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Tissue Residues Due to Ronidazole: Bioavailability of Residues in Swine Muscle on Ingestion by the Rat

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A substantial fraction of the radioactive residues present in muscle tissue from swine dosed with [*methyl*-^{14}C]ronidazole was absorbed and retained by the rat. This is characteristic of endogenous substances. On the other hand, drug-related residues characterized by methylamine liberation were poorly absorbed, apparently not metabolized and not covalently bound to rat tissue.

Ronidazole, (1-methyl-5-nitroimidazol-2-yl)methyl carbamate, is used in food animal production for controlling swine dysentery and for treatment of turkey blackhead. Although ronidazole disappears rapidly from edible tissues of the pig, radiocarbon from labeled ronidazole persists for prolonged periods (Wolf et al., 1983). Experiments in rats showed that most of the persistent tissue residues were derived from one- or two-carbon fragments of the drug (Wolf et al., 1983). However, a fraction of the residues may contain a drug-related moiety based on liberation of labeled methylamine when tissues of animals dosed with [*methyl*-^{14}C]ronidazole are subjected to strong acid hydrolysis.

When tissues containing radioactive endogenous substances are ingested, the radioactivity should be mostly absorbed and retained by the tissues of the dosed animal. The disposition of residues containing drug-related moieties is uncertain. This study was undertaken to determine the disposition of the radioactive residues in pig muscle on ingestion by the rat.

MATERIALS AND METHODS

Preparation of Swine Muscle Tissue. Muscle tissue from swine dosed with [*methyl*-^{14}C]ronidazole was obtained as described (Wolf et al., 1983). The animals were dosed once daily for 3 days. Seven days after the last dose, the pigs were slaughtered, and the muscle tissue was collected and homogenized by passing through a meat grinder several times. Convenient aliquots were packaged in polyethylene bags, frozen, and stored at −30 °C. The samples were thawed. Equal weights of tissue from three pigs were homogenized with 4 volumes of water, and the homogenate was freeze-dried.

Preparation of Rat Diets. The fluffy freeze-dried muscle was mixed with Purina Rat Chow at a ratio of 8 g of muscle tissue, which contained radioactive residues equivalent to 16 μg of ronidazole, and 10 g of Chow (by weight). A control diet was prepared with freeze-dried muscle from unmedicated pigs. After blending 16 μg of [*methyl*-^{14}C]ronidazole in 0.1 mL of ethanol was added to 18-g aliquots and each aliquot thoroughly blended. The specific activity of the ronidazole spike was 7.1 μCi/mg.

Animal Handling. Normal rats, male Charles River CD, weight approximately 250 g, were housed in individual metabolism cages equipped to collect separated excreta. However, fecal pellets may be washed with urine during collection. The cages were fitted with a specially designed feeding tunnel attached externally to the cage. The design is such that any spilled diet is readily collected without contamination with excreta.

Each animal was dosed with 18 g of the Chow–muscle mixture late in the day for 2 days. The total radioactivity ingested was determined by analysis of diet recovered daily from the feed compartment. On the second day each animal received 1 mL of an aqueous solution containing 2 μCi of ^{51}Cr EDTA complex to measure emptying of the GI tract. After the 2-day dosing period, each animal received 18 g of Purina Rat Chow daily for 2 days.

On the fifth day the animals were asphixiated with CO_2. After removal of the entire GI tract, the animals were skinned and the carcass homogenized by several passages through a meat grinder. Tissues samples were frozen and stored at −30 °C.

For collection of expired radioactivity, the metabolism cages were placed in plastic bags and the exiting air passed sequentially through absorption towers containing 1 N sulfuric acid and 2 N sodium hydroxide. The animals were dosed similarly except dosing with ^{51}Cr was omitted. In a control experiment urine, feces, and carcass were analyzed for methylamine liberation 48 h after dosing with 16 μg of [*methyl*-^{14}C]ronidazole.

Radiochemical Procedures. *Radioactive Ronidazole.* ^{14}C-Labeled methanol was converted to the methanesulfonate ester by treatment with methanesulfonic anhydride. Alkylation of 4-nitroimidazole yielded 1-[^{14}C]-methyl-5-nitroimidazole, which was converted to ronidazole by reaction with formaldehyde and transesterification with methyl carbamate. The product was found to be 99.4% pure by thin-layer chromatography using benzene–methanol (4:1) and benzene–dioxane–concentrated ammonium hydroxide (25:70:5). No single impurity accounted for

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Table I. Total Radioactivity Distribution in Rats Receiving [*methyl*-^{14}C]Ronidazole or Muscle Tissue from Swine Dosed with [*methyl*-^{14}C]Ronidazole and Recovery of ^{51}Cr[a] Radioactivity Expressed as Percent of Dose

	4[b]		5[b]		6[b]		av		1[b]		2[b]		3[b]		av	
	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}C	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr
urine																
24 h	9.43		14.57		11.59		11.86		18.27		15.08		17.18		16.84	
48 h	14.15	7.59	12.83	6.38	14.29	3.85	13.76	5.94	16.67	4.79	25.96	6.34	26.24	5.99	22.96	5.71
72 h	2.57	0.39	2.07	0.70	2.81	0.36	2.48	0.48	2.34	0.23	3.31	0.36	3.27	0.37	2.97	0.32
96 h	0.64	0.09	0.79	0.21	0.72	0.11	0.72	0.14	0.38	0.13	0.68	0.15	0.46	0.16	0.51	0.15
total	26.79	8.07	30.26	7.29	29.41	4.32	28.82	6.56	37.66	5.15	45.03	6.85	47.15	6.52	43.28	6.18
feces																
24 h	5.08		12.67		4.96		7.57		5.36		4.14		7.95		5.82	
48 h	24.70	92.28	25.11	86.70	24.93	95.65	24.91	91.54	34.26	92.29	25.00	77.95	27.68	94.53	28.98	88.26
72 h	5.05	1.10	3.89	6.44	4.48	2.80	4.47	3.45	7.50	2.16	16.84	10.64	10.29	5.14	11.54	5.98
96 h	0.41	0.12	0.83	0.37	1.01	0.31	0.75	0.27	0.97	0.23	1.34	0.74	0.49	0.34	0.93	0.44
total	35.25	93.50	42.50	93.51	35.38	98.76	37.71	95.26	48.09	94.68	47.32	89.33	46.39	100.01	47.27	94.68
GI tract[c]	1.31		1.75		1.64		1.57		0.09		0.12		0.11		0.11	
carcass[d]	19.93		19.84		20.28		20.02		1.44		2.53		3.64		2.54	
grand total	83.27	101.57	94.35	100.80	86.71	103.08	88.11	101.82	87.28	99.83	95.00	96.18	97.29	106.53	93.20	100.86

[a] Animals were fed a diet containing 8 g of freeze-dried pig muscle and 10 g of Rat Chow for 2 days and then 18 g of rat chow for an additional 2 days, and 1 mL of an aqueous solution containing 2 μCi of ^{51}Cr EDTA was administered after 24 h. Animals 1–3, received swine muscle from unmedicated pigs that had been spiked with 16 μg of [*methyl*-^{14}C]ronidazole. Animals 4–6 received swine muscle from pigs killed 7 days after receiving the last of three daily doses of [*methyl*-^{14}C]ronidazole. The total radioactivity content of the dried muscle was equivalent to 2.0 μg of ronidazole/g. [b] Animal number. [c] The entire GI tract and contents was analyzed. [d] Entire animal less GI tract and skin.

more than 0.3% of the total.

The ^{51}Cr EDTA complex was purchased from New England Nuclear.

Radioactivity Measurement. All tissue, diet, and fecal samples were homogenized with water to provide uniform slurries. About 0.5 g was weighed into paper combustion cups. After air-drying the samples were combusted with a Packard Model 306 sample oxidizer. Liquid samples from absorption traps and urine, 0.5 mL, were similarly treated. The $^{14}CO_2$ was absorbed in 8 mL of Carbosorb (Packard) and mixed with 10 mL of Permafluor V (Packard).

Samples of methylammonium *p*-toluenesulfonate were dissolved in methanol and counted in a phosphor mixture consisting of 4 g of Omniflour (Packard) in 700 mL of toluene and 300 mL of absolute ethanol. Samples were analyzed for ^{14}C content by using a Packard Model 3380 liquid scintillation spectrometer.

All counts were converted to dpm with external standardization and appropriate quench curves. Sample blanks from tissues and excreta from unmedicated animals similarly treated were used to determine net dpm. All determinations were replicated 2–4 times.

Duplicate 0.5-g samples of urine or fecal homogenate were analyzed for ^{51}Cr with a Packard Model 5360 gamma counter. Counts were related to a standard sample run at the same time to correct for counter efficiency and ^{51}Cr decay.

Reverse Isotope Dilution Analysis for Methylamine. Samples of pooled rat urine, feces, and carcass, as well as the swine muscle, were analyzed for labeled methylamine with a macroprocedure with methylammonium *p*-toluenesulfonate as carrier. Samples containing at least 10 000 dpm of ^{14}C radioactivity were added to 6 N hydrochloric acid by using at least 2.5 mL of acid/g (dry weight) of sample. Liquid samples were adjusted to 6 N with concentrated hydrochloric acid. After 50–100 mg of methylammonium *p*-toluenesulfonate was added, the mixture was deaerated by evacuating the frozen mixture and admitting N_2 gas to the tube. After two thawing, freezing, and evacuation operations, the evacuated tubes were sealed and heated at 120 °C for 16 h. The hydrolysates were made alkaline with concentration sodium hydroxide solution and then purged with nitrogen at 100 °C. Methylamine removed by this method was trapped in an alcoholic solution of *p*-toluenesulfonic acid. The resulting salt was recrystallized from acetonitrile to constant specific activity.

Table II. Recovery of Radioactivity from Rats Receiving [*methyl*-^{14}C]Ronidazole or Muscle Tissue from Swine Dosed with [*methyl*-^{14}C]Ronidazole,[a] Radioactivity Expressed as Percent of Dose

substance dosed	ronidazole	swine muscle residue
urine		
24 h	10.32	0.32
48 h	26.95	7.72
72 h	6.89	15.65
96 h	0.53	2.70
total	44.69	26.39
feces		
24 h	1.71	0.40
48 h	21.47	3.95
72 h	15.56	14.28
96 h	0.38	6.66
total	39.12	25.29
expired gas		
alkaline trap		
24 h	0.03	1.07
48 h	0.27	1.84
72 h	0.63	4.03
96 h	0.11	1.75
total	1.04	8.69
acidic trap		
24 h	0.79	0.69
48 h	0.22	0.61
72 h	0.85	0.87
86 h	0.41	0.34
total	2.27	2.51
GI tract	1.97	18.00
carcass	2.24	21.90
grand total	91.34	102.78

[a] The animals were handled and dosed as described in Table I. The entire cage was placed in a plastic bag. Air flow, about 1 L/h, was maintained by maintaining a slight vacuum on the end of the gas absorbtion train, which consisted of an acidic and alkaline trap, each containing about 100 mL of absorbing solution. The trap contents were replaced daily. The air inlet and outlet tubes were separated to ensure air circulation within the bag.

RESULTS

The total recovery for the rats in normal metabolism cages is in Table I and for those with expired air analysis

Table III. Reverse Isotope Dilution Analysis for $^{14}CH_3NH_2$ Liberated by Acid Hydrolysis of Lyophilized Swine Muscle and Urine, Feces, and Carcass of Rats Dosed with the Muscle Tissue[a]

sample	liberated $^{14}CH_3NH_2$, % of total radioact in sample[b]	liberated $^{14}CH_3NH_2$ recovered, % of that administered[d]
swine muscle	32.9	100
urine (24 h)	21	7.64
urine (48 h)	22	9.21
urine (72 h)	21	1.49
total		18.34
feces (24 h)	65	14.95
feces (48 h)	66	50.0
feces (72 h)	67	9.09
total		74.04
carcass	<0.5[c]	
total		92.38

[a] Samples from animals 4–6 of Table I were aliquoted by weight and analyzed for methylamine liberation as described under Materials and Methods. [b] Average of replicate determinations. [c] Samples were counted for 100 min. Detection limit estimated at ~0.5% of radioactivity in samples. [d] Calculation based on total dose of liberated $^{14}CH_3NH_2$ administered.

in Table II. Recovery of the ^{51}Cr averaged about 101%, indicating good collection of excretion samples. As only about 0.5% was obtained on day 4, the gastrointestinal (GI) tract contents do not contain medicated feed as this was discontinued the same day the ^{51}Cr dose was administered. Comparison of the ^{14}C dose indicates higher urinary, fecal, and overall recovery for the rats fed with diet containing ronidazole compared to those fed animals dosed with swine muscle and 93% for those dosed with ronidazole. When expired air was analyzed, about 11% of the administered dose was recovered from the rat dosed with swine muscle compared to about 3% for the animal dosed with ronidazole.

Analysis of excreta and carcass for methylamine liberating substances from swine muscle gave recovery of 92% for these substances with about 74% in the feces (Table III). A single dose of ronidazole by gavage gave a good recovery of total radioactivity. About 60% of the dose was recovered as ronidazole- or methylamine-liberating moieties (Table IV).

Table IV. Disposition of Total Radioactivity and Methylamine-Liberating Moieties in a Rat Dosed with [*methyl*-^{14}C]Ronidazole[a]

	total radioact, % of dose	methylamine liberated[b] % of dose	methylamine liberated[b] % of radioact
urine			
24 h	30.3	29.1	96.0
48 h	2.7	1.4	50.6
total	33.0	30.5	
feces			
24 h	49.2	27.3	55.5
48 h	8.9	NA	NA
total	58.1		
carcass[c]			
48 h	11.7	0.7	5.8
total	102.8	58.5	

[a] Dose administered by gavage, urine and feces. [b] Methylamine determined as described under Materials and Methods. Collected at 24 and 48 h. [c] Includes GI tract.

DISCUSSION

Analysis of the total radioactivity in the expired air was indicated when it became apparent that the recovery of radioactivity from the animals dosed with swine muscle containing residues due to ronidazole was lower than that from animals receiving the same diet spiked with ronidazole. Almost 8% more was expired from the animal doses with muscle residue. When this is included, the recovery from each experiment is about 100%.

Evidence that the residues in swine muscle consisted of two chemically and biologically distinct types has been presented (Wolf et al., 1983). This experiment confirms that finding and provides further evidence that a large fractionof the swine muscle residue is indeed due to o endogenous substances. This is evident from the much higher retention of radioactivity by the carcass and GI tract by the muscle residue dosed animals compared to those receiving ronidazole. In addition, the formation of a larger quantitiy of $^{14}CO_2$ observed with the muscle-dosed animals is a characteristic of utilization of endogenous substances as energy sources. Finally, the urinary excretion of the muscle-fed animals is about 15% lower than that observed when animals are dosed ronidazole. Thus, these results are all consistent with the hypothesis that a substanital fraction of the persistent residues in swine muscle tissue in indeed due to endogenous substances.

The observation that about 20–30%, depending on the time of analysis, of the total radioactivity present in swine muscle could be due to substances related to the drug has been presented (Wolf et al. 1983). This was based on the liberation of labeled methylamine on strong acid hydrolysis of the tissue. This method was found to produce nearly quantitive yields of methylamine from ronidazole, dimetridazole, and the ronidazole metabolite in which the 5-nitro group has been replaced with an acetylamino group. Thus, it seems likely that all derivatives of ronidazole containing the N–CH_3 moiety would liberate methylamine under similar treatment. It is the disposition of this fraction of the residue that is of toxicological concern.

These potentially drug related residues appear resistant to further, extensive metabolism as 92% of that dosed with recovered in the urine and feces. Metabolic reactions leading to degradation of the single-carbon fragment (methylamine) would result in disappearance of the methylamine-generating moieties. Thus, only a relatively small fraction, if any, of this residue could have been converted to endogenous metabolites.

A portion of these residues is absorbed. This amounts to at least 18% of the dose based on urine analysis. The total absorbed could be larger as part of that appearing in the feces might have been absorbed and excreted via the bile. As the fecal excretion represents a much larger fraction of the total than that observed with ronidazole, and it is known that higher molecular weight substances are excreted in the bile of rats, it appears that much of the swine muscle residue either is not absorbed or, if absorbed, is present as higher molecular weight substances resistant to further degradation and excreted via the bile.

That the "drug-related" tissue residues do not form "reactive intermediates" that have the capacity to couple with cellular macromolecular constituents is indicated by the lack of incorporation of the methylamine-generating moieties into the rat carcass. It has been proposed that the toxicity hazard of carcinogenic substances may be related to the propensity of the material to form covalently bound reaction products with cellular DNA and that the hazard of the bound matieral may be related to the capability of the bound substance to bind with DNA when administered in vivo (Jaggi et al., 1980). These authors demonstrated that aflatoxin B_1 binds to rat liver DNA but that the bound material (as well as soluble metabolites) did not bind to rat liver DNA on oral administration. From the known metabolic reactions of many substances,

the formation of a reactive intermediate and its reaction with a sensitive macromolecule alters the chemical nature of the substance in such a way that a second metabolic activation is either unlikely or impossible.

If the methylamine-liberating substances in swine muscle are capable of forming reactive intermediates that can bind to cellular macromolecules the formation of "carcass" residues would be expected on administration of the swine muscle to rats. The lack of detection of methylamine-liberating residues in the rat carcass indicates that the covalent binding potential of this fraction is lower than that of ronidazole (Wolf et al., 1984).

Administration of ronidazole at a dose of 16 μg gave carcass residues of 0.7% of the dose of methylamine-liberating residues. From these experiments it seems reasonable to conclude that the potential of the bound residues to form reactive intermediates that can bind to cellular macromolecules has been diminished by the primary activation-reaction sequence.

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Hydrolysis of Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone at Parts per Billion Levels in Aqueous Mediums

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Degradation rates of aldicarb, aldicarb sulfoxide, and aldicarb sulfone in aqueous solution were measured as part of a larger study to systematically investigate detoxification methodology for carbamate pesticides in drinking water. Gas chromatographic methods were developed for the study and are described. Pseudo-first-order rate constants for base hydrolysis were determined for 25-ppb solutions of each species at 15 °C. Second-order rate constants were calculated, and the order for rate of hydrolysis was found to be the same as that reported for ppm solutions: aldicarb sulfone > aldicarb sulfoxide > aldicarb. The temperature effect for hydrolysis of aldicarb sulfone was determined, and Arrhenius behavior was observed. An activation energy of 15.6 kcal/mol was calculated. Base hydrolysis rates of aldicarb sulfone in chlorinated water and in actual well water were measured and were found to be slower than those measured in distilled water. Data for rates of hydrolysis were extrapolated to hydroxide ion concentrations equivalent to environmental pH values, and good agreement with experimental data obtained in buffered pH solutions is shown.

Toxic metabolites of the pesticide aldicarb have been found in drinking water wells in several locations throughout the country including New York and Wisconsin (Rothschild et al., 1982). The most severe contamination still appears to be in New York State in eastern Suffolk County on Long Island (Guerrera, 1981; Zaki et al., 1982). Despite discontinued use of the pesticide since the 1979 growing season, it is still being found in groundwater wells at concentrations of total aldicarb greater than the New York State advisory guideline of 7 ppb. A recent study (Hansen and Spiegel, 1983) has predicted that the total aldicarb concentration will not remain above this level in groundwater beyond this decade. Even if these predictions are accurate, there is serious concern in the near future about the contamination of these wells and concern about aldicarb and other carbamate pesticides contaminating groundwater supplies in other areas in the country.

The high water solubility of aldicarb sulfoxide and aldicarb sulfone and their stability under some environmental conditions have made them a serious threat to groundwater. The degradation and leaching of aldicarb in various soil types have been studied by many researchers (Richey et al., 1977; Elgindi et al., 1978; Smelt et al., 1978, 1981; Bromilow et al., 1980; Bromilow and leistra, 1980). Early work by Richey et al. (1977) indicated extensive degradation of the aldicarb molecules in specific soils. Smelt et al. (1978) computed that 91-100% of the aldicarb applied to soil would be oxidized to the sulfoxide. They also showed that aldicarb sulfoxide and sulfone degraded considerably more slowly in deeper layers than in top layers of the soil profile; the degradation also varied with soil type. None of these studies predicted the environmental persistence of the sulfoxide and the sulfone in groundwater.

The work described herein is part of a broader investigation of the chemical degradation of aldicarb and its metabolites in water. The overall goal of this project is to develop detoxification methodology based on acid or base hydrolysis on reactive ion-exchange resin beds. This method has been shown to be effective in detoxifying organophosphate compounds (Janauer et al., 1981) and, if successful, will have an important application in the pro-

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