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FLUOROMETRIC DETERMINATION OF GUANISOQUIN IN BIOLOGICAL FLUIDS AND SOME OBSERVATIONS OF ITS PHYSIOLOGICAL DISPOSITION IN THE RAT AND THE DOG

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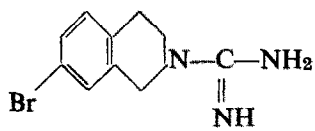
Abstract—A fluorometric method for determining guanisoquin in plasma, urine, and tissues, based upon the coupling of guanisoquin with ninhydrin in alkaline solution to form a highly fluorescent product, is described. Limited studies of the excretion and distribution in the rat and the dog indicated that the drug is very poorly absorbed orally, rapidly removed from the blood, and extensively bound to most tissues, with the exception of brain and adipose tissue.

GUANISOQUIN (7-bromo-1,2,3,4-tetrahydroisoquinoline-2-carboxamidine sulfate) has been reported to be an effective antihypertensive agent.[1] The hypotensive effect was considered to be primarily due to catecholamine depletion and blockade of sympathetic transmission at the postganglionic level. This report deals with the method of determination of guanisoquin in biological samples and some studies of the excretion, distribution, and metabolism of guanisoquin.



Guanisoquin

EXPERIMENTAL*

Assay procedure. Urine and plasma samples were diluted with water (1:5) prior to assay. Tissue samples were homogenized with nine parts of water in a Waring Blendor. Fecal samples were homogenized with six parts of water in a Waring Blendor. To the appropriate sample (2 ml), in a 50-ml glass-stoppered centrifuge tube, 0·5 ml of 0·2 N sodium hydroxide was added, followed by 8 ml of purified chloroform.† The tube

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† Distilled over sodium carbonate, passed through alumina and silica gel columns and then successively washed with 1 N HCl, 1 N NaOH, and finally water. Ethyl alcohol (0·5 ml/100 ml chloroform) was added, and it was stored at 4° in absence of light.

was shaken vigorously for 1 min by hand,* centrifuged, and the aqueous layer removed by aspiration. A portion (5 ml) of the chloroform layer was transferred into a 50-ml glass-stoppered centrifuge tube containing 8 ml of 0·5 N hydrochloric acid. The tube was shaken mechanically for 20 min, centrifuged, and 2 ml of the aqueous phase transferred into a 16 × 150 mm test tube containing 1 ml of 1% ninhydrin solution.† Potassium hydroxide solution‡ (1 ml) was added to the test tube and mixed immediately. Seven minutes after the addition of potassium hydroxide solution, 3 ml of the solution was transferred into a quartz cuvette and read in an Aminco-Bowman spectrophotofluorometer preset to a convenient sensitivity, with a quinine sulfate solution ($2{\cdot}57 \times 10^{-4}$M, 0·1 N sulfuric acid). The activation and fluorescence wavelengths were set at 400 and 510 mμ respectively. Drug concentrations in unknown samples were determined graphically from fluorescence readings on a calibration curve constructed by carrying known amounts of guanisoquin through the complete assay.

Paper chromatography. System 1: a descending system on Whatman 4 paper; the mobile phase is ethyl acetate:*n*-butanol:water (9:2:1). System 2: a descending system on Whatman 4 paper; the mobile plase is *n*-butanol:DEA:water.

Animals. Distribution and excretion experiments were carried out in male albino rats (Charles River) and female mongrel dogs maintained in metabolism cages with free access to food and water. Urine and feces were collected from the cages. For tissue distribution studies, the rats were sacrificed at various intervals by a blow on the head, and selected tissues were removed and immediately frozen.

RESULTS AND DISCUSSION

A number of reactions, such as the Jaffe,[2] the diacetyl-α-naphthol,[3] the Sakaguchi,[4, 5] and the sodium nitroprusside–potassium ferrocyanide,[6] have been used for the determination of guanidinium compounds. Unfortunately, these chromogenic reactions are nonspecific, and determination of the individual members of the guanidinium group requires chromatographic separation, chemical degradation, or specific enzymatic destruction of the compound.[7] The assay for guanisoquin described in this report was based on the knowledge that, in strongly alkaline media, the five-membered ring of ninhydrin opens to form *o*-carboxyphenylglyoxal,[8] which combines with the guanidine group to give a fluorescent product.[7] The order of fluorescent intensity of the adduct is N,N-disubstituted guanidine > N-monosubstituted guanidines > guanidine. The addition product of guanisoquin with ninhydrin in alkaline solution was found to be a highly fluorescent material with two sharp excitation peaks at 310 and 400 mμ. The two emission peaks have maxima at 410 and 510 mμ. The fluorescence due to the addition product, with an excitation wavelength of 400 mμ and fluorescence at 510 mμ, reached maximal intensity 5 min after KOH was added, and then declined very slowly (Fig. 1). The loss of fluorescence from 5 to 10 min after addition of KOH was negligible. It is best to keep the time of taking fluorimeter readings within this period, because the loss of fluorescence thereafter is significant. The fluorescence intensity obeyed Beer's law over the range of 1·0–25·0 μg/ml. The precision of the determinations within the above limits corresponds to deviations of less than 5 per

* Efficient and rapid extraction at this stage is imperative.

† Ninhydrin (1 g) in 95% ethyl alcohol (99 ml).

‡ Potassium hydroxide (10 g) in 95% ethyl alcohol (90 ml).

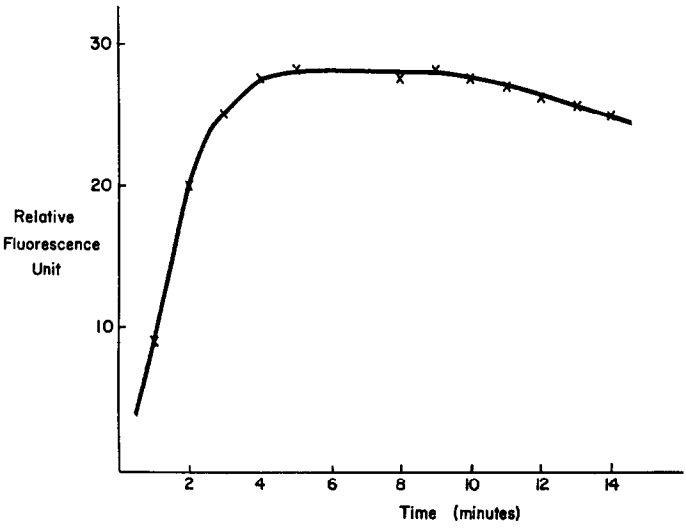

FIG. 1. Development of fluorescence of the guanisoquin-ninhydrin adduct with time.

cent. The method is sensitive enough to detect guanisoquin in concentrations as low as 0·3 μg/ml. Naturally occurring guanidinium compounds present in blood and urine that might interfere with guanisoquin determination are guanidinoacetic acid, guanidine, methylguanidine, creatine, and creatinine. The fluorescence intensities of these compounds in aqueous alkaline solution in the presence of ninhydrin are listed in Table 1, together with their normal values in human blood and urine.[5] Among these

TABLE 1. FLUORESCENCE OF NATURALLY OCCURRING GUANIDINES IN THE FLUORESCENT NINHYDRIN REACTION AND THEIR NORMAL AVERAGE VALUES IN BLOOD AND URINE OF ADULT MALE HUMAN

Compound	Relative* fluorescence unit	Blood† (mg/100 ml)	Urine† (mg/day)
Guanisoquin	7·0		
Guanidine	1·7	<0·04	<2
Methylguanidine	2·9	<0·02	<1
Creatinine	1·5	0·6	95-100% of picric acid value
Creatine‡	1·0	2·7	<150 mg/day

* Solution, 0·02 mg/100 ml.
† See Reference 5.
‡ Aqueous alkaline solution heated in boiling water bath for 1·5 hr.

interfering compounds, only guanidine and methylguanidine are soluble in chloroform to any appreciable extent, and the other interfering compounds are therefore removed by extraction of alkalized samples. Guanidine and methylguanidine present in blood and urine do not interfere with guanisoquin determination significantly, because of their low concentration and weak fluorescence. The fluorescences of the

reagent blank and varying tissue blanks were all negligible. The recovery of guanisoquin from urine, plasma, and tissue homogenates was greater than 65 per cent. The recovery from fecal samples was 55 per cent.

Plasma concentrations, urinary excretion, and biliary excretion of guanisoquin in the dog

The time course of plasma drug concentrations after i.v. administration of guanisoquin (25 mg/kg) is shown in Fig. 2. The drug was very rapidly removed from the

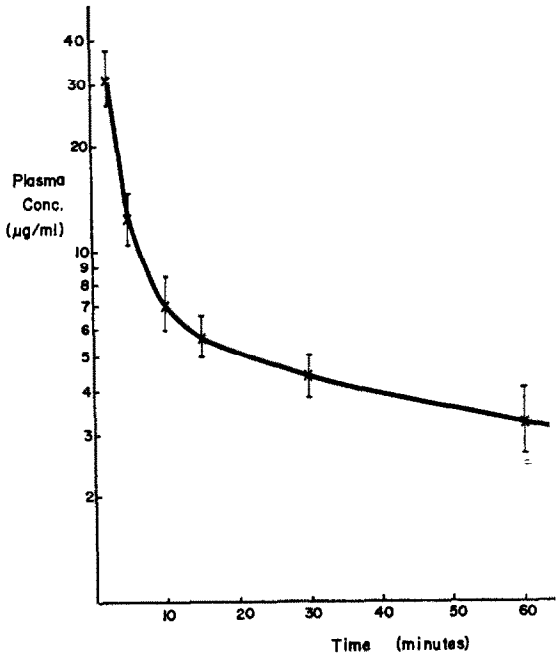

FIG. 2. Plasma concentration of guanisoquin in the dog after a dose of 25 mg/kg intravenously.

blood. Assumed that plasma volume is 35 ml/kg of body weight, it may be estimated that 10 min after an intravenous injection, less than 2 per cent of the administered dose remained in the blood of the dog. The rate of disappearance of guanisoquin was sharply reduced after 10 min, and the concentration in the blood declined exponentially with an estimated half-life of 1 hr. In view of this rapid removal. it is not surprising to find that after oral administration of guanisoquin (50 mg/kg) only a trace, (less than 0·5 μg/ml), of the drug was detected in the blood between 2 and 7 hr after administration, and essentially no drug was detected in the blood after 24 hr.

Indications that the rapid disappearance of guanisoquin from the blood was due to tissue localization rather than excretion or metabolism were obtained from excretion studies. The renal and biliary excretion after i.v. administration (25 mg/kg) of guanisoquin is shown in Table 2. At 2 hr, when less than 2 per cent of the administered drug

TABLE 2. RENAL AND BILIARY EXCRETION OF GUANISOQUIN BY DOGS RECEIVING 25 mg/kg INTRAVENOUSLY

Dog No.	Time after administration (min.)	Biliary excretion				Urinary excretion			
		Vol. (ml)	Concn (μg/ml)	Recovery		Vol. (ml)	Concn (μg/ml)	Recovery	
				(mg)	(%)			(mg)	(%)
1	0	1·10	0·0	0·000	0·000	4·3	0·0	0·0	0·0
	15	0·40	3·4	0·001	0·003	0·0	0·0	0·0	0·0
	30	0·12	21·5	0·003	0·001	0·0	0·0	0·0	0·0
	60	1·80	46·0	0·082	0·030	11·4	44·0	0·5	0·2
	120	3·00	104·0	0·312	0·110	37·0	190·0	7·0	2·6
				Total	0·141%			Total	2·8%
2	0	0·25	0·0	0·000	0·000	3·3	0·0	0·0	0·0
	15	1·10	4·0	0·003	0·001	0·8	2·5	0·0	0·0
	30	0·60	44·8	0·026	0·010	0·0	0·0	0·0	0·0
	60	1·0	50·0	0·50	0·020	5·0	120·0	0·6	0·2
				Total	0·031%			Total	0·2%

remained in the blood, less than 0·15 per cent of the dose had been excreted in the bile and less than 3 per cent excreted in the urine. There is the possibility, however, that being a strongly basic compound, significant amounts of administered guanisoquin might have diffused into the acidic stomach contents. Urinary excretion after oral administration (25 mg/kg) of guanisoquin is shown in Table 3. During the 5-day

TABLE 3. URINARY EXCRETION OF GUANISOQUIN BY DOGS RECEIVING 25 mg/kg ORALLY

Dog No.	Hours post dose	Guanisoquin excreted	
		(mg)	(%)
1	1	0·0	0·0
Weight—	2	1·3	0·7
7·7 kg	4	6·1	3·2
	6	10·2	5·3
	8	6·9	3·6
	24	2·2	1·1
	48	1·1	0·6
	72	3·2	1·7
	96	0·2	0·0
	120	1·1	0·6
		Total	16·8
2	0	0	0
Weight—	2	1·1	0·6
7·5 kg	4	2·8	1·5
	6	3·4	1·8
	8	0·8	0·4
	24	5·4	2·9
	48	6·3	3·4
	72	4·6	2·4
	96	1·2	0·6
	120	0·9	0·5
		Total	14·1

period, an average of 15·5 per cent of the administered dose was excreted in the urine A significant quantity of guanisoquin was still being excreted in the urine on the fifth day. Chromatography of chloroform and butanol extracts of alkalized urine concentrates were shown to contain guanisoquin. No other guanidinium compound (diacetyl-α-naphthol positive) was detected.

Excretion and tissue distribution of guanisoquin in the rat

Tissue concentrations of guanisoquin in the rat after i.v. administration (50 mg/kg) are shown in Table 4. In the rat, as in the dog, guanisoquin was very rapidly removed

TABLE 4. GUANISOQUIN TISSUE DISTRIBUTION IN THE RAT AFTER INTRAVENOUS ADMINISTRATION (50 mg/kg)

Time after administration (hr):	2		6		18	
Tissue*	Concn (μg/g)	Recovery (%)	Concn (μg/g)	Recovery (%)	Concn (μg/g)	Recovery (%)
Adrenal glands	150·0	0·05	87·0	0·0	60·0	0·0
Kidney	124·0	2·0	21·5	0·4	5·5	0·1
Heart	109·0	1·0	33·0	0·3	10·0	0·1
Liver	41·0	2·9	9·5	0·7	4·0	0·3
Muscle	26·5	0·3	17·0	0·3	8·0	0·1
Brain	9·0	0·1	<2·5	0·0	<2·5	0·0
Fat	6·0	0·1	5·5	0·0	5·0	0·1
Plasma	4·0	0·1	2·5	0·1	<0·5	0·0
Carcass†	44·7	55·7	29·0	32·5	16·8	21·6
Urine	360·0	6·6	280·0	25·7	230·0	31·9
Feces			120·0	4·0	252·0	14·0
Cage washings						1·6‡
Total in tissues		64·2		34·3		22·3
Total excreted		6·6		29·7		47·4
Total recovered		70·8		64·0		69·7

* Pooled samples from four rats for each time period.

† Less skin and hair.

‡ A total of 0·52 mg recovered from cage washings.

from the blood. Two hr after intravenous administration, less than 1 per cent remained in the blood. At this time the drug was highly localized in various tissues, with the notable exception of brain and fat. The failure of guanisoquin to affect brain norepinephrine levels[1] could thus be explained. The highest concentration was found in the adrenal gland, and the high level was maintained for the longest period of time in this tissue. The rate of disappearance of the drug from the body was reduced after 6 hr, and the levels in the body declined, with a half-life greater than 20 hr. Being a strongly basic compound, guanisoquin was not expected to be highly localized in tissues. However, guanethidine, also a guanidine, has been reported to be extensively localized in tissues.[9, 10] The retention of guanisoquin by heart and adrenal resembles the concentration pattern for exogenous norepinephrine[11] as well as for guanethidine. It would seen possible that guanisoquin, like guanethidine,[12] may be localized in norepinephrine-storage particles, which may be relevant for its mode of action.

It was noted that 18 per cent of the intravenously administered guanisoquin was excreted in the feces. It is not known whether the guanisoquin was introduced into the gastrointestinal tract by biliary excretion or by direct diffusion of the strongly basic compound into the acidic stomach contents.

The low concentration of the drug in blood made it impossible to study drug adsorption by measuring blood concentrations. The poor absorption of guanisoquin from the rat's alimentary tract was evident, however, from the excretion studies (Tables 4 and 5). The urinary excretion of guanisoquin after oral administration was

TABLE 5. EXCRETION OF GUANISOQUIN BY RATS RECEIVING 50 mg/kg ORALLY

Time after administration (hr)	Sample*	Recovery	
		(mg)	(%)
0–24	Feces	23·60	48·8
	Urine	0·24	0·5
24–72	Feces	9·97	20·6
	Urine	0·10	0·2
72–96	Feces	0·25	0·5
	Urine	<0·10	<0·2
96–120	Feces	0·15	0·3
	Urine	Trace	0·0
120–144	Feces	Trace	0·0
	Urine	Trace	0·0
		Total	71·1

Total in feces = 70·1%.
Total in urine = 0·9%.

* Pooled samples from four rats.

found to be less than 1 per cent, whereas greater than 32 per cent was excreted in the urine when the same dose was given intravenously. This conclusion is supported by the extensive fecal recovery observed after oral administration.

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