Studies on the Disposition of Fadrozole Hydrochloride (CGS 16949A) (Ⅱ): Absorption, Distribution, Metabolism and Excretion after Repeated Oral Administration to Rats

Satoru YAMAGAMI, Eiko KAWASAKI and Asao EGAWA

Pharmacokinetic Research Laboratory, Ciba-Geigy Japan Limited,

10-66 Miyuki-cho, Takarazuka-shi 665, Japan

Yoshio ESUMI, Katsuyuki HORI, Masao ISHIZAKI,

Takako HONDA and Ryoko NEMOTO

Tokai Research Laboratories, Daiichi Pure Chemicals Co., Ltd.,

2117 Muramatsu, Tokai-mura, Naka-gun, Ibaraki-ken 319-11, Japan

Key words ; Fadrozole hydrochloride (CGS 16949A), Rat, Repeated
administration, Absorption, Distribution, Metabolism, Excretion

Summary

The absorption, distribution, metabolism, and excretion of ^{14}C -fadrozole hydrochloride (^{14}C -CGS 16949A) were investigated during and after repeated oral administration to non-fasted female rats at a daily dose of 1mg/kg for 21 days.

1. The levels of radioactivity in the plasma (24hr after daily dosing) reached a steady state after the 15th dosing and was 3.1 times higher than that found after the 1st dosing. The peak concentration ($C_{\max}$) and the half-lives after 21st dosing were 477ng eq. of CGS 16949 (the base of CGS 16949A)/ml and 10hr from 4hr to 24hr and 65hr from 48hr to 168hr.

2. The excretion ratio of radioactivity in the urine and feces reached a steady state after the 4th dosing and the excretion ratio after the 21st dosing was similar to that after single dosing.

3. The levels of radioactivity in the tissues after the 7th dosing increased in comparison with those after single dosing and the radioactivity levels after the 14th dosing were similar to those after the 7th dosing. The radioactivity in aorta after the 21st dosing was 8.9 times higher than that after single dosing.

4. The relative amount of unchanged CGS 16949 in the plasma after the 21st dosing decreased and that of the metabolite with hydantoin type structure, MP2 increased, compared with those after single dosing. There were no significant differences between the concentration of CGS 16949 after single and the 21st dosing.

5. The relative amount of CGS 16949 and its metabolites in the urine for 24 hours after 21st dosing was not significantly different from that after single dosing.

INTRODUCTION

Fadrozole hydrochloride (CGS 16949A), 4-(5,6,7,8-tetrahydroimidazo [1,5-a]-pyridin-5-yl) benzonitrile monohydrochloride, is a potent, selective and non-steroidal competitive inhibitor of aromatase¹⁾, whose absorption, distribution, metabolism and excretion have been reported after a single oral administration of ¹⁴C-CGS 16949A to female rats²⁾.

Since CGS 16949A is administered repeatedly in the pharmacological studies and clinical trials, the absorption, distribution, metabolism and excretion of ¹⁴C-labelled CGS 16949A were investigated after repeated oral administration to non-fasted female rats in this study, and the steady state level of radioactivity in plasma, tissues and excreta and the accumulation of radioactivity were examined.

MATERIALS AND METHODS

1. Labelled Compound

¹⁴C-CGS 16949A was synthesized by Daiichi Pure Chemicals Co., Ltd. The labelled position of ¹⁴C-CGS 16949A is shown in Fig. 1. The specific radioactivity of the labelled compound was 3.86MBq/mg, and the radiochemical purity of the labelled compound was more than 97% during the experimental period; this was determined by thin-layer chromatography as described in a preceding paper²⁾.

Fig. 1 Chemical structure and labelled position of ¹⁴C-CGS 16949A

2. Experimental Animals

Sprague-Dawley strain SPF female rats (obtained from Charles River Japan, Inc.) at 7 weeks of age, weighing about 200g were used in this study.

Rats were acclimatized as described in the preceding paper²⁾ and were assigned to each test group. Three animals were assigned to each test group and one animal per group was used for whole body autoradiography.

3. Dose Level and Administration of the Test Article

¹⁴C-CGS 16949A mixed with unlabelled CGS 16949A was dissolved in distilled water and used to prepare a 0.5mg/ml dosing solution. The dosing solution of 2ml/kg was administered orally to rats by gavage once daily at a fixed time for 21days. The radioactivity dosed to rats was 1.93MBq/kg.

4. Measurement of Radioactivity

Radioactivity in each test sample was counted by a liquid scintillation counter (LSC-903 or LSC-3600, ALOKA) for 2min. The counting efficiency was corrected by the Channel Ratio method using external standard sources. Concentrations of radioactivity in plasma or tissues were expressed as ng eq. of CGS 16949 (the base of CGS 16949A)/ml or g.

5. Determination of Radioactivity Concentration in Plasma

Blood was withdrawn from the caudal vein of the animals 24hr after each administration and 5, 15 and 30min, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168hr after the 21st administration and immediately centrifuged (4°C, 1800 × g, 15min) to obtain plasma. The radioactivity concentration in the plasma was counted as described in the preceding report²⁾.

6. Determination of Excretion of Radioactivity in Urine and Feces

Urine and feces were collected for 24hours after each administration and for every 24 hours up to 168hr after the 21st administration. The radioactivity in the urine and feces was counted as described in the preceding report²⁾. The residual radioactivity in carcass at 168hr after the 21st doses was also measured as described in the preceding report²⁾.

7. Whole Body Autoradiography

Rats were sacrificed by ether inhalation 8, 24 and 168hr after the 21st administration. Whole body autoradiograms were prepared as described in the preceding report²⁾.

8. Determination of Radioactivity Concentration in Tissue

Rats were exsanguinated from the abdominal aorta under ether anesthesia 24hr after the 7th and 14th administration and 2, 8, 24, 72, 168, 336 and 672hr after the 21st administration. The tissues were excised and the concentrations of radioactivity in the plasma, blood and tissues counted as described in the preceding report²⁾.

9. Analysis of Metabolites

1) Samples for Analysis

Plasma samples (2, 8 and 24hr after the 21st administration), urinary and fecal samples (0-24hr after the 21st administration), liver and kidney samples (2, 8 and 24hr after the 21st administration) were used for the analysis of metabolites.

2) Analytical Methods

Each sample was pretreated by solid-phase extraction and each extract was evaporated into dryness. The residue was dissolved with a small amount of mobile phase and the solution was applied to HPLC system described in the previous report²⁾.

10. Calculation of Pharmacokinetic Parameters

The values of pharmacokinetic parameters were calculated from the individual plasma concentration-time curve of radioactivity in three animals. The values of area under the

concentration-time curve (AUC) were calculated by the trapezoidal method. Apparent half-life values ($T_{1/2}$) were estimated using the least square method of actual data.

Student t-test with two-tailed probabilities was used for the analysis of the significance of difference.

RESULTS

1. Radioactivity Concentration in Plasma

The radioactivity concentrations in the plasma (equivalent to CGS 16949) during repeated dosing are shown in Fig. 2. The radioactivity concentration in the plasma at 24hr after daily dosing reached a steady state after the 15th dosing and was 3.1times higher than that after the 1st dosing.

The radioactivity concentration in the plasma after the 21st dosing is shown in Fig. 3 compared with the level after single dosing²⁾. The radioactivity concentration in the plasma reached a peak (C_{max}) of 477ng eq. of CGS 16949/ml 1hr after the 21st administration and declined with half-lives of 10hr from 4hr to 24hr and 65hr from 48hr to 168 hr after the administration. The C_{max} was 1.3times higher and the half-life from 4hr to 24hr was 1.5times longer, respectively, than those in the single dosing group²⁾.

The pharmacokinetic parameters are also calculated from plasma concentration-time curve of radioactivity in each animal and are summarized by group in Table I.

2. Excretion of Radioactivity in Urine and Feces

Table II shows the cumulative excretions of radioactivity in the urine and feces during

Fig. 2 Plasma concentrations of radioactivity at 24hr after daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats
Data are expressed as the mean values $\pm$ S.E. for three animals.

Fig. 3 Plasma concentrations of radioactivity after a single and a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats
Data are expressed as the mean values $\pm$ S.E. for three animals.
Data on single dose are quoted from reference 2.

Table I Pharmacokinetic parameters of radioactivity in plasma after single and a 21 day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

	Pharmacokinetic parameters			
	$T_{\max}^{1)}$ (hr)	$C_{\max}^{2)}$	$T_{1/2}$ (hr)	$AUC^{3)}$
Single dose ⁴⁾	2	379 ± 56	6.8 ± 0.4 (4-24hr)	3.03 ± 0.22 (0-24hr) 3.32 ± 0.27 (0- ∞) ⁵⁾
21 doses	1 (1-2)	477 ± 19	10 ± 1 (4-24hr) 65 ± 6 (48-168hr)	5.09 ± 0.17 (0-24hr)

Data are expressed as the mean values $\pm$ S.E. for three animals.

1) : Time to peak concentration ($T_{\max}$) values are expressed as the median and range.

2) : ng eq. of CGS 16949/ml.

3) : μg eq. of CGS 16949 $\cdot$ hr/ml.

4) : Data on single dose are quoted from reference 2.

5) : AUC (0 - ∞) are calculated by the trapezoidal rule from 0hr to 24hr and extrapolated from 24hr to time infinity.

repeated dosing and up to 168hr after the 21st dosing. The excretion of radioactivity in the urine and feces reached a steady state after the 4th dosing.

The urinary, fecal and total excretions of radioactivity were 83.1, 12.8 and 95.9%, respectively, of the dose within 168hr after the 21st dosing. The residual radioactivity in carcass was 0.4% of the dose 168hr after the 21st dosing.

Table II Cumulative excretions of radioactivity in urine and feces during and after a 21 day period of daily oral administration of ^{14}C -CGS 16949
A 1 mg/kg to non-fasted female rats

Number of dosing	Excretion of radioactivity (% of cumulative dose)		
	Urine	Feces	Total
1	76.4 $\pm$ 0.9	9.1 $\pm$ 1.0	85.6 $\pm$ 0.1
2	80.3 $\pm$ 1.2	9.0 $\pm$ 0.5	89.3 $\pm$ 0.9
3	82.6 $\pm$ 1.0	9.3 $\pm$ 0.6	91.9 $\pm$ 0.8
4	83.1 $\pm$ 1.0	10.2 $\pm$ 0.5	93.3 $\pm$ 0.9
5	83.5 $\pm$ 1.0	10.2 $\pm$ 0.2	93.7 $\pm$ 0.8
6	83.1 $\pm$ 1.1	10.1 $\pm$ 0.2	93.2 $\pm$ 0.9
7	83.4 $\pm$ 1.0	10.1 $\pm$ 0.3	93.5 $\pm$ 0.7
8	83.4 $\pm$ 1.1	10.3 $\pm$ 0.2	93.7 $\pm$ 1.0
9	83.4 $\pm$ 1.1	10.6 $\pm$ 0.3	94.0 $\pm$ 0.8
10	83.1 $\pm$ 1.1	10.8 $\pm$ 0.4	93.8 $\pm$ 0.8
11	82.8 $\pm$ 1.1	11.2 $\pm$ 0.5	94.0 $\pm$ 0.7
12	82.5 $\pm$ 1.1	11.3 $\pm$ 0.3	93.9 $\pm$ 0.7
13	82.6 $\pm$ 1.1	11.7 $\pm$ 0.5	94.3 $\pm$ 0.6
14	82.5 $\pm$ 1.1	11.8 $\pm$ 0.5	94.3 $\pm$ 0.6
15	82.2 $\pm$ 1.1	12.1 $\pm$ 0.6	94.3 $\pm$ 0.5
16	82.0 $\pm$ 1.1	12.2 $\pm$ 0.6	94.3 $\pm$ 0.5
17	82.1 $\pm$ 1.0	12.2 $\pm$ 0.5	94.3 $\pm$ 0.4
18	82.0 $\pm$ 0.9	12.2 $\pm$ 0.5	94.2 $\pm$ 0.4
19	82.2 $\pm$ 1.0	12.4 $\pm$ 0.6	94.6 $\pm$ 0.4
21	82.0 $\pm$ 1.0	12.6 $\pm$ 0.6	94.6 $\pm$ 0.4
21 24*	82.5 $\pm$ 1.0	12.6 $\pm$ 0.6	95.1 $\pm$ 0.4
48*	82.9 $\pm$ 0.9	12.7 $\pm$ 0.6	95.6 $\pm$ 0.3
72*	83.0 $\pm$ 0.9	12.7 $\pm$ 0.6	95.7 $\pm$ 0.2
96*	83.1 $\pm$ 0.9	12.7 $\pm$ 0.6	95.8 $\pm$ 0.3
120*	83.1 $\pm$ 0.9	12.7 $\pm$ 0.6	95.8 $\pm$ 0.2
144*	83.1 $\pm$ 0.9	12.8 $\pm$ 0.6	95.9 $\pm$ 0.3
168*	83.1 $\pm$ 0.9	12.8 $\pm$ 0.6	95.9 $\pm$ 0.3

Residual radioactivity in carcass at 168hr after the 21st dosing : 0.4 $\pm$ 0.0% of 21 doses.

Data are expressed as the mean values $\pm$ S.E. for three animals.

* : Hours after the 21st dosing.

Samples were collected for 24 hours after daily dosing and for every 24 hours up to 168hr after the 21st dosing.

3. Whole Body Autoradiograms

The whole body autoradiograms after the 21st dosing are shown in Fig. 4. High levels of radioactivity were found in the adrenal gland, gastrointestinal contents, urine in urinary bladder, preputial gland, nasal cavity, bile in bile duct (radioactivity was detected in a autoradiogram which is not shown.), ligament, aorta and ovary at 8hr, followed by kidney, liver, mandibular gland, Harderian gland and skin. The radioactivity levels in the blood, brain and eyeball were the lowest.

Although overall radioactivity decreased at 24hr, high levels of radioactivity were still observed in the adrenal gland, nasal cavity, ligament, aorta and gastrointestinal contents,

Fig. 4 Autoradiograms showing the distribution of radioactivity after a 21 day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

1. Adrenal gland	10. Heart	19. Pituitary gland
2. Aorta	11. Intestinal contents	20. Preputial gland
3. Blood	12. Kidney	21. Skeletal muscle
4. Bone marrow	13. Ligament	22. Skin
5. Brain	14. Liver	23. Thymus
6. Brown fat	15. Lung	24. Thyroid
7. Eyeball	16. Mandibular gland	25. Urine in bladder
8. Gastric contents	17. Nasal cavity	26. Uterus
9. Harderian gland	18. Pancreas	

followed by kidney, skin, mandibular gland and liver.

At 168hr, overall radioactivity further decreased, but high levels of radioactivity were found in the nasal cavity, aorta and kidney, and low levels in the stomach, skin, adrenal gland, intestinal contents, thyroid, lung and liver.

Table III Tissue concentrations of radioactivity at 24hr after single and daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

Tissue	Concentration of radioactivity (ng eq. of CGS 16949/g or ml)			
	single dose	7 doses	14 doses	21 doses
Plasma	35 $\pm$ 2	63 $\pm$ 1	65 $\pm$ 3	69 $\pm$ 6
Blood	31 $\pm$ 1	61 $\pm$ 1	68 $\pm$ 4	81 $\pm$ 7
Cerebrum	23 $\pm$ 1	40 $\pm$ 2	46 $\pm$ 2	58 $\pm$ 7
Cerebellum	20 $\pm$ 1	36 $\pm$ 1	40 $\pm$ 3	50 $\pm$ 6
Spinal cord	21 $\pm$ 1	36 $\pm$ 1	38 $\pm$ 3	49 $\pm$ 6
Pituitary gland	46 $\pm$ 9	61 $\pm$ 6	72 $\pm$ 8	82 $\pm$ 12
Eyeball	26 $\pm$ 1	52 $\pm$ 2	55 $\pm$ 3	75 $\pm$ 8
Harderian gland	55 $\pm$ 2	93 $\pm$ 2	91 $\pm$ 9	110 $\pm$ 11
Thyroid	54 $\pm$ 4	147 $\pm$ 14	147 $\pm$ 5	218 $\pm$ 38
Mandibular ^{a)}	48 $\pm$ 2	80 $\pm$ 1	81 $\pm$ 9	98 $\pm$ 12
Lymph node	32 $\pm$ 1	55 $\pm$ 3	58 $\pm$ 2	70 $\pm$ 6
Thymus	28 $\pm$ 1	61 $\pm$ 9	43 $\pm$ 3	54 $\pm$ 4
Heart	38 $\pm$ 3	71 $\pm$ 3	78 $\pm$ 5	90 $\pm$ 6
Lung	75 $\pm$ 6	162 $\pm$ 6	187 $\pm$ 3	225 $\pm$ 6
Liver	145 $\pm$ 7	224 $\pm$ 9	211 $\pm$ 9	260 $\pm$ 26
Kidney	104 $\pm$ 1	188 $\pm$ 3	234 $\pm$ 16	275 $\pm$ 19
Adrenal gland	1226 $\pm$ 30	1470 $\pm$ 282	2076 $\pm$ 340	1622 $\pm$ 342
Spleen	39 $\pm$ 3	70 $\pm$ 1	76 $\pm$ 4	117 $\pm$ 11
Pancreas	61 $\pm$ 2	90 $\pm$ 1	93 $\pm$ 11	107 $\pm$ 11
Fat	13 $\pm$ 0	20 $\pm$ 2	20 $\pm$ 3	20 $\pm$ 2
Brown fat	46 $\pm$ 4	76 $\pm$ 6	79 $\pm$ 6	87 $\pm$ 2
Skeletal muscle	28 $\pm$ 0	47 $\pm$ 3	47 $\pm$ 4	64 $\pm$ 7
Skin	50 $\pm$ 13	164 $\pm$ 9	170 $\pm$ 7	201 $\pm$ 25
Bone marrow	37 $\pm$ 3	54 $\pm$ 1	55 $\pm$ 6	70 $\pm$ 4
Aorta	72 $\pm$ 9	196 $\pm$ 13	452 $\pm$ 34	639 $\pm$ 87
Sciatic nerve	31 $\pm$ 2	57 $\pm$ 3	69 $\pm$ 5	96 $\pm$ 7
Ovary	64 $\pm$ 14	117 $\pm$ 11	108 $\pm$ 16	121 $\pm$ 12
Uterus	34 $\pm$ 1	66 $\pm$ 10	61 $\pm$ 2	84 $\pm$ 13
Urinary bladder	40 $\pm$ 7	56 $\pm$ 7	69 $\pm$ 11	100 $\pm$ 17
Stomach	239 $\pm$ 13	292 $\pm$ 4	259 $\pm$ 13	344 $\pm$ 22
Duodenum	42 $\pm$ 6	52 $\pm$ 6	46 $\pm$ 10	79 $\pm$ 9
Ileum	97 $\pm$ 15	90 $\pm$ 9	68 $\pm$ 9	127 $\pm$ 4
Cecum	141 $\pm$ 20	150 $\pm$ 39	133 $\pm$ 45	234 $\pm$ 49
Colon	104 $\pm$ 2	110 $\pm$ 21	99 $\pm$ 30	98 $\pm$ 20

Data are expressed as the mean values $\pm$ S.E. for three animals.

a) : Mandibular gland.

Data on single dose are quoted from reference 2.

4. Radioactivity Concentration in Tissue

The radioactivity concentrations in the tissues 24hr after the 7th, 14th and 21st dosing are shown in Table III, compared with the level after a single administration²⁾. The radioactivity concentration after the 7th dosing increased, in comparison with the concentration after a single administration and the radioactivity levels after the 14th dosing were similar to those after the 7th dosing.

Table V Tissue concentrations of radioactivity after a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

Tissue	Concentration of radioactivity (ng eq. of CGS 16949/g ml)													
	2 hr		8 hr		24 hr		72 hr		168 hr		336 hr		672 hr	
Plasma	410±	24	235±	18	69±	6	18±	1	7±	0	N.D.		N.D.	
Blood	448±	25	245±	19	81±	7	37±	4	26±	1	15±	1	6±	0
Cerebrum	714±	28	237±	31	58±	7	24±	1	16±	2	8±	1	5±	0
Cerebellum	578±	23	202±	26	50±	6	22±	1	13±	2	7±	1	4±	0
Spinal cord	616±	26	208±	24	49±	6	23±	1	15±	2	8±	1	6±	0
Pituitary gland	1055±	122	365±	28	82±	12	N.D.		N.D.		N.D.		N.D.	
Eyeball	354±	9	202±	11	75±	8	33±	2	22±	2	12±	1	10±	0
Harderian gland	2101±	73	614±	41	110±	11	36±	2	20±	6	12±	2	10±	1
Thyroid	873±	70	440±	20	218±	38	91±	16	88±	11	57±	7	33±	6
Mandibular ^{a)}	2092±	304	671±	49	98±	12	28±	2	18±	3	8±	1	8±	1
Lymph node	666±	25	290±	27	70±	6	28±	3	18±	3	11±	1	10±	0
Thymus	610±	26	257±	23	54±	4	15±	1	9±	1	5±	1	4±	1
Heart	719±	15	324±	32	90±	6	37±	2	22±	3	11±	0	7±	1
Lung	1313±	65	593±	54	225±	6	154±	10	118±	5	94±	11	88±	1
Liver	2489±	69	1163±	94	260±	26	82±	5	44±	7	19±	3	11±	1
Kidney	1765±	59	878±	61	275±	19	170±	22	122±	2	53±	4	49±	2
Adrenal gland	12547±	2526	8406±	1956	1622±	342	347±	67	131±	54	64±	17	22±	1
Spleen	880±	30	401±	25	117±	11	69±	5	51±	2	48±	5	33±	4
Pancreas	1061±	48	463±	40	107±	11	29±	2	18±	4	8±	0	5±	0
Fat	287±	62	103±	11	20±	2	5±	0	4±	2	N.D.		N.D.	
Brown fat	670±	5	283±	19	87±	2	26±	2	17±	1	8±	2	5±	1
Skeletal muscle	494±	8	239±	14	64±	7	20±	3	12±	3	6±	0	5±	0
Skin	617±	13	356±	9	201±	25	142±	16	129±	12	92±	8	88±	6
Bone marrow	879±	100	355±	41	70±	4	22±	3	15±	2	N.D.		N.D.	
Aorta	1364±	117	1107±	90	639±	87	559±	23	524±	38	482±	13	446±	12
Sciatic nerve	673±	17	322±	20	96±	7	39±	2	34±	2	22±	2	21±	1
Ovary	1066±	139	577±	113	121±	12	44±	0	32±	5	24±	2	16±	1
Uterus	538±	10	265±	35	84±	13	29±	2	19±	2	11±	2	11±	1
Urinary bladder	974±	404	374±	64	100±	17	50±	4	38±	5	31±	2	31±	4
Stomach	2389±	73	733±	124	344±	22	213±	9	112±	28	65±	12	37±	5
Duodenum	637±	45	276±	50	79±	9	26±	3	21±	1	15±	2	13±	1
Ilem	653±	143	395±	68	127±	4	31±	2	22±	3	15±	2	11±	1
Cecum	596±	39	1052±	31	234±	49	39±	4	34±	3	22±	4	16±	2
Colon	594±	49	353±	56	98±	20	32±	2	26±	2	17±	2	16±	1

Data are expressed as the mean values $\pm$ S.E. of three animals.

a) : Mandibular gland.

N.D. : Not detected.

The radioactivity concentration in the skin 24hr after the 7th dosing was 3.3times higher than that after single dosing. The eyeball, thyroid, thymus, lung and aorta showed 2.0~2.7times higher concentrations than those after single dosing. Other tissues showed less than 2times of the concentrations after single dose.

The radioactivity concentrations in the aorta, thyroid and skin after the 21st dosing increased to 8.9, 4.0 and 4.0times higher concentrations, respectively, of those after

single dosing.

The radioactivity concentrations in the tissues after the 21st dosing are shown in Table IV.

At 2hr after the 21st dosing, the radioactivity concentration in the adrenal gland was the highest, which was 31times higher than the concentration in the plasma (410ng eq. of CGS 16949/ml). The liver, stomach, Harderian gland and mandibular gland showed 5~6 times higher radioactivity concentrations than that in the plasma. The radioactivity concentrations in the thymus, colon, cerebellum, cecum, uterus, skeletal muscle, blood, eyeball and fat were similar to that in the plasma.

At 8hr, the radioactivity concentration in the adrenal gland was the highest, which was 36times higher than that in the plasma (235ng eq. of CGS 16949/ml). The radioactivity in the liver and aorta showed 5times higher concentrations than that in plasma.

At 24hr, the concentration in the aorta decreased to 47% of the maximum. The radioactivity concentrations in the thymus, cerebellum, cerebrum, bone marrow, spinal cord, pituitary gland, fat, Harderian gland and mandibular gland showed 5-9% of each maximum concentration, and those in the other tissues were 10-33% of the maximum.

At 72hr, the aorta and skin showed 41 and 23%, respectively, of each maximum concentration. The radioactivity concentration in the pituitary gland was below the limit of detection at 72hr. At 168hr, the radioactivity concentration in the aorta and skin was 38 and 21% of the maximum, respectively, and that of thyroid, lung, kidney, eyeball, blood and spleen was 6-10%. The radioactivity of the other tissues decreased to less than 5% of those maximum concentrations.

At 672hr, the aorta and skin showed 33 and 14%, respectively, of each maximum concentration of radioactivity, and the lung, sciatic nerve and spleen showed 4-7% of the maximum. The radioactivity concentrations in the plasma, pituitary gland, fat and bone marrow were below the limit of detection. The radioactivity of the other tissues decreased to less than 3% of those maximum concentrations.

5. Metabolites

The HPLC chromatograms of metabolites in the plasma, urine and liver after the 21st dosing of ^{14}C -CGS 16949A 1mg/kg to female rats are shown in Fig. 5~7 and the relative amounts of metabolites in the plasma, urine, feces, liver and kidney are shown in Table VI~X. The unknown metabolites were named in order of retention time, as those in the single dosing group²⁾. The retention times of each fraction for metabolites in plasma, urine, feces, liver and kidney were summarized in Table V.

1) Metabolites in plasma

After repeated administration of ^{14}C -CGS 16949A 1mg/kg to female rats, the concentration of unchanged CGS 16949 was 198ng eq. of CGS 16949/ml at 2hr and accounted for 48.1% of total radioactivity (Table VI). CGP 45383 (trans-8-hydroxy metabolite), CGP 45384 (cis-8-hydroxy metabolite) and CGP 45385 (8-oxo metabolite) was observed 9.1%, 8.0% and 9.3%, respectively, of total radioactivity in plasma. The relative amount of the metabolite with hydantoin-type structure, MP2 was 9.6% of radioactivity in plasma at 2hr.

Table V Retention times of each fraction for metabolites in HPLC chromatograms for plasma, urine, feces, liver and kidney

Retention times (min)	Plasma	Urine	Feces	Liver	Kidney
4.0		MU a		ML a	
5.8		MU 1			
6.0			MF 1		
7.0				ML b	
8.0	MP 1	MU 2	MF 2	ML 1	MK 1
10.0		MU 3		ML 2	MK 2
11.0				ML c	
13.0		MU 4		ML 3	
14.5		NU 5		ML 4	
19.0	CGP 45384	CGP 45384	CGP 45384	CGP 45384	CGP 45384
20.0	CGP 45385	CGP 45385	CGP 45385	CGP 45385	CGP 45385
21.5	CGP 45383	CGP 45383	CGP 45383	CGP 45383	CGP 45383
23.0		MU 6		ML d	
24.0		MU b			
26.0	MP 2	MU 7	MF 3	ML 5	MK 3
28.0		MU 8		ML 6	MK a
31.0		MU c		ML 7	MK 4
36.0	CGS 16949	CGS 16949	CGS 16949	CGS 16949	CGS 16949

Each sample was obtained after a 21 day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats.

The analytical methods are described in the section "MATERIALS AND METHODS", 14. Analysis of metabolites in reference 2.

At 8hr, the relative amount of CGS 16949 decreased to 17.2% of radioactivity and its concentration was 42ng eq. of CGS 16949/ml. CGP 45383 decreased to 7.9% and CGP 45384 and CGP 45385 and MP2 increased to 13.6, 15.7 and 22.1%, respectively, of total radioactivity in plasma.

At 24hr, the radioactivity concentrations in the fractions corresponded to CGS 16949, CGP 45383 and CGP 45384 were below the limit of detection. The relative amount of CGP 45385 was 8.6% and that of MP2 increased to 35.4% of radioactivity at 24hr.

Compared with the data after single dosing²⁾, the relative amount of unchanged CGS 16949 decreased and that of MP2 increased. There were no significant differences between the concentration of CGS 16949 after single (2hr : 208 ± 23 (mean $\pm$ S.E.), 8hr : 35 ± 4 ng eq. of CGS 16949/ml) and the 21st dosing (2hr : 198 ± 19 , 8hr : 42 ± 10 ng eq. of CGS 16949/ml).

2) Metabolites in urine

The relative amounts of CGS 16949, CGP 45383, CGP 45384 and CGP 45385 were 13.2 %, 32.9%, 24.3% and 3.5% of urinary radioactivity, respectively (Table VII). Eleven kinds of unknown metabolites were found and each ratio was less than 7.9% of urinary radioactivity. The relative amounts of unchanged compound and main metabolites were similar to that found in the single dosing group²⁾.

3) Metabolites in feces

Fig. 5 HPLC chromatogram of radioactivity in plasma (2hr, 24hr) after a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

The relative amounts of CGS 16949, CGP 45383 and CGP 45384 were 6.6%, 28.0% and 6.7% of fecal radioactivity, respectively (Table VII). CGP 45385 was not detected. Two kinds of unknown metabolites, MF2 and MF3 accounted for 7.2% and 4.1% of radioactivity in feces, respectively.

Compared with the data after single dosing²⁾, the relative amount of CGS 16949 decreased and that of CGP 45383 increased.

4) Metabolites in liver

Table VI Composition of CGS 16949 and its metabolites in plasma after a 21 day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

CGS 16949 and its metabolites	% of radioactivity in plasma		
	2 hr	8 hr	24 hr
CGS 16949	48.1 ± 2.4	17.2 ± 3.0	N.D.
CGP 45383	9.1 ± 0.9	7.9 ± 0.3	N.D.
CGP 45384	8.0 ± 1.6	13.6 ± 1.2	N.D.
CGP 45385	9.3 ± 0.8	15.7 ± 1.2	8.6 ± 2.1
MP1	N.D.	N.D.	N.D.
MP2	9.6 ± 0.2	22.1 ± 1.3	35.4 ± 0.4
Others	9.7 ± 1.6	14.0 ± 1.4	30.9 ± 2.3
Recovery	93.8 ± 0.3	90.5 ± 0.1	74.9 ± 1.8

Data are expressed as the mean values $\pm$ S.E. for three animals.
N.D. : Not detected.

Fig. 6 HPLC chromatogram of radioactivity in urine (0-24hr) after a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

The radioactivity concentration of CGS 16949 in the liver at 2hr after 21st dosing was 1048ng eq. of CGS 16949/g, which corresponded to 42.2% of radioactivity in the liver (Table K). The relative amounts of CGP 45383, CGP 45384 and CGP 45385 were 4.8%, 6.7% and 2.3% of radioactivity, respectively.

Table VI Composition of CGS 16949 and its metabolites in urine (0-24hr) after a 21 day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

CGS 16949 and its metabolites	% of radioactivity in urine
CGS 16949	13.2 $\pm$ 1.3
CGP 45383	32.9 $\pm$ 0.5
CGP 45384	24.3 $\pm$ 0.6
CGP 45385	3.5 $\pm$ 0.5
MU1	1.8 $\pm$ 0.3
MU2	7.9 $\pm$ 0.8
MU3	1.2 $\pm$ 0.3
MU4	2.3 $\pm$ 0.3
MU5	1.0 $\pm$ 0.1
MU6	1.3 $\pm$ 0.0
MU7	3.7 $\pm$ 0.1
MU8	1.0 $\pm$ 0.1
MUa	0.4 $\pm$ 0.1
MUb	0.4 $\pm$ 0.1
MUc	0.4 $\pm$ 0.1
Others	4.2 $\pm$ 0.2
Recovery	99.5 $\pm$ 0.1

Data are expressed as the mean values $\pm$ S.E. for three animals.

Table VII Composition of CGS 16949 and its metabolites in feces (0-24hr) after a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

CGS 16949 and its metabolites	% of radioactivity in feces
CGS 16949	6.6 $\pm$ 0.7
CGP 45383	28.0 $\pm$ 3.7
CGP 45384	6.7 $\pm$ 1.5
CGP 45385	N.D.
MF1	N.D.
MF2	7.2 $\pm$ 1.3
MF3	4.1 $\pm$ 0.9
Others	32.7 $\pm$ 2.6
Recovery	85.4 $\pm$ 1.6

Data are expressed as the mean values $\pm$ S.E. for three animals.

N.D. : Not detected.

Fig. 7 HPLC chromatograms of radioactivity in liver (2hr, 24hr) after a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

At 8hr, the relative amount of CGS 16949 decreased to 23.1% of radioactivity and its concentration was 275ng eq. of CGS 16949/g. CGP 45383, CGP 45384 and CGP 45385 accounted for 6.2%, 14.3% and 2.9% of radioactivity, respectively, and the relative amounts of ML1, ML5 and ML6 were 2.5~9.4% of radioactivity.

Table K Composition of CGS 16949 and its metabolites in liver after a 21day period of daily oral administration of ^{14}C -CGS 16949A 1mg/kg to non-fasted female rats

CGS 16949 and its metabolites	% of radioactivity in liver		
	2 hr	8 hr	24 hr
CGS 16949	42.2 $\pm$ 1.9	23.1 $\pm$ 3.6	N.D.
CGP 45383	4.8 $\pm$ 0.5	6.2 $\pm$ 0.5	N.D.
CGP 45384	6.7 $\pm$ 0.4	14.3 $\pm$ 1.5	N.D.
CGP 45385	2.3 $\pm$ 0.4	2.9 $\pm$ 0.6	N.D.
ML1	4.2 $\pm$ 0.6	2.5 $\pm$ 0.3	N.D.
ML2	1.3 $\pm$ 0.4	N.D.	N.D.
ML3	N.D.	N.D.	N.D.
ML4	N.D.	N.D.	N.D.
ML5	8.7 $\pm$ 1.6	9.1 $\pm$ 0.3	14.0 $\pm$ 1.4
ML6	2.4 $\pm$ 0.2	9.4 $\pm$ 0.6	7.2 $\pm$ 0.4
ML7	0.9 $\pm$ 0.1	N.D.	N.D.
MLa	0.8 $\pm$ 0.4	N.D.	N.D.
MLb	4.2 $\pm$ 0.8	N.D.	N.D.
MLc	1.0 $\pm$ 0.2	N.D.	N.D.
MLd	1.2 $\pm$ 0.2	N.D.	N.D.
Other	13.2 $\pm$ 1.0	22.5 $\pm$ 3.6	55.2 $\pm$ 1.6
Recovery	93.8 $\pm$ 0.2	90.0 $\pm$ 0.4	76.4 $\pm$ 0.5

Data are expressed as the mean values $\pm$ S.E. for three animals.

N.D. : Not detected.

Table X Composition of CGS 16949 and its metabolites in kidney after a 21 day period of daily oral administration of ^{14}C -CGS 16949 A 1mg/kg to non-fasted female rats

CGS 16949 and its metabolites	% of radioactivity in kidney		
	2 hr	8 hr	24 hr
CGS 16949	53.5 $\pm$ 0.5	17.5 $\pm$ 1.0	N.D.
CGP 45383	9.4 $\pm$ 0.8	8.0 $\pm$ 1.0	N.D.
CGP 45384	5.1 $\pm$ 0.4	12.2 $\pm$ 1.5	N.D.
CGP 45385	3.6 $\pm$ 0.4	10.0 $\pm$ 1.9	N.D.
MK1	1.7 $\pm$ 0.1	N.D.	N.D.
MK2	N.D.	N.D.	N.D.
MK3	3.3 $\pm$ 0.9	8.6 $\pm$ 0.5	9.1 $\pm$ 0.2
MK4	N.D.	N.D.	N.D.
MKa	1.8 $\pm$ 0.5	N.D.	N.D.
Others	11.4 $\pm$ 0.6	25.0 $\pm$ 2.1	54.4 $\pm$ 1.4
Recovery	89.9 $\pm$ 0.8	81.4 $\pm$ 0.9	63.5 $\pm$ 1.2

Data are expressed as the mean values $\pm$ S.E. for three animals.

N.D. : Not detected.

At 24hr, the relative amounts of ML5 and ML6 were 14.0% and 7.2% of radioactivity, respectively.

Compared with the data after single dosing²⁾, the relative amount of unchanged CGS 16949 decreased.

5) Metabolites in kidney

The radioactivity concentration of CGS 16949 in the kidney at 2hr after 21st dosing was 946ng eq. of CGS 16949/g, which accounted for 53.5% of radioactivity in the kidney (Table X). The relative amounts of CGP 45383, CGP 45384 and CGP 45385 were 3.6~9.4% of radioactivity in the kidney.

At 8hr after 21st dosing, the radioactivity concentration of CGS 16949 decreased to 155 ng eq. of CGS 16949/g, which accounted for 17.5% of radioactivity. The relative amounts of CGP 45383, CGP 45384 and CGP 45385 were 8.0~12.2% of radioactivity. MK3 accounted for 8.6% of radioactivity in kidney.

At 24hr, unknown metabolite MK3 was found as 9.1% of radioactivity in kidney.

Compared with the data after single dosing²⁾, the relative amount of CGS 16949 decreased and that of MK3 increased after 21st dosing.

DISCUSSION

Larger variation of plasma concentration was found after 11th dosing in comparison with plasma concentration from 1st to 10th dosing (Fig. 2), but the reason is unclear. Since the plasma concentrations of radioactivity were almost the same at 15th, 17th, 19th and 20th dosing, it is suggested that the plasma concentration of radioactivity reaches a steady state after 15th dosing. The administration time (15times) to reach the steady state in the plasma concentration is roughly in accordance with that the radioactivity concentrations in many tissues including plasma and blood were similar at the 7th and 14th dosing (Table III), indicating that a steady state would be found by 15th dosing.

The radioactivity concentration in the aorta did not reach the steady state and increased with dosing. Further the elimination of radioactivity from the aorta was slow and the radioactivity concentration in the aorta was 33% of the maximum at 672hr after the 21st administration. Pfaar et al. (internal report) reported that the uptake of radioactivity into the fibrous connective tissues (collagen and elastin) of blood vessels was found after repeated oral administration of ¹⁴C-CGS 16949A to male rats. Referring that histidine can react with such aldimines by forming a covalent C-C bond between the imidazolyl moiety of histidine and the aldimine³⁾, they suggested that a similar reaction with the imidazole moiety of ¹⁴C-CGS 16949A may be responsible for the uptake of radioactivity in the walls of blood vessels. Therefore, remaining of radioactivity in the aorta (Table IV) may be caused by the binding of the radioactivity with collagen and/or elastin.

The relative amounts of the metabolites in the liver and kidney after the 21st dosing were changed and the relative amount of unchanged CGS 16949 was decreased, compared with that after single dosing²⁾. This might be due to the slower decline of metabolites compared with that of CGS 16949.

The urinary and fecal excretion of radioactivity reached almost a steady state after the 4th dosing and the each excretion ratio was similar to that after single dosing²⁾.

Compositions of CGS 16949 and its metabolites in urine after repeated dosing were similar to that of single dosing. After repeated dosing, some metabolites were found as several percentage in fecal radioactivity, although those relative amounts were N.D. levels after single dosing. The increase of the relative amount of some metabolites might be the result of actualization based on the pile of very small amount of metabolite. Relevantly the relative amount of CGS 16949 decreased after repeated administration. Therefore, repeated administration might not affect the excretion of CGS 16949 and its metabolites.

Since the relative amount of CGS 16949 and its main metabolites in the urine, as the main route of excretion, did not change significantly, the metabolism of CGS 16949 after repeated administration would be similar to that after single dosing.

The metabolites, MP2, MU7, ML5 and MK3 found in the plasma, urine, liver and kidney, respectively, have the same retention time (26min), indicating that these would be the identical compound.

REFERENCES

- 1) Schieweck, K., Bhatnagar, A.S. and Matter, A. : CGS 16949A, a New Nonsteroidal Aromatase Inhibitor : Effects on Hormone-dependent and -independent Tumors *in Vivo*. *Cancer research*, **48** : 834-838 (1988).
- 2) Yamagami, S., Kawasaki, E., Egawa, A., Esumi, Y., Hori, K., Ishizaki, M., Honda, T. and Moriki, H. : Studies on the disposition of fadrozole hydrochloride (CGS16949A) (I). Absorption, distribution, metabolism and excretion after a single oral administration to rats. *Xenobio. Metabol. and Dispos.*, **8** : 1097-1127 (1993).
- 3) Yamauchi, M., London, R.E., Guenat, C., Hasimoto, F. and Mechanic, G.L. : Structure and formulation of a stable histidine-based trifunctional cross-link in skin collagen. *J. Biol. Chem.*, **262** : 11428-11434 (1987).