RONIDAZOLE

IDENTITY

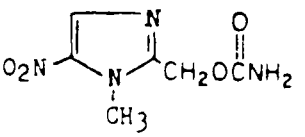
Chemical name: 1-methyl-5-nitroimidazole-2-methanol carbamate (ester)

carbamic acid (1-methyl-5-nitro-imidazol-2-yl)methyl ester

1-methyl-2-[(carbamoyloxy)methyl]-5-nitroimidazole
(1-methyl-5-nitroimidazole-2-yl)-methyl carbamate

Synonyms: Ridzol
Dugro
MCMN

Structural formula:



Molecular formula: $C_6H_8N_4O_4$

Molecular weight: 200.16

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: pale yellow crystals

Melting point: 167-169°C

(Windholz, 1983)

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Ronidazole is used in turkeys for the prevention and treatment of histomoniasis. The drug is also used in swine for the prevention and treatment of swine dysentery.

Dosages

Ronidazole is administered to swine at 0.006-0.008% in the feed for prevention and at 0.012% in the feed or 0.006% in the drinking water for 3 to 5 days for treatment.

The drug is administered to turkeys at 0.006-0.009% in the feed for prevention and at 0.012% in the feed or 0.004-0.006% in the drinking water for 7-14 days for treatment.

RADIOLABELED RESIDUE DEPLETION STUDIES

Turkeys

Three-week-old turkey poults were fed diets containing 0.006% 14C-ronidazole for four days. The radioactive forms of the drug used in the experiment had label in the N-methyl group or in the 2-position of the ring. The concentration of total residue of ronidazole was measured at various withdrawal times. The results are given in Table I. Beyond 21 days the levels of radioactivity in tissues of treated animals were indistinguishable from those in tissues of control animals. The residue depletion of ronidazole was not significantly different when birds were dosed with one radioactive form of ronidazole or the other. (Rosenblum, et al., 1972)

Table I. Concentration of Total Residue in Tissues of Turkeys Treated With 0.006% Ronidazole in the Diet (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	3.0	4.5	4.7	-
2	0.28	0.5	0.73	0.37
5	0.09	0.18	0.4	-
10	0.26	0.05	0.14	-
14	0.03	0	0.07	-
21	0.04	0	0	-

In a second experiment turkeys were maintained on a diet containing 0.006% 14C-ronidazole (labeled in the N-methyl or the 2-position of the ring) and sacrificed at various withdrawal times. Tissues from the birds were analyzed for total residue, as well as parent drug and its metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole, HMMNI). Ronidazole and the metabolite were determined with tlc and electrophoretic methods. The results are shown in Table II. The results of this study are similar to those described above both in terms of (1) the total residue values determined at the various withdrawal times, and (2) the comparability of the data obtained with each labeled form of ronidazole. (Rosenblum, et al., 1972) (Rosenblum, 1977)

Table II. Concentration of Total Residue, Ronidazole, and HMMNI in Turkeys Treated at 0.006% in the Feed (ppm)

Treatment	Tissue	Total Residue	Ronidazole	HMMNI
3 days on drug	Kidney	4.00	<0.03	0.0
N-methyl-14C	Liver	4.15	<0.02	0.0
0-withdrawal	Muscle	2.58	1.5	0.1
3 days on drug	Kidney	4.43	0.09	0.0
ring-2-14C	Liver	3.77	0.01	0.0
0-withdrawal	Muscle	2.05	1.6	0.03
3 days on drug	Kidney	0.9	0.0	0.0
ring-2-14C	Liver	0.38	0.0	0.0
2-day withdrawal	Muscle	0.15	0.007	<0.01
3 days on drug	Kidney	0.44	0.0	0.0
ring-2-14C	Liver	0.13	0.0	0.0
5-day withdrawal	Muscle	0.07	0.0	0.0

Swine

Swine weighing 20-30 kg and mixed as to sex were dosed with 14C-ronidazole labeled in the N-methyl group once daily for three days in a feed slurry. The dose was 7 mg/kg, which would be equivalent to a dose obtained from feed containing 0.006% ronidazole. Three animals were sacrificed at 0 (6 hours), 3, 7, 14, 28 and 42 days of withdrawal. Tissue samples taken from the animals were assayed for total radioactivity by combustion analysis. The results of the assays are given in Table III. (Wolf, et al., 1983a)

Table III. Total Residue in Tissues of Swine Treated With 14C-Ronidazole at 7 mg/kg (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	6.32	10.63	9.37	1.46
3	0.49	1.53	1.22	0.30
7	0.52	1.15	0.85	0.25
14	0.35	0.44	0.27	0.15
28	0.18	0.10	0.09	0.06
42	0.13	0.06	0.05	0.05

In another experiment, four barrows weighing approximately 20 kg were dosed with 14C-methyl-labeled ronidazole at 6.7 to 12 mg/kg for three days. The animals were sacrificed at 6 or 72 hours after the last dose and tissue samples were taken for radioassay. The results of the assays are presented in Table IV. (Wolf, et al., 1983a)

Table IV. Total Residue in Tissues of Swine Treated With 14C-Methyl-labeled Ronidazole (ppm)

Animal No. (withdrawal)	Dose mg/kg	Muscle	Liver	Kidney	Fat
19 (6 hours)	6.7	5.0	7.8	7.9	2.5
21 (6 hours)	12	8.6	12.3	11.9	1.3
17 (72 hours)	9.2	0.5	1.6	1.1	0.4
22 (72 hours)	12	1.1	2.4	2.5	0.2

Bioavailability of Swine Muscle Residue in Rat

Muscle tissue, obtained from pigs dosed with 14C-methyl-labeled ronidazole daily for 3 days and sacrificed 7 days after the last feeding, was homogenized with four volumes of water and freeze dried. The freeze-dried muscle was mixed with Purina rat chow at a ratio of 4 parts of muscle tissue (containing radioactivity equivalent to 16 μg of ronidazole) and 5 parts of chow. A control diet was prepared by blending 16 μg of $^{14}CH_3$-ronidazole with freeze-dried muscle from unmedicated pigs. Rats were fed with 18 g of the chow-muscle mixture late in the day for two days. Radioactivity was measured in the whole carcass minus skin and the GI tract.

The overall recovery of radioactive ronidazole was 102.78% for animals dosed with muscle from ronidazole-treated swine and 91.33% for those dosed with muscle spiked with ronidazole. The breakdown for the recovery of the radioactivity is given in Table V. (Wolf, et al., 1984a)

Table V. Recovery of Radioactivity from Rats Dosed with Labeled Ronidazole or Muscle From Swine Treated With Labeled Ronidazole, Expressed as a Percent of Dose

	Substance Dosed	
Sample	Ronidazole	Swine Muscle Residue
urine	44.69	26.39
feces	39.12	25.29
expired gas	3.31	11.20
GI tract	1.97	18.00
carcass	2.24	21.90
Total	91.33	102.78

It is of interest that the carcass and expired air of the muscle-treated rats contain a higher percentage of the radioactivity than do those of rats treated with ronidazole. Furthermore, it was found that 92% of the methylamine-releasing residues (i.e., compounds such as ronidazole or derivatives containing the N-methyl group) in the swine muscle was recovered in rat urine and feces, with <0.5% in the carcass. The sponsor of the drug believes that the results indicate retained radioactivity in tissues reflects more the

incorporation of the label into endogenous substances than the formation of 'drug-related' bound residue. (Wolf, et al., 1984a)

RESIDUE DEPLETION STUDIES

Swine

Two groups of three pigs, mixed as to sex and weighing about 120 lb, were administered 0.012% ronidazole through the drinking water for 7 days. One group was sacrificed with a 1-day withdrawal, the other with a 3-day withdrawal. Samples of muscle, liver, kidney and fat from each treated animal were taken for analysis. A differential pulse polarographic method with a sensitivity of 2 ppb was used to assay for ronidazole. Muscle tissues from animals sacrificed with a 1-day withdrawal were the only samples to show detectable residues of ronidazole, averaging 24 ppb. (Downing, et al., 1973)

Six groups of three pigs, mixed as to sex and weighing about 75 lb, were administered 0.012% ronidazole through the drinking water for 7 days. Groups were sacrificed at 0, 1, 3, 5, 7, or 9 days post dosing. Samples of muscle, liver, kidney and fat from each treated animal were taken for analysis with the differential pulse polarographic method described above. Detectable amounts of ronidazole were obtained on days 0 and 1 of withdrawal only; at all other withdrawal periods no ronidazole was found. The results for 0 and 1 day of withdrawal are summarized in Table VI. (Downing, et al., 1973) (Cala, et al., 1976)

Table VI. Concentrations of Ronidazole in Tissues of Swine Treated at 0.012% Through the Drinking Water (ppb) (ND = no detectable residue)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	3010	ND	14	58
1	80	ND	ND	ND

Six groups of three pigs, mixed as to sex and weighing about 25 lb, were fed a diet containing 0.009% ronidazole for 7 weeks (up until the pigs weighed about 75 lb). Groups were sacrificed at 0, 1, 3, 5, 7 or 9 days of withdrawal. Samples of tissues were assayed for ronidazole with the differential pulse polarographic method. As above, detectable amounts of ronidazole were obtained on days 0 and 1 of withdrawal only. The results are presented in Table VII. (Downing, et al., 1974)

Table VII. Concentrations of Ronidazole in Tissues of Swine Treated at 0.009% in the Feed For 7 Weeks (ppb) (ND = no detectable residue)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	612	ND	16	20
1	152	ND	6	4

Another experiment was conducted in a manner identical to that above, except the swine were fed the medicated diet for 12 weeks (up until the pigs weighed 175 lb). The results for this study are given in Table VIII. (Downing, et al., 1974)

Table VIII. Concentration of Ronidazole in Tissues of Swine Treated at 0.009% in the Feed for 12 Weeks (ppb) (ND = no detectable residue)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	409	ND	1.3	4
1	9	ND	ND	ND

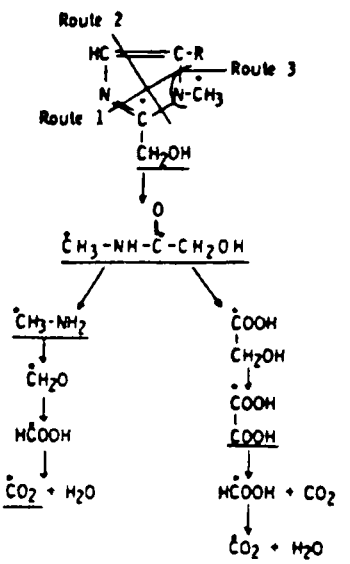
METABOLISM STUDIES

Turkeys

In a study of the metabolism of ronidazole in turkeys, birds were administered 0.006% 14C-ronidazole (labeled in the C-2 position of the ring or in the N-methyl group) through the feed for 3 days. Samples of tissues were analyzed for metabolites using paper electrophoresis and thin layer chromatography. Ronidazole and its metabolite HMMNI were identified in muscle from 0-withdrawal birds only. Glucuronide conjugates of ronidazole and HMMNI in muscle and urine were not detected. Analysis of the aqueous soluble extracts of liver, which contain approximately 80% of the total liver radioactivity, showed the presence of 14C-N-methylglycolamide, 14C-oxalic acid (from birds dosed with 2-14C-ronidazole), and 14C-methylamine. Evidence was also accumulated for the presence of various acids, including fumaric, succinic, glycolic, malic, α-ketoglutaric, and citric, in the extracts of liver from treated birds. Further, radioactivity was seen to be associated with amino acids in pooled liver samples from treated turkeys. The authors of this work contend that the bulk of radioactivity in the liver, therefore, proved to represent a wide variety of simple known substances commonly present in normal tissue (and, consequently, not of toxicological significance). (Rosenblum, et al., 1972) (Rosenblum, 1977)

The proposed pathways for the metabolism of ronidazole in turkeys are shown in Figure 1. Hydrolysis of the carbamate group leaves HMMNI. Ring scission may occur by several routes. Routes 1 and 2 lead to N-methylglycolamide, which in turn can be further metabolized to oxalic acid, methylamine and carbon dioxide. The nitro group is represented as R, because its exact status remains unknown. However, it has been suggested that the nitro group may simply hydrolyze or be reduced to the amine, which in turn can hydrolyze to the hydroxy group. (Rosenblum, et al., 1972)

Figure 1. Proposed Routes of Metabolism of Ronidazole in Turkeys.



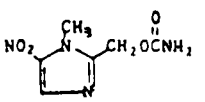
Swine

Studies similar to those carried out in turkeys were conducted in swine. Two 10-week-old barrows weighing approximately 20 kg were fed 14C-ronidazole, labeled in the N-methyl group, mixed in a slurry of feed and water at a rate of 6.7 mg/kg or 9.2 mg/kg per day for 3 days. One pig was sacrificed at 4 hours post last dose and the other at 72 hours following the final dose. Approximately 70-80% of the total radioactivity was recovered in urine, feces, GI tract contents and tissues. The authors speculate that the remainder of the radioactivity could be lost via exhalable material (methylamine, for example).

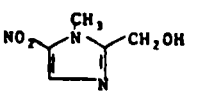
The amount of residue that was unextractable from muscle and liver of treated animals increased with time. Thus for the animal sacrificed at 4 hours, 74% of the radioactivity in liver and 16% in muscle was water soluble, while 28% and 14%, respectively, were insoluble. For the pig sacrificed at 72 hours, 26% of the radioactivity in liver and 27% in muscle was water soluble, while 71% and 65%, respectively, were insoluble. Moreover, when the cell components in tissue samples of the 72-hour sacrificed pig were examined, it was found that 53.6% of the total radioactivity in liver was associated with protein and about 10% each being distributed with the nucleic acid and lipid fractions. In muscle of that same animal, 58.3% of the total radioactivity was associated with protein and about 6% each in the nucleic acid and lipid fractions. The increase in the proportion of the radioactivity in the insoluble residue was taken as reflecting the incorporation of radioactive substances into macromolecular cellular constituents which have a relatively longer biological half-life than the lower molecular weight compounds present in the water-soluble fraction.

The examination of urine, muscle and liver for metabolites of ronidazole determined the presence of four components: ronidazole, HMMNI, imidazole and 1-methyl-2-carbamoyloxymethyl 1-5-acetamido-imidazole. With the exception of ronidazole at 4500 ppb and HMMNI at 95 ppb in muscle from the 4-hr sacrificed pig, none of the above compounds was present above 5 ppb in muscle or liver of either treated animal. In urine, the only two nitro-containing compounds found were ronidazole and HMMNI. The structures of the above compounds are shown in Figure 2. (Smith, et al., 1975)

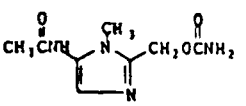
Figure 2. Metabolites of Ronidazole Found in Tissues of Treated Swine.



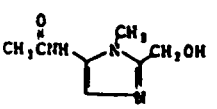
Ronidazole



1-methyl-2-hydroxymethyl-5-nitroimidazole



1-methyl-2-carbamoyloxymethyl-5-acetamidoimidazole



1-methyl-2-hydroxymethyl-5-acetamidoimidazole

The total residue depletion data in Table III show that, after a period of rapid elimination (day 0 to day 3 of withdrawal), residues persist in tissues for up to 42 days. These persistent residues were thought to be associated with cellular macromolecules. In fact, it was determined that about 60% of the radioactivity in muscle at 7 days of withdrawal was present in the protein fraction. Furthermore, that percentage did not change appreciably at 42 days of withdrawal. In order to estimate the amount of the persistent residue that was not due to incorporation of labeled material into endogenous substances, the researchers hydrolyzed tissue samples under acidic conditions to generate methylamine. Under those experimental conditions standards such as 14C-N-methyl-labeled ronidazole and dimetridazole gave quantitative yields of methylamine. Therefore, all metabolic products which have not been degraded to a single carbon fragment would yield radioactive methylamine. Tissue samples taken from animals sacrificed at 0 withdrawal showed that approximately 90% of the radioactivity present in muscle and 70% of that in liver liberates radioactive methylamine. Three days later, less than 30% of muscle radioactivity liberated methylamine. At this time, most of the methylamine liberating residues are present in the protein fraction. Since only 8-10% of the total radioactivity present in the 0-withdrawal sample remains after 3 days, and there is little change at 7

days after the last dose, it is clear that most of the methylamine generating substances have been eliminated within 3 days after the last dose. The authors concluded that the 70-80% of the residue which does not liberate methylamine represents incorporation of the 14C into endogenous substances. (Wolf, et al., 1983a)

Rats

Rats weighing 180-200 g were administered a single 10 mg/kg dose of ronidazole, labeled in one of four positions with 14C. Ronidazole was prepared with label in the N-methyl group; in the methylene group at the 2-position; in the 4- and 5-positions of the ring; and in the carbonyl group. Groups of three rats were sacrificed and tissues analyzed at 2, 4, 7, and 11 or 14 days after dosing. The results of the analyses are presented in Table IX.

Table IX. Concentration of Total Residue in Tissues of Rats Dosed with 14C-Ronidazole at 10 mg/kg (ppm)

Label Site	Withdrawal Time (days)	Liver	Muscle	Kidney	Fat
$-CH_3$	2	0.33	0.31	0.48	0.14
	4	0.27	0.26	0.41	0.13
	7	0.16	0.23	0.26	0.09
	11	0.11	0.18	0.17	0.07
$-CH_2-$	2	0.22	0.18	0.35	0.23
	4	0.10	0.13	0.26	0.14
	7	0.07	0.10	0.11	0.08
	14	0.02	0.05	0.04	0.06
4,5-ring	2	0.18	0.17	0.26	0.04
	4	0.10	0.11	0.14	0.03
	7	0.06	0.07	0.07	0.02
	14	0.03	0.06	0.04	0.02
-CO	2	0.40	0.12	0.19	0.07
	4	0.25	0.11	0.17	0.11
	7	0.12	0.07	0.08	0.07
	14	0,04	0.06	0.04	0.05

The varying levels of radioactivity in tissues from animals treated with different labels was taken by the authors as a clear indication that all of the residue cannot contain an intact N-methylimidazole nucleus. If the tissue residues contained only substances with an intact imidazole nucleus each label site should produce the same level of residues in each tissue at each analysis time, and parameters such as the $t_{1/2}$ of the residue would be identical.

The use of the methylamine generation test to determine the extent that the total residue may contain an intact imidazole nucleus, gave estimates of 10-30% of the total residue in muscle and liver at 7 and 11 days post dosing. These results are similar to those obtained for swine. (Wolf, et al., 1984b)

It is noteworthy that acetamide was identified as a metabolite in the urine of rats treated with ronidazole at 10 mg/kg. Acetamide, a known carcinogen, was found to be a breakdown product of metronidazole and its formation from ronidazole and dimetridazole seemed quite possible. (Alvaro, 1982)

EVALUATION OF THE PERSISTENT RESIDUE

General

The residue chemistry data summarized above gave evidence of persistent residues in the tissues of rats, turkeys and swine. Despite the good effort made in the metabolism studies that showed that ronidazole was extensively metabolized in vivo,

the exact nature of the total residue was undetermined. The data did indicate, however, that approximately 50-60% of the radioactivity remaining in tissues existed as protein-bound residues. Although a significant portion of that radioactivity was considered to be due to endogenous substances (and, therefore not of toxicological concern), the possibility that some of the radioactivity represented protein-bound metabolites containing the intact imidazole ring could not be discounted. Therefore, the sponsor of ronidazole, Merck Sharp and Dohme, undertook a series of studies aimed at determining the nature of the bound residue, the mechanism of its formation and the toxicological potential of ronidazole-bound residues.

The overall strategy for evaluating the safety of ronidazole protein-bound residues involved three phases. First, rat liver microsomes in an in vitro covalent binding system were used to study the mechanism of bound residue formation. Second, in vivo studies were conducted in the rat to permit a comparison with the residues formed in vitro and in swine to allow a comparison with the residue formed in the rat. Third, the Ames test was used to assess the potential mutagenicity of the bound residue. The results of those studies are highlighted below. (West, et al., 1982a) (West, et al., 1982b) (Miwa, et al., 1982) (Wolf, et al., 1983b) (Wislocki, et al., 1984a) (Wislocki, et al., 1984b) (Miwa, et al., 1984)

In Vitro Rat Studies

A number of important observations were made in the in vitro work:

(1) Anaerobic conditions are required for maximum binding and high oxygen concentrations inhibit covalent binding. Protein was found to be the primary binding target, with nucleic acid a very poor competitor. It was further observed that for every 20 molecules of ronidazole metabolized, only one (5%) alkylates microsomal protein. (West, et al., 1982a) (West, et al., 1982b)

(2) Cytochrome P-450 and P-450 reductase, in the presence of NADPH, catalyzes the reductive activation of ronidazole. (West, et al., 1982b)

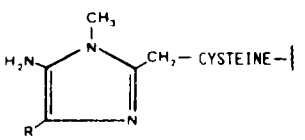
(3) The primary target of protein alkylation (nonspecific) is the cysteine thiol group. (West, et al., 1982b) (Miwa, et al., 1982)

(4) The major protein adduct retained the imidazole nucleus, but lost the carbamate group and the proton at the C-4 position. (Miwa, et al., 1982) (Miwa, et al., 1984)

(5) Although attachment of the cysteine thiol group can occur at the 2-methylene group or the 4-position of the ring, the studies suggest that the adduct occurs primarily at the 2-methylene group (see Figure 3 below). (Wislocki, et al., 1984a)

(6) The results of the studies suggest that the hydroxylamine derivative of ronidazole is the reactive species for covalent binding. (Miwa, et al., 1986b)

Figure 3. Generalized Structure for the Protein-bound Ronidazole Adduct.



In Vivo Rat and Swine Studies

Ronidazole labeled in different positions was administered to rats and swine. The animals were sacrificed at 6 hours post dosing and the protein-bound residues from liver and muscle were isolated for methylamine release experiments, oxalic acid formation determinations and chromatographic properties examination. Comparisons of the protein-bound residue from the in vitro rat microsomal system and the in vivo rat and swine systems, established the following: (1) HPLC analysis of acid hydrolyzed in

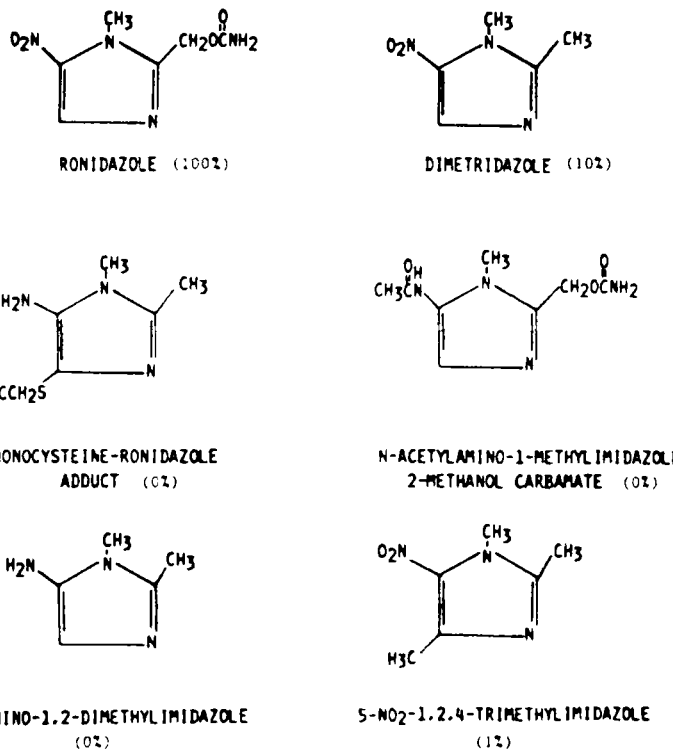
vitro and in vivo protein-bound residues obtained from 14C-ronidazole labeled in the 2-methylene position gave nearly identical radiochromatographic profiles. (2) Acid hydrolysis of the in vitro and in vivo residue samples from animals dosed with 14C-ronidazole labeled in the N-methyl group generated similar amounts of methylamine (rat microsome, 97%; rat in vivo, 76%; swine liver, 94% and muscle, 86%). Similarly, acid hydrolysis of samples from animals dosed with 4,5-ring-labeled ronidazole gave similar amounts of oxalic acid (rat microsomes, 10%; rat in vivo, 8.7%; swine liver, 9% and muscle, 6.5%). (3) All three protein-bound residue samples retain the intact imidazole nucleus but have lost the proton at the C-4 position. (Miwa, et al., 1984)

Toxicological Evaluation of Ronidazole-Protein Adduct

Because ronidazole is mutagenic in the Ames test, the systematic study of the mutagenicity of ronidazole and its derivatives was considered to be a logical approach to the assessment of the toxicological potential of the protein-bound residues. In particular, the Ames test was used to assess the activity of compounds structurally related to the bound residue to establish a structure-activity relationship, of free and bound microsomal metabolites of ronidazole, and of digests of protein-bound ronidazole adducts.

The mutagenic activity of a number of ronidazole-related compounds in the Ames test was examined. As shown in Figure 4, the removal of the carbamoyl group of ronidazole reduces the mutagenic activity by a factor of 10; substitution of an alkyl group at the 4-position further decreases the activity by another factor of 10. The reduction of the nitro group totally eliminates mutagenic activity. Based on these results and the observation that monocysteine-ronidazole adduct shows no mutagenic activity, it was concluded that the protein adduct would not be mutagenic. (Wislocki, et al., 1984b)

Figure 4. Mutagenic Activity of Ronidazole-related Compounds in the Ames Test Relative to Ronidazole (Taken as 100%)



Reduced metabolites from incubations of ronidazole were tested directly for mutagenicity in the Ames test without isolation to ensure that all metabolites were examined. Thus, ronidazole was incubated anaerobically with rat liver microsomes, NADPH-generating system, and cysteine for a prolonged period to achieve >99% metabolism. The supernatant fraction of the incubation, which contained residual ronidazole, reduced metabolites and their breakdown products, was separated from the microsomes and tested in the Ames test with and without the S-9 fraction. The supernatant was found to have little mutagenic activity and that was attributable to the residual ronidazole. These results were taken as demonstrating that the reduced metabolites and the cysteine adducts derived from ronidazole are non-mutagenic and that they cannot be activated by liver enzymes to mutagenic species. (Wislocki, et al., 1984b)

An attempt was made to determine whether mutagenic products could be released from the in vitro ronidazole-protein residues. Protease-treated ronidazole-bound residues were examined in the Ames test that had been modified to increase its sensitivity. No mutagenic activity was detected. In contrast, the addition of a few μg of ronidazole to the assay system containing maximum amounts of hydrolyzed protein samples produced mutagenic activity. (Miwa, et al., 1986a)

METHODS OF RESIDUE ANALYSIS

A combination tlc and differential pulse polarography method for ronidazole in the edible tissues of swine has been described. The assay involves extraction, purification with tlc and polarographic analysis. The method has a level of sensitivity of 2 ppb. HMMNI, the major metabolite of ronidazole, is poorly extracted in the initial step and then separated from ronidazole with tlc. The method, therefore, is specific for ronidazole. (Cala, et al., 1976)

APPRAISAL

The use of ronidazole at permitted concentrations in the feed of turkeys for 3 days produces residues that deplete below detectable levels by 5 days of withdrawal. The level of sensitivity of the assay used for ronidazole and HMMNI, though not specified, appeared to be in the 20-40 ppb range.

Tissues of swine treated with ronidazole through the feed or drinking water at prophylactic or therapeutic levels were free of detectable levels at 2 days of withdrawal. The level of sensitivity of the assay used for ronidazole was 2 ppb.

Although the residue depletion studies in turkeys and swine demonstrate that residues of parent ronidazole fall below detectable levels by 5 and 2 days of withdrawal, respectively, the radiotracer work shows that total residues persist to at least 21 days in turkeys and 42 days in swine. The residue chemistry data are not adequate for establishing a relationship between the total residue and a compound measured with a chemical assay.

The metabolism work indicates the two major pathways characteristic of the 5-nitroimidazoles: the oxidative and reductive routes. The reductive pathway, known to lead to ring scission and fragmentation, has also been implicated as producing the reactive intermediate that forms protein-bound residues containing the intact imidazole ring.

The most intriguing aspect of the data submitted for ronidazole unquestionably is the bound residue issue. Merck has conducted extensive research aimed at sensibly dealing with the toxicological significance of bound residues. In particular, the experimentation attempts to permit the prediction that the protein-bound residue would not be mutagenic and, therefore, not of significant toxicological concern.

The above work on the bound residue needs to be carefully considered in relation to the tolerance for ronidazole in edible tissues of food-producing animals to decide whether the bound residue can be reasonably assessed, and, consequently, discounted from the total residue of toxicological concern. The discounting of bound residues is particularly important for ronidazole, and the 5-nitroimidazoles generally, because it appears impossible to develop suitable regulatory analytical procedures to monitor for residues beyond a few days post treatment.

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