Absorption and Distribution of Oxafuradene in the Dog

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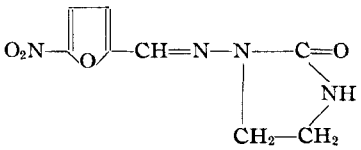
Studies on the absorption and distribution of oxafuradene, a chemotherapeutic nitrofuran, have been conducted in the dog. This drug is well-absorbed following peroral administration as a suspension or in capsule or tablet form. Oxafuradene is readily distributed into most body fluids following intravenous drug infusion. Similar concentrations of the drug are attained in thoracic lymph and plasma. Results are presented demonstrating placental transfer of the drug in the guinea pig, rabbit, dog, and sheep. Concentrations of oxafuradene attained in milk are consistently greater than concurrent plasma levels. Oxafuradene is found in bile and approximately 5 per cent of a dose is recovered in this fluid. The oxafuradene concentrations found in saliva and prostatic fluid increase with plasma levels. Drug concentrations appearing in aqueous humor and cerebrospinal fluid are comparable but lower than corresponding plasma concentrations. Correlation is obtained between oxafuradene concentrations measured chemically in these fluids and biological activity determined against *Escherichia coli.*

OXAFURADENE [1-(5-nitrofurfurylideneamino)-2-imidazolidinone], a chemotherapeutic nitrofuran, was synthesized in our laboratories (1). The structural formula is:



This drug has been found to be effective under *in vitro* conditions against bacterial species frequently involved in human urinary tract infections (2). Activity against these microorganisms has also been demonstrated in human and animal urine following oral administration of the drug (2, 3), indicating excretion of biologically active drug. These observations indicated that oxafuradene was of sufficient therapeutic interest to warrant an investigation of its absorption and distribution in the dog.

EXPERIMENTAL

Drug Characteristics.—Oxafuradene is a crystalline compound, yellow to orange in color, with a molecular weight of 224.18 and a decomposition point of 265 to 268°. The pH of an aqueous standard solution is 6.1. The following solubilities were obtained for the drug, in mg./L.: water, 100; chloroform, 180; nitromethane, 2000; plasma, 440; and serum, 515.

Although oxafuradene is essentially nonionizable, the aqueous solubility can be increased to approximately 2000 mg./L. by dissolving 100 mg. of the drug in 6 ml. of 10 *N* hydrochloric acid, adding 20 to 25 ml. of water, and *gradually* adjusting the pH of the final solution to 6.1 with sodium hydroxide.

The identity of the solubilized drug with authentic oxafuradene was shown by similar decomposition points, identical ultraviolet and infrared spectra, identical rates of formation of the phenylhydrazone derivative (4), similar chromatographic behavior on Whatman No. 1 paper for oxafuradene and the solubilized drug in 95% ethanol–*n*-butanol–0.5 *N* ammonium hydroxide, 1:4:1 (R_f 0.59, 0.58, respectively), in 95% ethanol–*n*-butanol–0.5 *N* acetic acid, 1:4:1 (R_f 0.55, 0.56, respectively), and equivalent inhibitory activity of the two forms against *Escherichia coli.*

It is well-established that protein binding, degree of ionization, and lipid solubility are major factors regulating drug transfer (5). Information on these characteristics was obtained for oxafuradene. The chloroform–buffer (phosphate 0.1 *M*, pH 7.4) partition coefficient of oxafuradene and the solubilized drug was determined as an index of lipid solubility (6). Plasma protein binding was measured by a dialysis method described earlier (6) using plasma samples collected from dogs dosed perorally with oxafuradene in carboxymethylcellulose (CMC) or intravenously with the solubilized drug. The oxafuradene plasma concentrations in these samples were comparable to those encountered in the distribution studies. The results are presented in Table I.

Drug Administration.—Oxafuradene was administered orally to dogs as a micronized crystalline material in gelatin capsules or as tablets containing 100 mg. The drug was also administered orally as a

TABLE I.—FACTORS REGULATING DRUG TRANSFER

pKa	Partition Coefficient Chloroform–Buffer	Plasma Protein Binding, %
12.0[a]	1.9	0
	1.8[b]	0[b]

[a] A true pKa value cannot be obtained for this drug since the pH value is so close to that of the weak titrating base used. The drug is therefore considered essentially nonionized. [b] Solubilized oxafuradene.

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suspension in 0.75% aqueous sodium CMC to fasted dogs. In addition, micronized drug suspended in 0.75% CMC was administered intraperitoneally to dogs. For intravenous injections or infusions, oxafuradene was usually administered as the solubilized form in a solution of 5% dextrose (pH 6.1).[1] These solutions ranged in drug concentration from 1.7 to 1.9 mg./ml. Drug solutions were infused intravenously at a rate of 1.91 ml./min. and at doses ranging from 2 to 15 mg./Kg./hr. using a Harvard pump. Suspensions also were prepared for intravenous injection by mixing dextrose with micronized oxafuradene (10:1) and dispersing with 5% dextrose to form a suspension which contained about 10 mg./ml. of the drug.

Collection of Body Fluids.—Sodium pentobarbital, administered either intravenously or intraperitoneally, served as anesthesia. Blood samples were usually collected concurrently with body fluid samples, by venipuncture or cardiac puncture, using heparin as an anticoagulant. Urine was obtained by a direct puncture of the bladder or by catheterization.

Oxafuradene was infused intravenously to dogs and thoracic lymph obtained by a procedure reported previously (7). The placental transfer of oxafuradene was investigated in guinea pigs, rabbits, dogs, and sheep in the third period of gestation utilizing methods reported earlier (8). Solutions of the solubilized drug were infused intravenously at concentrations ranging from 10 to 15 mg./Kg./hr. for 1 to 2 hr. and at the following rates: guinea pig 0.51, rabbit 0.764, dog 1.91, and sheep 3.82 ml./min. Degradation studies with oxafuradene and the solubilized drug were conducted with maternal and fetal kidney and liver slices from the guinea pig and dog using the procedure of Buzard and Conklin (8). Oxafuradene was given perorally in gelatin capsules or intravenously as the solubilized drug by a single injection to lactating bitches, and milk samples were obtained at designated intervals. For bile collection, the common bile duct was cannulated just cephalic to the entrance into the duodenum, the gall bladder emptied of all bile, and the cystic duct then ligated at the juncture with the common bile duct. This dog was administered oxafuradene intravenously, and hepatic bile samples were collected continuously by free flow. Prostatic fluid was obtained from dogs infused intravenously with oxafuradene by placing a ligature at the vesicourethral juncture. Pilocarpine hydrochloride was administered in saline intravenously in doses of 6 mg. to facilitate flow, and the interval between doses was governed by the volume of fluid collected. Both ureters were cannulated with polyethylene tubing (PE-90) and the urine pooled during collection. Since the administration of pilocarpine also augments the flow of saliva, samples of this fluid were collected whenever possible. Oxafuradene was infused intravenously to dogs and aqueous humor (AH) and cerebrospinal fluid (CSF) obtained as described by Buzard and Conklin (6).

[1] Although the solution of the acid-solubilized oxafuradene is hypertonic (about 1.2 meq. of sodium/ml.), similar hematocrits were obtained for dogs infused with solutions of either 5% dextrose, 0.9% saline, or the solubilized oxafuradene in 5% dextrose. During the investigation, no visible signs of toxicity or emesis were noted with any of the animals infused with the solubilized drug. In some dogs in which the infusion was maintained for longer than 2 hr., a slight diuretic effect was observed.

TABLE II.—CHROMATOGRAPHY OF PLASMA FROM DOSED DOG AND CONTROL PLASMA PLUS DRUG

Plasma Sample	R_f Acetic	R_f Basic
Extracted with 95% Ethanol		
Authentic	.52	.50
Exptl.	.52	.50
Extracted with Nitromethane		
Authentic	.51	.51
Exptl.	.50	.50
Authentic[a]	.52	.51
Exptl.[a]	.50	.50

[a] Conducted with solubilized oxafuradene.

Drug Assays.—Samples of AH, CSF, prostatic fluid, lymph, saliva, milk, and plasma were analyzed for oxafuradene by the colorimetric method of Buzard *et al.* (4) which measures the nitrofurfurylidene moiety. Oxafuradene recoveries from these fluids as determined by this colorimetric method ranged from 85 to 90%. Samples of control urine subjected to this colorimetric method yielded absorbance values which made it impossible to detect the oxafuradene. Apparently, urine pigments produced colored materials which interferred with the procedure. Bile samples were analyzed by a modification of the method of Herrett and Buzard (9).

A chromatographic comparison of plasma from experimental animals and of plasma to which the authentic drug had been added was conducted using a procedure reported previously (6). The R_f values obtained are reported in Table II.

Antibacterial activity against *E. coli* was determined by twofold serial dilutions in brain–heart infusion broth. Samples were inoculated to produce a final concentration of approximately 10^4 cells/ml., and incubated for 24 hr. at 37°. The minimal inhibitory concentration was defined as the lowest drug concentration which prevented visible growth. Some control body fluids exhibited antibacterial activity in dilutions as great as 1:4. The minimal inhibitory concentration for oxafuradene against *E. coli* (Es-2) was 0.5 mcg./ml.

RESULTS AND DISCUSSION

Absorption.—Oral administration of oxafuradene, as a suspension in CMC, to fasted dogs, produced plasma concentrations which were dose related (Fig. 1). Dog plasma concentrations obtained following

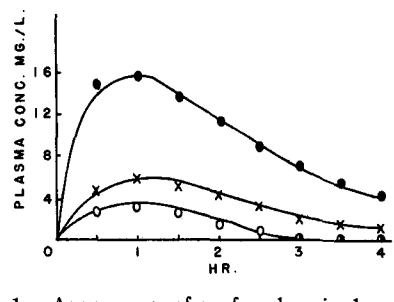

Fig. 1.—Appearance of oxafuradene in dog plasma after peroral administration of a suspension in CMC. Key: ○, 10 mg./Kg.; ×, 20 mg./Kg.; ●, 40 mg./Kg. (1 dog/dose).

TABLE III.—PERORAL ADMINISTRATION OF OXAFURADENE IN GELATIN CAPSULES

Time, hr.	Plasma Concn., mg./L. 10 mg./Kg. Fed	10 mg./Kg. Fasted	20 mg./Kg. Fed	20 mg./Kg. Fasted	40 mg./Kg. Fed	40 mg./Kg. Fasted
0.5	<0.4	<0.8	<0.8	1.9	<0.1	1.1
1.0	2.5	2.2	2.6	3.1	1.2	2.7
1.5	1.9	2.5	3.3	3.3	3.7	3.9
2.0	1.4	2.8	3.7	3.5	2.9	4.9
2.5	1.1	3.5	2.6	2.8	2.5	4.6
3.0	<0.6	3.7	1.6	2.8	2.0	3.7
3.5	<0.4	2.6	No sample	2.1	1.3	2.3
4.0	<0.3	2.0	<0.7	1.5	1.2	1.4

intraperitoneal injection at 40 mg./Kg. of oxafuradene as a suspension in CMC, were comparable to those attained orally at the same dose. Urine collected for 24 hr. from dogs given 10 mg./Kg. of oxafuradene suspended in CMC, perorally, contained 3–5% of the dose as determined by antibacterial activity.[2]

Peroral administration of the drug in gelatin capsules to dogs yielded plasma concentrations (Table III) which were similar to those obtained in dogs after oral administration of tablets containing oxafuradene (Table IV). In comparison, these concentrations were lower than those encountered with the drug suspensions (Fig. 1). The plasma concentrations in fed dogs were generally lower than those in fasted dogs (Table III).

The dose-response relationship illustrated in Fig. 1 established that a significant degree of absorption of oxafuradene occurred in the unanesthetized dog. Oxafuradene as a suspension in CMC was administered perorally at 20 mg./Kg. to an anesthetized dog and the plasma levels measured. The plasma concentrations obtained were comparable to those presented for the unanesthetized dog at the same dose (Fig. 1). In another anesthetized dog, the pylorus was ligated and the plasma concentrations determined following oral dosage of the drug at 20 mg./Kg. as a suspension. Since oxafuradene was not detected in the plasma under these conditions, it appears that the absorption of oxafuradene from the gastrointestinal tract of the dog is primarily intestinal absorption.

Distribution.—The disappearance of oxafuradene from the plasma of dogs, following a single intravenous dose of the solubilized drug, is characteristic of first-order kinetics (Fig. 2), with a plasma half-life for the drug of about 43 min. A suspension of oxafuradene in 5% dextrose, administered intravenously to dogs at 6 and 12 mg./Kg., produced plasma levels comparable to those obtained after intravenous dosage of the solubilized drug. Under these conditions, oxafuradene exhibited a slightly longer half-life (58 min.) than that observed with the solubilized drug (43 min.). This increase in half-life is probably due to some persistence of the drug in the crystalline state.

The work of Buzard *et al.* (7) established that certain nitrofuran derivatives are readily transported into the lymphatic circulation. Concentrations of oxafuradene which appeared rapidly in dog thoracic lymph following intravenous drug infusion at 5 mg./Kg./hr. closely paralleled concurrent drug plasma levels (Table V). These plasma and lymph samples exhibited activity against *E. coli* proportional to the chemically determined concentrations. A dose-response relationship occurred in both fluids collected from a dog in which successively higher doses of oxafuradene were infused at a constant fluid rate (Fig. 3). Following completion of the infusion,

TABLE IV.—PERORAL ADMINISTRATION OF OXAFURADENE IN TABLETS

Time, hr.	Plasma Concn.,[a] mg./L. Coated Tablet[b]	Uncoated Tablet[c]
0.5	0.0	<0.6
1.0	2.1	3.6
2.0	4.8	5.9
3.0	6.0	6.0
4.0	7.4	5.5

[a] Mean from two dogs; dose, 40 mg./Kg. [b] An orange, sugar-coated compressed tablet, weighing 324 mg. and containing 100 mg. of micronized oxafuradene (lot No. 63-11-13-1) from Pharmaceutical Research Division, Eaton Laboratories. [c] An uncoated compressed tablet, weighing 257 mg. and containing 100 mg. of oxafuradene, from Smith Kline & French Laboratories, Ltd.

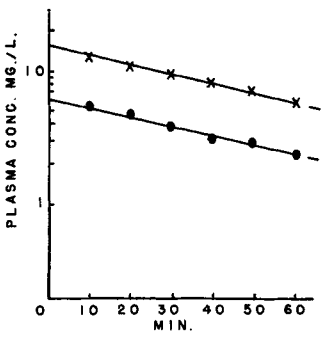

Fig. 2.—Disappearance of solubilized oxafuradene from dog plasma after intravenous injection. Key: ●, 6 mg./Kg.; ×, 12 mg./Kg. (1 dog/dose).

TABLE V.—OXAFURADENE IN THORACIC LYMPH[a]

Infusion, min.	Concn. Ratio, Lymph/Plasma	Reciprocal Inhibitory Dilution Lymph	Reciprocal Inhibitory Dilution Plasma
30		0	4
60	0.24	2	4
90	0.52	4	8
120	0.64	8	16
150	0.76	8	16
180	0.86	8	16
210	1.06		

[a] Lymph was collected over 30-min. intervals and the infusion stopped at 180 min.

[2] Biological cup plate procedure utilizing *E. coli* (unpublished data).

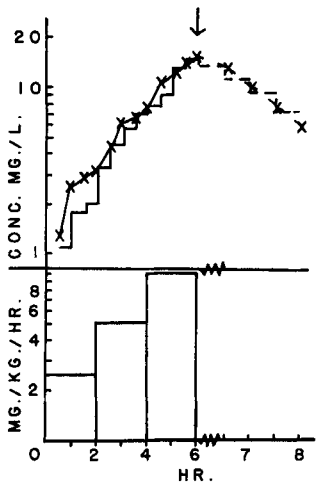

Fig. 3.—Plasma (X-X-X) and lymph (⌐¬) concentrations of oxafuradene during infusion of various dose rates (indicated in mg./Kg./hr. by blocks at bottom of figure) at 1.91 ml./min. Infusion stopped (broken line).

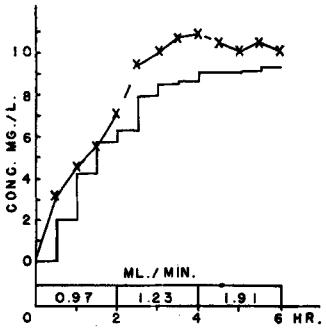

Fig. 4.—Plasma (X-X-X) and lymph (⌐¬) concentrations of oxafuradene during an infusion of 5 mg./Kg./hr. at varying rates of fluid administration.

Studies with nitrofurantoin[3] and furaltadone[4] in the guinea pig, rabbit, dog, and sheep have shown that the extent of transfer across the placenta is determined by the physical properties of the drug and the species-dependent placental type involved (8). An investigation of the placental transfer of intravenously infused oxafuradene in the same four species revealed that the drug appeared in the fetal circulation to some extent in all of the species studied (Table VI). Degradation studies conducted with maternal and fetal, kidney and liver slices from the guinea pig and dog established that in these tissues and species fetal tissue degradation of oxafuradene or the solubilized drug was less than that of maternal tissues. These results indicate that fetal plasma levels may be used as an index of placental transfer and are in agreement with work reported earlier with nitrofurantoin and furaltadone (8). Significant activity against *E. coli* was demonstrated with samples of maternal plasma and urine collected from these species (Table VI). In addition, antibacterial activity was also exhibited with fetal fluids from the dog and sheep, providing additional evidence for the placental transfer of the active drug.

It has been reported that nitrofuran derivatives enter other body fluids (10) in addition to plasma and lymph. Paul *et al.* (11) established that oxafuradene and certain other nitrofurans are found in the milk of lactating animals following drug administration. In the present studies, concentrations of oxafuradene in the milk of lactating dogs were greater than concurrent plasma concentrations, following either peroral or intravenous administration (Table VII). Although this suggests active secretion of the drug into this fluid, additional work will be necessary to substantiate this.

TABLE VII.—OXAFURADENE IN MILK

Dosage	Time, min.	Concn. Ratio, Milk/Plasma	Reciprocal Inhibitory Dilution, Milk
Dog *A*, 6 mg./Kg. i.v.	15	1.4	0
	30	1.6	0
	45	2.9	0
	60	3.1	0
Dog *B*, 30 mg./Kg. peroral	90	.7	0
	120	2.5	0
	180	3.3	0
	240	4.2	8
	360		16
	480		16
	600	5.5	32

TABLE VI.—OXAFURADENE IN FETAL FLUIDS[a]

Species	Mean Plasma Concn. Ratio, Fetal/Maternal	Reciprocal Inhibitory Dilution[b]			
		Plasma		Urine	
		Fetal	Maternal	Fetal	Maternal
Guinea pig	.32 ± .07	..	64	..	256
Rabbit	.23 ± .07	4	64	..	256
Dog	.37 ± .07	32	64	32	256
Sheep	.15	4	64	8	12

[a] The plasma concentrations ranged from 1.5 to 6.5 mg./L. for fetal, and from 9.2 to 18.5 mg./L. for maternal. [b] The data reported are the highest values obtained.

the half-lives of the drug in lymph (97 min.) and in plasma (76 min.) were not significantly different ($p > 0.05$). Intravenous drug infusion to a dog at 5 mg./Kg./hr., but with increases in the rate of fluid infusion, produced oxafuradene lymph levels which closely approximated plasma drug concentrations (Fig. 4). The similar concentrations attained in the lymph and plasma with oxafuradene agree with results reported by Buzard *et al.* (7), which showed that nitrofuran derivatives essentially unbound to plasma proteins are not restricted in their transfer into lymph.

[3] Trademarked as Furadantin by the Norwich Pharmacal Co.

[4] Trademarked as Valsyn by the Norwich Pharmacal Co.

Appreciable antibacterial activity of bile collected from animals administered nitrofuran derivatives, including oxafuradene, has been reported (11). The intravenous administration of oxafuradene resulted in high drug concentrations in hepatic bile (Fig. 5). About 5% of the dose was recovered within 5 hr. as determined colorimetrically. During drug infusion, the concentration of oxafuradene in bile was approximately 50 times that in plasma, and following the infusion, almost 100 times that in plasma, indicating possible biliary secretion of oxafuradene. A half-life of 54 min. was determined for the drug in plasma after a single injection and one of 54 min. after completion of the 3-hr. infusion. These values are in agreement with the half-life of 43 min. mentioned earlier for the drug in plasma following a single intravenous injection, but not in agreement with the half-life of 75 min. obtained for the drug after a 6-hr. infusion (Fig. 3). Antibacterial examination of the bile samples revealed significant activity against *E. coli* (Fig. 5) which increased correspondingly with bile concentrations measured chemically.

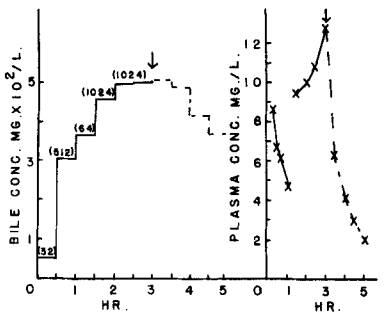

Fig. 5.—Plasma (X-X-X) and bile (⌐¬) concentrations of oxafuradene after a single intravenous injection at 6 mg./Kg. at zero time and during an infusion of 6 mg./Kg./hr. at 1.91 ml./min. from 60 to 180 min. Infusion stopped (broken line). Figure in parentheses is reciprocal inhibitory dilution against *E. coli*.

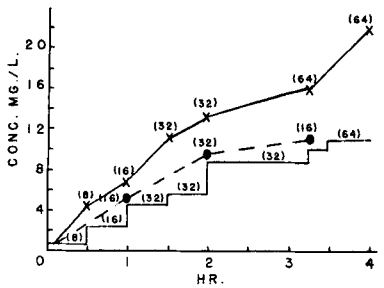

Fig. 6.—Plasma (X-X-X), saliva (●-●-●), and prostatic fluid (⌐¬) concentrations of oxafuradene during an infusion of 8 mg./Kg./hr. at a rate of 1.91 ml./min. Figure in parentheses is reciprocal inhibitory against *E. coli*.

Infusion of oxafuradene intravenously to dogs produced drug concentrations in saliva and prostatic fluid which increased with plasma concentration (Fig. 6). The final drug concentration ratio (fluid/plasma) for the results illustrated in Fig. 6 was 0.70 for saliva and 0.50 for prostatic fluid. Significant activity was obtained against *E. coli* with both fluids and plasma (Fig. 6) proportional to concentrations determined chemically. Urine samples collected during this study exhibited reciprocal inhibitory dilutions which ranged from 256 to 1024. Buzard *et al.* (12) reported concentrations of nitrofurantoin in the saliva of dogs receiving the drug intravenously.

Results reported recently by Buzard and Conklin (6) indicate that nitrofuran derivatives accumulate similarly in the aqueous humor and cerebrospinal fluid of the dog. In agreement with these data, comparable concentrations of oxafuradene were obtained in both fluids collected from dogs infused intravenously with the drug for 1 to 3 hr. at doses ranging from 5 to 12 mg./Kg./hr. The drug concentration ratio (fluid/plasma) was 0.29 ± 0.02 for AH and 0.28 ± 0.02 for CSF, when the plasma concentrations ranged from 5.4 to 15.5 mg./L.

Oxafuradene is essentially unbound to plasma proteins, nonionized at body pH, and lipid soluble. It has been reported that the passage of nitrofuran derivatives across body membranes is dependent upon the properties of the drug (6–8). Generally, it has been shown that the unbound, nonionized, lipid-soluble nitrofuran is most readily transferred. In agreement with this, the results presented show that oxafuradene readily enters most body fluids.

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