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Original Article

Comparative Metabolism of Procymidone in Rats and Mice

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The metabolic fates of procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide, Sumilex®] in rats and mice were examined. After [phenyl-^{14}C]procymidone was administered orally to male rats and mice at 100 mg/kg, the radiocarbon was rapidly and almost completely excreted into excreta of both animals, mainly into the urine, within 7 days after administration. ^{14}C-Levels in the blood of both species were fairly constant for 2–12 hr, reaching maximum at 12 hr in rats and at 2 hr in mice after administration. The ^{14}C-levels decreased with the biological half-lives of 12 hr in rats and 10 hr in mice from 8 to 72 hr after administration. In both species, the major metabolic reactions were oxidation of one of the methyl groups to carboxylic acid *via* hydroxymethyl and cleavage of the imide linkage. No marked species differences in metabolism were observed.

INTRODUCTION

Procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide, Sumilex®] is a fungicide for control of plant diseases such as gray mold and sclerotinia rot.[1-3]

The metabolism of procymidone in rats was studied by Mikami *et al.*,[4] but that in mice has not been studied yet. To compare the metabolism of procymidone in rats with that in mice in detail, the ^{14}C-excretion, ^{14}C-tissue distribution and biotransformation were examined in both species by single oral administration of [phenyl-^{14}C]procymidone.

MATERIALS AND METHODS

1. Designation of Compound

Abbreviations used in this report are shown in Table 1.

2. Chemicals

Procymidone was prepared in Sumitomo Chemical Co., Ltd., Japan. All the other chemicals used were of reagent or of analytical grade.

Procymidone uniformly labeled with ^{14}C at the phenyl carbons was synthesized in this laboratory (Fig. 1).[5,6] The specific activity of the ^{14}C-labeled compound was 844 MBq/mmol (22.8 mCi/mmol). The labeled compound was purified prior to use by TLC using hexane–acetone (3/1, v/v), whose radiochemical purity was more than 99% as determined by TLC using hexane–acetone (3/1, v/v) or dichloromethane.

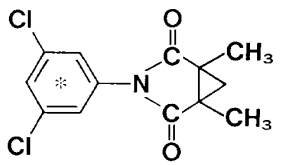
3. TLC

Precoated silica gel 60F_{254} chromatoplates (20×20 cm, 0.25 mm layer thickness, E. Merck, F.R.G.) were used for both analysis and purification of metabolites.

Rf values for the authentic standards were reported previously.[4] Unlabeled standards were located under UV light (Mitsumi Co., Ltd., Japan). Radioactive metabolites were detected by autoradiography using SB-5

Table 1 Procymidone and its metabolites.

Compound	Abbreviation
N-(3,5-Dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide	Procymidone
N-(3,5-Dichlorophenyl)-1-hydroxymethyl-2-methyl-cyclopropane-1,2-dicarboximide	Procymidone-OH
2-(3,5-Dichlorophenylcarbamoyl)-2-hydroxymethyl-1-methyl-cyclopropane-1-carboxylic acid	Procymidone-NHOH
2-(3,5-Dichlorophenylcarbamoyl)-1-hydroxymethyl-2-methyl-cyclopropane-1-carboxylic acid	Procymidone-NH'OH
N-(3,5-Dichlorophenyl)-1-carboxy-2-methylcyclo-propane-1,2-dicarboximide	Procymidone-COOH
2-(3,5-Dichlorophenylcarbamoyl)-1-methylcyclopropane-1,2-dicarboxylic acid	Procymidone-NH$(COOH)_2$
2-(3,5-Dichlorophenylcarbamoyl)-2-methylcyclopropane-1,1-dicarboxylic acid	Procymidone-NH'$(COOH)_2$
2-(3,5-Dichlorophenylcarbamoyl)-1,2-dimethylcyclopropane-1-carboxylic acid	Procymidone-NHCOOH
2-Methylcyclopropane-1,1,2-tricarboxylic acid	Cyclopropane-$(COOH)_3$



*Labeled position

Fig. 1 Chemical structure and labeled position of procymidone.

films (Kodak, U.S.A.).

4. Radioanalysis

Radioactivity in organosoluble fractions or urine was measured with a Tri-Carb® 460CD liquid scintillation spectrometer (Packard, U.S.A.), which gave dpm by the external standard method. Aliquots of samples were added to low potassium glass vials (Wheaton, U.S.A.) containing 10 ml of Emulsifier Scintillator 299™ (Packard, U.S.A.). Radioactivity in the homogenates of feces, tissues and unextractable fecal residues was quantified by the combustion method.[4] Radioactivity on the TLC plates was measured by scraping appropriate gel regions and counting in scintillation vials.[4]

5. Treatment of Animals

Caesarian derived (CD) Sprague-Dawley male rats and ICR male mice at 6 weeks old were purchased from Charles River Japan Inc. (Japan) and allowed to adjust to the environment for one week before dosing.

The labeled compound was dissolved in corn oil. Rats and mice were given a single oral dose of the labeled preparation at a rate of 100 mg/kg/5 ml through this study. To collect feces and urine separately, the treated animals were housed (rats: individually, mice: five mice/group) in all-glass metabolism cages (Metabolica-CO_2®, Sugiyamagen Iriki Co., Ltd., Tokyo, Japan). In the tissue level study, the treated animals were housed in aluminum cages.

All animals were supplied with a diet (CE-2, Clea Japan, Inc., Japan) and water *ad libitum* during the experiments.

The in-life portion of the studies was conducted under the following environmental conditions: room temperature, 23±2°C; relative humidity, 55±10%; ventilation, more than 10 air exchange/hr and artificial lighting, from 8:00 AM to 8:00 PM.

6. Analysis of Excreta and Tissues

Feces and urine were collected 1, 2, 3, 5 and 7 days after oral administration. The 0–2 day urine was combined and directly subjected to TLC analysis. The 0–2 day feces were extracted three times with methanol–water (9/1, v/v) and the extracts were combined, concentrated *in vacuo* and subjected to TLC analysis.

Animals were sacrificed on the 7th day after administration. In the ^{14}C-tissue distribution study, five male rats or mice were sacrificed 2, 4, 6, 8, 12, 24 and 72 hr after administration.

Tissue levels were given as parts per million equivalents (ppm) or parts per billion equivalents (ppb) of the dosed ^{14}C-labeled compound based on wet tissue weight.

7. Identification of Metabolites in Excreta and Tissues

Metabolites were tentatively identified by TLC co-chromatography with appropriate authentic standards as reported previously.[4]

RESULTS

1. ^{14}C-Excretion

In male rats or mice receiving a single oral dose of [phenyl-^{14}C]procymidone at 100 mg/kg, the radiocarbon was rapidly and almost completely eliminated in the excreta and the major excretion route of ^{14}C was urine (Fig. 2). On the first day, 59% and 92% of the dosed ^{14}C was excreted into the excreta of rats and mice, respectively. ^{14}C-recoveries within 7 days after administration were 96% in rats (feces: 13%, urine: 84%) and were 104% in mice (feces:

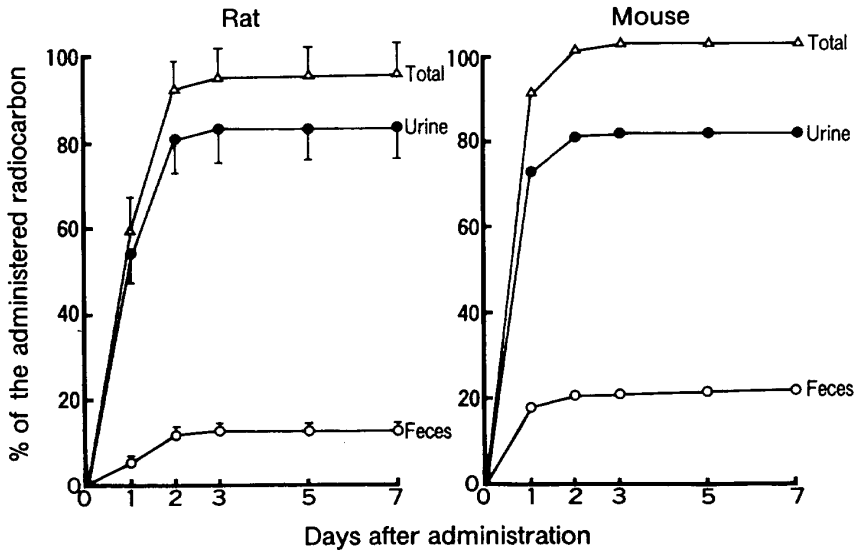


Fig. 2 Cumulative excretion of ^{14}C after single oral administration of ^{14}C-procymidone to rats and mice at 100 mg/kg.

Vertical bars represent standard deviation of five animals.

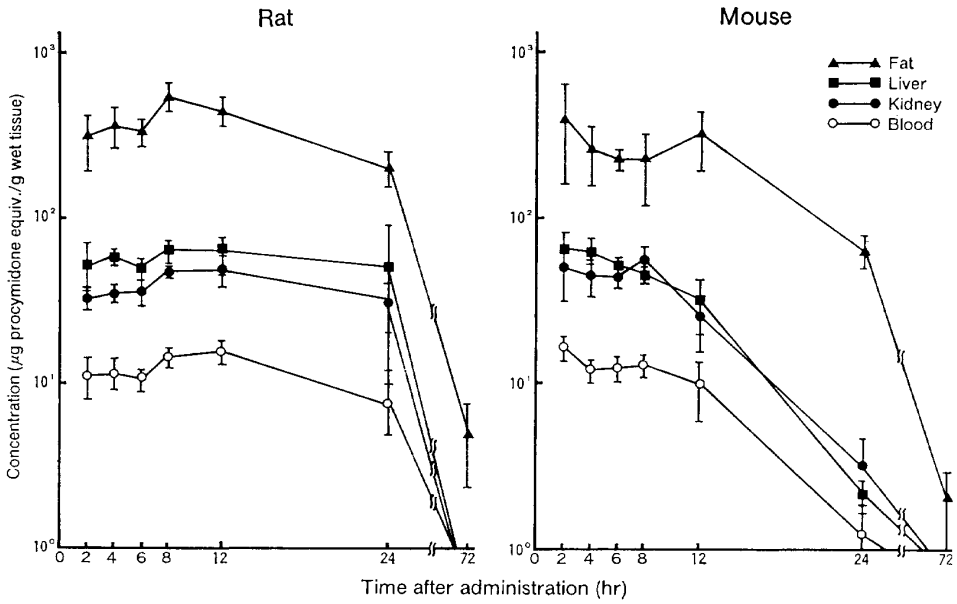


Fig. 3 Time course of ^{14}C-levels in the blood, fat, kidney and liver after single oral administration of ^{14}C-procymidone to rats and mice at 100 mg/kg.

Vertical bars represent standard deviation of five animals.

22%, urine: 82%).

2. *^{14}C-Tissue Distribution*

^{14}C-Levels in tissues of rats or mice receiving a single oral dose of [phenyl-^{14}C]procymidone at 100 mg/kg are shown in Fig. 3.

In rats, ^{14}C-levels in the blood, fat, kidney and liver were fairly constant for 2–24 hr and decreased rapidly after plateau. The maximal ^{14}C-levels in the blood, fat, kidney and liver were 15.4, 555, 49.1 and 66.5 ppm, respectively.

In mice, ^{14}C-levels in the blood, fat, kidney and liver retained a plateau for 2–12 hr and decreased rapidly after plateau. The maximal ^{14}C-levels in the blood, fat, kidney and liver were 16.6, 429, 57.8 and 67.1 ppm, respectively. Biological half-lives in various tissues of both species from 8 to 72 hr after administration are shown in Table 2.

3. *Amounts of Metabolites in Excreta and Tissues*

Table 3 shows the amounts (% of the dose) of urinary and fecal metabolites. In both species, major metabolites were procymidone-COOH, procymidone-NH(COOH)$_2$ and procymidone-NH'(COOH)$_2$. The sum amount of these metabolites in the urine and feces were 70% in rats and 62% in mice. Procymidone-OH, procymidone-NHOH and procymidone-NH'OH were also detected as minor metabolites. No attempt was made to distinguish procymidone-NH(COOH)$_2$ from procymidone-NH'(COOH)$_2$ and procymidone-NHOH from procymidone-NH'OH.

In both species, TLC analysis of methanol extracts from the blood, kidney and liver

Table 2 Biological half-lives ($T_{1/2}$) of ^{14}C in tissues after a single oral dose of [phenyl-^{14}C] procymidone to male SD rats and male ICR mice at 100 mg/kg.

Tissue	Half-life (hr)	
	Rats	Mice
	8–72 (hr)[a]	8–72 (hr)
Blood	12	10
Brain	12	9
Fat	10 (6–72 hr)	12
Kidney	10	10
Liver	9	10

[a] Time after administration.

Table 3 Relative amounts of identified metabolites in the urine and feces for 2 days after single oral administration of [phenyl-^{14}C]procymidone to male SD rats and ICR mice at 100 mg/kg.

Metabolite	% of the dosed ^{14}C					
	Rats[a]			Mice[b]		
	Feces		Urine	Feces		Urine
Procymidone	5.2±1.85		0.2±0.02	7.0		2.6
Procymidone-OH	1.1±0.51		3.2±0.52	2.1		7.1
Procymidone-NHOH	0.2±0.14	1.4±0.68	0.8±0.05	0.4	2.7	0.8	8.4
Procymidone-NH'OH	0.1±0.02		0.7±0.27	0.1		0.6
Procymidone-COOH	0.4±0.17		21.5±3.98	1.4		19.5
Procymidone-NH(COOH)$_2$	0.1±0.04	1.0±0.35	0.7±0.05	0.4	4.3	1.2	58.1
Procymidone-NH'(COOH)$_2$	0.5±0.16		46.5±4.23	2.5		37.4
Procymidone-NHCOOH	0.1±0.03		0.2±0.06	0.2		0.2
3,5-Dichloroaniline	N.D.[c]		N.D.	N.D.		0.0
Others	2.2±0.38		7.1±1.19	3.4		11.5
Unextractable ^{14}C	2.0±0.12			3.3		
Total	11.8±0.79		80.8±7.70	20.9		80.8

[a] Data shows the mean values±standard deviations of five rats.
[b] Urine and feces from five mice were collected.
[c] Not detected by autoradiography.

Table 4 ^{14}C-Levels and contents of procymidone and its metabolites in the blood, kidney and liver of SD rats and ICR mice given a single oral dose of [phenyl-^{14}C]procymidone at 100 mg/kg.

Tissue[a)]	Metabolite	μg Procymidone equiv./g wet tissue			
		Rat		Mouse	
		2 hr	24 hr	2 hr	24 hr
Blood	MeOH Extract				
	Procymidone	1.17	0.24	6.48	0.07
	Procymidone-OH	0 62	1.27	4.15	0 13
	Procymidone-NHOH	0.33 2.46	0.19 2.19	0 24 4.67	0.02 0.16
	Procymidone-NH'OH	1.51	0.73	0.28	0.01
	Procymidone-COOH	0.15	1.11	1.27	0.43
	Procymidone-NH(COOH)$_2$	0.04 0 89	0 05 3.40	0.50 2.95	N.D. 0.65
	Procymidone-NH'(COOH)$_2$	0.68	2 24	1.18	0.21
	Procymidone-NHCOOH	5.49	0.18	0.19	0.05
	3,5-Dichloroaniline	0.21	0.06	0.46	0.02
	Others	0.56	1.07	1.81	0.16
	Subtotal	10.8	7.14	16.6	1.09
	Unextractable ^{14}C	0.22	0.48	0.09	0.16
	Total	11.0	7.62	16.6	1.25
Kidney	MeOH Extract				
	Procymidone	16.1	1.23	18.4	0.59
	Procymidone-OH	5.20	5.51	15.3	0.49
	Procymidone-NHOH	0 33 5 86	0.21 6.29	1.03 16.7	0 04 0.56
	Procymidone-NH'OH	0 33	0 56	0 41	0 03
	Procymidone-COOH	4.26	12.5	3.97	0.67
	Procymidone-NH(COOH)$_2$	0 33 6.76	1.02 20.4	1.63 7.96	0 09 1.07
	Procymidone-NH'(COOH)$_2$	2 17	6 87	2 36	0 31
	Procymidone-NHCOOH	0 33	0 29	0.28	N.D.
	3,5-Dichloroaniline	1.45	0.13	1.03	0.02
	Others	3.45	2.00	5.48	0 39
	Subtotal	34 0	30.3	49 9	2.62
	Unextractable ^{14}C	0 28	0.80	0.30	0.47
	Total	34 3	31 1	50.2	3.10
Liver	MeOH Extract				
	Procymidone	37.7	0.71	35.9	0.52
	Procymidone-OH	6.12	7.94	15.7	0.20
	Procymidone-NHOH	0 11 6.33	4 33 12.5	0 34 16.8	0.04 0.35
	Procymidone-NH'OH	0.10	0 28	0.72	0.12
	Procymidone-COOH	0 65	0.73	2.88	0.26
	Procymidone-NH(COOH)$_2$	0.26 1.21	2.70 22.5	1.95 5.55	0 05 0.43
	Procymidone-NH'(COOH)$_2$	0 29	19.0	0 72	0.13
	Procymidone-NHCOOH	0 25	N.D.	0 21	0 03
	3,5-Dichloroaniline	0.47	N.D.	1.06	0 08
	Others	1.13	11.7	7.24	0.43
	Subtotal	47.1	47.5	66 8	1 84
	Unextractable ^{14}C	0.26	2.04	0.32	0 38
	Total	47.3	49 5	67.1	2.22

a) These tissues were analyzed together after combination of tissues from five animals.

revealed that major metabolites in these tissues were procymidone-COOH, procymidone-OH and intact procymidone (Table 4).

In rats, concentration of intact procymidone in the tissues reached maxima within 8 hr after administration and decreased in proportion to total ^{14}C-levels. The peak concentration of intact procymidone in the blood, kidney and liver were 4.90, 16.1 and 40.4 ppm, respectively.

In mice, concentrations of intact procymidone reached maxima within 2 hr after administration, and those of the metabolites within 12 hr after oral dosing and decreased in proportion to total ^{14}C-levels. The peak levels of procymidone in the blood, kidney and liver were 6.48, 18.4 and 35.9 ppm, respectively.

DISCUSSION

On single oral administration of [phenyl-^{14}C]procymidone to male rats or mice at 100 mg/kg, the radiocarbon was almost completely eliminated into the urine and feces within 7 days. In both species, the ^{14}C-excretion into the urine (rats: 84%, mice: 82%) was significantly larger than that into the feces (rats: 13%, mice: 22%). On the first day, ^{14}C-recoveries were 59% (feces: 5%, urine: 54%) in rats and 92% (feces: 18%, urine: 74%) in mice. These facts indicate that there is no marked species difference in ^{14}C-excretion between rats and mice, although it seems that ^{14}C is excreted somewhat faster in mice than in rats.

Plateau-like ^{14}C-levels were observed in the blood for 2–24 hr in rats and for 2–12 hr in mice. ^{14}C-Levels in the blood reached maxima at 12 hr in rats and at 2 hr in mice after administration and decreased with the biological half-lives of 12 hr in rats and 10 hr in mice from 8

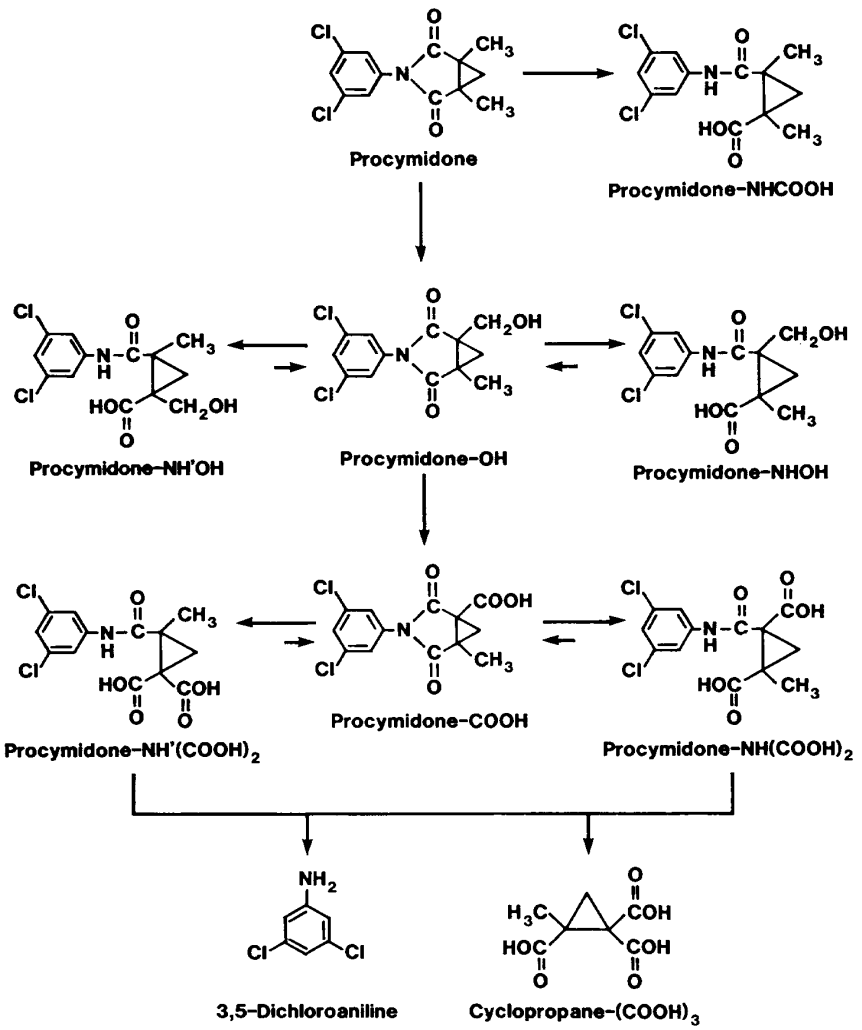


Fig. 4 Proposed metabolic pathways for procymidone in rats and mice.

to 72 hr after administration. The biological half-life in rats seemed to have somewhat faster phase after 24 hr following administration.

Concentration of intact procymidone in the blood reached maxima within 8 hr in rats and 2 hr in mice after administration and decreased in proportion to ^{14}C-levels.

Based on the identified metabolites in the excreta and tissues, major biotransformations of procymidone in rats were essentially the same as those reported previously[4]: 1) oxidation at one of the methyl groups to carboxylic acid *via* hydroxymethyl and 2) cleavage of the imide linkage. Similar biotransformations were observed in mice. Proposed metabolic pathways for procymidone in rats and mice are given in Fig. 4. In this study, cyclopropane-$(COOH)_3$ was not detected because [phenyl-^{14}C]procymidone was used.

These findings indicate that in both species, procymidone is readily absorbed from the gastrointestinal tract, distributed to various tissues, metabolized and excreted without any retention in the tissues and that there is no considerable species difference in metabolism between rats and mice.

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要　　約

プロシミドンのラットおよびマウスにおける比較代謝

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射場一彦，中塚　巌，吉武　彬，山田宏彦
宮本純之

プロシミドン [*N*-(3, 5-dichlorophenyl)-1, 2-dimethylcyclopropane-1, 2-dicarboximide] のラットおよびマウスにおける代謝運命を比較検討した．^{14}C 標識体を 100 mg/kg の投与量で1回経口投与したところ，投与 ^{14}C は，両種とも投与後7日以内にほぼ完全に排泄され，主要排泄経路は尿であった．血中 ^{14}C 濃度は，投与後2から12時間でプラトー状態を示した．ラットでは12時間で，マウスでは2時間で最高値に到達した．投与後8時間から72時間までラットで生物学的半減期12時間，マウスでは10時間で減少した．排泄物および組織中の同定代謝物からプロシミドンのラットおよびマウスにおける主要代謝反応は，メチル基のヒドロキシメチルを経由したカルボン酸への酸化およびイミド結合の開裂であった．プロシミドンの代謝において顕著な種差は認められなかった．