Relationship between Metabolism and Radioprotective Activity of 2-Phenylthiazolidine and Its *m*-Bromo Derivative

J. P. Fernandez,† Y. Robbe,† J. P. Chapat,*,† J. L. Chanal,‡ M. Genin,‡ H. Sentenac-Roumanou,§ and M. Fatome⊥

Laboratoire de Chimie Organique et Laboratoire de Physique, Faculté de Pharmacie, 34060 Montpellier-Cédex, Direction des Recherches, Etudes et Techniques, 75996 Paris-Armées, Division de Radiobiologie et Radioprotection, Centre de Recherche du Service de Santé des Armées 92141 Clamart, France. *Received July 19, 1982*

Molecular biotransformation of 2-phenylthiazolidine (1) and its *m*-bromo derivative (2) in the mouse is followed by autoradiographic studies and assessed by analysis of urinary metabolites. Cysteamine (4) is one of the metabolites of compounds 1 and 2. Radioprotective activity and efficacy over a period of time of 1, 2, and 4 correlate closely with distribution and metabolism.

We have previously reported[1,2] several highly effective thiazolidines as antiradiation agents. This activity, which has also been recognized by other authors,[3-5] may result either from the heterocyclic structure itself or from its metabolites, notably cysteamine or its derivatives. These may be liberated in vivo by hydrolysis (Scheme I), and they have a well-established radioprotective activity.[6-8] Although the studies performed in vitro indicate a radioprotective effect intrinsic to thiazolidines,[9-11] it appears necessary to examine their in vivo behavior under experimental conditions similar to those used for the determination of radioprotective effects in the mouse.

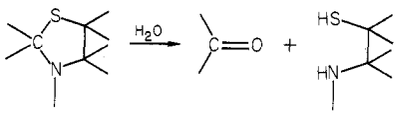
In order to investigate a possible relationship between radioprotective activity and the fate of thiazolidines in the animal, two compounds with very different radioprotective effects were selected: 2-phenylthiazolidine, which at 500 mg/kg gives 100% protection for up to 30 days in mice, after intraperitoneal administration 15 min before LD_{99} irradiation, and 2-(3-bromophenyl)thiazolidine, which at the same dosage gives only 20% protection under the same conditions. The two thiazolidines that were used incorporated a labeled isotope in the heterocyclic ring. The phenyl derivative was labeled at position 2 ([^{14}C]**1a**) or at position 1 [(^{35}S]**1b**), and the 3-bromophenyl derivative was labeled solely at position 1 ([^{35}S]**2**).

Molecular biotransformation of these two thiazolidines and of their primary putative metabolites (benzaldehyde, 3-bromobenzaldehyde, and cysteamine) was followed by autoradiographic studies of mouse sagittal sections. It was also monitored by assesment of urinary and fecal levels, along with analysis of urinary metabolites. All the labeled compounds were administered by the intraperitoneal route at the dosages indicated in Figure 1.

Results and Discussion

Autoradiographs of Thiazolidines (1a, 1b, and 2) and Their Putative Metabolites (3 and 4). The materials included in Table I (see paragraph at end of paper regarding supplementary material) show, at first a very rapid diffusion of 2-phenylthiazolidine and/or its metabolites [general impregnation by 2-phenyl[2-^{14}C]thiazolidine (**1a**) and 2-phenyl[^{35}S]thiazolidine (**1b**) after 15 min]. The differences in autoradiographic images for **1a** and **1b** at time intervals longer than 2 h indicate an at least partial hydrolysis of 2-phenylthiazolidine. Also, a very close correlation is revealed at 24 h between the autoradiographs resulting from administration of **1a** and those resulting from administration of [α-^{14}C]benzaldehyde (**3**) (putative metabolite); a similar result is observed from 2 to 24 h with **1b** and [^{35}S]cysteamine (**4**) (second putative metabolite).

Scheme I



There appears then a difference in elimination rates of metabolites labeled with ^{14}C (significant levels of radioactivity in kidneys and bladder 15 min after administration and almost total disappearence after 24 h) in comparison with the metabolites labeled with ^{35}S (general distribution with significant persistence after 24 h). Injection of 2-(3-bromophenyl)[^{35}S]thiazolidine (**2**) induces a considerably slower and more prolonged distribution, requiring at least 2 h to attain impregnation comparable to that observed at 15 min with **1b** and maintained for longer than 24 h.

Rates of Excretion of Urinary and Fecal Metabolites (Figure 1 and Table II). The observations relevant to 2-phenylthiazolidine in the autoradiographic series were confirmed. Figure 1 shows a more rapid and greater urinary excretion of the labeled metabolites from **1a** than **1b**. After 6 h, 41% of the former had been eliminated, compared with 21% of the latter, while after 30 h, 67% of the ^{14}C-label was recovered in the urine compared with 29% of the ^{35}S-labeled substances.

Comparison of the percentages of labeled metabolites excreted in the urine after administration of **3**, **4**, or 2-phenylthiazolidine (**1a,b**) indicates that hydrolysis is progressive and, apparently, only partial after 24 h because of a more rapid elimination in the first two cases. This implies the possibility of different locations for metabolites from **1a**, **1b**, **3**, and **4** and serves to explain the significant differences of urinary levels and their ratios (see Table II).

† Laboratoire de Chimie Organique, Faculté de Pharmacie.
‡ Laboratoire de Physique, Faculté de Pharmacie.
§ Direction des Recherches, Etudes et Techniques.
⊥ Division de Radiobiologie et Radioprotection.

(1) Fatome, M.; Poutrain, P.; Granger, R.; Orzalesi, H.; Robbe, Y.; Randon, M. *Chim. Ther.* **1970**, *5*, 812.
(2) Terol, A.; Fernandez, J. P.; Robbe, Y.; Chapat, J. P.; Granger, R.; Fatome, M.; Andrieu, L.; Sentenac-Roumanou, H. *Eur. J. Med. Chem.* **1978**, *13*, 149.
(3) Kaluszyner, A.; Czerniak, P.; Bergmann, E. D. *Radiat. Res.* **1961**, *14*, 23.
(4) Westland, R. D.; Lin, M. H.; Cooley, R. A., Jr.; Zwiesler, M. L.; Grenam, M. M. *J. Med. Chem.* **1973**, *16*, 328.
(5) Farmer, P. S.; Leung, C. C.; Lui, E. M. K. *J. Med. Chem.* **1973**, *16*, 411.
(6) Bacq, Z. M.; Hervé, A.; Fischer, P.; Lecomte, J.; Blavier, J.; Deschamps, G.; Le Bihan, H.; Rayet, P. *Arch. Int. Physiol.* **1951**, *59*, 442.
(7) Doherty, D. G.; Burnett, W. T., Jr.; Shapira, R. *Radiat. Res.* **1957**, *7*, 13.
(8) Bekkum, D. W.; Nieuwerkerk, H. T. M. *Int. J. Radiat. Biol.* **1963**, *7*, 473; *Chem. Abstr.* **1964**, *61*, 7325.
(9) Tondeur, R.; Sion, R.; Deray, E. *Bull. Soc. Chim. Fr.* **1964**, *10*, 2493.
(10) Granger, R.; Orzalesi, H.; Robbe, Y.; Chapat, J. P.; Terol, A.; Bitoun, J.; Simon, F. *Chim. Ther.* **1971**, *6*, 439.
(11) Vos, O.; Budke, L.; Fatome, M.; Van Hoo; Bonk, C. *Int. J. Radiat. Biol. Relat. Stud. Phys., Chim. Med.* **1981**, *39*, 291.

0022-2623/83/1826-1317$01.50/0

Table II. Urinary and Fecal Levels (Percent of Substances Excreted per Injected Dose[a])

	urine			
no.	0-6 h	0-24 h	24-30 h	feces: 0-30 h
1a	41.2 ± 3.7 (4)	62.7 ± 1.6 (5)[d]	4.0 ± 0.3 (5)	0.42 ± 0.05 (5)
1b	21.3 ± 1.6 (4)	26.4 ± 2.0 (4)[d]	3.0 ± 0.5 (4)	0.60 ± 0.08 (4)
2	8.9 ± 1.0 (4)	16.4 ± 0.5 (4)	4.0 ± 0.1 (4)	0.43 ± 0.11 (4)
3	52.6 ± 2.7 (4)	84.2 ± 2.1 (4)[b,d]	5.0 ± 0.4 (4)	0.43 ± 0.15 (4)
4	42.6 ± 2.0 (4)	55.7 ± 1.7 (5)[c,d]	4.1 ± 0.3 (5)	0.92 ± 0.07 (5)

[a] The injected dose is shown in Figure 1. Each value represents the mean of the number of experiments in parentheses plus or minus the standard error. [b] Significantly different from **1a** (0-24 h) ($p < 0.05$) as determined by the Mann Whitney-Wilcoxon test. [c] Significantly different from **1b** (0-24 h) ($p < 0.05$) as determined by the Mann Whitney-Wilcoxon test. [d] The ratio of the urinary excretion of ^{35}S-labeled metabolites differs significantly from that of the ^{14}C-labeled metabolites. For each ratio, the confidence limits were calculated ($p = 0.05$): $0.691 < C_{1a,3} < 0.799$; $0.395 < C_{1b,4} < 0.551$.

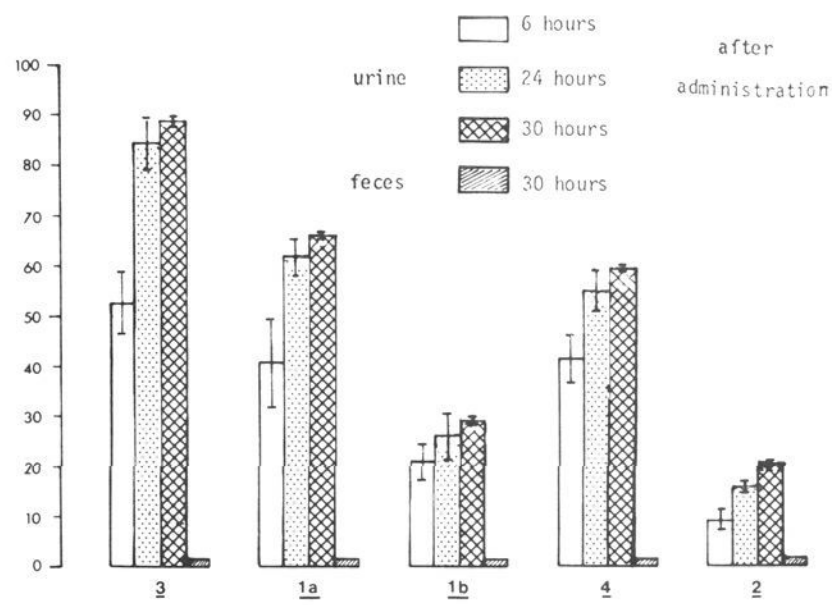


Figure 1. Urinary and fecal levels after administration of 2-phenylthiazolidine (**1a,b**; 500 mg/kg), 2-(3-bromophenyl)thiazolidine (**2**; 500 mg/kg), benzaldehyde (**3**; 250 mg/kg), and cysteamine (**4**; 175 mg/kg) to mice. Error margin denotes a 95% confidence interval for each dosage level.

In the case of **2**, urinary elimination remains slight (16%), in agreement with the considerable degree of retention demonstrated by means of autoradiography. Finally, it should be noted that fecal elimination of the derivatives investigated was almost negligible.

Urinary Metabolites in a 24-h Urine Sample of Compounds 1a,b and 2–4 (Analyzed by TLC Radiography) (Figure 2). Several radioactive metabolites were identified. After administration of **1a**, [α-^{14}C]benzoic acid was detected; hippuric acid was detected in more significant quantities, and only a very slight amount of **1a** (0.2%) (not shown in Figure 2) was detected. The first two metabolites probably originate in the benzaldehyde, which results from hydrolysis in vivo. However, administration of an equivalent quantity of **3** (250 mg/kg) is accompanied by the elimination of benzoic acid only, without hippuric acid. This difference in behavior may occur because in vivo benzoic acid formation takes place slowly enough to allow its conversion to hippuric acid.

With **1b** administration, chromatographic analysis indicates the presence of [^{35}S]cysteamine, [^{35}S]cystamine, [^{35}S]taurine, and [^{35}S]hypotaurine, as well as other unidentified radioactive substances, which are probably oxidation products. As before, only very slight quantities of 2-phenyl[^{35}S]thiazolidine are revealed. By comparison, cysteamine yields the same metabolites but in a slightly larger quantity. The different metabolites display a composition identical with that previously described.[12-14] In addition, *N*-acetyl[^{35}S]taurine is identified following [^{35}S]cysteamine administration. This behavior agrees with

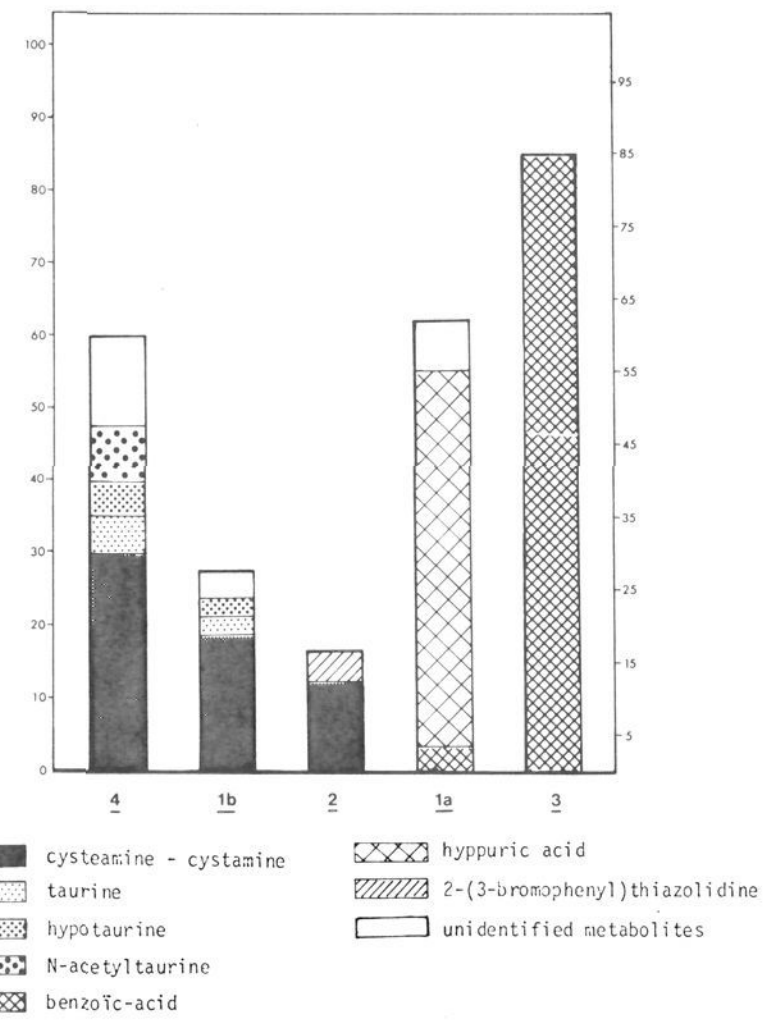


Figure 2. Percentage of different urinary metabolites from a 24-h urine sample. The drug dosage (in milligrams per kilogram) is mentioned in Figure 1.

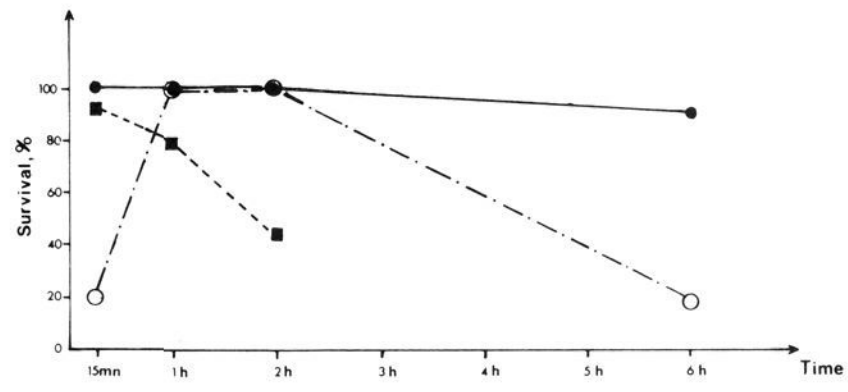


Figure 3. Radioprotective effects in mice of 2-phenylthiazolidine (●), 2-(3-bromophenyl)thiazolidine (○), and cysteamine (■) for time intervals ranging from 15 min to 6 between administration and irradiation.

(12) Eldjarn, L.; Nygaard, O. *Arch. Int. Phys.* **1954**, *67*, 476.
(13) Verly, W. G.; Bacq, Z. M.; Rayet, P.; Urbain, M. F. *Biochim. Biophys. Acta* **1954**, *13*, 233.
(14) Salvador, R. A.; Davison, C.; Smith, P. K. *J. Pharmacol. Expt. Ther.* **1957**, *121*, 258.
(15) Ulberg, S. *Acta Radiol., Suppl.* **1954**, *no. 18*, 1.
(16) Nelson, A.; Ulberg, S. *Acta Radiol.* **1960**, *53*, 305.

Table III. Radioprotective Activity of Phenylthiazolidines and Cysteamine[a]

no.	LD_{50}, mg/kg	drug dose,[b] mg/kg	% survival[c] at the following time intervals between administration and irradiation: 15 min	1 h	2 h	6 h
4	450	225	95	80	45	
1	1000	500	100	100	100	85
2	1000	500	20	100	100	20

[a] Carried out at the Centre de Recherche du Service de Santé des Armées (see Frossard, H.; Perles, R.; Fatome, M.; Holleville, P. *Radioprotection* 1971, *6*, 119). Radiation dosage was 883 rd from a ^{60}Co irradiator given at a rate of 50 R/min. Twenty mice were used at each dosage level. [b] Compounds were administered by the intraperitoneal route in water (**4**·HCl) or in miglyol 812 (1 and **2**). [c] Calculated from the number of mice surviving 30 days after irradiation. No 30-day survival among control mice was noted.

the difference in location, already mentioned.

In the case of **2**, we observed the elimination of a nonnegligible quantity of an unmetabolized compound, associated with slight amounts of metabolites (cysteamine plus cystamine); this results from a slower rate of hydrolysis of 2-(3-bromophenyl)thiazolidine than that of 2-phenylthiazolidine.

Relationship between Metabolism and Activity. Taking account of behavior differences in the compounds studied [2-phenylthiazolidine (**1**), 2-(3-bromophenyl)thiazolidine (**2**), and cysteamine (**4**)], the variations in their radioprotective effects were examined as a function of time (Table III). Figure 3 shows the results of this study for time intervals ranging from 15 min to 6 h between administration and irradiation. Cysteamine is characterized by an immediate but transient action, probably due to its rate of distribution and excretion. With 2-phenylthiazolidine, an immediate and prolonged radioprotective activity is the result of rapid diffusion and gradual liberation of cysteamine. The weak protective activity of **2** against irradiation 15 min after administration is related to inadequate diffusion and/or to the slow liberation of cysteamine. The maximum effect obtained between 1 and 2 h after administration probably results from an adequate quantity of free cysteamine, possibly associated with the specific activity of the 2-(3-bromophenyl)thiazolidine, which by then has adequately diffused. The loss of activity observed during the time interval from 2 to 6 h is probably due to a decline in the release of cysteamine from urinary excretion of nonhydrolyzed **2**. On the assumption that the bioavailability of **4** is identical with **1** and **2**, it appears that for identical doses administered (500 mg), the amount of cysteamine available for release by hydrolysis of 2-phenylthiazolidine (233 mg) is greater than that of its bromo derivative (150 mg).

It therefore appears that the radioprotective action of the thiazolidines tested must result from the cysteamine released by hydrolysis in vivo, but this does not necessarily preclude the possibility of a specific radioprotective action due to the heterocyclic structure.

Conclusion

The study of the metabolism of two phenylthiazolidines with different radioprotective activities indicates that the heterocyclic structure plays the role of vector vis a vis cysteamine. The radioprotective activity of these compounds is linked to their rates of diffusion, hydrolysis, and retention. The relative occurrence of these three factors still awaits specific elucidation. Moreover, a radioprotective action inherent in the heterocyclic structure cannot be ruled out.

The differences concerning the in vivo behavior of these compounds should permit control of the duration of their radioprotective effect.

Experimental Section

Radiochemical Compounds. The labeled thiazolidines **1a,b** and **2** were prepared starting from [α-^{14}C]benzaldehyde (**3**) or [^{35}S]cysteamine (**4**) (Radiochemical Centre, Amersham). Radiochemical purity was obtained by TLC analysis on a silica plate (Merck F 254; eluant: chloroform–methanol–acetic acid, 50:50:2). The relative proportion of labeled compound was determined after elution, air-drying of the plate, development with radiographic Kodak Kodirex film (exposure time 3 days), by scraping off the spots and the absorbent phase, which are then subjected to liquid scintillation counting (Packard Tricarb 3320). Compounds **1a,b** and **2** had specific activities of 192, 504, and 393 μCi/mmol, respectively, and showed radiochemical purity greater than 98%.

Autoradiographs. All labeled compounds were administered in Miglyol 812 (0.4 mL per animal) by the intraperitoneal route (500 mg/kg of compounds **1a,b** and **2**, 250 mg/kg of compound **3**, and 175 mg/kg of compound **4**). Pairs of mice were simultaneously sacrificed by immersion in liquid nitrogen at 15 min, 2 h, 4 h, 6 h, and 24 h after administration of the compounds. The animals were then kept for 1 week at −30 °C, and sagittal sections were then made (Zeiss low-temperature microtome). These were then positioned on Kodak Kodirex film for periods of 3 to 6 weeks after labeling.

Urinary and Fecal Levels. After administration of the labeled compounds (see above) to the mice, they were placed in separate metabolic cages and fed normally. Urine samples were taken at 6, 24 and 30 h and then subjected to liquid scintillation counting. The quantity of radioactivity present in feces was determined according to the same principle but after pounding.

Analysis of Urinary Metabolites. The urine samples collected during a 24-h period were subjected to analysis by TLC on a silica plate (Merck F 254; eluant: chloroform–methanol–acetic acid, 50:50:2). The number of labeled metabolites and their relative proportions were determined (see above) with radiographic Kodak Kodirex film (exposure time 1 week). Some metabolites were identified by mass spectrometry (LKB 2091 instrument). They were obtained after chromatographic separation on a plate by scraping off the adsorbent phase (silica) and extracting with absolute ethanol. After evaporation of the solvent, analysis by MS (70 eV) was carried out. The following metabolites were identified in this way: cysteamine m/e 0.24 ($M^{+\cdot}$, 77), 0.50 (M − 33), 0.22 (M − 34), 1.00 (M − 47); hypotaurine, m/e 0.21 ($M^{+\cdot}$, 109), 0.48 (M − 30), 0.84 (M − 65), 0.54 (M − 66), 1.00 (M − 79); *N*-acetyltaurine, m/e 0.19 (M^{+}; 167), 0.11 (M − 15), 0.22 (M − 81), 0.69 (M − 107), 1.00 (M − 124); benzoic acid, m/e 0.81 (M^{+}; 122), 1.00 (M − 17), 0.70 (M − 45), 0.49 (M − 51).

In addition, the presence of cysteamine associated with cystamine, taurine, acetyltaurine, hippuric acid, and also benzoic acid was demonstrated by TLC and comparison with controls.

Acknowledgment. The authors are grateful for Professor R. Granger for his continued interest and for his stimulating discussion in this work. We also thank G. Subra for technical assistance. Support by a DRET grant is acknowledged.

Registry No. **1**, 4569-82-8; **1a**, 86146-91-0; **1b**, 86146-92-1; **2**, 67086-84-4; **3**, 100-52-7; **4**, 60-23-1; cystamine, 51-85-4; taurine, 107-35-7; hypotaurine, 300-84-5; *N*-acetyltaurine, 19213-70-8; benzoic acid, 65-85-0; hippuric acid, 495-69-2.

Supplementary Material Available: Table I containing autoradiographs of mouse sagittal sections after administration of 2-phenylthiazolidine (**1a,b**), 3-(2-bromophenyl)thiazolidine (**2**), benzaldehyde (**3**), and cysteamine (**4**) (1 page). Ordering information is given on any current masthead page.

(17) Miglyol 812: neutral oil, mixture of triglycerides, from Dyna France S.A., Paris.