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Distribution of Dibenzoxazepines Bearing the Carboxamide or Other Side Chains in Ocular and Other Tissues of Dogs

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Abstract □ The distribution of four substituted dibenzoxazepines in tissues of dogs was examined 7–14 days after oral administration to intact dogs and 7 hr. after intravenous administration to dogs with externalized bile ducts. 4-[3-(7-Chloro-5,11-dihydrodibenz-[*b,e*][1,4]oxazepin-5-yl)propyl]-1-piperazine ethanol hydrochloride, its trifluoromethyl analog, and 5-[(2-dimethylamino)ethyl]-5,11-dihydrodibenz[*b,e*][1,4]oxazepine maleate were present in organs in greater concentrations than in blood, particularly in the brain, liver, lungs, and melanin-containing portion of the eye consisting of the combined retina, choroid, and sclera. These same compounds were bound to various extents to melanin granules of beef eyeball *in vitro*. 7-Chloro-5,11-dihydrodibenz[*b,e*][1,4]oxazepine-5-carboxamide, which bears a carboxamide substituent, was neither localized in any tissues of dogs, relative to concentrations in blood, nor bound to melanin granules *in vitro*. It is concluded that the presence of the carboxamide side chain alters the affinity of 7-chloro-5,11-dihydrodibenz[*b,e*][1,4]oxazepine-5-carboxamide for tissues, especially those containing melanin.

Keyphrases □ Dibenzoxazepines, carboxamide and other side chains—tissue distribution, affinity for melanin, dogs □ Carboxamide-substituted dibenzoxazepines—tissue distribution in dogs, affinity for melanin □ Tissue distribution—dibenzoxazepines, melanin affinity, dogs □ Melanin tissue distribution—dibenzoxazepines, dogs □ Ocular tissue distribution—dibenzoxazepines, dogs

It is generally accepted that substituted phenothiazines can adversely affect the eye and skin when large amounts are ingested chronically (1–3). Some compounds, like chlorpromazine, produce primarily opacities of the lens and cornea, whereas others, like thioridazine, can produce a loss of vision by their effect on the retina. Because of these past findings, studies were conducted with some substituted dibenzoxazepines that have exhibited CNS activity in animals (4, 5). The results of these studies show that the presence of a carboxamide side chain alters the localization of the compound and/or its metabolites in the tissues of dogs, including the melanin-containing portion of the eye, as well as the binding of the parent molecules to melanin granules of beef eyeball *in vitro*.

METHODS AND MATERIALS

Purity and Specific Activity of Compounds—The radioactive compounds studied had the following chemical names, radiochemical purities, and specific activities, respectively: I, 4-[3-(7-chloro-5,11-dihydrodibenz[*b,e*][1,4]oxazepin-5-yl)propyl]-1-piperazine-$^{14}C_2$-ethanol hydrochloride, 96%, 21.6 μc./mg.; II, 4-[3-(7-(trifluoromethyl)-5,11-dihydrodibenz[*b,e*][1,4]oxazepin-5-yl)propyl]-1-piperazine-$^{14}C_2$-ethanol hydrochloride, 99%, 24.9 μc./mg.; III, 5-[(2-dimethylamino)ethyl-1,2-$^{14}C_2$]-5,11-dihydrodibenz-[*b,e*][1,4]oxazepine maleate, 99%, 6.9 μc./mg.; and IV, 7-chloro-5,11-dihydrodibenz[*b,e*][1,4]oxazepine-5-^{14}C-carboxamide, 99%, 5.0 μc./mg.

Surgical Preparation of Dogs—Purebred male beagles were anesthetized with 30 mg./kg. of sodium pentobarbital administered intravenously. A catheter was inserted into the bladder for the collection of urine. The radial vein was then cannulated, and infusion of the following solution was begun at the rate of 3 ml./min.: mannitol (100 g.), potassium dihydrogen phosphate (200 mg.), potassium hydrogen phosphate (900 mg.), sodium pentobarbital (25.5 mg./kg. of body weight), and sufficient water to make 2 l. Mannitol was included to ensure an adequate flow of urine. A midline incision was made, and the entrance to the gallbladder was clamped at its juncture with the common bile duct. A polyethylene

catheter was then inserted about 1.27 cm. (0.5 in.) into the common bile duct. The catheter was tied in place, and the midline incision was closed with wound clips.

Analysis of Blood—A sample (0.2 ml.) of heparinized blood was digested in 0.5 ml. of 1 *N* NaOH by heating overnight at 80°. The digest was then bleached with 30% hydrogen peroxide, neutralized with 0.2 ml. of 2-ethylhexanoic acid, and counted with 15 ml. of scintillator (6).

Collection and Analysis of Urine, Bile, and Feces—Purebred beagles (9–11 kg.) and monkeys, *Macaca mulatta* (3.2–4.1 kg.), were fasted overnight prior to drug administration and were housed in metabolism cages[1] designed to allow the separate collection of urine and feces. All animals were dosed between 8 and 10 a.m. Samples of feces were homogenized with 2–3 volumes of methanol. About 50–100 mg. of homogenate was transferred to a weighed scintillation vial, and 1 ml. of a solubilizer[2] was added. The sample was shaken for at least 1 hr., bleached with 30% hydrogen peroxide, and counted in 15 ml. of scintillator (6). Samples of urine and bile were counted directly in 15 ml. of scintillator (6).

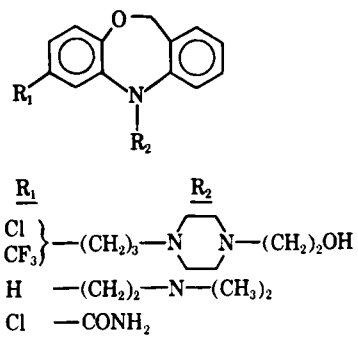
Analysis of Tissue—Samples of brain tissue were homogenized in water, using an all-glass Potter–Elvehjem homogenizer; about 200 mg. of homogenate was transferred to a weighed scintillation vial and digested in 2 ml. of solubilizer. Certain portions of the eye (lens, cornea, aqueous and vitreous humors, and combined retina, choroid, and sclera) were digested directly in 2 ml. of solubilizer. Similarly, representative portions of omental fat, skin (without hair), and the adrenal gland were digested directly in the solubilizer. Samples of tissue from the heart, kidneys, liver, lungs, thigh muscles, and testes were analyzed by first grinding the tissue in a meat grinder and then solubilizing about 100 mg. of the well-mixed sample in 2 ml. of solubilizer. All samples of tissue were counted in 15 ml. of toluene scintillator solution, which contained 5 g. 2,5-diphenyloxazole and 0.3 g. 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene/l. of toluene. Since these analytical methods are normally reproducible to within 5%, replicate determinations were not routinely performed.

Binding to Melanin Granules *In Vitro*—Melanin granules were prepared from the eyes of oxen, according to the method of Potts (7). Since IV is only slightly soluble in water (I, II, and III are readily water soluble), *all* compounds were first dissolved in 100% dimethylformamide. Then a stock solution of each drug at a concentration of 3.5×10^{-3} *M* in 10% dimethylformamide–90% 0.0165 *M* sodium phosphate buffer, pH 6.0, was prepared. The final incubation mixtures, for drug concentrations of 0.75×10^{-4} and 1.5×10^{-4} *M*, contained 2.15 and 4.30 ml. of the stock drug solution and 6.85 and 4.70 ml. of buffer, respectively, and 1 ml. of a suspension of melanin granules (7.1 mg. dry weight/ml.). Thus, the final concentration of dimethylformamide was 2.15% at 0.75×10^{-4} *M* and 4.30% at 1.5×10^{-4} *M*. The final pH was about 6.9. Samples were incubated, with shaking, at 25° for 30 min. and then centrifuged at 35,000×*g* for 15 min. Aliquots of the resulting supernate and of a control sample prepared with water, instead of with melanin granules, were counted in Bray's scintillator. Control experiments with I, II, and III, carried out in an entirely aqueous medium, indicated that the presence of dimethylformamide at the concentrations employed did not appreciably alter the binding of these compounds to melanin granules.

Counting of Samples—Radioactivity in each sample was measured by a liquid scintillation spectrometer[3]. Counting efficiency was determined with automatic external standardization and the use of previously prepared quench curves.

RESULTS

Dogs with externalized bile ducts were dosed intravenously, each with one of the four dibenzoxazepines (5 mg./kg.). The single animals treated with each compound were sacrificed 7 hr. after they had been dosed, and selected tissues were examined for their content of radioactivity (Table I). After the administration of I, II, and III, radioactivity, considered relative to that present in the blood, was highly localized in tissues. The highest concentrations of radioactivity were located in the pigmented portion of the eye,



Compound	R_1	R_2
I	Cl	$-(CH_2)_3-N\langle\rangle N-(CH_2)_2OH$
II	CF_3	
III	H	$-(CH_2)_2-N-(CH_3)_2$
IV	Cl	$-CONH_2$

structures of substituted dibenzoxazepines

consisting of the combined retina, choroid, and sclera, and in the liver and lungs. Most of the other tissues contained levels of radioactivity that were several times those present in the blood but not as high as those just mentioned. By contrast, levels of radioactivity in most of the tissues of the dog that had been dosed with IV were actually lower than those present in the blood, except for the liver and kidneys, the organs of excretion. For IV, there was no localization of radioactivity in the combined retina, choroid, and sclera.

The distribution of radioactivity was examined in selected tissues of intact animals that had been dosed orally with each of the four dibenzoxazepines. The animals were sacrificed 7 or 14 days after being dosed, at which time the residual levels of radioactivity in tissues were determined (Table II). For I, both dogs and monkeys were studied. As it had been after the intravenous administration of I, radioactivity was still localized in certain tissues relative to that present in the blood, most notably in the combined retina, choroid, and sclera of both dog and monkey. For II, radioactivity was localized in the pigmented portion of the eye and in most of the other tissues as it was after the administration of I. The apparent half-life for the elimination of radioactivity from these tissues was about 7 days for most of the tissues and substantially longer for the combined retina, choroid, and sclera. After the administration of III, radioactivity also persisted in the pigmented portion of the eye and in several of the other tissues. The distribution of residual radioactivity in tissues of dogs following the administration of IV, however, was quite different from that observed for the other three dibenzoxazepines. Here, with the marginal exception of the cornea, none of the tissues contained levels of radioactivity higher than that present in the blood and, in most cases, only traces of radioactivity were present.

The distribution of radioactivity after the oral administration of IV was also studied in tissues of intact dogs that were sacrificed 3.5 hr. after being dosed (Table III). Once again, the distribution

Table I—Distribution of Radioactivity in Tissues of Dogs with Externalized Bile Ducts 7 hr. after Intravenous Dosing

Tissue	I[a,b]	II[a,b]	III[a,b]	IV[a,b,c]
	mcg. Drug Equivalents/g. Tissue			
Brain	2.90	3.66	1.86	0.52
Lungs	12.30	24.56	7.43	1.15
Omental fat	0.64	1.57	3.17	1.27
Skin	1.03	1.36	1.94	1.04
Kidneys	3.44	4.83	3.41	1.76
Liver	11.43	14.87	7.67	4.77
Testes	5.60	8.04	5.51	0.90
Heart	2.38	2.95	1.81	0.87
Adrenals	3.19	5.18	4.47	1.17
Skeletal muscle	1.04	1.92	0.96	0.84
Cornea	0.55	0.70	2.35	0.39
Lens	0.13	0.10	1.15	0.35
Aqueous humor	2.34[d]	2.36[d]	0.05	0.15
Vitreous humor			4.31	0.08
Combined retina, choroid, and sclera	23.14	15.01	19.03	0.50
Blood, mcg./ml.	0.45	0.65	0.65	0.98

[a] Five mg./kg. as a 5-min. infusion into the radial vein. [b] Male. [c] Drug administered in 9.6 ml. of 50% aqueous dimethyl sulfoxide. [d] Aqueous and vitreous humors were combined before analysis.

[1] Porter-Mathews Co.
[2] NCS Solubilizer, Amersham-Searle.
[3] Packard Tri-Carb, model 3380.

Table II—Residual Radioactivity in Tissues of Dogs and Monkeys after Oral Administration of Some Substituted Dibenzoxazepines

Tissue	I (14 Days[a]) Dog[b]	I (14 Days[a]) Monkey[c]	II (7 Days), Dog[b]	II (14 Days), Dog[b]	III (7 Days), Dog[b]	IV (7 Days), Dog[d]
	mcg. ± *SE* Drug Equivalents/g. Tissue					
Brain	0.30 ± 0.07	0.067 ± 0.011	0.21 ± 0.00	0.14 ± 0.01	0.016 ± 0.008	0.007 ± 0.007
Lungs	0.34 ± 0.05	0.13 ± 0.03	0.38 ± 0.02	0.19 ± 0.03	0.128 ± 0.040	0.078 ± 0.045
Omental fat	0.12 ± 0.01	0.061 ± 0.013	0.35 ± 0.14	0.15 ± 0.03	0.274 ± 0.253	0.000
Skin	0.16 ± 0.02	0.14 ± 0.03	0.36 ± 0.04	0.18 ± 0.01	0.118 ± 0.020	0.032 ± 0.018
Kidneys	0.33 ± 0.04	0.78 ± 0.67	0.46 ± 0.04	0.22 ± 0.02	0.153 ± 0.011	0.016 ± 0.009
Liver	1.41 ± 0.24	0.69 ± 0.18	2.17 ± 0.03	1.03 ± 0.11	0.510 ± 0.092	0.24 ± 0.04
Skeletal muscle	0.22 ± 0.03	0.11 ± 0.01	0.23 ± 0.02	0.14 ± 0.01	0.049 ± 0.025	0.000
Cornea	0.12 ± 0.01	0.063 ± 0.01	0.15 ± 0.02	0.094 ± 0.009	0.000	0.000
Lens	0.049 ± 0.004	0.032 ± 0.001	0.056 ± 0.005	0.040 ± 0.002	0.008 ± 0.001	0.021 ± 0.009
Aqueous humor and vitreous humor	0.075 ± 0.006[e]	0.033 ± 0.016[e]	0.10 ± 0.01[e]	0.13 ± 0.04[e]	0.000	0.000
Combined retina, choroid, and sclera	0.96 ± 0.28[f]	3.96 ± 1.40	1.04 ± 0.24[f]	0.80 ± 0.05[f]	0.308 ± 0.054[f]	0.070 ± 0.041
Blood, mcg./ml.	0.12 ± 0.02	0.067 ± 0.010	0.15 ± 0.01	0.066 ± 0.013	0.043 ± 0.005	0.15 ± 0.01

[a] The time when the animals were sacrificed is cited in parentheses. [b] Dogs were given 5 mg./kg. by gavage. Two dogs of each sex were employed, except for III for which one male and two females were used. [c] Monkeys (one of each sex) were given 2.5 mg./kg. by nasogastric intubation. [d] Dogs (two of each sex) were given 10 mg./kg. by capsule. [e] Aqueous and vitreous humors were combined before analysis. [f] Concentrations in the combined retina, choroid, and sclera were divided by the concentrations in blood. These ratios were statistically different ($p < 0.025$) by Student's t test from concentrations found in dogs dosed with IV.

of radioactivity found in the tissues of these dogs was very different from that seen for the other substituted dibenzoxazepines. Here, only the liver and kidneys contained levels of radioactivity that were higher than that present in the blood. Radioactivity was rather uniformly distributed in most of the other well-perfused tissues, with the exception of the brain in which low levels were found. Thus, the results obtained with intact dogs dosed orally were similar to those found with the dog having the externalized bile duct that had been dosed intravenously. This finding suggests that the administration of IV intravenously in 50% aqueous dimethyl sulfoxide did not influence the distribution of IV in tissues of dogs.

To determine whether the mode of excretion of the four dibenzoxazepines had any bearing on the distribution of the parent compound or its metabolites in tissues, the excretion of radioactivity in the urine and feces or bile was examined following the administration of each compound (Table IV). The data show that dogs with externalized bile ducts that had been dosed intravenously excreted radioactivity in the urine and bile in a manner very similar to that found in the urine and feces of the dogs dosed orally. Dogs dosed with the nonpiperazine-containing compounds, III and IV, excreted much more radioactivity in the urine than did dogs dosed with I and II, compounds substituted with the piperazine-containing side chains.

To provide additional evidence for the affinity of the four substituted dibenzoxazepines for the melanin-containing portion of the eye, each radioactive parent dibenzoxazepine was incubated *in vitro* with melanin granules from beef eyeball (Table V). Whereas I, II, and III were significantly bound to the malanin granules, IV was bound only to an extent that is within the error of the method.

Table III—Distribution of IV and/or Its Metabolites in Tissues of Intact Dogs

Tissue	Male[a], mcg./g.	Female[a], mcg./g.	Mean, mcg./g.
Brain	0.12	0.046	0.083
Lungs	0.73	0.62	0.68
Omental fat	0.42	0.17	0.30
Skin	0.92	0.53	0.73
Kidneys	4.12	1.87	3.00
Liver	6.47	4.18	5.33
Testes	0.52	—	0.52[b]
Heart	0.56	0.33	0.45
Adrenals	0.59	0.38	0.49
Skeletal muscle	0.51	0.29	0.40
Cornea	0.19	0.13	0.16
Lens	0.070	0.036	0.053
Aqueous humor	0.15	0.088	0.12
Vitreous humor	0.062	0.083	0.073
Combined retina, choroid, and sclera	0.46	0.36	0.41
Blood, mcg./ml.	1.21	0.72	0.97

[a] Dogs were given 10 mg./kg. of IV in a gelatin capsule. The dogs were sacrificed 3.5 hr. after dosing. [b] Single value only.

DISCUSSION

A number of studies showed that chlorpromazine (8–10) and other phenothiazines (11) are localized in various tissues, particularly in the brain, lungs, and liver. The substituted dibenzoxazepines, I, II, and III, and the dibenzoxepin, doxepin (12), are all localized in tissues of dogs to various degrees. In addition, all of these compounds bind to the pigmented portion of the eye. Bolt and Forrest (13) reported on the interaction of chlorpromazine and 7-hydroxychlorpromazine with melanin. The persistence of radioactivity in the fetal and maternal eyes, but not in other tissues, of mice given ^{35}S-chlorpromazine was also reported (14). It is not known if a common mechanism underlies the formation of cataracts and the development of retinopathy. However, chlorpromazine-induced cataracts have been studied experimentally in both albino and pigmented guinea pigs (15). On the basis of those studies, it was concluded that the development of cataracts had no apparent relationship to the presence of melanin. Thus, the binding of compounds to melanin apparently should not be considered as an indicator of potential cataractogenesis.

The excretion of the dibenzoxazepines reported in the present studies appears to follow the pattern established for the phenothiazines (16). Thus, those compounds substituted with piperazine-containing side chains (I and II) were excreted primarily in the feces or bile, whereas the excretion of a compound substituted with the aliphatic amine side chain (III) was shifted toward the urinary route. Compound IV, which bears the carboxamide side chain, was appreciably excreted in the urine after intravenous administration. After oral dosing, IV is incompletely absorbed; nevertheless, 23% of the dose was excreted in the urine, a considerably larger fraction than that excreted in the urine of the dogs dosed with I and II (5–9% of the dose) (17).

An earlier report described the metabolism of I and II in dogs (17). No significant differences could be found in the excretion, distribution, or biotransformation of these two compounds, which differ by the fact that I contains a chloro substituent on the tricyclic ring system whereas II bears a trifluoromethyl group. The binding of these two compounds to melanin granules *in vitro* was comparable. Likewise, III, but not IV, bound to melanin granules *in vitro*. Interestingly, I, II, III, and IV all appear to undergo hydroxylation on the tricyclic ring system, since mass spectral studies of metabolites of I and II showed the presence of the hydroxyl group (17), and other experiments in this laboratory indicated that glucuronide conjugates of all four compounds were excreted in the bile of dogs. For the phenothiazines, chlorpromazine (9) and fluphenazine (18), 7-hydroxylation has been identified as a prominent metabolic pathway. Additional findings in this laboratory showed

Table IV—Excretion of Radioactivity by Dogs after Oral or Intravenous Administration of Some Substituted Dibenzoxazepines

Compound[a]	Dose, mg./kg.	Route[b]	Time of Sacrifice	Percent of Dose ± *SE*		
				Urine	Feces or (Bile)	Total
I (1)	5	i.v.[c]	7 hr.	5.82	(68.01)	73.83
I (4)	5	p.o.	14 days	9.30 ± 1.07	83.28 ± 4.80	92.58 ± 4.50
II (1)	5	i.v.[c]	7 hr.	4.25	(59.58)	63.83
II (4)	5	p.o.	14 days	5.34 ± 0.50	80.88 ± 2.86	86.22 ± 2.42
III (1)	5	i.v.[c]	7 hr.	35.40	(22.76)	58.16
III (2)	5	p.o.	7 days	55.04 ± 9.47	33.96 ± 7.52	89.00 ± 1.95
IV (1)	5	i.v.[c]	7 hr.	31.55	(46.60)	78.15
IV (4)	10	p.o.	7 days	23.25 ± 0.71	62.12 ± 4.15	85.37 ± 3.74

[a] The number of dogs is indicated in parentheses. [b] All drugs were administered orally in aqueous solution by gavage, except IV which was given in capsules. Intravenous dosing for all drugs except IV was as an aqueous solution infused over a 5- or 10-min. interval. IV was administered in 9.6 ml. of 50% aqueous dimethyl sulfoxide. [c] These dogs had their bile ducts externalized.

Table V—Binding of Some Substituted Dibenzoxazepines to Beef Eyeball Melanin Granules *In Vitro*

Compound	Percent Bound[a]	
	0.75×10^{-4} *M*	1.5×10^{-4} *M*
I	52.6	40.1
II	44.1	35.8
III	23.5	19.5
IV	1.3	1.1

[a] The numbers cited are the average of two or three replicate determinations with the same granule preparation. The percent bound is based on the total radioactivity present in the incubation mixture.

that little, if any, unchanged I, II, III, or IV was excreted in the urine and bile of dogs dosed intravenously. Thus, it seems likely that I, II, and III can bind to melanin *in vitro* and that metabolites of these same compounds can bind to the melanin-containing portion of the eye *in vivo*.

In animals, IV has antidepressive, rather than neuroleptic, properties (5) that are similar to some of those exhibited by doxepin and amitriptyline. The latter two compounds bind to melanin, although not to the extent found for chlorpromazine (12), an electron donor that forms charge-transfer complexes with melanin (9, 19, 20). It is suggested that the carboxamide side chain in some way alters the affinity of IV and its metabolites for tissues, including those that contain melanin.

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