Biochemical Pharmacology, 1962, Vol. 11, pp. 747–753. Pergamon Press Ltd., Printed in Great Britain.

# SOME ASPECTS OF THE PHARMACOLOGY OF QUININE IN THE DOGFISH

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(*Received* 23 *February* 1962; *accepted* 16 *April* 1962)

**Abstract**—The pharmacology of quinine has been studied in the dogfish, *Squalus acanthias*. Quinine was concentrated approximately 100-fold in the red and white blood cells, as compared with the plasma. This probably was not due to nonionic diffusion. Quinine was also highly concentrated in the acid pericardial fluid in a manner consistent with nonionic diffusion. Brain and muscle also contained high concentrations of quinine. Moderate binding to plasma proteins was demonstrated. Slow elimination of quinine was also observed.

THE PHARMACOLOGICAL disposition of quinine was studied in detail during World War II after the development of methods for its quantitative assay in biological fluids. The distribution, metabolism, and excretion of this compound have been studied in avian and mammalian species.[1–4] It seemed of interest to us to determine some aspects of the pharmacology of quinine in a lower vertebrate, the spiny dogfish. In this marine Elasmobranch, quinine was highly concentrated in the circulating blood cells, in the acid pericardial fluid, and in muscle and brain. Binding to plasma protein was moderate, and quinine left the plasma at a relatively slow rate.

## MATERIALS AND METHODS

The dogfish (*S. acanthias*) were freshly caught and kept in a live-fish car in Laboratory Cove or in a tank with a rapid flow of sea water. USP quinine dihydrochloride (10–20 mg/ml, pH 5) was injected into the paravertebral muscles of the dogfish between the dorsal fins. The samples were collected and prepared for analysis in the following manner. Ventricular fluid* (VF) was obtained by direct puncture of the cerebellum after removal of a portion of the cartilagenous skull. Extradural fluid* (EDF) was obtained by percutaneous puncture of the cartilage in the dorsal midline 3 cm caudal to the tip of the nose. Pericardial fluid* (PCF) was obtained after a midline incision just caudal to the xiphoid process. The heart could be located by a finger inserted into this incision. The needle was passed between the finger and the heart. One or more ml of clear fluid was aspirated. Plasma (PL), whole blood (WB), buffy coat (BC), and red cells (RBC) were obtained by puncture of the dorsal artery or vein of the tail. The following procedure was used. The blood vessel was entered with an 18-gauge needle attached to a heparinized syringe. After a free flow of blood was established, the first syringe was removed and its contents used for whole-blood analysis. A second syringe was attached to the needle and the blood withdrawn with

* Information on the nature of these fluids has been published.[5, 6]

a minimum of negative pressure. The blood in this syringe was gently transferred to plastic tubes. These were kept in a controlled-temperature water bath until centrifugation in a temperature-controlled centrifuge for 60 min at 1200 × *g*. The top portion of the plasma was then removed without disturbance of the buffy coat and served as the plasma sample. The remainder of the plasma was removed and discarded. The large buffy coat—a mixture of leukocytes, erythrocytes, and plasma—was aspirated. The packed red cells were then sampled. Muscle and brain samples (300–900 mg) were blotted, weighed, and homogenized in 3% NaOH.

The VF, EDF, PCF, BC, RBC, and plasma samples were diluted from 1 : 30 to 1 : 150 with 3% *m*-phosphoric (MPA) acid and centrifuged. The concentration of quinine in the sample was then determined fluorometrically by the method of Brodie and Udenfriend.[7] Since all the fluid samples contained salts that quench the fluorescence of quinine, blanks and standards (using samples from fish not injected with quinine) accompanied each set of determinations. Quinine concentrations were calculated on the basis of these standards. The muscle and brain samples in 30 ml of 3% NaOH were extracted with 1 vol of benzene. The quinine was then re-extracted from the benzene into 3% MPA, and the fluorescence of the MPA solution was measured as above. Blanks and standards accompanied each experimental set of determinations.

The distribution between buffer and benzene of the fluorescent material from dogfish plasma 18 hr after quinine administration was compared with authentic quinine at pH 5·5, 6·8, and 7·8.

At pH 5·5 the buffer/benzene ratio with authentic quinine was 19·0, with dogfish "quinine," 20·0. At pH 6·8 comparable figures were 0·62 and 0·60; at pH 7·8, 0·21 and 0·21. Further, all fluorescence in the sample of dogfish "quinine" was quenched by excess NaCl.

Protein binding of quinine was determined by the Rehberg technic[6] or by equilibrium dialysis of plasma containing quinine against Elasmobranch saline.

Measurement of pH in blood, VF, and PCF was made with a Beckman G pH meter and a 32066 glass electrode at 15–17 °C.

## RESULTS

The toxicity of quinine given intramuscularly to dogfish is shown in Table 1. At 50 mg of quinine/kg the pregnant female dogfish frequently aborted.

TABLE 1. TOXICITY OF QUININE FOR THE DOGFISH

Intramuscular administration; observation period, 18 hr

| Dose of quinine (mg/kg) | No. dead/no. injected |
|---|---|
| 40 | 0/12 |
| 50 | 1/8 |
| 60 | 1/3 |
| 75 | 2/5 |
| 100 | 3/3 |

The study on the distribution of quinine between buffer and benzene suggests that there was little or no metabolism of quinine by the dogfish into fluorescent metabolites. Metabolism to nonfluorescent compounds, of course, would not have been detected.

Plasma protein binding was performed in 13 experiments. At plasma quinine concentrations of 0·5 to 2 μg/ml 30–50 per cent of plasma quinine was nondiffusible.

Quinine found in whole blood corresponded to the quinine added (Table 2). When

TABLE 2. RECOVERY OF QUININE ADDED TO WHOLE BLOOD *in vitro*

| Whole blood (μg/ml added) | Whole blood (μg/ml found) | Plasma (μg/ml found) | Cells/ plasma* |
|---|---|---|---|
| Experiment 1 | | | |
| 17·3 | 17·1<br>17·0<br>16·5 | | |
| Experiment 2 | | | |
| 4·8 | | 1·4 | 12 |
| 9·0 | | 3·0 | 11 |
| 16·7 | | 8·6 | 6 |

* Assumed 100% recovery and a hematocrit of 20%.

the plasma of blood to which quinine had been added was analyzed, however, little quinine was found in the plasma. The hematocrit of the whole blood used was 20 per cent (a normal value for the dogfish), and it could be calculated that the cells contained 6 to 12 times the concentration of quinine found in the plasma.

This finding prompted the study of the relative concentration of quinine in plasma, red blood cells, and buffy coat after administration to dogfish. The results in Table 3

TABLE 3. DISTRIBUTION OF QUININE AMONG PLASMA, RED CELLS, AND BUFFY COAT 18 HR AFTER INJECTION

Blood handled at 0–3 °C

| Dogfish number | Dose of quinine (mg/kg) | Concentration of quinine (μg/ml) | | | | Hematocrit |
|---|---|---|---|---|---|---|
| | | Whole blood | Plasma | Red cells | Buffy coat | |
| 1 | 25 | 7·2 | 0·1 | 18 | 20 | |
| 2 | 50 | 16 | 0·4 | 49 | 83 | 22 |
| 3 | 50 | 13 | 0·6 | 54 | 38 | 23 |
| 4 | 75 | 26 | 0·3 | 93 | 105 | 18 |

were obtained. From 63 to 350 times as much quinine was found in the blood cells as in plasma. Since the blood was handled at 0–3 °C, and the normal temperature of the dogfish was about 15–16 °C, this experiment was repeated. The blood was divided immediately after withdrawal and half was processed at 0–3 °, the other half at 15–17 °C. At both temperatures (Table 4) high cell to plasma quinine ratios were

found. In each instance higher plasma concentrations and lower red cell quinine concentrations were found at the higher temperature.

Similar studies were performed in which blood from dogfish was held and processed at room temperature or at 30 ° as well as at 0–3 °C. At 30° quinine floods out of the cells into the plasma (Table 5). It is important to note that the whole blood values were constant, irrespective of time and temperature, as were the plasma values when the separated plasma was stored.

TABLE 4. DISTRIBUTION OF QUININE BETWEEN PLASMA AND RED CELLS WHEN HANDLING AND CENTRIFUGATION WERE PERFORMED AT 0·3° AND 15–17 °C

| Dogfish number | Dose of quinine (mg/kg) | Whole blood | Quinine concentration (μg/ml) | | | |
|---|---|---|---|---|---|---|
| | | | At 0–3 °C* | | At 15–17 °C* | |
| | | | Plasma | Red cells | Plasma | Red cells |
| 5 | 25 | 10·0 | 0·1 | 36 | 0·2 | 23 |
| 6 | 25 | 8·0 | 0·04 | 67 | 0·08 | 55 |
| 7 | 85 | 40·0 | 0·8 | 250 | 1·5 | 192 |
| 8 | 85 | 24·0 | 1·3 | 145 | 2·2 | 97 |

* Temperature at which the plasma and red cells were held during the process of separation.

TABLE 5. EFFECT OF TEMPERATURE ON THE DISTRIBUTION OF QUININE BETWEEN PLASMA AND WHOLE BLOOD OF A DOGFISH 18 HR AFTER 80 MG OF QUININE/KG

| Time after withdrawal of blood before centrifugation* | Quinine concentration (μg/ml)† | | | |
|---|---|---|---|---|
| | Temp. 0–4 °C | | Temp. 30 °C | |
| | WB | Pl | WB | Pl |
| 3 min | 39 | 0·8 | | |
| 15 min | | 0·9 | | 3·6 |
| 60 min | 42 | 1·1 | 41 | 5·4 |

* Plasma removed at 3 min and re-assayed at 60 min after being kept at 0–4 or 30 °C contained 0·9 μg quinine/ml. Centrifugation was performed at 0–2 °C.

† WB = whole blood, Pl = plasma.

In light of these findings, it was impracticable to determine directly the distribution of quinine between plasma and the pericardial fluid and the other fluids of the dogfish.* The pH of ventricular fluid and of EDF was virtually identical and differed from plasma by only 0·05 pH unit. Therefore, the concentration in VF or EDF should be representative of the plasma quinine available for diffusion and the ratio quinine in PCF/(quinine in VF (or EDF)) at equilibrium should approximate the ratio quinine in PCF/quinine in plasma water. The results in 33 dogfish are shown in Table 6. Quinine is highly concentrated within PCF. The rate of attainment of

* If the true WB/PL quinine ratio is 200/1, and 1 per cent of the cells is destroyed, then the plasma quinine concentration will increase approximately 3-fold.

equilibrium is fairly slow. The pH of the PCF of five fish was 5·6, 5·9, 6·0, 6·1, and 6·3, wherea sventricular fluid, EDF and plasma pH in these same fish was 7·55–7·65.

The lower ratios obtained when the quinine concentration in PCF was referred to EDF rather than to VF is probably due to the presence of protein in EDF[6] that would bind additional drug and result in an EDF/PL ratio greater than 1. It was found

TABLE 6. RELATIVE CONCENTRATION OF QUININE IN PERICARDIAL FLUID*

| Time after quinine (hr) | N | $C_{PCF}/C_{VF}$ Mean | $C_{PCF}/C_{VF}$ Range | $C_{PCF}/C_{EDF}$ Mean | $C_{PCF}/C_{EDF}$ Range |
|---|---|---|---|---|---|
| 1 | 3 | 1·1 | 0·8–15 | | |
| 4 | 5 | 5·0 | 3–7 | | |
| 12 | 3 | 10 | 8–13 | 12 | 10–13 |
| 16–18 | 12 | 17 | 2–20 | 12 | 2–26 |
| 24–48 | 10 | 24 | 6–90 | 19 | 6–56 |

* N is the number of fish. $C_{PCF}$, $C_{VF}$, and $C_{EDF}$ refer to quinine concentration in pericardial, ventricular, and extradural fluids, respectively.

TABLE 7. DISTRIBUTION OF QUININE IN DOGFISH 18 HR AFTER INTRAMUSCULAR INJECTION OF 50 MG/KG

| Sample | Quinine (μg/ml*) Fish 9 | Fish 10 |
|---|---|---|
| Whole blood | | 15·0 |
| Plasma | 4·8 | 1·0 |
| Extradural fluid | 0·5 | 0·6 |
| Ventricular fluid | 0·2 | 0·3 |
| Pericardial fluid | 2·0 | 13·0 |
| Brain | 11·0 | 5·1 |
| Muscle | 29·0 | 24·0 |

* Wet weight for brain and muscle; no precautions taken to prevent blood cell destruction; i.e. the blood was handled at room temperature.

experimentally that the mean EDF/VF ratio in these fish was greater than 1 after 18 hr.

An estimation of the plasma half-life of quinine can be made if VF is used as an index of free plasma quinine. Data from 15 fish are shown in Fig. 1. Quinine in VF disappears slowly in the dogfish, the half-life being about 10–12 hr.

Quinine concentrations in muscle and brain were determined 18 hr after intramuscular drug administration (Table 7). Muscle contained more quinine than did brain, although in both tissues quinine was concentrated relative to VF and EDF. In fish 9, and to a lesser extent in fish 10, no precautions were taken in handling the the blood, and it is interesting to note the low VF/PL ratios of about 0·06 and 0·02.

## DISCUSSION

The salient points of the pharmacology of quinine in the dogfish are its concentration in the red and white blood cells, its moderate binding to plasma proteins, its slow elimination, and its concentration in pericardial fluid, brain, and muscle.

The concentration in blood cells is particularly worthy of consideration. Red cells of dogfish are nucleated. Avian red cells, also nucleated, have been reported to contain

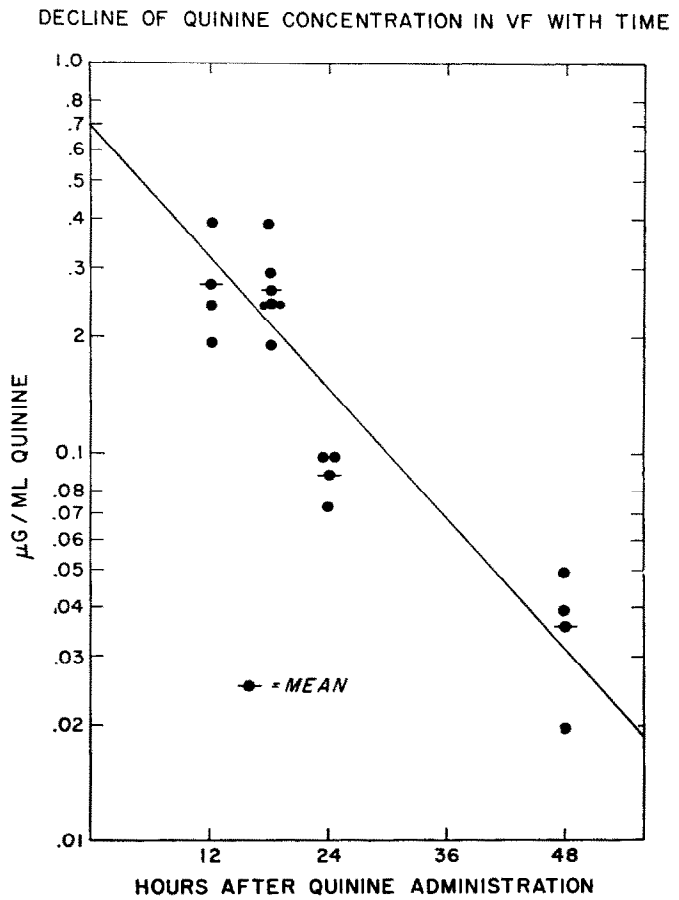


FIG. 1. Decline in quinine concentration in ventricular fluid with time after intramuscular administration of 40–50 mg quinine/kg to dogfish.

up to three times the plasma concentration of quinine.[4] In these reports no mention was made of special precautions taken in drawing and handling blood. Mammalian red cells reportedly concentrate twice as much quinine as does plasma.[2] The high degree of plasma protein binding in the mammals and birds (about 90 per cent) may, if diffusible drug is important, impede the binding to or concentration in cells. The role of the intracellular pH in red blood cells of fish is unclear. If nonionic diffusion

occurs,* an intracellular pH of 5·6 (plasma pH = 7·6) would lead to a cell/plasma ratio of 80/1. If calculated only on diffusible drug this might approach 160/1. Both of these values are in the range of those observed. The VF/brain ratio of 33/1 in experiment 1 and that of 25/1 in experiment 2 could be accounted for by an intracellular brain pH of 6·0–6·3. Such pH gradients, however, seem most unlikely, since estimates[9] of intracellular pH generally are between 6·8 and 7·1. Therefore, as with quinacrine in mammals,[10] some binding to cell constituents must be involved.

For the explanation of the high concentration of quinine in PCF, however, it seems clear that the pH gradients observed are of adequate magnitude. The range of values for PCF/VF quinine at 24–48 hr was 6–90. If nonionic diffusion, a VF pH of 7·60, and a pK of quinine of 8·4 are assumed this would represent a range of pH values for PCF of 7·25 to 5·55. This is consistent with the data reported here and elsewhere.[5] The relatively high concentrations of quinine in pericardial fluid of dogfish is therefore one of the most striking examples of the effects of nonionic diffusion.

The lower plasma protein binding of quinine in the dogfish, compared with mammals, may be explained by the absence of albumin in dogfish plasma.[6] It is interesting though, that the proteins of the dogfish plasma, presumably globulins, are able to bind significant amounts of quinine.

Attention should be drawn to the need for precaution in handling biological samples, particularly blood, prior to analysis. Throughout the early phases of this study, when few precautions were taken, plasma concentrations were high and variable, and quinine seemed to be relatively excluded from the fluids sampled. Even with utmost precaution, as little as 1 per cent cell destruction can markedly alter the plasma concentration in samples of dogfish blood containing quinine.

* Nonionic diffusion refers to a situation in which a membrane separating two compartments (inside (*i*) and outside (*o*)) is permeable to the un-ionized form of a drug, and is impermeable to the ionized moiety. The ratio of total (ionized + un-ionized) drug between these two compartments will be the inverse of the ratio of the (pH-dependent) fraction of un-ionized drug in each compartment. If $R$ = the ratio of total drug $i/o$, then, for basic drugs, the Jacobs equation will define the relationship:

$$R = (1 + 10^{pKa-pHi})/(1 + 10^{pKa-pHo}).$$

For a complete discussion see Milne *et al.*[8]

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