixed with 15% buffered formalin (pH 6.8), embedded in paraffin, sectioned, stained with hematoxylin-eosin and then closely examined using a light microscope. The eyeballs were fixed with a combined glutaraldehyde/formalin fixative and then refixed in 15% buffered formalin (pH 6.8). The testes and epididymides were fixed with Bouin's solution. The eyeballs, testes and epididymides were then processed the same as the other tissues described above and examined histopathologically.

Toxicokinetics

Blood samples (approx. 0.5 ml/point) were collected from the limb vein with heparin used as the anti-coagulant on the first treatment day, on the midpoint

day of the treatment period, and on the final treatment day.

For the 4-week oral administration study of WSA, samples were obtained prior to treatment and 1, 2, 4, 7 and 24 hr after administration on Days 0 and 27. On Day 13, samples were obtained prior to treatment and 4 hr following administration.

For the 2-week intravenous continuous infusion study of WSA, samples were obtained prior to treatment, 1, 2, 4, 7 and 24 hr after the start of 2-hr continuous infusion on Days 0 and 13. On Day 7, samples were obtained prior to treatment and 2 hr after the start of 2-hr continuous infusion.

For the 2-week oral administration of the active metabolite study, samples were obtained prior to treatment and 1, 2, 4, 7 and 24 hr after administration on Days 0, 7 and 13.

Immediately after the collection of each specimen, citric acid and paraoxon were added to facilitate the stabilization of the WSA. Samples were centrifuged and plasma level profiles of WSA, its active metabolite and its pro-moiety were measured using LC/MS/MS (PE SCIEX API, Applied Biosystems, CA, USA).

Statistical analysis

The data collected were expressed as the mean $\pm$ S.D.

Statistical analysis was performed using Pitman test (Lehmann, 1975) for the hematology and blood chemistry values, as well as the organ weights. The *p* values less than 0.05 ($p < 0.05$) were regarded as being statistically significant compared to the control group.

RESULTS

General conditions

At the 90 mg/kg level of WSA via oral administration, two female monkeys expired on Days 16 and 25, respectively, both exhibiting severe emaciation. Severe to moderate emaciation, with a remarkable reduction of both body weight and food consumption was observed in the 90 mg/kg WSA and the active metabolite treatment groups. Vomiting, soft stool or diarrhea, and a decrease in spontaneous motor activity were occasionally observed slightly in the 30 mg/kg group receiving oral administration of WSA, while these signs were described as moderate in the 60 and 90 mg/kg groups receiving oral or intravenous administration of WSA and the active metabolite.

Major toxicity effects elicited by WSA

The primary or most significant toxicological changes initiated by WSA were hepatotoxicity and toxic effects exerted upon the endocrinological system, specifically on the adrenals. The resulting data from this study are discussed below and are considered to be indicative of significant toxicity.

1. Liver toxicity

Data collected from this study that indicate the liver toxicity parameters measured are summarized in Tables 2-1, 2-2 and 2-3 (Tables 2-1, 2-2 and 2-3).

1) Oral administration of WSA

An increase in the liver weight, hypertrophy of hepatocytes and fatty changes in the hepatocytes were detected and documented by the completion of the treatment period. These alterations were very slight for the 30 mg/kg and slight for the 90 mg/kg group. Paralleling these morphological changes, a decrease in the total cholesterol level and an increase in the triglyceride level in the serum were observed on Days 13 and 27. These parameters are indicators of hepatotoxicity and were more pronounced in the male than in the female monkeys. There was no detectable hepatotoxicity in the 10 mg/kg WSA group (Table 2-1).

2) Intravenous continuous infusion of WSA

An increase in the liver weight, hypertrophy of the hepatocytes and/or eosinophilic changes in the perilobular hepatocyte were detected and documented by the completion of the treatment period. These alterations were appraised as very slight for the female and slight for the male monkeys from the 60 mg/kg group. Again, paralleling these morphological changes, a decrease in the total cholesterol level and an increase in the triglyceride level in the serum were observed on Day 13. For the 30 mg/kg group, a slight increase in the liver weight and an increase in the serum triglyceride level were recorded in the male monkeys, and the fatty changes in the centrilobular hepatocytes were also observed as very slight in the female monkeys. Overall, the indicators of hepatotoxicity were more severe for the male than for the female monkeys. There was no detectable drug-related hepatotoxicity observed in the 10 mg/kg group (Table 2-2).

3) Oral administration of the active metabolite

An increase in the liver weight, hypertrophy of the hepatocytes and/or fatty changes in the hepatocytes were detected and documented by the completion of the treatment period. These alterations were observed to be very slight for the 90 mg/kg group. Paralleling

these morphological changes, a decrease in the total cholesterol level and an increase of triglyceride level in the serum were recorded on Day 13. Furthermore, a decrease in the total cholesterol level was observed in the male monkey from the 30 mg/kg group. These indicators of hepatotoxicity were more severe for the male than for the female monkeys. There was no detectable hepatotoxicity in the 10 mg/kg group (Table 2-3).

2. Endocrinological toxicities

Data collected from this study indicates endocrinological toxicity, observed primarily in the adrenals as summarized in Tables 3-1, 3-2 and 3-3 (Tables 3-1, 3-2 and 3-3).

1) Oral administration of WSA

An increase in the adrenal weight and a thickening of the zona fasciculata with an increase of lipid droplets were observed in both male and female monkeys from the 90 mg/kg group. An increase in the adrenal weight was measured and recorded in male from the 30 mg/kg group. There was no detectable endocrinological toxicity observed in the 10 mg/kg group (Table 3-1).

2) Intravenous continuous infusion of WSA

An increase in the adrenal weight was measured and recorded in both the male and female monkeys from the 60 mg/kg group. Furthermore, there was a very slight increase of lipid droplets in the cortical cells in the zona fasciculata in the male monkey from the 60 mg/kg group. In the 30 mg/kg WSA group, a slight increase in the adrenal weight and an increase in the lipid droplets was very slightly observed in a female monkey. There was no detectable endocrinological toxicity in the 10 mg/kg group (Table 3-2).

3) Oral administration of the active metabolite

An increase in the adrenal weight was measured and recorded in both male and female monkey from the 90 mg/kg group. There was a very slight increase of lipid droplets in the cortical cells in the zona fasciculata in the male monkey from the 90 mg/kg group and in the female monkey from the 30 mg/kg group. There was no detectable endocrinological toxicity found in the 10 mg/kg group (Table 3-3).

3. Other toxicities

1) Other changes in the monkeys that expired during the 4-week oral toxicity study

In the monkeys that expired in the 90 mg/kg group, an opportunistic bacterial infection that led to a

Table 2-1. Changes in the hepatotoxicity parameters – Oral administration of WSA (Four-week study).

Oral WSA dose (mg/kg)		Control		10		30		90	
Sex		Male	Female	Male	Female	Male	Female	Male	Female
No. of animals		4	4	4	4	4	4	4	4
Item (Unit) \	Expired animals	Day		0		0		0	
Blood chemistry									
GOT (IU/L)	0	41.0 ± 9.4	56.3 ± 14.7	43.8 ± 13.9	49.5 ± 18.3	42.5 ± 12.1	50.0 ± 25.0	51.5 ± 23.9	46.8 ± 16.2
	13	58.0 ± 22.8	62.5 ± 35.2	37.3 ± 14.6	48.8 ± 18.4	43.8 ± 9.8	29.0 ± 10.3	47.3 ± 7.9	58.5 ± 54.5
	27	49.5 ± 13.3	34.3 ± 7.6	37.0 ± 6.6	36.0 ± 9.5	38.8 ± 8.8	28.5 ± 10.2	34.8 ± 14.4	38.0
GPT (IU/L)	0	33.0 ± 8.3	67.0 ± 37.4	46.3 ± 13.1	72.8 ± 34.0	36.8 ± 12.2	60.8 ± 24.5	45.8 ± 7.4	50.0 ± 26.1
	13	45.3 ± 17.9	71.0 ± 27.0	42.8 ± 10.3	62.3 ± 14.4	53.3 ± 8.4	48.5 ± 14.8	49.0 ± 11.6	48.8 ± 13.2
	27	37.0 ± 9.3	60.8 ± 24.5	43.0 ± 20.0	63.3 ± 28.9	41.3 ± 12.6	40.5 ± 11.4	51.3 ± 25.9	44.0
TG (mg/dL)	0	26.5 ± 11.4	33.3 ± 5.5	39.8 ± 23.6	28.8 ± 4.5	36.0 ± 18.9	44.0 ± 12.2	37.0 ± 13.0	33.8 ± 12.0
	13	44.3 ± 14.2	32.8 ± 5.0	61.3 ± 24.5	37.8 ± 8.2	57.8 ± 22.2	63.0 ± 8.8**	71.5 ± 32.8	63.0 ± 12.7**
	27	32.0 ± 10.7	30.0 ± 6.6	54.0 ± 13.6	40.8 ± 4.9	61.0 ± 17.2	67.0 ± 15.4*	172.5 ± 63.3**	82.0**
T-CHO (mg/dL)	0	90.5 ± 7.5	97.3 ± 3.6	121.8 ± 24.3	106.5 ± 9.1	96.8 ± 11.3	153.0 ± 13.9**	118.8 ± 23.4	133.5 ± 32.9*
	13	104.3 ± 12.4	100.8 ± 7.3	126.8 ± 33.5	112.3 ± 4.6	80.5 ± 11.0	125.3 ± 21.7	65.8 ± 18.7	81.5 ± 22.1
	27	100.3 ± 13.5	96.8 ± 4.0	124.0 ± 20.3	110.5 ± 3.4	78.0 ± 6.4	123.0 ± 35.0	81.0 ± 11.6	59.5
No. of animals #		3	3	3	3	3	3	3	3
Liver weight									
Absolute weight (g)	27	81.97 ± 13.38	64.73 ± 4.34	92.13 ± 6.93	72.03 ± 3.34	114.17 ± 11.05	80.13 ± 5.69*	138.63 ± 31.19	132.4
		17.96 ± 1.70	19.49 ± 0.63	21.08 ± 1.99	22.45 ± 0.69	25.82 ± 1.76**	26.12 ± 4.85*	35.99 ± 2.86**	44.9
Histopathology of liver									
Fatty change of centri/peri lobular hepatocytes	Grade								Surviving
	1	0	0	0	0	0	0	0	1
Hypertrophy of hepatocytes	2	0	0	0	0	0	0	1	1
	1	0	0	0	0	2	2	3	0
	2	0	0	0	0	1	0	0	0
									Expired

Values represent the mean ± SD, Statistical significance of treatment-control difference : * $p \leq 0.05$, ** $p \leq 0.01$.

Grade 1: very slight, Grade 2: slight.

Three animals/sex/dose were euthanized at the end of the treatment period and one animal/sex/dose was euthanized at the end of the recovery period.

Table 2-2. Changes in the hepatotoxicity parameters – Intravenous continuous infusion of WSA (Two-week study).

Intravenous infusion WSA dose (mg/kg)		Control		10		30		60	
Sex		Male	Female	Male	Female	Male	Female	Male	Female
No. of animals	Day	4	4	4	4	4	4	4	4
Item (Unit) \ Expired animals		0	0	0	0	0	0	0	0
Blood chemistry									
GOT (IU/L)	0	32.5 ± 3.3	36.3 ± 2.2	33.3 ± 2.5	32.3 ± 2.9	30.3 ± 5.4	41.5 ± 13.0	29.5 ± 7.5	31.0 ± 6.1
	13	31.8 ± 10.1	29.8 ± 2.2	35.5 ± 9.7	31.0 ± 5.5	28.3 ± 2.6	29.5 ± 5.8	29.8 ± 5.7	26.8 ± 6.2
GPT (IU/L)	0	50.3 ± 19.6	56.5 ± 12.1	31.8 ± 10.5	41.5 ± 22.7	29.8 ± 7.7	39.8 ± 19.5	23.5 ± 6.5	46.5 ± 22.2
	13	45.8 ± 26.2	27.8 ± 4.9	22.8 ± 5.8	35.8 ± 14.4	30.5 ± 7.3	32.3 ± 29.4	23.3 ± 5.1	33.0 ± 10.4
TG (mg/dL)	0	40.3 ± 23.9	67.3 ± 61.3	27.8 ± 18.5	71.3 ± 46.7	26.0 ± 13.6	53.8 ± 41.1	26.5 ± 8.2	55.5 ± 22.2
	13	51.8 ± 17.9	52.3 ± 17.2	54.8 ± 22.8	48.3 ± 14.9	102.0 ± 30.3	62.5 ± 16.4	181.5 ± 78.0*	87.3 ± 33.4
T-CHO (mg/dL)	0	145.8 ± 17.5	152.5 ± 32.2	139.0 ± 18.5	138.5 ± 14.2	131.0 ± 39.1	102.8 ± 11.4	109.5 ± 35.0	138.0 ± 23.1
	13	151.0 ± 41.9	139.5 ± 18.4	132.3 ± 11.2	140.3 ± 25.6	107.8 ± 14.2	94.3 ± 8.4*	81.3 ± 16.4	98.5 ± 15.5*
No. of animals #		3	3	3	3	3	3	3	3
Liver weight									
Absolute weight (g)	13	91.27 ± 12.61	63.50 ± 4.62	88.37 ± 13.91	84.83 ± 16.14	105.60 ± 12.08	83.37 ± 3.96	134.33 ± 22.08	86.53 ± 12.94
		21.97 ± 3.52	21.20 ± 1.35	24.27 ± 3.23	27.87 ± 2.89 ^{b)}	27.63 ± 3.74	27.60 ± 2.17	36.57 ± 7.83	32.80 ± 6.81
Histopathology of liver									
Grade									
Eosinophilic change of	1	0	0	0	0	0	0	0	0
Perilobular hepatocytes	2	0	0	0	0	0	0	1	0
Fatty change of centrilobular	1	0	0	0	0	0	1	0	0
Hepatocytes									
Hypertrophy of hepatocytes	1	0	0	0	0	0	0	1	1

Values represent the mean ± SD, Statistical significance of treatment-control difference : * $p \leq 0.05$, ** $p \leq 0.01$; Grade 1: very slight, Grade 2: slight.

^{b)} one female monkey, enlargement of liver due to parasite infestation (no drug-related changes observed).

Three animals/sex/dose were euthanized at the end of the treatment period and one animal/sex/dose was euthanized at the end of the recovery period.

Table 2-3. Changes in the hepatotoxicity parameters – Oral administration of the active metabolite of WSA (Two-week study).

Oral active metabolite dose (mg/kg)		Control		10		30		90		
Sex		Male	Female	Male	Female	Male	Female	Male	Female	
No. of animals		1	1	1	1	1	1	1	1	
Item (Unit) \ Expired animals	Day	0	0	0	0	0	0	0	0	
Blood chemistry										
GOT (IU/L)	0	31	26	30	20	25	37	33	34	
	13	22	40	20	24	22	17	25	23	
GPT (IU/L)	0	22	59	43	21	18	76	23	29	
	13	26	91	50	28	29	21	27	52	
TG (mg/dL)	0	11	33	32	51	24	35	34	51	
	13	18	20	45	57	46	34	119	86	
T-CHO (mg/dL)	0	143	173	155	119	136	146	140	152	
	13	144	180	113	113	84	132	59	111	
Liver weight										
Absolute weight (g)	Grade	75.8	56.3	110.5	67.3	105.5	72.6	138.4	86.2	
		17.6	17.6	22.6	21.0	23.4	20.7	30.8	30.8	
Histopathology of liver										
Vacuolation of Ito cells		1	0	0	0	0	0	0	0	
Eosinophilic change of perilobular hepatocytes		1	0	0	0	0	0	0	0	
Fatty change of centrilobular hepatocytes		2	0	0	0	0	0	0	0	
		1	1	0	0	0	1	0	0	
		2	0	0	0	0	0	0	1	
Hypertrophy of hepatocytes		1	0	0	0	0	0	0	0	
Grade 1: very slight, Grade 2: slight.										

Table 3-1. Changes in the endocrinological toxicity (adrenal) parameters – Oral administration of WSA (Four-week study).

Oral WSA dose (mg/kg)		Control		10		10		30		30		90	
Sex		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of animals		4	4	4	4	4	4	4	4	4	4	4	4
Item (Unit)	Expired animals	Day	0	0	0	0	0	0	0	0	0	0	2 (day 16, 24)
Blood chemistry													
TG (mg/dL)	0	26.5 ± 11.4	33.3 ± 5.5	39.8 ± 23.6	28.8 ± 4.5	36.0 ± 18.9	44.0 ± 12.2	37.0 ± 13.0	44.0 ± 12.2	37.0 ± 13.0	44.0 ± 12.2	33.8 ± 12.0	33.8 ± 12.0
	13	44.3 ± 14.2	32.8 ± 5.0	61.3 ± 24.5	37.8 ± 8.2	57.8 ± 22.2	63.0 ± 8.8**	71.5 ± 32.8	63.0 ± 8.8**	71.5 ± 32.8	63.0 ± 8.8**	63.0 ± 12.7**	63.0 ± 12.7**
	27	32.0 ± 10.7	30.0 ± 6.6	54.0 ± 13.6	40.8 ± 4.9	61.0 ± 17.2	67.0 ± 15.4*	172.5 ± 63.3**	67.0 ± 15.4*	172.5 ± 63.3**	67.0 ± 15.4*	82.0**	82.0**
No. of animals #		3	3	3	3	3	3	3	3	3	3	3	3
Adrenal weight													
Absolute weight (mg)	Right	237.0 ± 23.0	217.0 ± 40.0	190.0 ± 10.0	267.0 ± 29.0	310.0 ± 26.0*	287.0 ± 67.0	307.0 ± 40.0*	287.0 ± 67.0	307.0 ± 40.0*	287.0 ± 67.0	420	420
	Left	283.0 ± 15.0	297.0 ± 61.0	227.0 ± 6.0	330.0 ± 20.0	353.0 ± 50.0	370.0 ± 50.0	430.0 ± 62.0**	370.0 ± 50.0	430.0 ± 62.0**	370.0 ± 50.0	570	570
Relative weight (mg/kg BW)	Right	52.3 ± 5.1	65.7 ± 14.3	43.3 ± 2.9*	83.0 ± 7.2	70.3 ± 3.8**	91.3 ± 11.2	80.3 ± 2.5**	91.3 ± 11.2	80.3 ± 2.5**	91.3 ± 11.2	142	142
	Left	62.3 ± 6.1	89.7 ± 19.6	50.2 ± 5.6	102.7 ± 3.2	79.7 ± 10.2*	120.7 ± 23.6	112.7 ± 4.9**	120.7 ± 23.6	112.7 ± 4.9**	120.7 ± 23.6	193	193
Histopathology of adrenals													
Thickening of zona fascicula (Incl. increase of lipid droplets)	Grade 1	0	0	0	0	0	0	0	0	0	0	0	1
	Grade 2	0	0	0	0	0	0	0	0	0	0	1	1

Values represent the mean ± SD, Statistical significance of treatment-control difference : * $p \leq 0.05$, ** $p \leq 0.01$.

Grade 1: very slight, Grade 2: slight.

Three animals/sex/dose were euthanized at the end of the treatment period and one animal/sex/dose was euthanized at the end of the recovery period.

Table 3-2. Changes in the endocrinological toxicity (adrenal) parameters – Intravenous continuous infusion of WSA (Two-week study).

Intravenous infusion dose (mg/kg)		Control		Control		10		10		30		30		60		60	
Sex		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of animals	Day	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Item (Unit) \ Expired animals	Day	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood chemistry																	
TG (mg/dL)	0	40.3 ± 23.9	67.3 ± 61.3	27.8 ± 18.5	71.3 ± 46.7	26.0 ± 13.6	53.8 ± 41.1	26.5 ± 8.2	55.5 ± 22.2								
	13	51.8 ± 17.9	52.3 ± 17.2	54.8 ± 22.8	48.3 ± 14.9	102.0 ± 30.3	62.5 ± 16.4	181.5 ± 78.0*	87.3 ± 33.4								
No. of animals #		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Adrenal weight																	
Absolute weight (mg)	Right	250.0 ± 75.3	237.3 ± 37.9	258.3 ± 11.2	303.3 ± 99.8	300.7 ± 23.3	273.3 ± 64.0	535.0 ± 156.6	367.0 ± 19.9								
	Left	327.3 ± 60.2	342.0 ± 78.3	319.7 ± 25.6	325.0 ± 76.7	349.3 ± 34.4	393.3 ± 109.5	624.7 ± 129.2	413.0 ± 47.7								
Relative weight (mg/kg BW)	Right	60.3 ± 18.4	79.3 ± 12.1	71.3 ± 2.9	99.3 ± 26.9	78.3 ± 7.4	91.0 ± 25.7	146.0 ± 50.6	138.0 ± 11.3								
	Left	79.0 ± 16.8	115.0 ± 28.1	88.0 ± 7.8	106.3 ± 15.7	91.3 ± 11.8	129.7 ± 34.6	170.0 ± 42.9	154.7 ± 13.2								
Histopathology of adrenals																	
Increase of lipid droplets in cortical cells in zona fasciculata	Grade																
	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0

Values represent the mean ± SD, Statistical significance of treatment-control difference : * $p \leq 0.05$, ** $p \leq 0.01$.

Grade 1: very slight, Grade 2: slight.

Three animals/sex/dose were euthanized at the end of the treatment period and one animal/sex/dose was euthanized at the end of the recovery period.

Table 3-3. Changes in the endocrinological toxicity (adrenal) parameters – Oral administration of the active metabolite of WSA (Two-week study).

Oral active metabolite dose (mg/kg)		Control		Control		10		10		30		30		90		90	
Sex		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of animals		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Item (Unit) \ Expired animals	Day	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood chemistry																	
TG (mg/dL)	0	18	33	32	51	24	35	34	51	34	35	34	51	34	35	34	51
	13	11	20	45	57	46	34	119	86								
Adrenal weight	13																
Absolute weight (mg)	Right	242	257	391	293	266	262	444	336								
	Left	279	310	368	351	393	371	567	473								
Relative weight (mg/kg BW)	Right	56.0	80.0	79.8	92.0	59.0	75.0	99.0	120.0								
	Left	65.0	97.0	75.1	110.0	87.0	106.0	126.0	169.0								
Histopathology of adrenals	Grade																
Increase of lipid droplets in cortical cells in zona fasciculata	1	0	0	0	0	0	1	1	0								

Grade 1: very slight, Grade2: slight.

deterioration in these animals' body condition was observed and considered to be a secondary change such as the increases in the serum values for BUN and creatinine, renal tubule degeneration, atrophy of lymphoid tissues and so on.

Toxicokinetics

The plasma concentrations of the WSA, the active metabolite and the pro-moiety were closely examined throughout the treatment period. The results obtained are shown in Table 4.

After the completion of the intravenous continuous infusion of WSA, systemic exposure to WSA was relatively low and brief due to the rapid conversion of WSA to the active metabolite. The metabolic pathway of WSA in the plasma is shown in Fig. 2.

As shown in Table 4, the systemic exposure of the monkeys to the active metabolite was high, during the repeated infusion and oral administration period. The AUC of the active metabolite increased in nearly a dose-proportional pattern. These results suggest that the WSA was efficiently absorbed and converted at all concentrations tested. Systemic exposure to the active metabolite was high (AUC in i.v. (Male/Female): WSA: 3.04/2.62, 9.34/7.76, 10.1/17.9 $\mu\text{g}\cdot\text{hr}/\text{ml}$; active metabolite: 31.0/34.1, 100.0/85.0, 190.0/230.0 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 10, 30 and 60 mg/kg, respectively) and the AUC of the active metabolite increased in more than a dose-proportional manner in the high dosage group. No substantial accumulation of WSA, the active metabolite or the pro-moiety was observed during the repeated administration period. The levels of the C_{max} and the AUC of the active metabolite exhibited dose-dependent values during the administration period for the 10

mg/kg and 30 mg/kg groups. However, in the groups of monkeys administered the highest WSA concentrations, the C_{max} and the AUC of the active metabolite were increased. This was attributable to a decreasing metabolism due to the deteriorating physical condition of these monkeys (Days 13 and 27). In particular, in the 4-week oral administration study, the monkeys that expired from the 90 mg/kg group had a delayed T_{max} (surviving monkeys: 2 - 7hr, dying monkeys: 7 - 24 hr) and higher AUC values (surviving monkeys: 30 - 66.6 $\mu\text{g}\cdot\text{hr}/\text{ml}$, dying monkeys: 128 - 189 $\mu\text{g}\cdot\text{hr}/\text{ml}$) than the surviving female monkeys.

In the 2-week oral administration study of the active metabolite in monkeys, the plasma concentrations of the active metabolite was very comparable to values from the oral administration of WSA study (Table 4, Fig. 2).

DISCUSSION

Toxicity studies of a new water-soluble azole (WSA) were performed in cynomolgus monkeys given WSA by oral and intravenous administration (infusion). The purpose of these studies was to estimate and evaluate toxicities when the WSA was administered both orally and intravenously, and to compare the toxicity of WSA to the active metabolite exposure.

In the repeated administration studies over 2-week or 4-week periods, the toxicological profiles of WSA were observed primarily in the liver and adrenals, the common and expected situation for azole compounds (Nishikawa *et al.*, 1984; Buchi *et al.*, 1986; Van Cauteren *et al.*, 1987, 1991; Tachibana *et al.*, 1987; Davey, 1990).

Fig. 2. Metabolic pathway of WSA in the plasma.

Table 4. Toxicokinetics of the active metabolite after the administrations of WSA or the active metabolite of WSA (Two and four-week studies).

Toxicokinetics of the active metabolite after 2-hr i.v. infusion and p.o. administration of WSA to cynomolgus monkeys (n=4)						
Study duration	Route	Dose (mg/kg/day)	Sampling day	C _{max} (μg/ml) Male / Female	AUC ₀₋₂₄ (μg•hr/ml) Male / Female	T _{max} (hr) Male / Female
4 weeks	p.o.	10	0	1.92±0.75 / 1.60±0.47	18.1±5.7 / 18.7±2.6	3.50±1.00 / 3.50±1.00
			13	1.76±0.36 ²⁾ / 1.63±0.17 ²⁾	—	—
			27	1.69±0.47 / 1.91±0.14	19.3±4.3 / 19.7±2.5	3.00±1.15 / 2.00±0.00
		30	0	3.98±1.05 / 4.06±1.35	55.5±41.9 / 55.6±24.6	5.50±1.73 / 4.75±1.50
			13	6.04±1.02 ²⁾ / 5.45±0.70 ²⁾	—	—
			27	5.32±0.99 / 4.68±0.31	74.3±12.6 / 57.2±7.6	6.25±1.50 / 4.75±1.50
		90	0	15.0±7.0 / 6.21±3.59	241±105 / 103±70	7.00±0.00 / 10.0±9.60
			13	25.0±9.51 ²⁾ / 18.5±7.04 ²⁾	—	—
			27	18.2±3.6 / 12.2 ⁴⁾	283±72 / 212 ⁴⁾	6.25±1.50 / 7.00 ⁴⁾
		10	0	2.96±0.37 / 3.25±1.35	31.0±7.8 / 34.1±17.6	2.00±0.00 / 2.50±1.00
			7	3.68±0.72 ¹⁾ / 2.74±1.69 ¹⁾	—	—
			13	3.49±0.26 / 2.37±1.59	30.8±6.6 / 21.4±12.4	2.00±0.00 / 2.50±1.00
2 weeks	i.v. infusion for 2 hr	30	0	8.88±2.10 / 8.07±1.28	100±7 / 85.0±12.8	2.00±0.00 / 2.50±1.00
			7	11.7±1.00 ¹⁾ / 8.65±1.69 ¹⁾	—	—
			13	10.8±1.3 / 8.80±1.17	127±10 / 99.7±24.1	2.00±0.00 / 2.00±0.00
		60	0	13.3±1.9 / 17.1±2.2	190±23 / 230±31	3.00±1.15 / 2.50±1.00
			7	34.2±12.04 ¹⁾ / 26.6±19.62 ¹⁾	—	—
			13	29.8±17.7 / 19.7±5.2 ³⁾	479±341 / 253±64 ³⁾	3.00±1.15 / 2.67±1.15
Toxicokinetics of the active metabolite to cynomolgus monkeys (n=1)						
2 weeks	p.o.	10	0	1.33 / 1.02	17.9 / 11.8	2.00 / 2.00
			7	2.08 / 2.52	25.1 / 29.5	4.00 / 4.00
			13	1.67 / 1.40	19.8 / 18.6	4.00 / 4.00
		30	0	1.94 / 1.55	30.9 / 26.0	4.00 / 4.00
			7	3.95 / 3.58	54.1 / 64.6	4.00 / 2.00
			13	2.55 / 2.37	32.0 / 45.4	4.00 / 7.00
		90	0	4.56 / 13.6	46.6 / 251	24.00 / 15.5
			7	21.9 / 9.04	469 / 203	7.00 / 4.00
			13	23.3 / 6.03	508 / 78.7	4.00 / 4.00

Values represent the mean ± SD, —: Not calculated,

¹⁾ Concentrations at 2 hr, ²⁾ Concentrations at 4 hr, ³⁾ n = 3, ⁴⁾ n = 2.C_{max}: maximum concentration; AUC: area under the concentration-time curve; T_{max}: time to the maximum concentration.

A summary of the main toxicities detected in this study is shown in Table 5. In the 4-week WSA oral administration study, the lethal dose was determined to be 90 mg/kg. At the 90 mg/kg level, two female monkeys expired on Days 16 and 25. In the 2-week intravenous continuous infusion study, no monkeys from any of the treatment groups died. However, the higher WSA dosage groups from both of these studies (oral and intravenous) exhibited marked behavioral changes in their general conditions, such as a decreases in their food consumption and body weight, vomiting, a decrease in spontaneous motor activity and loose stool/diarrhea.

For the plasma concentration of the active metabolite at 10 and 30 mg/kg levels, the $C_{\max}$ and AUC had nearly constant values that demonstrated dose-proportionality over all administration periods in all of our studies, including the direct administration of the active metabolite. However, with higher doses, the systemic exposure to the active metabolite increased, resulting in a pattern beyond the dose-proportionality for these indices. Specifically, the $C_{\max}$ and AUC of the active metabolite increased after Day 13. The higher plasma concentrations observed in the late phases of the high-dose regimen could be a result of or be enhanced by the deteriorating physical condition of the monkeys and the decrease in animal metabolism.

Liver toxicities were observed in the 90 mg/kg group in the 4-week oral WSA administration study and in the 60 mg/kg group in the 2-week intravenous continuous infusion study. In the 2-week oral administration study of the active metabolite, the 90 mg/kg group demonstrated the same level of toxicity. Furthermore, the plasma concentration of the active metabolite, which displayed toxic effects, was almost the same value in both of these studies.

The following changes were detected and considered to be indicative of liver toxicity. For blood chemistries, an increase in the TG and a decrease in the T-CHO level from Day 14 were observed, and an increase in the liver weight measurement and histopathological changes was observed in the liver (hypertrophy and fatty and/or eosinophilic changes in the hepatocytes) at the end of the administration period.

Endocrinological toxicities were detected in the 90 mg/kg from the 4-week oral WSA administration study and in the 60 mg/kg group from the 2-week intravenous continuous infusion study. The endocrinological toxicities observed in this study were an increase in the adrenal weight measurements and histopathological change in the adrenals such as thickening of the

zona fasciculata and increased lipid droplets. These toxicity indicators were observed very slightly in the 30 mg/kg group at the end of the administration period. Increased lipid droplets were detected in the 2-week administration study. However, in the 4-week administration study, the adrenals displayed both a thickening of the zona fasciculata and increased lipid droplets. The presence of this additional toxicity indicator, specifically, the thickening of the zona fasciculata, may be dependent on the length of the exposure period.

In the oral administration study, the endocrinological toxicity for male and female monkeys was of comparable severity, but for intravenous continuous infusion study, the males treated with 60 mg/kg exhibited more severe toxicity than the female monkeys. In these studies, the values for $C_{\max}$ and AUC were very similar for both male and female monkeys, except for the $C_{\max}$ and AUC values for the 60 mg/kg group from the 2-week infusion study. At the 60 mg/kg level, the $C_{\max}$ and AUC values were higher for the males than for the female monkeys. The $C_{\max}$ and AUC values on Day 13 were almost identical for the females treated with 60 mg/kg via intravenous administration as were found in the male and female monkeys treated with 90 mg/kg of WSA via oral administration. In addition, the observed changes in the biochemical and morphological parameters were nearly identical for both the 60 and 90 mg/kg groups. The $C_{\max}$ and AUC values for the intravenous infusion group were approximately 2 times higher than the levels from the oral administration at the low and middle dosage levels. This suggested that the toxicities may be correlated with the $C_{\max}$ and AUC values of the active metabolite.

After intravenous continuous infusion of WSA, the systemic exposure to WSA was relatively low due to its rapid conversion to the active metabolite, and the AUC increased as the concentration of the administered dose increased. This indicates that the WSA was converted rapidly to the active metabolite after its administration.

In the intravenous continuous infusion study, WSA was converted rapidly into the active metabolite. The oral bioavailability of the active metabolite was good, approximately 87%, in the monkeys, and the $T_{1/2}$ of the active metabolite was 12.8 hr (Nakano *et al.*, 2000).

Overall, in these repeated administration WSA studies, given orally and intravenously, the toxicity was observed to primarily affect the liver and adrenals. The results are consistent with earlier research on other azole compounds, such fluconazole and itraconazole

Table 5 Summary of the primary sites and parameters indicating toxicity due to WSA administration (Two- and four-week studies).

Study type	Dose level (mg/kg)	Sex	As active metabolite			Intensity								
			Day	C _{max} (μg/ml)	AUC (μg•hr/ml)	Day	Liver toxicity			Endocrinological tox. (adrenals)		Other toxicity		
							TG	T-CHO	OW	Histo. ¹⁾	OW	Histo. ²⁾		
4-week p.o.	10	Male	0	1.92	18.1	13	—	—	—	—	—	—	Anemia —	
		13*	1.76	n.d.	27		—	—	—	—	—	—		—
		27	1.69	19.3	27		—	—	—	—	—	—		—
	Female	0	1.60	19.1	13	—	—	—	—	—	—	—	—	
		13*	1.63	n.d.		27	—	—	—	—	—	—		—
		27	1.91	19.7		27	—	—	—	—	—	—		—
30	Male	0	3.98	55.5	13	—	—	—	—	—	—	—	—	
		13*	6.04	n.d.		27	—	—	—	—	—	—		—
		27	5.32	74.3		27	—	—	(↑)	±a	(↑)	—		—
	Female	0	4.06	55.6	13	—	—	—	—	—	±#	—	—	
		13*	5.45	n.d.		27	—	—	—	—	—	—		—
		27	4.68	57.2		27	—	—	(↑)	±a	—	—		—
2-week i.v.	90	Male	0	15.00	241.0	13	(↑)	(↓)	↑#	±a#	↑#	±#	—	
		13*	24.98	n.d.	27		↑	(↓)	↑	+	↑	+	—	
		27	18.20	281.0	27		—	—	—	—	—	—	—	
	Female	0	6.21	103.0	13	—	(↓)	↑#	±b#	(↑)#	—	—	—	
		13*	18.55	n.d.		27	↑	↓	↑	+	↑	+		±
		27	12.20	212.0		27	—	—	—	—	—	—		—
infusion	10	Male	0	2.96	31.0	13	—	—	—	—	—	—	—	
		13	3.49	30.8	13		—	—	—	—	—	—	—	
		0	3.25	34.1	13		—	—	—	—	—	—	—	
	Female	13	2.37	21.4	13	—	—	—	—	—	—	—	—	
		0	8.88	100.0		13	↑	—	(↑) ³⁾	—	—	—		—
		13	10.80	127.0		13	—	—	—	—	—	—		—
60	Female	0	8.07	85.0	13	—	—	—	±b	(↑) ³⁾	±	—	—	
		13	8.80	99.7		13	—	—	—	—	—	—		—
		0	13.30	190.0		13	↑	↑	(↓)	↑	↑	±		—
	Male	13	29.80	479.0	13	↑	↑	(↓)	↑	↑	±	—	—	
		0	17.10	230.0		13	↑	(↓)	(↑)	±a	↑	—		±
		13	19.70	253.0		13	—	—	—	—	—	—		—

(↑): very slight increase, ↑: slight increase, ↑↑: moderate increase; (↓): very slight decrease, ↓: slight decrease, ↓↓: moderate decrease.

¹⁾ ±a: Hypertrophy in hepatocytes, ±b: Fatty change in hepatocytes or vacuolation of Ito cells, +: Hypertrophy in hepatocytes and eosinophilic changes in hepatocytes.²⁾ Thickening of zona fasciculata (including an increase of lipid droplets): ± very slight, + slight, ++ moderate.³⁾ One animal exhibited an increase in organ weight.

#: The results from the 2-week oral toxicity study of the active metabolite of WSA, *: C4h, n.d.: not done.

(Bodey, 1992; Davey, 1990; Tachibana *et al.*, 1987; Van Cauteren *et al.*, 1987). The toxicological intensity of WSA can be described as nearly comparable to these older azole compounds, based upon the therapeutic ratio.

No specific toxicity, other than liver and adrenal, was detected, which has been a common result in studies of other azole compounds.

In addition, by maintaining the concentration of the active metabolite of WSA at effective and non-toxic levels using intravenous continuous infusion, the safety range would be expected to be wider than for intravenous bolus administration alone. Furthermore, clinical application using intravenous continuous infusion would be an effective first intensive treatment, followed by oral administration.

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