TECHNICAL NOTE

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Chloroquine Distribution in Postmortem Cases

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ABSTRACT: Chloroquine concentrations in blood and tissues were examined in overdose and non-overdose cases to determine appropriate ranges for interpretation. Twenty-nine literature overdose cases and 8 non-overdose literature cases were compared with this laboratory's findings. The results indicate significant postmortem redistribution of chloroquine. Combining this laboratory's results and the literature results indicates that using a liver concentration of 150 mg/kg as a cutoff between overdose and non-overdose concentrations properly identified 30 of the 34 published cases containing liver chloroquine and 19 of the 20 presented cases.

KEYWORDS: pathology and biology, chloroquine, tissue-drug concentrations, blood-drug concentrations

Chloroquine is the most widely prescribed antimalarial drug in the world today. It is also used in the treatment of rheumatoid arthritis [*1*]. The therapeutic range of chloroquine in plasma is approximately 10^{-8} to 10^{-7} *M* (0.003 to 0.03 mg/L) for antimalarial

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activity and approximately 10^{-6} *M* (0.3 mg/L) for treating rheumatoid arthritis. Its mechanism of action is believed to be related to its ability to accumulate in acidic subcellular compartments, such as food vacuoles, of the malarial parasite and to eliminate it by interrupting its intake of nutrients. Other mechanisms, such as inhibition of phospholipid metabolism, may also be involved in its antimalarial activity [*2*].

Chloroquine may be administered orally or parenterally. Following a single 300-mg intravenous dose, peak plasma chloroquine concentrations ranged from 0.478 to 1.2 mg/L. Much lower peak concentrations were found after oral administration (0.055 to 0.102 mg/L). The drug was detectable in plasma for at least 23 days after use [*3*]. Chloroquine is extensively metabolized in man, forming desmethylated, *N*-oxide and carboxylic acid metabolites. Monodesmethylchloroquine possesses activity similar to that of the parent drug against *Plasmodium falciparum* [*4*]. Urinary recovery of chloroquine is about 42 to 47% for the unchanged drug and 7 to 12% for monodesmethylchloroquine [*5*]. Dose-dependent pharmacokinetics were reported by one group [*6*], but this was not substantiated when more specific chromatographic assays were used.

Because of its widespread use and availability, it is not surprising that chloroquine has been involved in a large number of fatal overdoses [*7–18*]. Chloroquine concentrations in blood, urine, and tissues from selected cases are given in Table 1. In addition, tissue distribution data from eight cases in which the cause of death was not chloroquine intoxication are given in Table 2 [*7*].

This laboratory performs toxicological analyses on all military aircraft fatalities and mishaps to determine whether any toxicant could have influenced the accident. The information in Tables 1 and 2 is of particular significance in these investigations as chloroquine is one of the most frequently encountered drugs. However, the literature data on blood and tissue concentrations of chloroquine in non-overdose cases are still too limited to assist in interpretation of our results. The following is a collection of data obtained over the past several years on the distribution of chloroquine in non-drug-related fatalities.

Experimental Procedure

Specimens were sent to the Division of Forensic Toxicology at the Armed Forces Institute of Pathology (AFIP) by military pathologists worldwide. The decedents were on active duty in the military at the time of death. Due to the traumatic manners of their deaths and the fact that the autopsies were performed at remote locations, the integrity of the specimens could not be assured. Analyses were performed on the specimens as received regardless of their condition.

Two millilitres of biological fluid or 2 g of homogenized tissue were alkalinized with 2 mL of 0.1*N* potassium hydroxide after the addition of an internal standard (SKF-525A). The samples were then extracted with 15 mL of chlorobutane and, after shaking and centrifugation, the organic layer was removed and extracted with 3 mL of 0.2*N* sulfuric acid. The acid layer was removed, alkalinized with 2*N* potassium hydroxide, and extracted with 5 mL of chlorobutane. The chlorobutane layer was evaporated to dryness, and the residue was reconstituted with 100 μL of methanol and chromatographed [*19*].

A Hewlett-Packard 5880 gas chromatograph with a nitrogen phosphorus detector was used for chloroquine analysis. The helium carrier gas flow was 1 mL/min, using a J & W DB-5 15 m by 0.25-mm by 0.2-μm capillary column. The injector temperature was 260°C and the detector temperature was 300°C. The oven temperature began at 110°C, was held for 1 min, increased at 20°C/min to 200°C, was held for 1 min, increased at 10°C/min to 280°C, and was held for 10 min. A Hewlett-Packard 5890 gas chromatograph with a 5970 mass selective detector was used for chloroquine confirmation. The same gas chromatograph parameters just listed were used.

TABLE 1—*Chloroquine tissue distribution in overdose cases.*

Case No.	Blood, mg/L	Urine, mg/L	Liver, mg/kg	Kidney, mg/kg	Brain, mg/kg	Spleen, mg/kg	Method[a]	Reference
1	–	–	–	84	10	–	S	7
2	–	–	900	470	11	–	S	7
3	–	–	145	11	65	–	U	8
4	–	–	700	630	–	–	U	9
5	–	–	2.3	0.4	–	–	U	9
6	–	–	200	110	8.8	–	U	9
7	–	–	290	170	2.8	–	U	9
8	–	–	750	300	7.7	–	U	9
9	–	–	410	240	9.4	–	U	9
10	–	–	220	180	10	–	U	9
11	30	–	280	640	–	260	U	9
12	–	–	540	290	13	–	U	9
13	30	1700	300	170	26	–	U	9
14	3	–	150	–	–	–	F	10
15	24	–	230	160	42	400	S	11
16	–	–	280	180	11	170	S	11
17	51	–	140	30	1	290	S	11
18	21	–	–	160	24	–	S	11
19	16	20	175	70	–	–	S	12
20	12	68	344	300	–	–	S	12
21	14	–	370	–	–	–	U	13
22	–	–	650	–	–	–	U	13
23	–	–	320	–	–	–	U	13
24	8	–	247	–	–	–	U	13
25	–	–	254	91	–	–	U	14
26	4	–	71	33	–	–	S	15
27	66	–	–	864	–	–	S	16
28	33	370	169	110	–	–	G	17
29	17	516	1110	1690	90	1000	L	18

[a]Key to abbreviations:
S = spectrophotometry.
G = gas chromatography.
L = liquid chromatography.
F = spectrofluorometry.
U = not reported.
– = no data given.

TABLE 2—*Chloroquine tissue distribution in non-overdose cases.*

Case No.	Blood, mg/L	Urine, mg/L	Liver, mg/kg	Kidney, mg/kg	Brain, mg/kg	Spleen, mg/kg	Method	Reference
1	–[a]	–	58	–	7.3	–	S	7
2	–	–	57	–	6.9	25	S	7
3	–	–	6.2	0.6	0.5	–	S	7
4	–	–	24	–	3	–	S	7
5	–	–	13	3.2	1	–	S	7
6	–	–	5.5	3.4	0.7	–	S	7
7	–	–	4.3	2	1.7	–	S	7
8	–	–	2.9	5.8	2	–	S	7

[a]No data given.

Results and Discussion

Data are presented in Table 3 from 28 cases in which toxicological analysis identified chloroquine in postmortem specimens. In all cases, the causes of death were clearly established at autopsy. Twenty-three of the 28 were aircraft fatalities; the deaths resulted from multiple injuries in airplane or helicopter crashes. The causes of death in the remaining 5 cases were multiple injuries from a mortar explosion, motor vehicle accident, boating accident, gunshot wound to the head, and choking. In each case, the presence of chloroquine was interpreted as an incidental finding unrelated to the cause of death.

Although chloroquine was not involved in the deaths of these individuals, large blood concentrations of the drug were often found when their blood was assayed. Concentrations greater than 3 mg/L were found in 9 of the 16 cases in which the blood was quantitated. This is approximately one order of magnitude greater than the expected therapeutic concentrations. The mean blood concentration of the 16 cases was 5.2 mg/L (range, 0.3 to 14 mg/L). No information in the literature was found on chloroquine concentrations in postmortem blood specimens where death occurred from causes other than chloroquine overdose. In the overdose cases presented in Table 1, the average blood chloroquine concentration was 24 mg/L (range, 3 to 66 mg/L), which is substantially higher than the average blood concentration found in the presented AFIP cases.

In an attempt to provide greater information for interpretation of these concentrations, the tissue distributions of chloroquine in the AFIP cases were compared with previously published data in both overdose and therapeutic cases (Tables 1 and 2). Most of the literature chloroquine analyses were performed by spectrophotometry, and the AFIP chloroquine analyses were performed by gas chromatography. Chloroquine metabolites were not measured in the AFIP cases. While it is recognized that the less specific spectrophotometric method might result in higher concentrations, the interpretive value of the comparison is still valid. For example, liver concentrations of chloroquine in the AFIP cases ranged from 0.6 to 203 mg/kg, with an average concentration of 74 mg/kg. In previously published cases, the average liver concentration was 355 mg/kg (range, 2.3 to 1110 mg/kg) in overdoses and 25 mg/kg (range, 2.9 to 57 mg/kg) in therapeutic cases. If previously published therapeutic cases were combined with the current cases, an average of 59 mg/kg would be obtained. The ratio of overdose to therapeutic cases is about 6, which is a similar ratio to what was found in blood concentrations (4.5). Furthermore, if 200 mg/kg were used as a cutoff for identifying overdose cases, 19 of 20 AFIP cases and 27 out of 34 cases published in the literature would be correctly identified. Likewise, if a 150-mg/kg cutoff were used, 19 of the 20 AFIP cases and 30 of 34 published cases would be properly identified.

A different statistical pattern is observed if brain concentrations are studied. The average brain chloroquine concentration is 2.9 mg/kg (range, 0.5 to 7.3 mg/kg) in previously published non-overdose cases and 18 mg/kg (range, 1 to 90 mg/kg) in previously published overdose cases. In the AFIP cases, the average brain concentration was 16 mg/kg (7.3 to 66 mg/kg), which is much closer to that of overdose cases than to non-overdose cases.

One question that arises from these data is whether the postmortem blood concentrations of chloroquine are an accurate indication of drug concentrations at death. Drugs such as tricyclic antidepressants have been shown to produce higher postmortem blood concentrations than are seen antemortem [*20*]. This postmortem increase in tricyclic antidepressants may be due to release of the drug from the liver [*21*]. Chloroquine also appears in high concentrations in the liver, and postmortem release could be possible. One way to approach this problem is to compare blood concentrations obtained in non-fatal cases sent to this laboratory with concentrations obtained in fatal cases. Thirty-one non-fatal cases were positive for chloroquine in urine specimens. The blood was negative for chloroquine in 14 cases (limit of quantitation, 0.05 mg/L). The remaining 17 cases

TABLE 3—*Chloroquine distribution in non-overdose AFIP cases.*

Case No.	Blood, mg/L	Liver, mg/kg	Lung, mg/kg	Kidney, mg/kg	Spleen, mg/kg	Brain, mg/kg
1	–	203	13	21	–	66
2	–	61	11	10	8.5	7.3
3	–	128	61	–	40	8.8
4	–	143	29	16	14	7.5
5	11	131	31	27	–	–
6	6.4	101	66	19	–	–
7	–	2.7	0.9	1.3	–	–
8	14	87	–	–	–	10
9	8.4	76	–	–	–	–
10	3.9	–	–	–	–	–
11	1.3	–	–	–	–	–
12	–	1.0	–	–	–	–
13	–	8.1	–	–	–	–
14	2.1	–	–	–	–	–
15	6.3	–	–	–	–	–
16	2.9	–	–	–	–	–
17	1.7	–	–	–	–	–
18	0.9	–	–	–	–	–
19	0.4	–	–	–	–	–
20	–	53	–	–	–	–
21	–	83	–	–	–	–
22	9.4	99	–	–	–	–
23	–	28	–	–	–	–
24	–	0.6	–	–	0.7	–
25	9.5	102	8.8	9.4	2.0	38
26	0.3	120	2.4	9.9	9.7	12
27	–	26	28	1.1	14	12
28	–	30	52	44	–	36
Average	5.2	74	28	15	13	16
Standard deviation	4.4	56	23	13	13	12

had an average chloroquine concentration of 0.16 mg/L (range, 0.05 to 0.51 mg/L). This is well within the reported therapeutic range and is approximately 30 times less than blood concentrations measured in non-overdose fatalities. This vast difference between "antemortem therapeutic" and "postmortem therapeutic" strongly suggests some sort of postmortem redistribution of chloroquine. These data are supported by the findings of Rouzioux et al. [*22*], who found a factor of 10 increase in postmortem blood chloroquine concentrations in comparison with antemortem drug concentrations.

The blood and liver concentrations observed in the AFIP cases and previously published chloroquine cases are summarized in Table 4. The table demonstrates that there is no overlap between antemortem non-overdose blood and postmortem overdose blood spec-

TABLE 4—*Blood and liver chloroquine concentrations.*

	Blood Concentration, Mean (Range), mg/L	Liver Concentration, Mean (Range), mg/kg
Antemortem therapeutic	0.16 (0.05–0.51)	. . .
Postmortem therapeutic	5.2 (0.3–14)	59 (0.6–203)
Postmortem overdose	24 (3–66)	355 (2.3–1110)

imens, but that there is overlap between postmortem non-overdose and postmortem overdose cases.

On the basis of the above discussion, the following conclusions can be made:

1. Postmortem blood concentrations of chloroquine may be elevated relative to antemortem concentrations, and this elevation does not necessarily mean that a drug overdose or intoxication has occurred.
2. Liver tissue might be a more suitable specimen for interpretation of postmortem chloroquine concentrations. A liver concentration greater than 150 mg/kg would most likely indicate a drug overdose.
3. Brain tissue is not a suitable specimen for interpretation, as there is significant overlap between overdose and non-overdose cases in brain concentrations.

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