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Metabolism of Drugs. LXXIII.[1] The Metabolic Fate of Nitrofuran Derivatives. (1) Studies on the Absorption and Excretion

Kiyoshi Tatsumi, Toshio Ou, Hidetoshi Yoshimura and Hisao Tsukamoto

Faculty of Pharmaceutical Sciences, Kyushu University[2]

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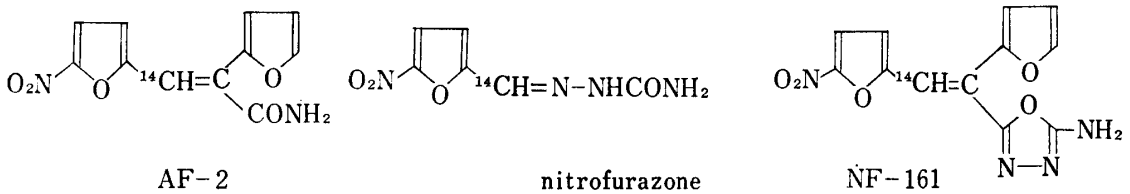
The absorption and excretion of ^{14}C-labeled 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), 2-amino-5[2-(5-nitro-2-furyl)-1-(2-furyl)vinyl]-1,3,4-oxadiazole (NF-161) and 5-nitro-2-furfural semicarbazone (nitrofurazone) were examined following oral administration to rats. In the cases of AF-2 and NF-161 the radioactivity was mainly recovered in feces, whereas in the case of nitrofurazone it was found more in urine than in feces. In all the cases, the summation of the radioactivity which was excreted in urine and feces 96 hr after drug administration, was approximately equal to the administered radioactivity.

It was also found that the hepatobiliary system played a considerably important role in the excretion of these labeled drugs. Considering this biliary excretion, the percentages of the radioactivity absorbed from gastrointestinal tract were calculated to be 59, 33 and 88% for AF-2, NF-161 and nitrofurazone, respectively.

The unchanged compounds recovered in urine, feces and bile were very small amount in all the cases. In the feces of rats given NF-161, however, about 20% of the dose was excreted unchanged.

The finding of antibacterial activity of nitrofuran derivatives by Dodd and Stillman[3] has been followed by their wide use in the treatment of diseases of animals and man or in the preservation of food. In Japan, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) instead of 5-nitro-2-furfural semicarbazone (nitrofurazone) is currently being used as food preservative and 2-amino-5[2-(5-nitro-2-furyl)-1-(2-furyl)vinyl]-1,3,4-oxadiazole (NF-161) is recently demonstrated to be effective for the treatment of respiratory diseases of chickens.[4]

Early works[5] presented a number of results on the distribution, excretion and metabolic degradation of various kinds of nitrofuran derivatives, most of which have –CH=NR in their side chain. However, no report concerning the *in vivo* metabolic fate of vinyl type nitrofuran derivatives having $-CH=C\langle^{R'}_{R}$ in their side chain, such as AF-2 and NF-161 mentioned above, has been made so far.



AF-2 nitrofurazone NF-161

Chart 1. Radioactive Nitrofuran Derivatives

1) Part LXXII: H. Yoshimura, M. Mori and K. Oguri, *Chem. Pharm. Bull.* (Tokyo), **18**, 2548 (1970).
2) Location: *Katakasu, Fukuoka.*
3) M.C. Dodd and W.B. Stillman, *J. Pharmacol. Exptl. Therap.*, **82**, 11 (1944).
4) S. Ota, S. Watanabe and C. Kuniyasu, *Nat. Inst. Anim. Hlth. Quart.*, **10**, 1 (1970).
5) M.F. Paul, H.E. Paul, R.C. Bender, F. Kopko, C.M. Harrington, V.R. Ells and J.A. Buzard, *Antibiot. Chemotherapy*, **10**, 287 (1960); H.E. Paul, V.R. Ells, F. Kopko and R.C. Bender, *J. Med. Pharm. Chem.*, **2**, 563 (1960); J.A. Buzard, J.D. Conklin, E. O'Keefe and M.F. Paul, *J. Pharmacol. Exptl. Therap.*, **131**, 38 (1961); J. Olivard, S. Valenti and J.A. Buzard, *J. Med. Pharm. Chem.*, **5**, 524 (1962); R.J. Herrett, C.W. Williams, J.P. Heotis and J.A. Buzard, *J. Agr. Food. Chem.*, **15**, 433 (1967).

The present inves tigation was initiated in order to learn the comparative metabolic fate of AF-2 and NF-161, together with nitrofurazone in rats utilizing radioactive compounds.

Experimental

Materials——The ^{14}C-labeled compounds, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (acrylamide-3-^{14}C), 2-amino-5[2-(5-nitro-2-furyl)-1-(2-furyl)vinyl]-1,3,4-oxadiazole (vinyl-2-^{14}C) and 5-nitro-2-furfural semicarbazone (formyl-^{14}C) were kindly supplied by Ueno Pharmaceutical Co., Ltd. The radioactive purity of these labeled compounds was established by thin-layer chromatography (solvent system: AcOEt–hexane–AcOH, 12:8:1).

Administration of Drugs——Donryu male rats weighing 150 g were used in this study. The animals were fasted overnight prior to administration of drugs. ^{14}C-AF-2 (0.15 μCi/mg), ^{14}C-NF-161 (0.086 μCi/mg) and ^{14}C-nitrofurazone (0.12 μCi/mg) were suspended in 10% gum arabic and administered orally at a dose of 100 mg/kg in all experiments otherwise indicated. After medication, each animal was placed in an individual metabolism cage. Urine and feces were collected over a 4-day period, and ^{14}C in urine and feces was measured daily. In the experiment designed for determining ^{14}C of the expired CO_2, each animal was individually housed in a Roth metabolism cage.

Treatment of Rats——Rats were anesthetized with urethane (1.4 g/kg, *i.p.*). The common bile duct was exposed through a small mid-line incision in abdominal wall, and a polyethylene cannula (PE 10 Clay-Adams catheter) was inserted and tied in position according to the method of Abou-El-Makarem.[6] The animals were kept under restraint for 24 hr with injection of 5% glucose–0.9% NaCl mixed solution and kept warm with a 60 W lamp. After recovery from the operation, the animals were given single oral dose of drug. Bile and urine were collected over a 2-day period, and ^{14}C in bile and urine was measured daily. The animals were killed by decapitation 48 hr after medication. The gastrointestinal tracts were removed from the treated animals and quickly homogenized with Waring blender for counting.

In order to investigate the reabsorption of ^{14}C excreted in bile from the gastrointestinal tract, 4 ml of the radioactive bile was re-fed by stomach tube to a cannulated rat. ^{14}C-Content of the bile and urine newly collected for 24 hr was then measured.

Radioisotope Methods of Analysis——The radioactivity of all samples was measured using an Aloka Liquid Scintillation Spectrometer (Model 502, Japan Radiation & Medical Electronics, Inc., Tokyo) and was corrected for quenching by an internal standard method using ^{14}C-toluene standard. The samples of urine and bile were exactly diluted to 150 ml and 10 ml with distilled water, respectively. Aliquots of urine and bile were counted in a *p*-dioxane phosphor consisting of 60 g naphthalene, 4 g PPO, 0.2 g POPOP, 100 ml methanol, 20 ml ethyleneglycol and *p*-dioxane to make 1 liter. Feces (150 mg, wet weight) and tissue homogenates (150 mg) were oxidized to $^{14}CO_2$ with the oxidation mixture which was prepared according to the method of Van Slyke and Folch.[7] Expired and oxidized $^{14}CO_2$ was trapped in 2-methoxyethanol–ethanolamine (2:1) according to the method of Jeffay, *et al.*[8] Aliquots of the above solution were counted in a toluene phosphor consisting of 0.55% PPO in 2-methoxyethanol–toluene (1:2). The other materials were also counted in a toluene phosphor prepared by dissolving 4 g PPO and 0.1 g POPOP in 1 liter of toluene. Radioactivity on thin-layer radiochromatograms was determined by either an Aloka Thin-layer Radiochromatogram Scanner (Japan Radiation & Medical Electronics, Inc., Tokyo) or a scintillation counter after 0.5 cm bands of silica gel were scraped from the plate into vials containing 10 ml of toluene phosphor.

Quantitative Determination of Unchanged Compounds——The unchanged compounds in urine and bile were determined by reversed dilution analysis as follows. An aliquot (20 ml) of urine or bile was extracted three times with equal volume of AcOEt by shaking mechanically. After centrifugation, the organic phase was removed and evaporated to dryness *in vacuo*.

The preliminary studies showed that under this condition, AF-2, NF-161 and nitrofurazone, which were added to urine and bile, were extracted into AcOEt in the recovery of 92%, 90% and 94%, respectively.

To the residue was exactly added 100 mg of carrier drug and the mixture was recrystallized repeatedly from benzene (AF-2), dioxane (NF-161) or ethanol (nitrofurazone), until its specific radioactivity became constant. The radioactivity of the unchanged compounds excreted in urine and bile was calculated by the equation described in the previous paper.[9]

The determination of the unchanged compounds in feces was carried out spectrophotometrically. Feces (1 g, wet weight) was homogenized in 2 ml of N-dimethylformamide using a Waring blender. In the case of AF-2 and NF-161, an aliquot (500 mg) of the homogenate was extracted with 10 ml of toluene by shaking

6) M.M. Abou-El-Makarem, P. Millburn, R.L. Smith and R.T. Williams, *Biochem. J.*, **105**, 1269 (1967).
7) D.D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).
8) H. Jeffay and J. Alvarez, *Anal. Chem.*, 33, 612 (1961).
9) K. Tatsumi, N. Arima, C. Yamato, H. Yoshimura and H. Tsukamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1253 (1970).

mechanically for 1 hr. After centrifugation, the organic phase was removed and measured at 395 mμ for AF-2 and at 425 mμ for NF-161. In the case of nitrofurazone, an aliquot (500 mg) of the homogenate was extracted with 15 ml of 75% N-dimethylformamide by shaking as described above. After centrifugation, 10 ml of the organic phase was removed and allowed to react with phenylhydrazine according to the method of Herrett, *et al.*[10] The concentration of nitrofurazone was measured at 440 mμ.

The preliminary studies showed that under this condition, AF-2, NF-161 and nitrofurazone which were added to feces, were recovered quantitatively 100%, 103% and 101%, respectively.

Result

Excretion of Radioactivity in Urine and Feces

After oral drug administration, the urine and feces were collected over a period of 4 days, and the samples of 0—24 hr, 24—48 hr, 48—72 hr and 72—96 hr were counted for radioactivity.

Table I. Percent Recovery of ^{14}C in Urine and Feces of Rats after Oral Administration of ^{14}C-Nitrofuran Derivatives

Time (hr)	AF-2		NF-161		Nitrofurazone	
	Urine	Feces	Urine	Feces	Urine	Feces
0—24	19.5	74.6	9.8	52.3	61.2	26.2
24—48	1.5	4.3	2.8	27.3	4.1	7.3
48—72	0.6	0.5	trace	2.8	0.6	1.2
72—96	0.3	0.3	trace	trace	0.2	0.2
Total	21.9	79.7	12.6	82.4	66.1	34.9

Values in Table represent the average of percent ^{14}C recoveries for four rats.

As can be seen in Table I, most of the radioactivity administered was recovered during the first 48 hr in all the cases. It was also found that the major pathway of excretion in the cases of AF-2 and NF-161 was by way into feces, while that in the case of nitrofurazone was by way into urine.

Excretion of Radioactivity in Expired Carbon Dioxide

The expired carbon dioxide was trapped over a period of 4 days, and the aliquots of 0—3 hr, 3—6 hr, 6—9 hr, 9—24 hr, 24—48 hr, 48—72 hr and 72—92 hr carbon dioxide absorbents were counted for radioactivity.

Table II. Percent Recovery of ^{14}C in Expired Carbon Dioxide after Oral Administration of ^{14}C-Nitrofuran Derivatives

Time (hr)	0—3	3—6	6—9	9—24	24—48	48—72	72—96	Total
AF-2	0.08	0.10	0.11	0.11	0.04	0.05	0.03	0.52
NF-161	0.05	0.03	0.06	0.04	0.01	trace	trace	0.19
Nitrofurazone	0.08	0.24	0.31	0.41	0.10	0.07	trace	1.21

Values in Table represent a typical result obtained by one rat.

Table II shows that only 0.2—1.2% of the administered ^{14}C was recovered in expired carbon dioxide.

10) R.J. Herrett and J.A. Buzard, *Anal. Chem.*, **32**, 1676 (1960).

Excretion of Radioactivity in Urine and Bile of Cannulated Rats

After oral drug administration, the urine and bile were collected over a period of 2 days. The aliquots of 0—24 hr and 24—48 hr urine and bile were counted for radioactivity. Since little or no feces was excreted during a period of this experiment, the cannulated rats were killed at 48 hr after medication. The gastrointestinal tract was isolated and homogenized by a Waring blender together with its content. An aliquot of homogenate was oxidized as described in method, followed by counting the radioactivity of $^{14}CO_2$.

Table III. Percent Recovery of ^{14}C in Urine, Bile and Gastrointestinal Tract after Oral Administration of ^{14}C-Nitrofuran Derivatives

Time (hr)	AF-2			NF-161			Nitrofurazone		
	Urine	Bile	G	Urine	Bile	G	Urine	Bile	G[a]
0—24	18.5	29.4	—	10.6	13.1	—	50.2	22.9	—
24—48	1.9	9.2	—	3.9	5.1	—	10.4	4.4	—
Total	20.4	38.6	43.3	14.5	18.2	68.8	60.6	27.3	12.2

a) G=gastrointestinal tract and its content
Values inTable represent the average of percent ^{14}C recoveries for two rats.

As can be seen in Table III, the highest level of radioactivity in bile was found in the cannulated rats given AF-2. In the cases of AF-2 and NF-161, the radioactivity was higher in bile than in urine, but in the case of nitrofurazone this correlation was the reverse. The summation of recovery in bile and in gastrointestinal tract was nearly equal to that in feces, which was shown in Table I. From these facts, it seems reasonable to consider that the radioactivity found in this gastrointestinal tract was corresponded to that of unabsorbed material.

Enterohepatic Recirculation

After oral administration of radioactive bile, the urine and bile were collected from the newly cannulated rat over a period of 24 hr, and the aliquots of urine and bile were counted for radioactivity.

Table IV. Percent Recovery of ^{14}C in Urine and Bile after Oral Administration of Radioactive Bile

	AF-2	NF-161	Nitrofurazone
Urine	2.3	7.1	26.3
Bile	trace	2.5	1.4

Values in Table represent a typical result obtained by one rat.

Results in Table IV show that the radioactivity in bile was reabsorbed to a slight extent from the intestinal tract of the cannulated rats.

Quantitative Determination of Unchanged Compounds in Urine, Feces and Bile

Table V shows the result of quantitative determination of each compound excreted unchanged into the 24 hr urine and feces, which had been collected in order to examine the excretion of the radioactivity in non-cannulated rats as described in Table I. The determination was also carried out on the 24 hr bile, which had been collected from the cannulated rats.

As can be seen in Table V, unchanged compounds recovered in urine, feces and bile were very small amount in all the cases, except the feces of rats given NF-161.

Table V. Percentage of Unchanged Compounds recovered in Urine, Feces and Bile after Oral Administration of ^{14}C-Nitrofuran Derivatives

	AF-2	NF-161	Nitrofurazone
Urine	0.05	0.04	0.08
Feces	trace	20.80	trace
Bile	0.07	0.31	0.23

Values in Table represent the average of percentage of unchanged compounds for two rats.

Discussion

By the present studies on excretion of radioactive nitrofuran derivatives, it was found that after oral administration the radioactivity was mainly recovered in feces in the cases of AF-2 and NF-161, whereas in the case of nitrofurazone it was counted more in urine than in feces. In order to elucidate how much of the radioactivity in feces was attributable to that which was excreted in bile, the biliary excretion of the administered radioactivity was further investigated using the cannulated rats. As indicated in Table III, the recovery of the radioactivity in bile was 38.6, 18.2 and 27.3% for AF-2, NF-161 and nitrofurazone, respectively.

Furthermore, the summation of the radioactivity in bile and gastrointestinal tract shown in Table III was found to be approximately equal to that recovered in feces shown in Table I, in all the cases. From these findings and the facts that reabsorption of the radioactive compounds in bile from the gastrointestinal tract of the cannulated rat was not very much as shown in Table IV, it should be concluded that nitrofurazone was absorbed from the gastrointestinal tract of rats to a greater extent (about 88%), AF-2 to a lesser extent (about 59%) and NF-161 to a least extent (about 33%). It is interesting to note that such varieties with regard to absorption after oral dosage may be ascribed partly to a rate of dissolution and partly to a rate of degradation of these compounds in gastrointestinal tract.

In the present studies with rat, very little AF-2, NF-161 or nitrofurazone was recovered unchanged in urine and bile. However, fecal excretion varied from a trace with AF-2 and nitrofurazone up to 21% of the dose with NF-161. These results in feces very likely reflect the different solubility of NF-161 and the others, because the solubility[11] of NF-161, AF-2 and nitrofurazone in water is 3 μg/ml, 125 μg/ml and 238 μg/ml, respectively.

An antimicrobial agent which is not readily absorbed and is inactibated by destruction in gastrointestinal tract may be unadequate in the treatment of systemic infection, but useful as food preservative. On the basis of the present results, it would appear that AF-2 has preferable properties as food preservative if this compound is not metabolized into any toxic substance in animal body. Further studies on the toxicity of these metabolites should be necessary in future.

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11) K. Tatsumi, T. Ou, H. Yoshimura and H. Tsukamoto, unpublished data.