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BIOCHEMICAL PROPERTIES OF ANTI-INFLAMMATORY DRUGS—VI.

THE EFFECTS OF CHLOROQUINE (RESOCHIN), MEPACRINE (QUINACRINE) AND SOME OF THEIR POTENTIAL METABOLITES ON CARTILAGE METABOLISM AND OXIDATIVE PHOSPHORYLATION

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Abstract—Chloroquine, hydroxychloroquine and mepacrine inhibited the *in vivo* incorporation of ^{35}S into cartilage polysaccharide sulphates only after these drugs had been administered in high doses to rats for several days. Desethylchloroquine and 4-amino-7-chloroquinoline inhibited the metabolism of ^{35}S in cartilage *in vivo* within 24 hr.

Desethylchloroquine and 4-amino-7-chloroquinoline were faster-acting drugs than chloroquine in depressing the incorporation of ^{35}S into mucopolysaccharide sulphates by cartilage slices *in vitro*.

Of the heterocyclic (basic) drugs examined, only mepacrine and 4-amino-7-chloroquinoline significantly uncoupled oxidative phosphorylation. Other potential metabolites of chloroquine, which might be formed by degradation of the 4-diethylamino-1-methylbutylamino side chain, had no effect on oxidative phosphorylation.

Indomethacin and gold sodium thiosulphate (Sanochrysin) were potent inhibitors of mucopolysaccharide sulphate biosynthesis in cartilage and uncoupled oxidative phosphorylation.

MANY different classes of anti-inflammatory (antirheumatic) drugs depress the metabolism of the connective tissues.[1] Some of these drugs e.g. salicylates, phenylbutazone and indomethacin, uncouple oxidative phosphorylation (i.e. ATP* biogenesis linked to respiration) thereby depressing ATP-linked processes in these tissues such as *in vitro* and *in vivo* mucopolysaccharide sulphate biosynthesis.[2–4] Other antirheumatic drugs such as chloroquine (resochin, SN 7618) and related antimalarials, or hydrocortisone and related 11-oxycorticosteroids, also inhibit mucopolysaccharide biosynthesis *in vitro*[5] and *in vivo*[6] but they are distinguishable from the uncoupling (antirheumatic) drugs by their delayed action *in vitro*.[5] Chloroquine has no effect on the metabolism of rat rib cartilage *in vivo* in short term (24 hr) experiments, in contradistinction to other classes of anti-inflammatory drugs including steroids,[4] and is

* Abbreviations used in this article: ATP = adenosine-5′-triphosphate; dihydroxychloroquine = 7 chloro-4-[1-methyl-4-di(β-hydroxyethyl) aminobutyl-amino]-quinoline, cf. hydroxychloroquine in Fig. 1; indomethacin = 1-p. chlorobenzoyl-5-methoxy-2-methylindole-3-acetic acid; S_i = inorganic sulphate; P_i = inorganic phosphate; Porg. = organic phosphate; Rivanol (Ethodin) = 6:9 diaminoethoxyacridinium lactate; Tris = tris (hydroxymethyl) aminomethane.

known to be a slow-acting antirheumatic drug in man. This raises the question of whether or not chloroquine metabolites might be responsible for some of the pharmacological properties attributable to the drug.

We have now examined the effects of chloroquine, some related antimalarials (Fig. 1) and some potential chloroquine metabolites (Fig. 2) on (i) *in vivo* cartilage metabolism after sustained administration of these compounds to rats and (ii) *in vitro* cartilage metabolism, principally by using ^{35}S as a radiotracer. Drug action on oxidative phosphorylation was also investigated using isolated liver mitochondria.

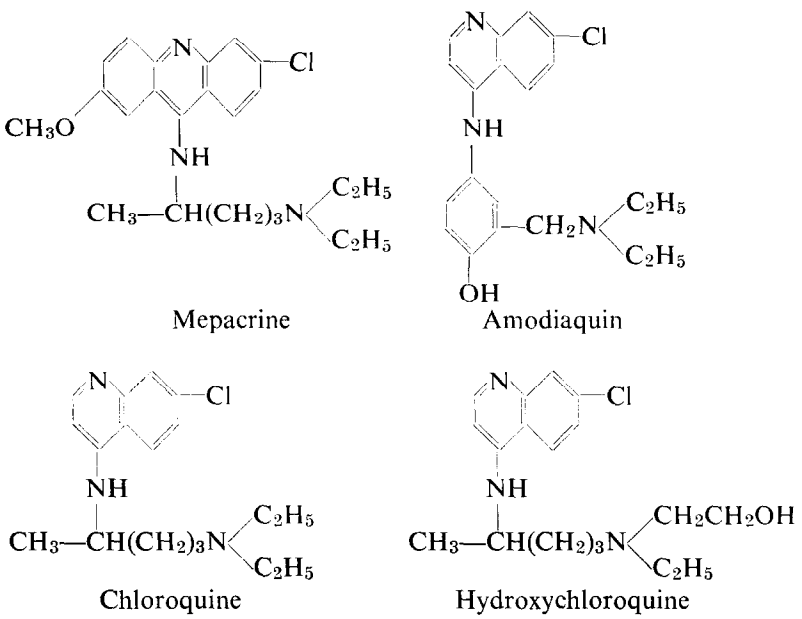

FIG. 1. Structures of some antirheumatic antimalarial drugs.

EXPERIMENTAL

The sources of the compounds were: chloroquine phosphate ('Avloclor'), Pharmaceuticals Division, I.C.I. Ltd., Wilmslow, Cheshire; chloroquine sulphate ('Nivaquine') May & Baker Ltd., Dagenham, Essex; hydroxychloroquine and its sulphate ('Plaquenil'), Winthrop Laboratories Ltd., Newcastle-upon-Tyne; hydroxychloroquine phosphate and monodesethylchloroquine (Win. 10,002; SN 13588), Dr. A. R. Surrey, Sterling-Winthrop Institute, Rensselaer, N.Y., U.S.A.; dihydroxychloroquine, Dr. R. Rutman, University of Pennsylvania, Philadelphia, U.S.A.; N-(7-chloro-4-quinolyl)-alanine (SKF 71244) and N-(7-chloro-4-quinolyl)-4-aminobutyric acid (SKF 71363), Dr. G. J. Durant, Smith, Kline & French Laboratories, Welwyn Garden City, Herts; amodiaquin (camoquine) and the mono- and di- N-oxides of mepacrine (quinacrine) and of amodiaquin, Dr. L. F. Elslager, Parke, Davis & Co., Ann Arbor, Michigan, U.S.A.; 4-amino-7-chloroquinoline, Dr. A. W. Nineham, May & Baker Ltd , Dagenham, Essex; 7-chloro-4-hydroxyquinoline, Dr. J. S. Lowe, I.C.I. Ltd , Alderley Edge Cheshire; acridone, Dr. R. M. Acheson, Oxford; gold sodium thiosulphate ('Sanochrysin'), Platinum Chemicals Ltd., Asbury Park, N.J., U.S.A.; indomethacin, Dr. L. H. Sarett, Merck, Sharp & Dohme Ltd., Rahway, N. J.,U.S.A.

Radiochemicals were purchased from the Radiochemical Centre, Amersham, Bucks. Other compounds were obtained from commercial supply houses and where necessary, recrystallised before use.

In vivo *experiments*

Drugs were administered intraperitoneally to Sprague-Dawley or Wistar rats (150–200 g) either as aqueous solutions of the phosphate salts or as solutions of the free bases in either 0·2 M hydrochloric acid or in an aqueous emulsion with 1% (v/v) Tween 20. They were administered either simultaneously with, or for several days (as a single daily dose) before and then simultaneously with, the injection of sodium sulphate-^{35}S. Urine voided by these animals during the following 24 hr was collected and processed as described,[4] to identify and assay the urinary ester sulphates-^{35}S. The animals were killed after 24 hr and the radioactivity of the costal cartilage mucopolysaccharide sulphates determined after papain digestion of their rib cages.[4] Cartilage polyuronides extracted by papain digestion were determined with an orcinol reagent.[2]

In vitro *experiments*

(i) Calf costal cartilage slices (40 mg total dry wt. or greater) were incubated with the drugs, added as aqueous solutions (together with the equivalent of sodium bicarbonate, if necessary) or from N,N-dimethylformamide (50μl), and sodium sulphate-^{35}S or glucose-U-^{14}C in at least 5 ml of a Krebs-Ringer-phosphate buffer (without sulphate ions) with shaking at 37°. Slices were also incubated with sodium phosphate-^{32}P in a Krebs-Ringer-Tris hydrochloride buffer, pH 7·4. Radioactivity of cartilage fractions and respiratory carbon dioxide was determined as described.[2, 5]

(ii) Oxidative phosphorylation was studied in the presence of drugs (added as above) using rat liver mitochondria respiring with succinate or α-oxoglutarate as substrates (initial concentration = 10 mM), as detailed.[7]

RESULTS

Drug action on cartilage metabolism in vivo (*Table* 1)

Chloroquine diphosphate had no effect upon the incorporation of ^{35}S from inorganic sulphate (S_i) into costal cartilage polysaccharides when administered simultaneously with $^{35}S_i$ or for 3 days before and then simultaneously with $^{35}S_i$. When given daily for seven days before the $^{35}S_i$, chloroquine did depress the incorporation of ^{35}S into cartilage polysaccharides of Wistar rats but only when given in high doses (100 mg/kg/day). Higher doses proved lethal to two strains of rats available to us (Sprague-Dawley and Wistar: the former was more susceptible to chloroquine).

Hydroxychloroquine phosphate was likewise toxic at doses exceeding 100 mg/kg for both rat strains. When given at daily doses of 100 mg/kg, hydroxychloroquine only affected ^{35}S incorporation into costal cartilage polysaccharides of Wistar rats when given for at least one week before administering $^{35}S_i$.

Some potential metabolites of chloroquine (Fig. 2) and of hydroxychloroquine were also administered to Sprague-Dawley rats at doses equivalent to one of 100 mg/kg chloroquine diphosphate. Two of these, N-desethylchloroquine and 4-amino-7 chloroquinoline (III, Fig. 2) did affect ^{35}S incorporation within 24 hr. Extending the period of administration before the $^{35}S_i$ was injected did not appear to increase the

TABLE 1. EFFECT OF CHLOROQUINE AND SOME OF ITS POTENTIAL METABOLITES ON (A) URINARY ESTER SULPHATE EXCRETION, AND (B) INCORPORATION OF $^{35}S_i$ INTO RIB CARTILAGE POLYSACCHARIDE SULPHATES (PS) IN RATS

$^{35}S_i$ administered on last day of drug administration. Data with S.M.D. from groups of 4 rats.

Compound injected	Dose (mg/kg/day) + duration (days)	New ester sulphates in urine	Ester sulphates-^{35}S as % total ^{35}S in urine: with drug	control	% of control	Cartilage PS-^{35}S (c.p.m./30 μg): with drug	control	% of control
Chloroquine diphosphate	100 × 1	—	17·2 ± 4·4	13·5 ± 2·1	127	81 ± 19	79 ± 8	102
	100 × 1	(+)	26·7 ± 8·0	27·0 ± 1·8	99	316 ± 13	280 ± 16	113
	100 × 4	—	26·6 ± 2·8	23·1 ± 4·4	115	207 ± 4	225 ± 22	92
	100 × 4	—	29·0 ± 5·8	21·9 ± 5·7	133	91 ± 2	85 ± 10	107
	100 × 8	—	15·3 ± 2·4	18·8 ± 2·6	82	45 ± 14	62 ± 5	73
	100 × 8	—	28·7 ± 6·5	15·9 ± 0·8	180	140 ± 32	182 ± 43	72
Hydroxychloroquine	65 × 1	—	22·4 ± 4·8	18·5 ± 3·7	121	95 ± 8	88 ± 6	108
	65 × 3	—	16·2 ± 4·1	17·3 ± 1·5	94	106 ± 15	100 ± 19	106
	65 × 9	—	17·1 ± 1·8	13·0 ± 2·4	132	46 ± 2	58 ± 6	79
Desethyl-chloroquine	60 × 1	—	11·6 ± 1·0	11·5 ± 1·7	101	107 ± 2·8	177 ± 35	60
	60 × 1	—	*	*	—	64 ± 19	88 ± 6	73
	60 × 4	—	17·4 ± 4·0	12·0 ± 3·9	145	178 ± 35	285 ± 43	62
N-(7-Chloroquinolin-4-yl)-alanine	49 × 1	—	23·7 ± 3·9	18·1 ± 3·4	131	48 ± 11	50 ± 7	96
	49 × 1	—	15·4 ± 3·4	22·8 ± 3·3	68	121 ± 21	125 ± 9	97
4-Amino-7-chloroquinoline	32 × 1	+	28·4 ± 3·1	21·9 ± 5·1	130	69 ± 8	92 ± 9	75
	32 × 1		*	*	—	86 ± 2	104 ± 3	83
	32 × 4	+ +	36·3 ± 3·6	14·8 ± 1·6	245	80 ± 30	126 ± 5	63
Mepacrine dihydrochloride	40 × 1	—	14·9 ± 2·3	14·5 ± 2·4	103	310 ± 20	292 ± 24	106
	40 × 4	—	13·6 ± 1·3	12·5 ± 3·0	109	59 ± 7	79 ± 14	75
	40 × 8	—	9·5 ± 7·3	15·5 ± 3·2	61	24 ± 18	77 ± 25	31

* not measured

magnitude of this drug effect, for a given daily dose. A third potential metabolite, N-(7-chloro-4-quinolyl)-alanine (II, Fig. 2) was apparently without any effect, when similarly administered.

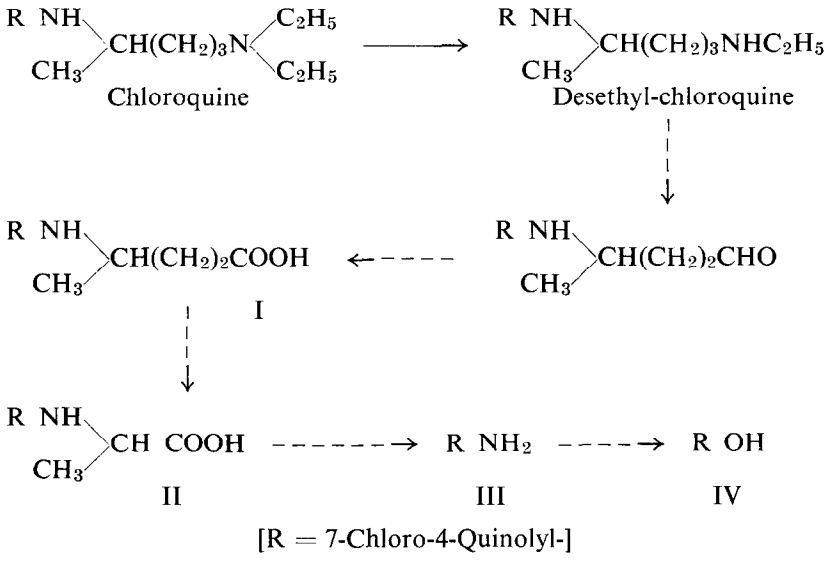

FIG. 2. A scheme for the possible metabolic degradation of chloroquine *in vivo*.

Mepacrine (quinacrine, atebrine) dihydrochloride had no effect in an acute dose of 100 mg/kg, although it is a more potent drug than chloroquine and hydroxychloroquine *in vitro* (Table 2). Like the latter quinoline bases, mepacrine did inhibit ^{35}S-metabolism by costal cartilage *in vivo* when administered for several days prior to the $^{35}S_i$.

There was no consistent increase in the amount of ^{35}S excreted in the urinary ester sulphate fraction, when ^{35}S incorporation into the cartilage polysaccharides was depressed by one of these compounds; except in the case of 4-amino-7-chlorquinoline (III). None of the other compounds listed in Table 1 when administered to our experimental rats, caused excretion of a new sulphurylated component in urine (see Ref. 4). Therefore the reduction in ^{35}S incorporation into cartilage polysaccharides must be ascribed to drug action on cartilage metabolism, rather than to the removal of ^{35}S by metabolism of the drug concerned (with the formation of sulphated excretion products), except in the possible instance of 4-amino-7-chloroquinoline. Here, the reduction in ^{35}S incorporated by the cartilage could possibly reflect a diversion of ^{35}S into other metabolic pathways since the ester sulphate-^{35}S content of the urine did rise considerably and at least one new ^{35}S-containing component was identified in the urine by two-dimensional paper chromatography[4] after administering the aminochloroquinoline.

Drug action on cartilage metabolism in vitro (*Table* 2)

The incorporation of ^{35}S from inorganic sulphate (S_i) into the polysaccharide sulphates of cartilage serves as one index of cartilage metabolism in the presence of the

drugs. Table 2 shows the effect of some antimalarials and of some other antirheumatic drugs (phenylbutazone, indomethacin, gold sodium thiosulphate) on the ^{35}S-labelling of the polysaccharide sulphates within cartilage slices, when these drugs were pre-incubated with cartilage slices for periods of up to 2 hr before adding $^{35}S_i$ to the incubation medium. Drugs known to be capable of uncoupling oxidative phosphorylation (2,4-dinitrophenol, salicylates, phenylbutazone) rapidly inhibit the incorporation of ^{35}S into the polysaccharide sulphates.[5] Table 2 shows that other anti-inflammatory drugs such as indomethacin and gold sodium thiosulphate (Sanochrysin), which also uncouple oxidative phosphorylation in isolated liver mitochondria,[3, 8] rapidly inhibit cartilage ^{35}S metabolism. Of the antimalarial drugs, only mepacrine had a similar rapid effect; this drug also uncouples oxidative phosphorylation at mM concentrations (see below and Refs. 9, 10).

TABLE 2. EFFECT OF SOME DRUGS ON THE INCORPORATION OF $^{35}S_i$ INTO CARTILAGE POLYSACCHARIDE SULPHATES (PS) *IN VITRO*

Cartilage slices were pre-incubated with drugs for varying time intervals at 37° before addition of $^{35}S_i$ to incubation medium: ^{35}S incorporation into PS measured after 1 hr further incubation.

PS-^{35}S computed as percentage PS-^{35}S in controls (no drugs) which had not been pre-incubated (marked * in table).

Buffer medium	Compound	Concn. (mM)	PS-^{35}S after pre-incubation for 0 hr (%)	1 hr (%)	2 hr (%)
Phosphate	None	—	100*	95	72
	Chloroquine phosphate	1·0	100	85	20
	Hydroxychloroquine phosphate	1·0	100	100	20
	Dihydroxychloroquine	1·0	100	90	33
	Desethyl-chloroquine	1·0	90	50	16
	N-(7-Chloro-4-quinolin-4-yl)-alanine	1·0	94	83	77
	4-Amino-7-chloro-quinoline	1·0	42	37	44
Phosphate	Mepacrine	0·8	50	35	12
	Mepacrine-N^{10}-oxide	0·8	100	65	20
	Mepacrine-N^{ω}-oxide	0·8	110	70	23
	Mepacrine-10,N^{ω}-dioxide	0·8	100	85	48
Tris	None	—	100*	100	
	Amodiaquin dioxide	0·4	85	64	
	Mepacrine dioxide	0·8	100	80	
	Hydroxychloroquine	1·0	100	100	
Phosphate	Phenylbutazone	0·5	48		
	Indomethacin	0·5	42		
	Gold sodium thiosulphate	0·5	40		
	(Sodium thiosulphate	2·5	70)		

All the quinoline congeners of chloroquine examined here, exhibited the same delayed effect upon cartilage metabolism as chloroquine itself.[5] In a limited series of experiments desethylchloroquine acted more rapidly than chloroquine in inhibiting polysaccharide sulphation but when the preincubation period was extended (to 3 hr

or greater), these two drugs appeared to be approximately equipotent. Two more polar derivatives, mono- and di- hydroxy-chloroquine were even slower acting than chloroquine *in vitro*. The potential chloroquine metabolite II was inactive but metabolite III did have a rapid effect on ^{35}S metabolism.

The N-oxides of mepacrine were slower-acting and less potent than mepacrine in inhibiting polysaccharide sulphation; the N^{10}, $^{\omega}$-dioxide being rather less active than either the N^{10} or N^{ω}-monoxides. Cartilage slices were stained much less on incubation with the dioxide, than with the monoxides or with mepacrine itself; indicating that the dioxide was not taken up so readily as the parent amine.

These observations on the relative potencies of chloroquine and its ω-hydroxy derivatives and of mepacrine and its N-oxides, emphasize the importance of lipophilic character in determining *in vitro* drug activity of antimalarials upon connective tissue metabolism. (The same parallelism between relative lipophilic character and *in vitro* activity has been discerned for other classes of anti-inflammatory drugs[5]). Some further evidence of this point was obtained as follows.

TABLE 3. EFFECT OF SOME DRUGS ON THE PROPORTION OF ^{35}S IN CARTILAGE SLICES WHICH WAS NOT PRECIPITATED WITH RIVANOL

^{35}S released from slices (incubated with $^{35}S_i$ for 1 hr) after papain digestion. Rivanol-soluble fraction was mainly inorganic sulphate ('$^{35}S_i$').

Buffer medium	Drug	Conc. (mM)	'$^{35}S_i$'/total ^{35}S in slices after pre-incubation for 0 hr (%)	1 hr (%)
Phosphate	None	—	4·6	3·8
	Chloroquine	1·0	5·5	8·8
	Hydroxychloroquine	1·0	4·6	7·0
	Dihydroxychloroquine	1·0	5·4	6·9
	Desethyl-chloroquine	1·0	8·7	15·3
	Mepacrine	0·8	15·0	18·0
	Mepacrine-10-oxide	0·8	6·7	9·2
	Mepacrine-N^{ω}-oxide	0·8	4·3	6·9
	Mepacrine-10,N^{ω}-dioxide	0·8	4·8	4·6
Tris	None	—	4·7	3·6
	Amodiaquin-1-oxide	<0·4	6·9	7·0
	Amodiaquin-N^{ω}-oxide	0·4	6·0	5·1
	Amodiaquin-1,N^{ω}-dioxide	0·4	4·6	5·0

Although chloroquine has a delayed effect upon polysaccharide sulphation in cartilage slices, it has a more rapid effect upon that fraction of the ^{35}S content of cartilage slices which is not precipitated with Rivanol (a quantitative precipitant of microquantities of polysaccharide sulphates[11, 12]): this fraction is mainly inorganic sulphate[11] though part of it may be derived from labile organic sulphates e.g. 'active sulphate', PAPS. Table 3 shows that the proportion of the total ^{35}S, contained in thoroughly washed cartilage slices after incubation with $^{35}S_i$ and the drugs, which is Rivanol-soluble ("$^{35}S_i$") varied with the different drugs. It was greatest with desethyl-chloroquine and decreased in the order: chloroquine, hydroxychloroquine, dihydroxy-chloroquine; with the related acridine bases, this ratio ($^{35}S_i$/total ^{35}S) decreased in the

order: mepacrine monoxides, mepacrine dioxide. This parallels the relative activities of both these quinoline and acridine bases in inhibiting polysaccharide sulphation.

These latter observations suggested that the mepacrine N^{10}-oxide might be a more active drug than the N^{ω}-oxide, i.e. the terminal diethylamino group being more important than the other tertiary nitrogen (nuclear N^{10}) in determining the drug activity *in vitro* of mepacrine, an acridine drug. Some supporting evidence was provided from studies of the relative activities of the mono-N-oxides of amodiaquin, a quinoline drug. For these experiments, a Tris-hydrochloride buffer, pH 7·4 had to be used instead of the conventional phosphate buffer in the Krebs-Ringer medium, because the amodiaquins were precipitated by phosphate ions at pH 7·4. Even in the modified incubation medium, i.e. with Tris, amodiaquin itself was too insoluble for adequate testing. At less than 0·4 mM, (a saturated solution of) amodiaquin-l-oxide had a greater effect on the ($^{35}S_i$/total ^{35}S) ratio than did 0·4 mM. amodiaquin-N^{ω}-oxide.

The comparatively water-soluble amodiaquin-1, N^{ω}-dioxide was the only drug of this amodiaquin series that could be compared adequately with the mepacrine series. It stained the cartilage slices less strongly than the amodiaquin-l-oxide (cf. mepacrine dioxides and monoxides) and was more potent than the mepacrine dioxide when tested in the Krebs-Ringer-Tris incubation medium (Table 3). Substituting a Tris-hydrochloride buffer for the phosphate buffer did not seem to render the tissues any more drug-sensitive (altering the chondrocyte permeability etc), since hydroxychloroquine did not inhibit cartilage metabolism any more rapidly when it was incubated with the cartilage slices in the Tris medium than when it was incubated with the slices in the phosphate medium.

The effect of some of these drugs upon oxidative phosphorylation in cartilage slices was studied indirectly by (a) comparing glucose-U-^{14}C oxidation to $^{14}CO_2$ by cartilage slices incubated with and without added drugs in the Krebs-Ringer phosphate medium, and (b) measuring the incorporation of ^{32}P from inorganic phosphate (P_i) into an organic phosphate fraction (P_{org}), by slices incubated with and without drugs in the Krebs-Ringer-Tris medium. The results showed that only 1mM mepacrine, of the

TABLE 4. EFFECT OF DRUGS ON (A) THE INCORPORATION OF $^{32}P_i$ INTO ORGANIC PHOSPHATES ($P_{org.}$), AND (B) OXIDATION OF GLUCOSE-U-^{14}C TO $^{14}CO_2$; BY CARTILAGE SLICES *IN VITRO*

$^{32}P_{org.}$ and $^{14}CO_2$ computed as % of $^{32}P_{org.}$ and $^{14}CO_2$ in controls without drugs preincubated for the same period of time. Incubation period with $^{32}P_i$ or glucose—^{14}C was 50 min. at 37°.

Drug	Conc. (mM)	$^{32}P_{org.}$ after pre-incubation for 0 hr (%)	1 hr (%)	2 hr (%)	$^{14}CO_2$ after pre-incubation for 0 hr (%)	1 hr (%)
None		100	100	100	100	100
Chloroquine sulphate	1·0	85	90	90	95	105
Hydroxychloroquine sulphate	1·0	93	85		100	100
Desethyl-chloroquine	1·0	94	86	100	90	100
Mepacrine dihydrochloride	0·8	78	55		80	40
Mepacrine-10,N^{ω}-dioxide	1·0	100	100	84		
Amodiaquin dihydrochloride	<0·4	83	82			
Indomethacin	0·8	30	25		25	
Gold sodium thiosulphate	1·0	75	45		50	

basic drugs listed in tables 1 and 2, significantly inhibited glucose oxidation and the labelling of the organic phosphate fraction with ^{32}P, i.e. phosphorylation (Table 4).

Effective uncoupling drugs such as indomethacin and gold sodium thiosulphate (2mM) strongly inhibited the incorporation of ^{32}P into the organic phosphates. At high drug levels, these two drugs (like mepacrine) also inhibited glucose catabolism by cartilage slices; either by inhibiting respiration, or by preventing the phosphorylation of glucose (which depends on a supply of ATP) which initiates glycolysis and glucose oxidation.

Although the interpretation of this effect of mepacrine on glucose oxidation is uncertain, the fact that none of the quinoline or acridine bases tested (other than mepacrine) had any significant effect on ^{32}P or glucose metabolism, suggests that none of these other basic drugs has any rapid action on the energy-yielding mechanisms within chondrocytes i.e. oxidative phosphorylation or glycolysis.

Drug action on mitochondrial respiration and oxidative phosphorylation

We could not confirm reports[13, 14] that chloroquine strongly inhibits the respiration of mouse liver homogenates or phosphorylating rat liver mitochondria when incubated with either α-oxoglutarate of pyruvate (10 mM, with or without 0·5 mM fumarate) Respiration linked to succinate or citrate oxidation was also insensitive to chloroquine. With each of these 4 oxidisable substrates, the oxygen uptake by the mitochondria in the presence of 2·5 mM chloroquine sulphate, hydroxychloroquine sulphate and mepacrine hydrochloride was never less than 70 per cent of the oxygen uptake by mitochondria without added drugs, after 10 min incubation at 30°.

Phosphorylation (ATP biosynthesis) coupled to the mitochondrial oxidation of succinate, citrate, pyruvate or α-oxoglutarate, was not affected by chloroquine sulphate at concentrations up to 5 mM, confirming previous observations.[13, 15] Phosphorylation linked to succinate or α-oxoglutarate oxidation was also insensitive to each of the following: 5 mM hydroxychloroquine, 2·5 mM desethyl-chloroquine, dihydroxychloroquine, and 4-hydroxyquinoline, saturated solutions of amodiaquin, acridone and 7-chloro-4-hydroxyquinoline (IV, Fig. 2), 2 mM N-(7-chloro-4-quinolyl) alanine (II, Fig. 2.) and N-(7′-chloro-4′-quinolyl)-4-aminobutyric acid (desmethyl-I, Fig. 2.). Mepacrine and 4-amino-7-chloroquinoline (III, Fig. 2.) uncoupled oxidative phosphorylation at concentrations greater than 2 mM but did not inhibit the yeast hexokinase, used to trap newly synthesized ATP as glucose-6-phosphate, at 5 mM. Mepacrine N^{10} and N^{ω}-oxides and mepacrine-N^{10}, N^{ω}-dioxide had no effect upon oxidative phosphorylation at 3 mM.

DISCUSSION

Chloroquine and hydroxychloroquine depressed the metabolism of at least one connective tissue *in vivo*, namely rat costal cartilage—but only after an appreciable time lag. This might not be considered significant in view of the known toxicity of these two drugs for rats[17, 18] and the high doses of these drugs that we used.

One distinctive property of chloroquine is that it has little effect on the well-being of small animals when given in sublethal doses,[19] in contrast to sublethal doses of many other antimalarial or toxic drugs. We certainly had the impression that both chloroquine and hydroxychloroquine had little or no toxic effect upon the rats we used, when given at doses not exceeding 100 mg/kg. Since these two drugs are also

slow-acting anti-rheumatic agents in man, the delayed effects of these drugs in rats then become rather more significant.

In short-term experiments, chloroquine has been shown not to affect the incorporation of ^{35}S into the macromolecular (mucopolysaccharide) components of cartilage,[4] skin[20] and granulation tissue,[21] in contradistinction to other antirheumatic drugs such as phenylbutazone and hydrocortisone which depress ^{35}S metabolism in these connective tissues *in vivo* within 24 hr of their administration. Conventional (short-term) anti-oedema and anti-erythema assays for anti-inflammatory activity have also indicated that chloroquine is set apart from other antirheumatic drugs.[1, 22]

This failure of chloroquine to exhibit the same biochemical and pharmacological properties (including antirheumatic activity) as other antirheumatic drugs in short-term experiments, could be readily explained if (i) chloroquine only accumulated rather slowly in the connective or reticulo-endothelial tissues, and/or if (ii) chloroquine is not itself an anti-inflammatory drug but is metabolised *in vivo* to (an)other compound(s) with anti-inflammatory activity.

Newbould found that chloroquine had no effect upon the induction of an experimental arthritis in rats, which is mediated through the lymph nodes.[22] Other workers have found that the immune response in guinea pigs and rabbits was not affected by chloroquine.[6, 23] Adam and Houba noted that chloroquine would inhibit granuloma development in rats but only if the granuloma were not sterile.[24] These various findings suggest that (a) the connective tissues rather than the reticulo-endothelial system may be the target for chloroquine action and (b) that biotransformation products of chloroquine might be the active drugs *in vivo*. Metabolites of another anti-inflammatory drug, amidopyrine, uncouple oxidative phosphorylation[16]—a property of many anti-inflammatory drugs[2, 3, 8]—but not of amidopyrine itself. It therefore seemed worthwhile to study the properties of some potential chloroquine metabolites, both *in vivo* and *in vitro*, to see whether any of these compounds might affect cartilage-metabolism more rapidly than chloroquine and/or uncouple oxidative phosphorylation.

A possible pathway of chloroquine metabolism, depicted in Fig. 2, is indicated by reports of the formation of desethylchlorquine in man and rabbits[25] and the tentative identification of more completely degraded transformation products such as 4-amino-7-chloroquinoline in human tissues,[26] after chloroquine administration. This scheme only involves well-known drug transformations such as oxidative dealkylation and deamination and β-oxidation of aliphatic acids, with the exception of the last deamination reaction (III→IV, Fig. 2). This last potential transformation is of interest, since certain 4-hydroxyquinoline derivatives are effective drugs against avian (but not human) malaria,[27] and might be facilitated by tautomerism of the amine (III) to the (N-hydro) 4-imine. The general pattern of drug metabolism in vertebrates suggests that compounds susceptible to oxidation either in an aromatic ring or in an alkyl side chain, are metabolised by routes involving degradation of the side chain rather than oxidation of the nucleus[28] (and see Ref. 25). However, there is evidence that 8-aminoquinoline drugs such as Pamoquine and Pentaquine may undergo nuclear hydroxylation *in vivo*, yielding metabolites with antimalarial activity (e.g. quinoline-5,6-quinones).[29, 30] (We have not studied any nuclear oxidation products of chloroquine in this present survey).

The N-oxides of mepacrine and amodiaquin (camoquin)[31] were included in this investigation because certain quinoline oxides are potent respiratory inhibitors.[32] A teritary amine can be oxidised biologically to the corresponding amine oxide[33] and the heterocyclic sulphur atom in a promazaine may be similarly oxidised *in vivo* to the corresponding sulphoxide.[34]

CONCLUSIONS

Desethylchloroquine is certainly a faster-acting (and more potent?) drug than chloroquine *in vitro* and *in vivo* as regards its effect on the metabolism of one connective tissue. None of the other potential chloroquine metabolites investigated in this study would seem to fulfil the role of an unambiguous pharmacologically-active metabolite, which might be responsible for the antirheumatic activity of chloroquine itself.

The acidic degradation products (e.g. II) do not uncouple oxidative phosphorylation and so do not resemble acidic anti-inflammatory drugs such as phenylbutazone and indomethacin: furthermore these acidic metabolites (I, II) do not exhibit any significant anti-inflammatory activity in experimental animals (Mr. G. M. Smith and Dr. R. G. Spickett, personal communication). 4-Amino-7-chloroquinoline, the one chloroquine metabolite which does uncouple oxidative phosphorylation, is less potent than salicylic acid in this respect. However, it is inactive in experimental arthritis assays when given orally (Dr. B. B. Newbould, personal communication) although we found it to be a moderately active drug in its effect on cartilage metabolism *in vivo* when injected into rats. Kuroda[26] did find this amine in human peripheral tissues following chloroquine ingestion. It would be interesting to know if this compound has any local anti-inflammatory activity when injected into experimental granulomae, especially since this amine is the only potential metabolite common to both chloroquine and amodiaquin. Amodiaquin, like chloroquine, exhibits slow-acting antirheumatic properties in man.[35]

Our findings also suggest that amine-oxide derivatives of chloroquine, or its biological degradation products, would be less active drugs than the parent amines (deoxy compounds) as regards their effect upon the metabolism of connective tissues *in vivo*.

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