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Metabolism of chloroquine-3-^{14}C in the rhesus monkey

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IN AN earlier communication[1] the metabolism of chloroquine-4-^{14}C was studied in 3 rhesus and 1 squirrel monkey (all females). In the former species, 48–62 per cent of the label was excreted in the urine within 96 hr after an i.m. injection of a 4 mg/kg dose, and about 2 per cent was excreted in the feces. The tissues obtained on sacrificing the animals at 96 hr postmedication accounted for 28–42 per cent of the dose, bringing the total recovered to 91–96 per cent. In terms of concentration, the increasing order in the tissues was generally brain, muscle, skin, heart, bone marrow = spleen = kidney, lung, pituitary = liver, adrenal, and retina-choroid, but as per cent of dose, only skin, bone marrow, liver, and muscle were important repositories. No attempt was made to characterize the tissue residues, but it was found for both plasma and urine that only about 1/3 of the label was extractable by methods applicable to chloroquine (CQ) analysis. The remaining label in the urine was extractable at pH 5–6 with isopropanol, on saturation with sodium sulfate, and the principal compound in the extract was tentatively identified as 4-(7-chloroquinolyl)-γ-amino-*n*-valeric acid. The purpose of the present study has been to confirm and extend these observations. The development of an improved method for the extraction of acids of the type named above has facilitated the study.[2]

EXPERIMENTAL

Chloroquine-3-^{14}C (sp. act., 8·25 μc/mg base) was obtained from the New England Nuclear Co., Boston, Mass. It was administered intramuscularly to two female rhesus monkeys in doses of 3·6–3·8 mg/kg, after dilution with inactive carrier to give specific activities of 4·1 (monkey B) or 5·5 (monkey A) μc/mg base. These doses were injected in 2 ml water, with enough phosphoric acid added to effect solution. The animals were then handled and the various biological materials were analyzed, exactly as previously described,[1] the period between medication and sacrifice again being 96 hr. The analytical data are presented in tabular form: Table 1 presents the absorption–excretion data; Table 2. presents the data on tissues distribution at necropsy.

In addition to the tabulated data, the following problems were studied: (a) the nature of the labeled compounds in the liver at necropsy; (b) the distribution of the urinary excretory products among CQ and its various known metabolites; and (c) a positive identification of the urinary carboxylic acid metabolite. Problem (a) was studied by extracting an aqueous homogenate of the livers with ether, first at pH 5·5 and then at pH 11·0. The latter extract was evaporated, the residue was taken up in methanol, spotted on Brinkman F-254 silica gel thin-layer plates, and the chromatogram was developed with an ethyl acetate:isopropanol:ammonia system.[1] The location of the various spots and their content of labeled quinoline derivatives were determined with a gas flow counter. The distribution of the excretory products was studied by extracting the urine samples at pH 10, first with ethylene dichloride, and then with butanol.[2] The extracts were evaporated *in vacuo*, and the residue from the former solvent was subjected to thin-layer chromatographic analysis as for the liver extracts. The amount of label in each of the two solvent extracts also was determined by liquid scintillation counting. The third item, i.e. the nature of the carboxylic acid metabolite, was studied by administering 50 mg/kg of unlabeled CQ sulfate orally to another female rhesus monkey, and collecting urine for the next 24 hr. (Direct u.v. spectrophotometric analysis of this urine sample showed that it contained the equivalent of 24 per cent of the dose.) The butanol-extractable materials in this sample were separated as previously described,[2] the solvent was evaporated *in vacuo*, and the residue was esterified with methanol with a boron trifluoride catalyst. The esters were extracted from water into ether at pH 7·5 after which they were reduced with lithium aluminium hydride.[1] The alcohols so formed were converted to their trimethylsilyl ethers and were subjected to gas chromatographic analysis with 4 ft 3·8 per cent SE-30 columns at 220°.

RESULTS

In addition to the data presented in Tables 1 and 2, the following results were obtained relating to the 3 specific problems listed above:

(a) In the livers of both monkeys, desethyl-CQ accounted for about 63 per cent of the extractable labeled bases, unchanged CQ accounted for 26 per cent, bisdesethyl-CQ accounted for 8·5 per cent, and traces of labeled carboxylic acid derivatives were present. (See Ref. 1 for the structures of these compounds.)

TABLE 1. ABSORPTION AND EXCRETION OF ^{14}C IN RHESUS MONKEYS AFTER AN INTRAMUSCULAR DOSE (3·6–3·8 MG/KG) OF CHLOROQUINE-3-^{14}C

Plasma levels (μg base/L.)*

Time post-medication (hr)	Monkey A	Monkey B
3	268	256
6	210	166
24	85	50
48	93	50
96	29	45

Urinary and fecal excretion (% of dose)

Time post-medication (hr)	Monkey A			Monkey B		
	U	F	T	U	F	T
0–24	20·5	1·0	21·5	36·2	2·6	38·8
24–48	17·0	0·6‡	17·6	18·9	4·7	23·6
48–72	7·5	0·4	7·9	9·2	2·2‡	11·4
72–96	7·1	§	7·1	6·2	2·1	8·3
0–96	52·1	2·0	54·1	70·5	11·6	82·1

* Calculated from sp. act. of the dose administered.

† The headings U, F, and T refer to urinary, fecal, and total excretion.

‡ Three ml of castor oil was administered at 72 hr to prevent constipation.

§ No feces excreted during this period.

(b) In the urine extracts of monkey A, only traces of labeled compounds in the area R_f 0·68 to 0·74 were found. These could have been either 4-amino-7-chloroquinoline or the 4′-alcohol.[3] Unchanged CQ accounted for 5/6 of the labeled bases in both monkeys during the 0–24-hr period, but this fraction had decreased to 2/3 in the 72–96-hr period. In the meantime, the desethyl metabolite had increased from about 1/10 of the bases to 1/4; traces of bisdesethyl-CQ were present in two samples, again from monkey A only. The butanol-extractable labeled components exceeded the ethylene dichloride-extractable (using the latter solvent first[2]) by 14/5 in monkey B, but in monkey A the corresponding ratio was about 5/6. These ratios showed little change with time after medication.

(c) The butanol-extractable labeled compounds were readily esterified, but proved difficult to purify because of the low initial carboxylic acid concentrations. However, the esters obtained from the monkey that received the large dose of the unlabeled drug were readily purified. On reduction of these esters and conversion of the alcohols to trimethylsilyl ethers, the ether of the unknown alcohol was found to have a retention time identical to that of the ether of known 7-chloro-4(4-hydroxy)-methyl-butylamino)quinoline[4] (m.p. of the reference alcohol was 179–180°, uncorrected). No evidence of the presence of the trimethylsilyl ether of 7-chloro-4-[(2-hydroxy-1-methyl)-ethylamino]-quinoline was seen. This alcohol would have been formed in the reduction process if 4-(7-chloroquinolyl)-α-alanine,

suggested by Whitehouse and Böstrom as a metabolite of CQ,[5] had been present in the urine. The reference alcohol in this case (m.p. 206–210°, uncorrected; per cent N = 11·80; per cent Cl = 15·03) was prepared for us by Dr. D. Bailey.

TABLE 2. TISSUE DISTRIBUTION OF ^{14}C IN RHESUS MONKEYS 96 HR AFTER AN INTRAMUSCULAR DOSE (3·6–3·8 MG/KG) OF CHLOROQUINE-3-^{14}C

	Amount found			
	(As μg CQ base/g*)		(As % of dose)	
Tissue	Monkey A	Monkey B	Monkey A	Monkey B
Adrenal	16·7	3·2	0·1	<0·05
Bile			0·3	0·1
Bone Marrow†	1·6	2·2	1·9	2·6
Brain	1·3	0·6	0·6	0·3
Fat‡	0·2	0·7	0·3	
Heart	8·9	1·2	0·7	0·1
Kidney	4·8	2·7	0·4	0·3
Liver	23·0	7·8	11·1	4·1
Lung	8·1	4·6	1·4	0·7
Muscle§	0·9	0·4	10·4	3·5
Pituitary	16·9	5·2	<0·05	<0·05
Skin and hair‖	0·4	0·4	1·1	1·2
Spleen	3·9	1·7	0·1	<0·05
Intestinal tract			1·6	1·5
Total in tissues analyzed			30·0	14·5

* Calculated from the sp. act. of the dose administered.

† Estimated as 5% of body wt. The sample from Monkey A was in poor condition when removed.

‡ Estimated normally as 8% of body wt. However, Monkey B was virtually devoid of fat, and the sample analyzed contained a considerable amount of blood.

§ Estimated as 43% of body wt.

‖ Estimated as 12% of body wt.

COMMENT

As shown in the tables, nearly 97 per cent of the administered dose was recovered from monkey B, but only 84 per cent was recovered from monkey A. However, the total excretion of the label by monkey A was more in line with that observed earlier[1] than was the case for monkey B. The plasma level data permit only a very rough calculation of the half-life (30–36 hr), but the excretion data indicate a half-life of 1·6 days for monkey B and 3·4 days for monkey A. (The previous estimate was 3·2 days.[1]) The tissue concentrations for both monkeys were of the order one would expect from their respective rates of excretion, but the order of increasing tissue concentrations differed slightly from that observed earlier;[1] i.e. it was generally skin, brain, muscle, bone marrow, spleen, heart, adrenal, pituitary, and liver. However, again, only liver and muscle were really important repositories of the drug.

It has been demonstrated herein that the metabolism of CQ in the monkey differs from that in man in two important respects: (1) the principal metabolite is the 5-carbon side chain carboxylic acid, whereas in man[2] only a very small fraction of the dose is excreted in this form and (2) there is a gradual shift in the urinary metabolites from CQ to desethyl-CQ, whereas in man these constituents remain in a ratio of 3:1 both during and following an extended medication period.

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The biotransformation of isosorbide dinitrate in dogs and humans*

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There has been interest recently in the metabolic fate of the various organic nitrate coronary vasodilators and the mechanism by which they are inactivated. Nitroglycerin has been reported to be partially metabolized to inorganic nitrite by rabbit liver homogenates.[1] Needleman and Krantz[2] have shown that nitroglycerin is metabolized to 1,2 and 1,3-dinitroglycerin, and these compounds are resistant to further degradation. They appear as urinary metabolites after the administration of nitroglycerin and are less active than the parent compound as coronary vasodilators. Pentaerythritol tetranitrate has been shown to be denitrated[3] to pentaerythritol and its mononitrate. Previous work in this laboratory† has shown that 1-chloro-2,3-propandiol dinitrate is metabolized to a mixture of the mononitrates and is excreted as their glucuronic acid conjugates. It was of interest to see if isosorbide dinitrate (ISD) is denitrated also, and if humans as well as dogs can metabolize it.

ISD was supplied as Isordil® by Ives Laboratories, Inc. Three female mongrel dogs were fasted for 24 hr and the urine was collected as a control. The dogs were allowed food for 24 hr and on the next day 100 mg of ISD was administered orally. The dogs were again fasted for 24 hr and the urine was collected for 24 hr after the administration of the compound. Nonfasted 24-hr urine specimens were taken from each of 10 patients on a regimen of from 80–160 mg ISD/day. The test urines from both dogs and humans were evaporated to a volume of about 50 ml at 40° *in vacuo*. Each 50-ml sample was then extracted three times with 150 ml of ethyl acetate and the extracts were pooled. The solvent was then dried over anhydrous sodium sulfate, evaporated to dryness at 40° *in vacuo*, and the residue taken up in 0·5 ml of ethyl acetate. A 10-μl portion of the extracts of control dog urines, of the test dog urines, and of the test human urines was spotted on chromatographic plates coated with silica gel (0·25 mm) which had been activated at 100° for 30 min. The plates were developed by ascending techniques with two solvent systems, one a benzene:ethyl acetate (1:1) mixture, and the second a 2-propanol: ammonium hydroxide (4:1) mixture. The plates from each solvent system were sprayed with a 1% diphenylamine in methanol solution and exposed to an intense source of ultraviolet light for 5 min, for the purpose of visualizing the organic nitrate esters.

Table 1 gives the R_f values of the urine samples and reference compounds in a benzene:ethyl acetate solvent system sprayed with 1% diphenylamine, and the R_f values for a 2-propanol: am-

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