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A COMPARISON OF THE CONCENTRATIONS OF CERTAIN NITROFURANS IN THE AQUEOUS HUMOUR AND CEREBROSPINAL FLUID OF THE DOG

BY

J. A. BUZARD AND J. D. CONKLIN

From the Biological Research Division, The Norwich Pharmacal Company, Norwich, New York

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The penetration of various substances into cerebrospinal fluid has been studied extensively (Mayer, Maickel & Brodie, 1959; Rall, Stabenau & Zubrod, 1959; Brodie, Kurz & Schanker, 1960; Rall & Zubrod, 1962) while only a number of isolated reports of the transfer of certain drugs into the aqueous humour have appeared (Langham, 1951; Bleeker & Maas, 1958; Furgiuele, Sery & Leopold, 1960). In a few instances, limited results suggest that the concentrations of drugs attained in the aqueous humour and cerebrospinal fluid may be quite similar (Constant, 1961; Dayton, Brand, Taller & Mark, 1961). In this study, the concurrent concentrations in both the aqueous humour and cerebrospinal fluid of the dog have been determined for a series of nitrofuran derivatives. The results show that these two fluids accumulate these drugs similarly, with drug-to-drug differences which may be related to their physical properties.

METHODS

Mongrel dogs of either sex, weighing from 20 to 30 kg, were used. The method of obtaining aqueous humour was similar to that of Krause & Yudkin (1930). Instead of ether and amytal anaesthesia, pentobarbitone sodium was used and no local anaesthetic agent was applied to the eyeball. The sclerocorneal junction in the upper and outer quadrant of the eye was pierced with a 27-gauge, 0.5-in. needle attached to a 2-ml. syringe. Samples of cerebrospinal fluid were taken from the cisterna magna with a 17-gauge, 3.5-in. needle attached to a 50-ml. syringe. Blood was taken by venepuncture with heparinized syringes, and the plasma was removed by immediate centrifugation.

The drugs were infused intravenously into a saphenous vein at doses ranging from 4 to 10 mg/kg/hr, and samples of aqueous humour, cerebrospinal fluid and blood were collected at the end of the infusion period. The infusion studies were carried out with solutions of the sodium salts of nitrofurantoin [1-(5-nitrofurfurylideneamino)hydantoin], NF-160 [5-methyl-1-(5-nitrofurfurylideneamino)hydantoin] and NF-183 [5-butyl-1-(5-nitrofurfurylideneamino)hydantoin] in 5% dextrose solution. The *p*H of such solutions ranged from 6.0 to 8.0. Furaltadone [(±)-5-morpholinomethyl-3-(5-nitrofurfurylideneamino)oxazolid-2-one] and its optical isomers (NF-602, laevorotatory, and NF-409, dextrorotatory) and NF-189 [1-(2-dimethylaminoethyl)-1-(5-nitrofurfurylideneamino)urea] were administered as solutions (*p*H 6.0) of the hydrochloride in 5% dextrose solution. The structures of these compounds are shown in Table 4.

All drug solutions were infused at a rate of 1.91 ml./min. All infusions were carried out for at least 1 hr in order to ensure adequate opportunity for transfer. A blood sample was taken before and after obtaining the aqueous humour and cerebrospinal fluid. Not more than 15 min elapsed between samples, and the concentrations of these two samples were within 10% of each other. The plasma concentration reported

represents an average of these two samples. Control samples of aqueous humour and cerebrospinal fluid were taken from a dog after a 3-hr infusion of 5% dextrose. A sample of control plasma was collected from each dog at the beginning of every experiment.

In each experiment the aqueous humour collected from both eyes was pooled. The average amount of aqueous humour removed from each eye during one experiment was 0.8 ml., while 6.0 to 8.0 ml. of cerebrospinal fluid was withdrawn in most experiments. An interval of at least 30 days was maintained between experiments to allow each dog to recuperate. Although re-use of animals for such studies has been avoided on general principles by some (Davson, 1956), no evidence of significant changes in distribution ratios was demonstrable in the dogs used in the present studies. The results obtained originally, and in the same animals 30 days later, are presented in Table 1.

TABLE 1

DRUG CONCENTRATION RATIOS IN AQUEOUS HUMOUR (AH) AND CEREBROSPINAL FLUID (CSF) COMPARED WITH PLASMA, AND VALUES FOR THE TEST REPEATED AFTER 30 DAYS

	Concentration ratio (after 4 hr infusion)			
	AH/Plasma		CSF/Plasma	
Compound	Original	30 days	Original	30 days
Nitrofurantoin	0·07	0·09	0·07	0·07
NF–189	0·23	0·28	0·24	0·26
NF–183	0·06	0·05	0·16	0·13
NF–160	0·21	0·13	0·25	0·18
Furaltadone	0·35	0·39	0·46	0·48

The samples of aqueous humour, cerebrospinal fluid and plasma were analysed by the colorimetric method of Buzard, Vrablic & Paul (1956). This method measures the nitrofurfurylidene moiety common to all the drugs used (Buzard, Conklin, O'Keefe & Paul, 1961b). The specificity of this method was established by chromatographic comparison of plasma from experimental animals and of control plasma to which the authentic compound had been added. To a 1.0-ml. sample of plasma withdrawn after a 4-hr infusion, 4.0 ml. of 95% ethanol was added and the supernatant fluid was reduced to 1.0 ml. by evaporation. Samples of control dog plasma to which authentic drug had been added were treated similarly. A 0.5-ml. aliquot of each was spotted on Whatman No. 1 paper and subjected to ascending chromatography for 15 hr at room temperature. The solvents used were: 95% ethanol, *n*-butanol and 0.5 N-acetic acid (1 : 4 : 1);

TABLE 2

R_F VALUES FROM PAPER CHROMATOGRAPHY OF PLASMA CONTAINING NITROFURANS

On six of sixty separate chromatographic strips, a barely discernible yellow spot, not intensified by ammonia, was also visible in the experimental samples. This is believed to be a non-nitrofuran metabolite, similar to that reported with other nitrofurans, which does not react in the phenylhydrazone method (Olivard, Valenti & Buzard, 1962)

		R_F in solvents	
Compound	Plasma	Acidic	Basic
Nitrofurantoin	Experimental	0·41	0·16
	Authentic	0·43	0·16
NF–189	Experimental	0·24	0·80
	Authentic	0·26	0·77
NF–183	Experimental	0·89	0·60
	Authentic	0·82	0·58
NF–160	Experimental	0·67	0·33
	Authentic	0·68	0·35
Furaltadone	Experimental	0·60	0·69
	Authentic	0·59	0·68

and 95% ethanol, *n*-butanol and 0.5 N-ammonium hydroxide (1 : 4 : 1). After drying, the papers were exposed to ammonia vapour to render the nitrofuran spots visible (Janovsky, 1891). The R_F for the single spot seen with each drug under the conditions described is given in Table 2.

The plasma protein binding of furaltadone, nitrofurantoin, NF-160 and NF-183 in the dog was reported previously (Buzard, Conklin & Buller, 1961a). Using this same procedure, the plasma binding of NF-189 was estimated on samples withdrawn at the end of a 60-min constant infusion of the drug and was determined at each of two plasma concentrations, representative of the concentrations encountered in the investigation. Proof of the stability of the drug in this procedure was obtained by simultaneously incubating an aliquot of the original plasma sample in a glass tube in the dialysis bath. This sample retained 98% of its original concentration.

The chloroform-buffer partition coefficient was determined for each compound and used as an index of lipid solubility. The solvent was purified by washing with equal volumes of 0.1 M-phosphate buffer, *p*H 7.4. Solutions of the compounds in the chloroform-washed 0.1 M-phosphate buffer, *p*H 7.4, were shaken in closed glass tubes with an equal volume of buffer-washed chloroform for 30 min. The phases were separated by centrifugation and the distribution ratio determined from the concentration of drug remaining in the separated phases.

RESULTS

The passage of certain nitrofurans into the cerebrospinal fluid of the dog has been established (Paul, Paul, Bender, Kopko, Harrington, Ells & Buzard, 1960). Preliminary studies with nitrofurantoin and furaltadone showed that both compounds also are capable of entering the aqueous humour of the dog (Table 3). These results indicate that both fluids

TABLE 3
NITROFURANTOIN AND FURALTADONE IN AQUEOUS HUMOUR

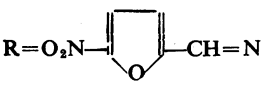
Compound	Experiment	Infusion time (hr)	Concentration in Aqueous humour (mg/l.)	Concentration in Plasma (mg/l.)
Nitrofurantoin	Dog 30	1	0·8	6·9
	Dog 25	2	1·2	16·7
Furaltadone	Dog 25	1	1·5	8·0
	Dog 12	2	4·3	12·3

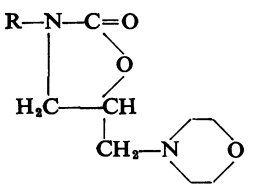
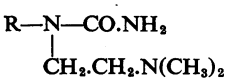
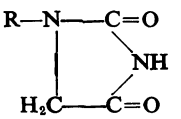
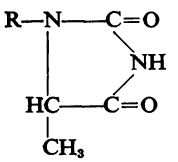
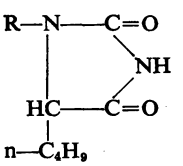
accumulate the drugs similarly and that a difference exists between the rates of accumulation of nitrofurantoin and furaltadone in aqueous humour, comparable to that observed with both compounds in cerebrospinal fluid.

These two compounds, and three others with intermediate physical properties (Table 4), were selected for further study. Simultaneous comparisons of the concentrations of the five nitrofurans in the aqueous humour and cerebrospinal fluid were then made (Table 5). With all five compounds, the concentrations attained in the aqueous humour and cerebrospinal fluid were less than those observed in the plasma. For each drug, the concentrations in the aqueous humour and cerebrospinal fluid were quite similar, in agreement with reports on other drugs (Constant, 1961; Dayton *et al.*, 1961).

Furaltadone, the nitrofuran which exhibited the highest aqueous humour/plasma and cerebrospinal fluid/plasma ratios, is a racemic mixture of two optically active forms. The effect of this variable was investigated by studying NF-409 (dextrorotatory) and NF-602

TABLE 4
PHYSICAL PROPERTIES OF SELECTED NITROFURANS



Compound	Structure	*pKa*	Plasma binding (%)	Water solubility (mg/l.)	Partition coefficient chloroform/buffer
Furaltadone		5·0	5	753	15·0
NF-189		6·4	0	1,975	2·5
Nitrofurantoin		7·2	12	201	0·1
NF-160		7·4	42	183	0·8
NF-183		7·6	80	70	43·2

(laevorotatory). All three of these forms behaved similarly (Table 6), suggesting that optical specificity is not involved in the regulation of aqueous humour and cerebrospinal fluid concentrations.

Several authors have suggested, on the basis of observations with unrelated compounds that entry into the aqueous humour may be a function of factors such as the protein binding, lipid solubility and dissociation constant of the drug (Langham, 1951; Furgiuele *et al.*, 1960). That these factors govern the transfer of drugs into the cerebrospinal fluid has been well established (Mayer *et al.*, 1959; Rall *et al.*, 1959; Brodie *et al.*, 1960).

As shown by the results in Table 5, differences in the drug concentration ratios of aqueous humour and cerebrospinal fluid to plasma exist among these compounds. When a correction for plasma protein binding is applied (Table 7), the concentration ratios become more nearly the same, indicating that plasma protein binding is a major factor governing the concentra-

TABLE 5

CONCENTRATION OF SELECTED NITROFURANS IN THE AQUEOUS HUMOUR (AH) AND CEREBROSPINAL FLUID (CSF) OF THE DOG

Each compound was studied in four dogs infused respectively for 1, 2, 3 or 4 hr

Compound	Time (hr)	Concentration (mg/l.) in Plasma	AH	CSF	Ratio AH/plasma	Ratio CSF/plasma
Furaltadone	1	9·1	2·1	3·7	0·23	0·41
	2	12·1	4·2	4·6	0·35	0·38
	3	14·2	6·3	5·7	0·44	0·40
	4	19·9	6·7	7·8	0·34	0·39
					Mean 0·34	Mean 0·40
NF–189	1	7·4	2·0	1·5	0·27	0·20
	2	9·5	3·0	2·2	0·32	0·23
	3	14·5	3·6	2·5	0·25	0·17
	4	11·6	2·7	3·2	0·23	0·28
					Mean 0·27	Mean 0·22
Nitrofurantoin	1	21·5	2·2	1·1	0·10	0·05
	2	24·0	1·2	0·9	0·05	0·04
	3	33·5	4·2	1·6	0·13	0·05
	4	55·0	3·8	4·8	0·07	0·09
					Mean 0·09	Mean 0·06
NF–160	1	10·8	1·7	1·1	0·16	0·10
	2	13·4	2·0	1·6	0·15	0·12
	3	17·5	2·5	2·2	0·14	0·13
	4	17·9	3·7	2·4	0·21	0·13
					Mean 0·16	Mean 0·12
NF–183	1	13·9	1·2	1·0	0·09	0·07
	2	21·9	1·3	1·5	0·06	0·07
	3	27·8	1·5	1·8	0·05	0·07
	4	25·2	1·4	1·2	0·06	0·05
					Mean 0·06	Mean 0·06

TABLE 6

COMPARISON OF THE CONCENTRATION OF THE OPTICAL ISOMERS OF FURALTADONE IN THE AQUEOUS HUMOUR (AH) AND CEREBROSPINAL FLUID (CSF)

Furaltadone values are from Table 5, and are means and standard deviations. The results with NF–602 and NF–409 represent experiments with one dog for each compound, infused for 3 hr, and are not significantly different from those obtained with furaltadone ($P>0·05$)

Compound	Concentration ratio AH/Plasma	CSF/Plasma
Furaltadone (racemic)	0·34±0·05	0·40±0·01
NF–602 (laevo-form)	0·36	0·47
NF–409 (dextro-form)	0·40	0·44

tion of these drugs in both fluids. The minor differences in ratios remaining after this correction appear to be related primarily to the lipid solubility of the unbound drug (Table 7).

It has been pointed out that drug concentrations attained in cerebrospinal fluid are regulated by the rates of entry and exit of the drug (Davson, 1956; Pappenheimer, Heisey & Jordan, 1961; Rall & Zubrod, 1962; Schanker, Prockop, Schou & Sisodia, 1962). Since drug-to-drug differences in the concentration ratios of the aqueous humour and cerebrospinal fluid to plasma were obtained, the rates of disappearance for some of the drugs from both fluids were determined.

TABLE 7

CONCENTRATION OF NITROFURANS IN THE AQUEOUS HUMOUR (AH) AND CEREBROSPINAL FLUID (CSF) OF THE DOG WITH CORRECTION FOR PLASMA PROTEIN BINDING

The ratios (means and standard deviations) were derived from the unbound plasma concentration as calculated from the "% binding" values in Table 4 and the total plasma concentration given in Table 5. Partition coefficients are rounded off from values in Table 4, because the partition coefficient is, at best, an index of *in vivo* lipid solubility. Un-ionized percentages refer to *p*H 7·4

Compound	Average ratio		Partition coefficient (range)	Un-ionized (%)
	AH/unbound plasma	CSF/unbound plasma		
Furaltadone	0·36±0·05	0·42±0·01	>10	100
NF-183	0·32±0·04	0·32±0·03		60
NF-160	0·29±0·03	0·21±0·01	1 to 10	50
NF-189	0·27±0·02	0·22±0·03		90
Nitrofurantoin	0·10±0·02	0·06±0·02	<1	40

Three of the compounds, representing extremes in physical properties, were infused intravenously for 4 hr. At 30 min after the infusion was completed, a cannula was inserted into the cisterna magna, a 1.0-ml. sample of cerebrospinal fluid was taken and aqueous humour was removed from one eye. This procedure was repeated, using the other eye, 30 min later. Cerebrospinal fluid samples were also taken at 30-min intervals for the next hour. With this procedure, the rate of disappearance of the compounds, expressed as %/min, were for aqueous humour: 0.59 (furaltadone), 0.61 (nitrofurantoin) and 0.67 (NF-183); and for cerebrospinal fluid: 0.54 (furaltadone), 0.57 (nitrofurantoin) and 0.58 (NF-183). The results of this study show that these compounds apparently leave the aqueous humour and cerebrospinal fluid at similar rates.

DISCUSSION

The purpose of this study was to provide a simultaneous comparison of the accumulation of drugs by the aqueous humour and cerebrospinal fluid. The results presented show that, while a given drug was accumulated equally by the aqueous humour and the cerebrospinal fluid, each drug accumulated in these fluids at a rate which was primarily related to the extent of plasma binding. The degree of lipid solubility of the drug was related to the rate of accumulation of unbound drug. The three compounds with the most different physical properties disappeared from the aqueous humour and cerebrospinal fluid at essentially the same rate, suggesting that the observed differences in accumulation were a result of differences in rates of entry into these fluids.

The disappearance rates of these compounds from aqueous humour and cerebrospinal fluid can be used to estimate the turnover rates for the fluids. The turnover rates thus obtained for the cerebrospinal fluid (0.54 to 0.58%/min) are in good agreement with estimates obtained in the dog of 0.4 to 0.7%/min with ^{24}Na (Wang, 1948; Davson & Spaziani, 1960) or inulin (Schanker *et al.*, 1962), suggesting that these drugs leave by diffusion and are not subject to active transport processes as are some drugs (Pappenheimer *et al.*, 1961). Very few results have been published on the rate of turnover of aqueous humour in the dog. Wang (1948) reported a value of 2.5%/min in the anaesthetized dog, using ^{24}Na, while a value of 1.3%/min has been reported in the unanaesthetized dog (Davson, 1956). The results from these compounds, based on only two points, indicate an aqueous humour turnover rate of about 0.6%/min. The results with nitrofurantoin and

furaltadone also indicate that lipid solubility may not be a major factor in the passage of drugs out of the aqueous humour or the cerebrospinal fluid, in agreement with Mayer, Maickel & Brodie (1960) and Davson & Spaziani (1960).

While conclusions regarding the mechanism of egress of these compounds from the aqueous humour and cerebrospinal fluid cannot be drawn from the present study, it is of interest that the suggestion of Pappenheimer *et al.* (1961), that a weak acid transport system, comparable to that of the proximal renal tubule, exists for the secretion of weak acids out of the cerebrospinal fluid is not supported by these studies. Nitrofurantoin, a weak acid shown to be secreted by the proximal renal tubule transport system (Buzard, Bender, Nohle, Humphrey & Paul, 1962) was apparently removed from the cerebrospinal fluid at the bulk flow rate. Furaltadone, a weak base not subject to renal tubular secretion (unpublished results), was removed from the cerebrospinal fluid at about the same rate. A detailed analysis of the rates and mechanism of egress of these compounds from the aqueous humour and cerebrospinal fluid will be the subject of further studies.

SUMMARY

1. An investigation of the concentration of a series of nitrofuran derivatives in the aqueous humour and cerebrospinal fluid of the dog revealed that both fluids accumulate the drugs similarly.

2. Differences in individual drug concentration were noted which may be related to the physical properties of the drugs.

3. Results are also presented which suggest that these compounds disappear similarly from both the aqueous humour and the cerebrospinal fluid, despite their differing properties.

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