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Urinary Metabolites of 1-(3-Mercapto-2-D-methyl-1-oxopropyl)-L-proline (SQ-14225), a New Antihypertensive Agent, in Rats and Dogs

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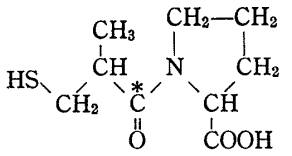
The *in vivo* metabolism of 1-(3-mercapto-2-D-methyl-1-oxopropyl)-L-proline (SQ-14225) was investigated in rats and dogs. SQ-14225 labeled with ^{14}C at the mercaptoacyl moiety was administered orally and the metabolites in the 24 hr urine were identified by comparing their *Rf* values with those of authentic samples. Unchanged SQ-14225 was trapped *via* its sulfhydryl group by the previous addition of N-ethylmaleimide to prevent air oxidation. In both animal species, most of the radioactivity was excreted as unchanged SQ-14225 and its disulfide (SQ-14551). A significant amount of the urinary radioactivity was accounted for by mixed disulfides, among which the disulfide with L-cysteine (Cys-SQ-14225) was predominant. N-Acetyl-Cys-SQ-14225 was also detected as a minor component, but the amount of the mixed disulfide with glutathione (GS-SQ-14225) was negligible. S-Methyl-SQ-14225 and its sulfoxide were also recognized. As judged from the negligible incorporation of radioactivity into collagen after administration of SQ-14225-^{3}H labeled at the proline moiety, the metabolic cleavage of the amide bond in SQ-14225 was not significant.

Keywords——urinary metabolite; rat; dog; sulfhydryl compound; mixed disulfide; antihypertensive agent; SQ-14225

1-(3-Mercapto-2-D-methyl-1-oxopropyl)-L-proline (SQ-14225) is a strong inhibitor of angiotensin-converting enzyme and was developed in the Squibb Institute for Medical Research, USA, as an antihypertensive agent (Fig. 1).[2] Significant effects against hypertension have been recognized clinically[3] as well as in experimental animals.



*: the position labeled with ^{14}C

Fig. 1. The Structure of 1-(3-Mercapto-2-D-methyl-1-oxopropyl)-L-proline (SQ-14225)

In this paper, the urinary metabolites of SQ-14225 after oral administration to rats and dogs were identified by chromatographic comparison with synthesized authentic samples. Most of the radioactivity was excreted as unchanged SQ-14225, but some metabolites were identified. The major urinary metabolites were the disulfides with SQ-14225 itself (SQ-14551) and/or with other endogenous sulfhydryl compounds. The amide bond in SQ-14225 was shown to be metabolically stable.

Materials and Methods

Labeled Compounds——1-(3-Mercapto-2-D-methyl-1-oxopropyl)-L-proline (SQ-14225) labeled with ^{14}C was obtained from Dai-ichi Pure Chemicals Co., Ltd. (Fig. 1).[4] The specific radioactivity was 14.7 μCi/mg (3.2 mCi/mmol) and the radiochemical purity was determined by a reverse isotope dilution method to be 98.4%. SQ-14225-proline-^{3}H was obtained from the Squibb Institute for Medical Research, USA. The specific radioactivity was 119.2 μCi/mg (25.9 mCi/mmol) and the radiochemical purity was 101% (reverse isotope dilution method). L-Proline-2,3,4,5-^{3}H was obtained from New England Nuclear Corp. (Boston, USA). The specific radioactivity was 964 mCi/mg (111.0 Ci/mmol) and the radiochemical purity was over 99% as determined by cellulose thin-layer chromatography (TLC) using sec-butanol saturated with water as a developing solvent.

Materilas——Non-radioactive SQ-14225 was obtained from the Squibb Institute for Medical Research, USA. Precoated silica gel thin-layer plates (0.25 mm of thickness, F_{254}) were purchased from Merck Co., Ltd. Other chemicals were commercial products of analytical-reagent grade.

Collection of Urine and Separation of the Urinary Metabolites——An aqueous solution of SQ-14225-^{14}C was administered orally to three male rats of Sprague-Dawley strain and five male beagle dogs at doses of 5 and 25 mg/kg, respectively. The animals were housed separately in metabolic cages and urine samples were collected during the first 24 hr period in the presence of N-ethylmaleimide (NEM) to prevent the spontaneous disulfide formation of SQ-14225.[5] A stock solution of NEM in acetone (250 mg/ml) was used and 500 mg (2 ml) and 125 mg (0.5 ml) were added to the urine collection vessels for each dog and rat, respectively. The potassium phosphate buffer (2 M, pH 6.0) was also added previously to maintain the pH of the collected urine at 6.0 (5 ml and 0.5 ml for dog and rat, respectively). After the application of authentic samples, 10 μl portions of the urine samples were spotted on the silica gel thin-layer plate and developed with the following solvent systems: A, benzene–acetic acid (3: 1);[5] B, *n*-butanol–acetic acid–water (4: 1: 1). With solvent A, good separation between S-methyl-SQ-14225, SQ-14225-NEM adduct and SQ-14551 was obtained but the polar metabolites remained at the origin. Solvent B gave a good separation between polar metabolites. The developed plates were dried completely and brought into contact with X-ray films for 2 weeks to obtain the autoradiograms. The radioactive spots were identified by comparing the *Rf* values with those of authentic samples, scraped into counting vials and supplemented with 10 ml of toluene–ethanol liquid scintillator (500 ml of toluene, 500 ml of ethanol, 200 mg of dimethyl POPOP and 8g of PPO). The radioactivity was measured in a Packard model 3380 liquid scintillation spectrophotometer. In the experiments to compare chromatographically the partially purified metabolites with the authentic samples, three additional solvent systems were used: C, isopropanol–water (7: 3); D, ethanol–28% aqueous ammonia (7: 3); E, chloroform–methanol–14% aqueous ammonia (2: 2: 1).

Preparation of Authentic Samples

The Disulfide of SQ-14225 (SQ-14551)——SQ-14225 (1.55 g) was dissolved in 30 ml of water and cooled on an ice-bath, then a saturated solution of iodine in methanol was added until the yellowish tinge of excess iodine was obtained. The reaction mixture gave a negative response to Ellman reagent[6] and was kept overnight at 4°. The precipitated SQ-14551 was filtered off and recrystallized from ethanol–water (1.25 g). *Anal.* Calcd for $C_{18}H_{28}N_2O_6S_2$: C, 49.98; H, 6.52; N, 6.48; S, 14.82. Found: C, 49.74; H, 6.46; N, 6.55; S, 14.79. NMR: 1.2 (3H, d, $-CH_3$ in $HSCH_2CHCH_3CO$), 2.0—2.2 (4H, m, $-CH_2$ in proline), 2.7—3.0 (3H, m, $-CH$ and $-CH_2$ in $HSCH_2CHCH_3CO$), 3.8 (2H, m, $-CH_2$ in proline) and 4.5 (1H, m, $-CH$ in proline).

Mixed Disulfide of SQ-14225 with Cysteine (Cys-SQ-14225)——L-Cysteine (1.2 g) and SQ-14225 (200 mg) were dissolved in 5 ml of water (molar ratio; 10: 1), then iodine (1.3 g) was added and the mixture was stirred slowly. A saturated solution of iodine was added to complete the reaction. The precipitated L-cystine was removed by centrifugation. The supernatant was loaded on a Dowex 1 anion exchange column (Cl^- form, 3×5 cm) and eluted with distilled water. The eluate was concentrated to 3 ml *in vacuo* and the precipitated L-cystine was removed by filtration. The concentrated eluate was loaded on a Sephadex LH-20 column (2.6×35 cm, ethanol suspension) and eluted with ethanol (10 ml/fraction). Each fraction was checked by silica gel TLC with the solvent system: *n*-butanol–acetic acid–water (4: 1: 1). After color development with ninhydrin, the fractions containing Cys-SQ-14225 were collected, evaporated to dryness *in vacuo* and redissolved in 3 ml of water. The residual L-cystine was precipitated by adjusting the pH to 7.0 with ammonium hydroxide and the crude solution of Cys-SQ-14225 was loaded on a Dowex 1 anion exchange column (Cl^- form, 3×5 cm). The column was washed with 200 ml of water, then Cys-SQ-14225 was eluted with 0.5 N HCl. The fractions containing Cys-SQ-14225 were concentrated *in vacuo* to provide a white powder (150 mg), which gave a single spot on silica gel TLC. NMR: 1.2 (3H, d, $-CH_3$ in $HSCH_2CHCH_3CO$), 2.0—2.2 (4H, m, $-CH_2$ in proline), 2.7—3.0 (3H, m, $-CH$ and $-CH_2$ in $HSCH_2CHCH_3CO$), 3.1 (2H, d, $-CH_2$ in cysteine), 3.8 (2H, m, $-CH_2$ in proline), 4.5 (2H, m, $-CH$ in proline and cysteine).

S-Methyl-SQ-14225——Sodium hydride (0.13 g) was added slowly to 10 ml of ethanol and then 0.5 g of SQ-14225 was dissolved in this solution. Next, 0.5 ml of methyl iodide was added and the whole was stirred for 5 min at room temperature. The absence of free sulfhydryl groups was confirmed by means of the Ellman reagent, then the reaction mixture was poured into 50 ml of water and the pH of the solution was adjusted to 2.0 with dilute hydrochloric acid. S-Methyl-SQ-14225 was extracted 3 times with 100 ml of ethyl acetate. The organic layer was evaporated to dryness, redissolved in 3 ml of ethanol, loaded on a Sephadex LH-20 column (2.6×35 cm, ethanol suspension) and eluted with ethanol (10 ml/fraction). The fractions containing S-methyl-SQ-14225 were identified by silica gel TLC (iodine color development) and evaporated to dryness *in vacuo* (250 mg). Mass spectrum of the methyl ester: *m/e* 245 (M^+). NMR: 1.2 (3H, d, $-CH_3$ in $-SCH_2CHCH_3CO$), 2.0 (3H, s, $-SCH_3$), 2.0—2.2 (4H, m, $-CH_2$ in proline), 2.8—3.0 (3H, m, $-CH$ and $-CH_2$ in $CH_3SCH_2CHCH_3CO$), 3.8 (2H, m, $-CH_2$ in proline) and 4.5 (1H, m, $-CH$ in proline).

S-Methyl-SQ-14225 Sulfoxide——S-Methyl-SQ-14225 (0.1 g) was dissolved in 4 ml of methylene chloride and then an equimolar amount of *m*-chloroperbenzoic acid (74.6 mg) was added. After 20 min, the reaction mixture was evaporated to dryness *in vacuo*, redissolved in 3 ml of benzene, and loaded on a silica gel column (0.7×3 cm, benzene suspension). The column was washed with ethyl acetate, then S-methyl-SQ-14225 sulfoxide was eluted with methanol. The methanol eluate was evaporated to dryness, redissolved in 2 ml of ethanol, loaded on a Sephadex LH-20 column (0.7×20 cm, ethanol suspension) and eluted with ethanol (2 ml/fraction). S-Methyl-SQ-14225 sulfoxide was obtained as a colorless oil (50 mg). Mass spectrum of the methyl ester: *m/e* 261 (M^+). NMR: 1.2 (3H, d, $-CH_3$ in $-SOCH_2CHCH_3CO$), 2.0—2.2 (4H, m, $-CH_2$ in

proline), 2.7 (3H, s, $-OSCH_3$), 2.8—3.3 (3H, m, -CH and $-CH_2$ in $CH_3SOCH_2CHCH_3CO$), 3.7 (2H, m, $-CH_2$ in proline) and 4.5 (1H, m, -CH in proline).

Radioactive Mixed Disulfides of SQ-14225——To compare them chromatographically with the urinary metabolites, the radioactive mixed disulfides of SQ-14225 with glutathione, L-cysteine and N-acetyl-L-cysteine were prepared by principally the same method as Cys-SQ-14225. Each endogenous sulfhydryl compound (20 mg) was mixed with SQ-14225-^{14}C (20 μg, 0.3 μCi). A saturated solution of iodine in methanol was added to the mixture until a yellow tinge persisted. The precipitated L-cystine was removed by centrifugation. No precipitate was observed in the case of glutathione or N-acetyl-L-cysteine. The supernatant was stocked in a deep freezer (−17°) as an authentic mixed disulfide for TLC.

Treatment of the Urine with Dithiothreitol (DTT)——To determine whether the urine contains mixed disulfides of SQ-14225 with endogenous sulfhydryl compounds or not, the urine was effectively reduced with 10 mM dithiothreitol (DTT). Urine samples obtained after oral administration of SQ-14225-^{14}C to dogs (0.5 ml) were treated with 0.5 ml of 20 mM DTT (final 10 mM) and the mixture was incubated for 30 min at 37°. Next, 25 mg of NEM (acetone solution) was added and the solution was analyzed by TLC with the solvent system; benzene–acetic acid (3: 1).

Incorporation of Radioactivity into Dermal Collagen after the Administration of Proline-^{3}H and SQ-14225-proline-^{3}H——To determine whether the metabolic cleavage of the amide bond of SQ-14225 occurred or not, the radioactive uptake into the dermal collagen was compared from SQ-14225-proline-^{3}H and proline-^{3}H. Four male rats of the Sprague-Dawley strain weighing 70 g were given SQ-14225-^{3}H orally for 3 days (53 μCi/body/day, total 159 μCi). Proline-^{3}H was administered once to five rats intraperitoneally (116 μCi/body). Eleven days after the first administration of the drugs, the rats were depilated then exsanguinated. The collagen fractions were obtained according to Tsurufuji's method.[7] The dermal tissues were excised, and the fat and vessels were remaved. The tissues were minced with scissors and suspended in 100 ml of cold water to extract water-soluble components. The extraction was carried out for three days changing the water every day. The dermal tissues were resuspended in 50 ml of water and autoclaved at 120° for 9 hr to obtain a colloidal solution of collagen. The collagen solutions were filtered and the volume was adjusted to 50 ml. The radioactivity of an aliquot of the collagen solution (0.5 ml) was measured in a Packard model 3380 liquid scintillation spectrophotometer after the addition of toluene–ethanol scintillator (10 ml).

Results

Identification of Mixed Disulfides

The TLC-autoradiograms of rat and dog urine are represented schematically in Fig. 2. The unchanged SQ-14225 (NEM adduct) is the major spot, followed by the spot of the disulfide of SQ-14225 itself (SQ-14551). In both animal species, two metabolites were observed as adjacent spots near the origin (M-I and M-II). A fairly significant spot was observed at *Rf* 0.09 in the rat urine (M-III). M-III was negligible in the dog, however, so that there is some species difference. In both animal species, small amount of radioactivity were detected at the highest *Rf* (0.6) corresponding to free SQ-14225 and/or S-methyl-SQ-14225, which cannot be separated with the solvent system: benzene–acetic acid (3: 1). This spot was tentatively designated as M-IV. To examine whether these unknown metabolites are mixed disulfides or not, the dog urine was treated with dithiothreitol (DTT). The urine samples which contained a large amount of the metabolites around the origin (M-I and M-II) were selected. As shown in Table I, the spots of M-I and M-II disappeared after

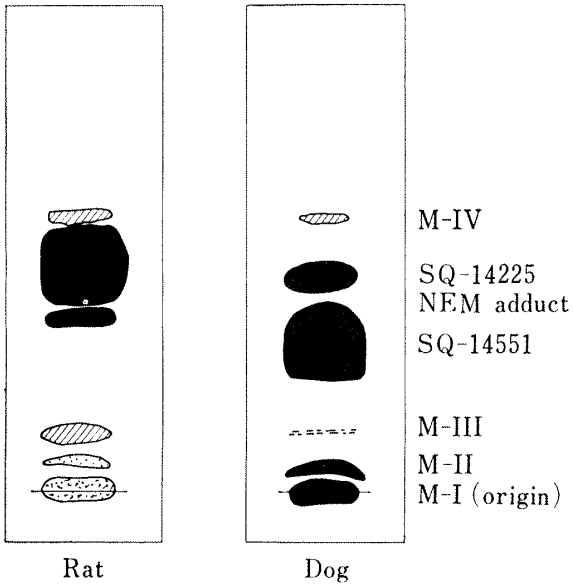


Fig. 2. Schematic Representation of TLC-Autoradiograms of Urinary Metabolites (0—24 hr) after Oral Administration of SQ-14225-^{14}C to Rats and Dogs

Table I. Effect of Dithiotheritol (DTT) on the Urinary Metabolites after Oral Administration of SQ-14225-^{14}C to Doges (25 mg/kg)

	% to the radioactivity (0—24 hr urine)			
	Dog A		Dog B	
	Control	DTT-treated	Control	DTT-treated
Origin (M-I and M-II)	33.60	3.28	23.79	5.09
M-III	Trace	Trace	Trace	Trace
SQ-14551	45.63	17.59	54.54	23.41
SQ-14225 (NEM adduct)	14.13	71.96	15.78	66.33
M-IV	5.85	5.65	5.73	4.86

DTT treatment, with a concomitant increase of SQ-14225 (NEM adduct), demonstrating that M-I and M-II are mixed disulfides. M-IV was not affected by the DTT treatment. Because of the low content of M-III in the dog urine, M-III was extracted and concentrated by preparative TLC from the rat urine and treated with DTT. The results showed that M-III is not a mixed disulfide. Thus, M-I and M-II, the mixed disulfides, were compared with authentic radioactive mixed disulfides by silica gel TLC. In five solvent systems, M-I was separated into one major component and a minor component. The former coincided with the mixed disulfide with cysteine (Cys–SQ-14225) and the latter was identified as the mixed disulfide with glutathione (GS–SQ-14225). M-II was separated into two adjacent spots, which were difficult to isolate. The spot of higher *Rf* value coincided with the mixed disulfide of SQ-14225 with N-acetyl-cysteine on TLC using five solvent systems. The identity of the other spot in M-II is unknown. These results show that SQ-14225 was excreted into the urine partly as mixed disulfides in which Cys–SQ-14225 was the major metabolite, followed by N-acetyl-Cys–SQ-14225 and a negligible amount of GS–SQ-14225.

Identification of S-Methyl-SQ-14225 and Its Sulfoxide

In order to determine whether M-IV has a free sulfhydryl group or not, M-IV was partially purified by preparative TLC and reacted with excess NEM (25 mg/ml), because M-IV has the same *Rf* value as free SQ-14225 and might be SQ-14225 which had not reacted with NEM. M-IV was shown to be inert to NEM treatment, without any formation of SQ-14225 NEM adduct. The inertness of M-IV to oxidation with iodine was also demonstrated in the presence of an excess of non-radioactive SQ-14225. In the iodine oxidation experiment, a non-radioactive spot of SQ-14551 was detected under UV light, while the radioactive spot of M-IV remained at the original position. Thus, M-IV was shown to have no sulfhydryl group. M-IV coincided with an authentic sample of S-methyl-SQ-14225 on TLC using five solvent systems and thus identified as S-methyl-SQ-14225.

As for the structure of M-III, it was not a mixed disulfide, and its *Rf* value (0.09) suggested that it is a polar metabolite. In the experiment to concentrate S-methyl-SQ-14225 from the dog urine by preparative TLC, a small amount of a new radioactive spot which coincided with M-III was recognized when the plates were exposed to the air for several days after development. This suggested that M-III is an oxidized S-methyl-SQ-14225. M-III was identified as S-methyl-SQ-14225 sulfoxide by comparison with the authentic compound in TLC using five different solvent systems.

Comparison of the Radioactive Uptake into the Collagen Fraction from Proline-^{3}H and SQ-14225-^{3}H

It is known that proline is well taken up into dermal collagen.[7] Thus, in order to examine whether the amide bond of SQ-14225 is cleaved or not, the radioactivity in the dermal collagen fraction was compared after the administration of equal radioactivity of proline-^{3}H or SQ-14225-^{3}H to rats. As shown in Table II, considerable radioactivity (5×10^{5} dpm/g) was

Table II. Incorporation of the Radioactivity into the Dermal Collagen Fraction of Rats after Administration of SQ-14225-^{3}H and Proline-^{3}H

	Radioactivity of dermal collagen fraction dpm/g-skin	
SQ-14225-^{3}H (*p.o.*)	796±135	$n=4$
Proline -^{3}H (*i.p.*)	492167±79174	$n=5$

Table III. *Rf* Values of SQ-14225 and Its Metabolites

Solvent system	Compounds							
	S-Methyl-SQ-14225	Free SQ-14225	SQ-14225 NEM adduct	SQ-14551	S-Methyl-SQ-14225 sulfoxide	N-Acetyl-Cys-SQ-14225	Cys-SQ-14225	GS-SQ-14225
Benzene–acetic acid (3 : 1)	0.46	0.46	0.37	0.25	0.09	0.05	0.00	0.00
n-Butanol–acetic acid–water (4 : 1 : 1)	0.75	0.74	0.68	0.62	0.37	0.45	0.36	0.15
Isopropanol–water (7 : 3)	0.78	0.78	0.78	0.73	0.54	0.72	0.66	0.54
Ethanol–28% Aq. ammonia (7 : 3)	0.83	0.74	0.84	0.77	0.65	0.75	0.66	0.53
Chloroform–methanol–14% aq. ammonia (2 : 2 : 1)	0.92	0.91	0.92	0.91	0.83	0.88	0.86	0.83

observed after the administration of proline, demonstrating that this is a sensitive method to detect the release of free proline. On the other hand, the radioactivity in the collagen was almost negligible (8×10^2 dpm/g) after the administration of SQ-14225, showing that no significant hydrolysis had occurred.

Quantification of the Urinary Metabolites after Oral Administration of SQ-14225-^{14}C to Rats and Dogs

In Table III, the *Rf* values of SQ-14225 and its metabolites in five developing solvents are listed. SQ-14225 (NEM adduct), SQ-14551, S-methyl-SQ-14225, S-methyl-SQ-14225

Table IV. Urinary Metabolites after Oral Administration of SQ-14225-^{14}C to Rats and Dogs

	% of the urinary metabolites			
	Rats ($n=3$, 5 mg/kg)		Dogs ($n=4$, 25 mg/kg)	
	% to radioactivity	% to dose	% to radioactivity	% to dose
S–Methyl–SQ-14225[a)]	2.72±0.56	1.78±0.35	3.89±0.62	2.75±0.40
SQ-14225 NEM adduct[a)]	72.19±3.17	47.39±4.09	38.65±8.65	28.08±7.06
SQ-14551[a)]	10.97±2.04	7.10±1.09	35.62±6.96	25.12±4.54
S–Methyl–SQ-14225 sulfoxide[a)]	5.00±0.51	3.25±0.23	1.76±0.31	1.24±0.21
N–AcetylCys–SQ-14225[a,c)]	3.63±0.48	2.35±0.21	3.75±0.86	2.66±0.59
Cys–SQ-14225[b)]	2.12±0.21	1.38±0.10	10.12±2.40	7.28±1.79
GS–SQ-14225[b)]	2.19±0.22	1.42±0.09	0.95±0.16	0.66±0.10

a) Solvent system A: benzene–acetic acid (3: 1).
b) Solvent system B: *n*-butanol–acetic acid–water (4: 1: 1).
c) Contains unknown mixed disulfide.

sulfoxide and N-acetyl-Cys–SQ-14225 were separated well in solvent system A: benzene–acetic acid (3: 1). In solvent A, however, the mixed disulfides remained at the origin, except for N-acetyl-Cys–SQ-14225, and had to be quantified in other solvent. Solvent system B [*n*-butanol–acetic acid–water (4: 1: 1)] gave good separation between the mixed disulfides, although the spots of Cys–SQ-14225 and S-methyl-SQ-14225 sulfoxide were too close to be evaluated separately. Thus, SQ-14225 (NEM adduct), SQ-14551, S-methyl-SQ-14225, S-methyl-SQ-14225 sulfoxide and N-acetyl-Cys–SQ-14225 in the urine were determined with solvent system A and Cys–SQ-14225 and GS–SQ-14225 were determined with solvent system B. The quantity of Cys–SQ-14225 was obtained accurately by subtracting the amount of S-methyl-SQ-14225 sulfoxide in solvent A from that of Cys–SQ-14225 in solvent B. Table IV lists the percentages of the urinary metabolites obtained after oral administration of SQ-14225-^{14}C to rats and dogs.

Discussion

SQ-14225 has two significant structural characteristics: a free sulfhydryl group and an amide bond with the endogenous amino acid, L-proline, in the molecule. When SQ-14225-^{14}C was administered to rats and dogs, several radioactive metabolites as well as unchanged SQ-14225 were excreted into the urine. The chemical structures of the following metabolites were determined by comparing them chromatographically with authentic standards; S-methyl-SQ-14225, S-methyl-SQ-14225 sulfoxide, GS–SQ-14225, Cys–SQ-14225, N-acetyl-Cys–SQ-14225 and SQ-14551. Hydrolysis of the amide bond was revealed to be negligible by the findings in the present experiments that almost all the urinary radioactivity (>95%) was accounted for by metabolites retaining the amide bond, and practically no radioactive uptake was observed into the dermal collagen fraction after the administration of SQ-14225 labeled with ^{3}H in the proline moiety. SQ-14225 and SQ-14551, the symmetrical disulfide of SQ-14225, were the major constituents in the urine and together they accounted for 83% and 73% of the urinary radioactivity in rats and dogs, respectively. Minor species difference, however, was observed between rats and dogs. In the dog urine, the amounts of SQ-14225 and SQ-14551 were roughly equal, while in the rat, the urinary radioactivity level of unchanged SQ-14225 was 72% but that of SQ-14551 was only 10%. The rats appear to have a higher reducing ability for the disulfide bond than the dogs. In addition, the levels of mixed disulfides of SQ-14225 with endogenous sulfhydryl compounds (Cys–SQ-14225, N–Acetyl–Cys–SQ–14225) were significant in the dog urine, whereas S-methyl-SQ-14225 sulfoxide was a very minor component. On the other hand, the amount of S-methyl-SQ-14225 sulfoxide was greater than that of the mixed disulfides in the rat urine. The difference in the amount of S-methyl-SQ-14225 sulfoxide between the two animal species might be explained by a higher activity of the mixed function oxidase system in the rat than in the dog.[8]

Overall, these results indicate that the main metabolic pathway of SQ-14225 *in vivo* is the modification of the sulfhydryl group. The precise mechanism of SQ-14225 metabolism is now under investigation and will be reported elsewhere.

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