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Effects of antimalarial drugs on interleukin 1-induced cartilage proteoglycan degradation in-vitro

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Previous studies having shown that chloroquine and hydroxychloroquine could reduce interleukin 1 (IL-1)-induced cartilage degradation in-vitro, the effects of a range of antimalarial drugs on the cartilage proteoglycan degrading actions of porcine leucocyte (pI 4·8) α-interleukin 1 (syn. catabolin) have been examined using the standard bovine nasal cartilage culture system. The anti-IL-1 effects in this system were specific to several aminoquinoline and aminoacridine analogues having a side chain with a tertiary amino group similar to that of chloroquine. Aminoquinoline compounds devoid of this side chain and the tertiary amino, as well as pyrimidines or biguanides with antimalarial activity were without effect. Mefloquine, the most potent of the compounds active against porcine α-IL-1, was only equipotent with chloroquine and its hydroxyanalogue against human recombinant α-IL-1. This suggests that there may be subtle differences in the receptors for these drugs and interleukins in bovine cartilage. The results provide further evidence for the specificity and utility of antimalarial drugs in the treatment of chronic inflammatory conditions, especially in relation to actions on IL-1.

Interleukin 1 (IL-1), which is produced by mononuclear leucocytes, comprises a group of protein inflammatory mediators that are important in many chronic and some acute inflammatory reactions (Dinarello 1984). Among the effects induced by IL-1 are stimulation of acute phase protein production, fever, T- and B-lymphocyte proliferation and maturation, T-lymphocyte chemotaxis, neutrophil activation and neutrophilia, bone resorption, fibroblast proliferation, prostaglandin production, and destruction of cartilage matrix proteoglycans, collagens and other connective tissue proteins (Mizel et al 1981; Dingle 1983; Miossec et al 1984; Dinarello 1984; Gowen et al 1984; Saklatvala et al 1984; Krakauer et al 1985). The cartilage degrading effects of IL-1 were ascribed to a molecular species termed 'catabolin' (Jasin & Dingle 1981; Dingle 1983) which has now been shown to have the properties of α-IL-1 (Saklatvala et al 1984). Cartilage degradation in-vivo is dependent, in part, on leucocytes accumulated at inflamed sites (Sedgwick et al 1984) and thus might be due to the IL-1 derived from these cells (Billingham & Rushton 1985).

The release of IL-1 or of catabolin-like activity (ILA) from synovial tissue in culture is inhibited in-vitro by relatively high concentrations of corticosteroids (Sheppeard et al 1982) and some, but not all, non-steroidal anti-inflammatory (NSAI) drugs (Sheppeard et al 1982; Herman et al 1984; Dingle 1985). Recently, it was found that proteoglycan degradation induced by porcine IL-1 in bovine nasal cartilage could be inhibited in-vitro by therapeutic concentrations of hydroxychloroquine, other NSAI drugs being virtually ineffective in acceptable therapeutic concentrations (Rainsford 1985). In view of the relatively selective effects of hydroxychloroquine in that system (Rainsford 1985), and the current interest in antimalarials as potential antirheumatic agents, a study of the effects of various antimalarials was initiated to determine the relationship between chemical structure and effect on IL-1-induced cartilage proteoglycan degradation in-vitro.

METHODS

Bovine nasal septum cartilage from freshly (<1 h) slaughtered adult cattle was dissected under sterile conditions and transverse slices (1·5 mm thick) cut from which discs (2 mm in diameter) were subsequently obtained with a sterile leather punch. The cartilage discs were pre-incubated for 1–2 days in Microtiter 92 well dishes in the presence of 200 μl Dulbecco's modified Eagle's medium (DMEM) containing penicillin (200 u ml^{-1}), streptomycin (200 u ml^{-1}), and 5% foetal calf serum (FCS) in an atmosphere of 5% CO_2 in air (Saklatvala et al 1984). The medium was then changed and the drugs or solvent mixtures (see Table 1), together with chromatofocussed pI 4·8 porcine leucocyte α-IL-1 (gift of Dr J. Saklatvala, Strangeways Research Laboratory, prepared as described by Saklatvala et al 1984), or human recombinant r-α-IL-1 (Genzyme Corp., Boston, MA, USA) were added. Sufficient IL-1 was

added to give at least a four-fold increase in proteoglycan degradation above that in control cultures. Incubations with drug or solvent mixtures were performed in octuplicate for 4 days. Control cultures (without IL-1) had drugs alone or solvent mixtures added. Drug or solvent cytotoxicity was determined by comparing the release of glycosaminoglycans (GAGs) (see later for methods) from those cultures to which the drugs or solvent mixtures had been added, with GAG release in controls (in DMEM ± 5% FCS).

Cartilage proteoglycan degradation was determined by measurement of the content of GAGs in the media compared with that in papain-digested cartilage (Saklatvala et al 1984). The assay of GAGs was performed using the dimethyl-methylene blue (Serva) technique of Farndale et al (1982). The drugs used did not interfere with this assay.

Drugs

All cinchona alkaloids (group I, Table 1), simple quinolines (group IV, Table 1), quinacrine and aminoquinolines (group II, Table 1) were purchased from Aldrich. Chloroquine sulphate was purchased from Sigma. Chloroquine base (II) and hydroxychloroquine were donated by Sterling-Winthrop (UK). Mefloquine (I) and *N*-methyl mefloquine were donated by Roche Products Ltd (Welwyn Garden City, UK). ZK-20674 (III) [7-chloro-4-4′-pyrrolidino-1′-methylbutylamino) quinoline] was a gift from the Walter Reed Army Institute of Research (Washington, DC, USA).

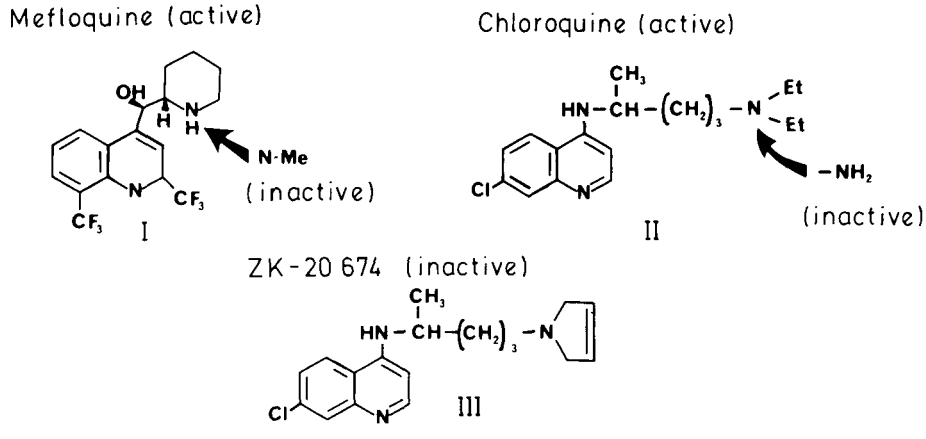
The two biguanides, cycloguanil and chloroguanide (group VI, Table 1) were provided by ICI (Alderley Park, UK).

The compounds were dissolved either in media or in ethanolic solutions as described in Table 1 and footnotes to Fig. 1. No significant effects occurred on GAG release with ethanol to 0·02% final concentration (Table 1).

RESULTS

The data in Table 1 and Fig. 1a show that inhibition of porcine α-IL-1-induced cartlage degradation was obtained only with those antimalarial compounds having either an aminoquinoline, quinoline methanol or aminoacridine structure and an unsubstituted tertiary amino group on their side chain. With the quinine analogues (i.e. cinchona alkaloids) there was also an apparent requirement for a 6-methoxy substituent on the quinoline ring (Table 1). Thus, quinine and quinidine (i.e. 8*S*:9*R* and 8*R*:9*S* isomers, respectively, with a 6-methoxy substituent) inhibited IL-1 actions whereas the corresponding unsubstituted isomers, cinchonidine and cinchonine, were inactive (Table 1). This suggests a requirement for an electron-withdrawing substituent on the aminoquinoline ring. Other active aminoquinolines also have electron-withdrawing substituents (Table 1).

The specificity defined by the aminoquinoline or aminoacridine structure described is illustrated by the inactivity of antimalarial pyrimidines and biguanides (Table 1) in the test. No inhibitory activity was found with desethyl chloroqune, the *N*-pyrrolidine derivative of chloroquine (ZK 20674), and *N*-methyl mefloquine (Table 1, cf. I–III) even though chloroquine and the quinoline-methanol mefloquine, were both potent inhibitors of IL-1-induced cartilage degradation (Fig. 1a). Conversion to a primary



I–III. Structures of mefloquine and chloroquine, both inhibitors of IL-1-induced cartilage degradation, and those of *N*-substituted derivatives (i.e. of groups attached to respective quinoline moieties) which were found inactive as anti-IL-1 agents. The structure of ZK-20674 is also given.

Table 1. Effects of antimalarial compounds on interleukin 1-induced cartilage degradation in-vitro.

	Compound	Concn (μM)	Proportion GAGs released (mean ± s.d.) with IL-1	Proportion GAGs released (mean ± s.d.) no IL-1	(% change)
I.	4-Aminoquinolines:				
	Amodiaquine[b]	100	68·1 ± 5·8*	25·4 ± 7·9	(−43·5)
		20	72·4 ± 9·1*	15·8 ± 8·0	(−25·1)
	Cinchonine[a]	100	86·6 ± 9·7	16·8 ± 6·5	
		20	94·7 ± 14·7	11·8 ± 5·4	
	Cinchonidine[a]	100	82·8 ± 18·1	15·5 ± 4·7	
		20	85·5 ± 17·6	16·0 ± 6·0	
	Quinine[a]	100	13·4 ± 6·1*	21·2 ± 4·9	(*ca* −100)
	sulphate	50	62·7 ± 12·3*	18·1 ± 5·7	(−36·3)
		10	94·0 ± 9·4	20·3 ± 15·1	
	Quinidine[d]	100	21·2 ± 5·1*	17·8 ± 5·6	(−95·1)
	sulphate	20	90·5 ± 7·2	14·8 ± 11·1	
	Desethyl[b]	100	86·4 ± 11·1	15·0 ± 4·2	
	chloroquine	20	92·3 ± 14·5	31·6 ± 18·9	
	N-Methylmefloquine[b]	100	90·2 ± 14·8	17·3 ± 6·9	
		20	86·6 ± 7·9	12·1 ± 5·3	
II.	8-Aminoquinolines:				
	Primaquine[b]	100	55·6 ± 11·7*	18·3 ± 7·7	(−50·7)
		20	84·3 ± 18·5	14·1 ± 4·9	
	Pamaquine[b]	100	62·8 ± 2·0*	20·5 ± 13·3	(−44·1)
	naphthoate	20	91·9 ± 5·9	25·4 ± 14·0	
III.	9-Aminoquinacridine:				
	Quinacrine[b]	100	21·4 ± 7·7*	11·6 ± 5·4	(−87·0)
		20	80·0 ± 15·6	12·1 ± 9·1	
IV.	Simple quinolines:				
	8-Aminoquinoline[c]	100	82·4 ± 14·7	12·6 ± 3·1	
		20	95·3 ± 1·3	11·6 ± 6·1	
	4-Aminoquinaldine[c]	100	91·2 ± 1·7	18·2 ± 3·5	
		20	94·1 ± 2·1	11·1 ± 4·4	
	4,7-Dichloroquinoline[b]	100	90·5 ± 2·1	11·2 ± 3·6	
		20	95·1 ± 1·3	19·7 ± 11·8	
	6-Amino, 2-phenyl,[c]	100	91·8 ± 2·2	25·0 ± 18·1	
	4-quinolol	20	94·8 ± 1·0	8·1 ± 2·1	
V.	Pyrimidines:				
	Pyrimethamine[b]	100	77·7 ± 11·7	17·0 ± 7·9	
		20	76·8 ± 15·6	13·0 ± 3·8	
	Trimethoprim[b]	100	83·1 ± 10·4	21·5 ± 3·7	
		20	74·5 ± 12·6	15·8 ± 5·4	
VI.	Biguanides:				
	Cycloguanil[b]	100	69·5 ± 32·7	14·1 ± 4·5	
		10	85·3 ± 18·0	23·3 ± 9·1	
		5	87·0 ± 17·4	17·4 ± 5·1	
	Chlorguanide[b]	100	84·1 ± 7·8	15·9 ± 8·7	
		10	94·8 ± 1·5	28·3 ± 9·9	
		5	85·4 ± 11·5	18·6 ± 6·7	
	Control DMEM alone		93·1 ± 2·1	23·2 ± 4·9	
	EtOH 0·02%		90·6 ± 2·8	15·0 ± 4·5	

Dissolved [a] in medium alone, [b] initially in ethanol then diluted to 0·02% final concentration in medium, [c] by preparing HCl salts then dilution into medium.

Proportion of GAGs released determined from:

$$\frac{\text{GAGs in Medium}}{\text{Total of GAGs in medium + disc}}$$

* Denotes significant reduction cf. control ($P < 0{\cdot}05$; Mann-Whitney U test). Values shown in brackets denote % reduction calculated as:

$$\frac{\text{Proportion of GAGs released}\left(\left[\begin{matrix}\text{with IL-1}\\+\text{ drug}\end{matrix}\right]-\left[\begin{matrix}\text{no IL-1}\\+\text{ drug}\end{matrix}\right]\right)}{\text{Proportion of GAGs}\left(\left[\begin{matrix}\text{with IL-1}\\+\text{ drug}\\\text{solvent}\end{matrix}\right]-\left[\begin{matrix}\text{no IL-1}\\+\text{ drug}\\\text{solvent}\end{matrix}\right]\right)}\times\frac{100}{1}$$

amine results in loss of activity (i.e. in desethyl chloroquine) suggesting specific electronic features inherent in the tertiary N are required for activity of chloroquine or mefloquine. The lack of effects of the pyrrolidine derivative of chloroquine (ZK-20674) and *N*-methyl mefloquine derivatives suggests that the tertiary nitrogen of the side chain attached to the quinoline ring must be relatively exposed, i.e. unhindered, for inhibitory activity.

The requirement for a side chain having a structure like that on chloroquine is also indicated by the lack of inhibitory effects of those simple aminoquinolines devoid of such a side chain with a tertiary amino group (group IV, Table 1).

The most potent inhibitor of porcine α-IL-1-induced cartilage degradation was undoubtedly mefloquine (Fig. 1a). This drug had an IC50 ca 20 μM and was about twice as potent as chloroquine and hydroxychloroquine (IC50 ca 50–60 μM). Mefloquine is appreciably more liposoluble than chloroquine and its congeners. Thus, relative liposolubility might be considered a feature determining the potency of inhibitory actions against porcine α-IL-1-induced cartilage degradation. However, as shown in Fig. 1b, this enhanced inhibitory activity of mefloquine (cf. chloroquine and hydroxychloroquine) is not seen in those cartilage cultures exposed to the human recombinant α-IL-1. Here the inhibitory activity of mefloquine is about equipotent with that of chloroquine. These differences in effect against porcine compared with human IL-1s might well reflect subtle differences in interactions with postulated receptors for both the IL-1s as well as for the drugs.

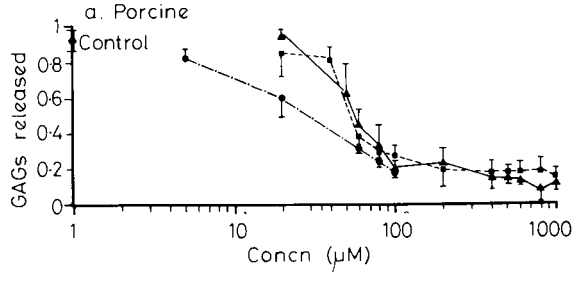


FIG. 1a. Concentration–response effects of (▲) chloroquine, (■) hydroxychloroquine and (●) mefloquine in the inhibition of porcine leucocyte α-IL-1-induced destruction of bovine nasal cartilage proteoglycans after 4 days culture. Each point represents the mean of 8 determinations and the bar is the s.d. Values for the effects of drug alone (i.e. in absence of IL-1) not shown but all were within a mean value of ca 0·2. Effects on cartilage proteoglycan destruction are shown as the proportion of release of glycosaminoglycans (GAGs) i.e. GAGs in medium/GAGs in medium + GAGs in cartilage disc.

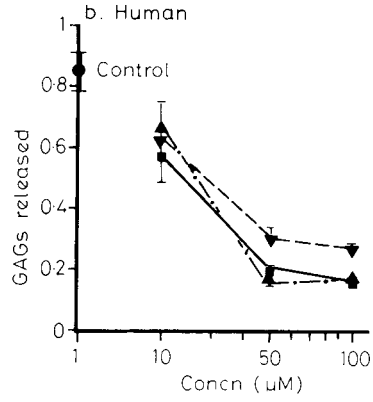


FIG. 1b. Effects of (■) chloroquine, (▼) hydroxychloroquine and (▲) mefloquine on the cartilage proteoglycan destruction induced by human recombinant α-IL-1. Conditions of system as described in a. Human α IL-1 (25 units ml^{-1}) added.

The results show that there is an appreciable structural specificity for inhibitory activity against α-IL-1-induced cartilage degradation. Several biochemical effects might be responsible for the inhibitory actions seen with the chloroquine-like drugs, including (i) inhibition of the release of lysosomal enzymes, (ii) effects on release and actions of inflammatory eicosanoids with effects on phospholipid metabolism, (iii) inhibition of glucose metabolism and (iv) inhibition of DNA and protein synthesis following intercalation of drug with DNA (Manku & Horribin 1976; Matsuzawa & Hostetler 1980; Larsson et al 1981; Pappu & Hausser 1981; Riches et al 1981; Kelman et al 1981; Authi & Traynor 1982; Read & Trist 1982; Bhattacharyya et al 1983; Puustinen et al 1983; Wolfe 1983; McChesney & Fitch 1984). Appreciable uptake of chloroquine and related drugs into cartilage occurs (Larsson et al 1981) thus favouring biochemical effects in this tissue.

Inhibition of eicosanoid metabolism would not on its own seem especially important since cyclooxygenase and/or lipoxygenase inhibitors are not very effective in preventing IL-1-induced cartilage degradation in-vitro (Rainsford 1985; unpublished studies). Inhibition of DNA synthesis, while not itself considered a major effect of chloroquine in relation to its antimalarial actions (because of it only being a weak intercalator with DNA, Wilson & Jones 1981), could nonetheless be a factor where transcription of DNA is evident, such as from the effects of IL-1. Evidence for IL-1-induced transcription has been shown by the fact that inhibitors of DNA synthesis prevent IL-1-induced cartilage des-

truction in the model system employed herein (Rainsford 1985). Hence it has been suggested that cartilage proteoglycan and collagen destruction might be mediated by IL-1 causing induction of the synthesis of those enzymes responsible for degradation of these macromolecules (Rainsford 1985). Since inhibitors of the synthesis of DNA precursors (i.e. pyrimidines) are ineffective in preventing IL-1-induced cartilage proteoglycan degradation (Table 1), this suggests that specific interaction of chloroquine and related drugs with DNA is required to prevent induction of the synthesis of cartilage-degrading enzymes. In addition, it is also conceivable that those other drug effects outlined in (i) and (ii) above may further add to the anti-IL-1 actions of the aminoquinoline antimalarials.

The present studies provide evidence of the potential of chloroquine and related antimalarial drugs to modify disease by controlling the effects of IL-1. In-vivo studies are needed to establish the relative importance of the anti-IL-1 effects of antimalarials. Since a range of inactive, as well as active, compounds has been identified as a result of the present work, this may provide a useful basis for selectively influencing IL-1 effects in-vivo and in-vitro.

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