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ANTI-PROLIFERATIVE PROPERTIES OF CLOZIC,* A DISEASE-MODIFYING ANTI-ARTHRITIC AGENT

STEPHEN J. FOSTER,† MARGARET E. MCCORMICK and CAROLINE J. CUNLIFFE
Imperial Chemical Industries plc, Pharmaceuticals Division, Bioscience I Department, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

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Abstract—Cytostatic drugs have been used in the treatment of rheumatoid arthritis but are of limited clinical application due to their severe toxic side-effects. We have discovered that 'Clozic' (ICI 55897), an agent with disease-modifying properties in rheumatoid arthritis patients, inhibits the growth of a variety of mammalian cell types including a matrix-secreting cell culture derived from neonatal rat hearts. The inhibition of growth was reversible and no loss of cell viability occurred when measured by lactate dehydrogenase released into the medium or by vital staining, suggesting a cytostatic rather than a cytotoxic mechanism. Cytostatic activity was observed at ICI 55897 concentrations within the reported therapeutic plasma concentration range and was related to the concentration of unbound compound, since the effect could be reduced by increasing the albumin concentration in the medium. Other oxyalkanoic acids inhibited cell growth. Their inhibitory potency correlated with lipophilicity. The anti-proliferative potencies of *R* and *S* enantiomers of two oxyalkanoic acids containing asymmetric centres were similar. These observations suggest that the anti-proliferative effect of the oxyalkanoic acids is due to their interaction with lipophilic cell target sites.

It is well known that a number of toxic agents exhibit therapeutic side-effects including suppression of inflammatory disease. Anti-cancer agents, such as cyclophosphamide, methotrexate, chlorambucil and azathioprine, have been tried in rheumatoid arthritis with varying degrees of success. It has been suggested that their efficacy is related to an immunosuppressive action. Alternatively, inhibition of cell growth in the rheumatoid pannus could explain their efficacy. It is perhaps more than coincidence, therefore, that disease-modifying agents used in rheumatoid arthritis inhibit the growth of cell cultures. Thus, sodium aurothiomalate [1] and D-penicillamine [2] inhibit the proliferation of human rheumatoid synovial cells *in vitro* and the synthesis of connective tissue macromolecules in these cultures. The concentrations of gold found to inhibit cell proliferation *in vitro* were equivalent to serum gold concentrations in rheumatoid arthritis patients during chrysotherapy [1, 3]. With D-penicillamine, however, although mucopolysaccharide biosynthesis was depressed at therapeutic concentrations, anti-proliferative effects were achieved only at higher concentrations [2].

ICI 55897 ('Clozic') {2-[4-(4-chloro-phenyl)-benzyloxy]-2-methylpropionic acid} was of interest as a potential anti-arthritic agent. This agent inhibits the acute phase protein response in the chronic stage of a modified adjuvant arthritis in the rat without having anti-inflammatory or analgesic properties [4–6]. It lowers the acute phase protein levels and erythrocyte sedimentation rate in rheumatoid arthritis patients [7] and compared favourably with gold in clinical trials [8]. Unfortunately during the course of this work ICI 55897 was withdrawn from development as an anti-rheumatic drug following a review of all the clinical and animal data. This resulted in the conclusion that the overall ratio of efficacy to safety was not adequate to justify further development. However, since there have been so few drugs with some proven disease-modifying activity for use in rheumatoid arthritis, it is important to investigate the mode of action of this agent.

In this paper we describe the effect of ICI 55897 and some related oxyalkanoic acids on the proliferation of a matrix-secreting cell culture, derived from neonatal rat hearts. The anti-proliferative effect was reversible and occurred within the concentration range measured in the plasma of rheumatoid arthritis patients undergoing therapy with ICI 55897. The relevance of the anti-proliferative effect of ICI 55897 is discussed with respect to its anti-arthritic action.

MATERIALS AND METHODS

Compounds and drugs. For convenience the following compounds (Fig. 1) are referred to by numbers: ICI 55897, trade mark Clozic {2-[4-(4-chlorophenyl)benzyloxy]-2-methylpropionic acid}; ICI 107030 {2-[4-(4-chlorophenyl)benzyloxy]-2-phenylacetic acid}; ICI 115432 {2-[4-(4-chlorophenyl)benzyloxy]-2-phenylbutyric acid}. The *R*(−) and *S*(+) enantiomers of ICI 107030 were >98% optically pure and the *R*(+) and *S*(−) enantiomers of ICI 115432 were >90% optically pure [9]. These compounds and clofibric acid were prepared at ICI, Pharmaceuticals Division, as were the following radiolabelled compounds: 2-[4-(4-chlorophenyl)benzyloxy]-2-methyl-[1-^{14}C]propionic acid (4.77 μCi/mg); 2-[4-(4-chlorophenyl)benzyloxy]-2-phenyl-[3-^{14}C]butyric acid (12.3 μCi/mg); 2-(4-chloro[U-^{14}C]phenoxy)-2-methylpropionic acid

* 'Clozic' is a trade mark and the property of Imperial Chemical Industries plc.

† To whom correspondence should be addressed.

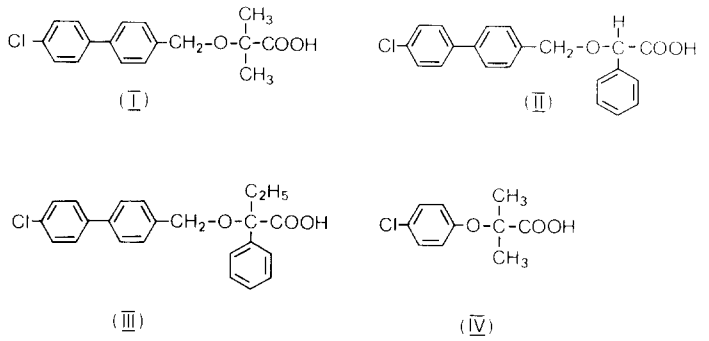

Fig. 1. Structures of oxyalkanoic acids tested for anti-proliferative activity: (I) ICI 55897; (II) ICI 107030; (III) ICI 115432; (IV) clofibric acid.

(7.3 μCi/mg). *N*-[1-^{14}C]Hexadecane (reference standard), [methyl-^{3}H]thymidine (44 Ci/mmole) and PCS liquid scintillation cocktail were obtained from the Radiochemical Centre (Amersham, U.K.). Drugs were obtained from the following suppliers: ketoprofen (May & Baker, Dagenham, U.K.); ibuprofen and flurbiprofen (Boots, Nottingham, U.K.); aspirin, phenylbutazone and indomethacin (Sigma). Benoxaprofen was extracted from tablets ['Opren' (Dista)]. Other materials were obtained from the following sources: Eagle's minimal essential medium (EMEM), trypsin, glutamine and foetal calf serum (Flow Laboratories, Irvine, U.K.); tryptose phosphate broth (Difco Laboratories, Detroit, MI); streptomycin, pancreatin, bacterial collagenase and bovine serum albumin fraction V (Sigma U.K.); penicillin G (Glaxo, Greenford, U.K.); Isoton II balanced electrolyte solution (Coulter Electronics, Luton, U.K.).

Compounds were incorporated into media as follows. Concentrated methanol solutions of the compounds were added dropwise to continuously stirred foetal calf serum at 37°. The remaining media components were added slowly to produce the required final concentrations of compound and 20% (v/v) serum, and then neutralised by titration with 1 N NaOH. The same volume of methanol was added to control media. All solutions were sterilised by filtration. This procedure was necessary to prevent precipitation of the oxyalkanoic acids as their calcium salts and was adopted as standard for all compounds.

Heart cell cultures. Heart cell cultures were isolated using a slight modification of the method described by Jones *et al.* [10]. Twenty-four hearts were aseptically removed from 1-day-old Charles River rats and placed into calcium- and magnesium-free Earle's balanced salts solution (CMFS). The hearts were cut into small pieces and washed twice with CMFS. Tissue fragments were resuspended in 0.1% (w/v) trypsin in CMFS and slowly stirred at room temperature. The first harvest of cells, obtained after 10 min, was discarded. The next four successive 30-min harvests were collected and the cells obtained by centrifugation (1000 *g*, 5 min). Cells were resuspended in EMEM containing 10% (v/v) foetal calf serum, 10% (v/v) tryptose phosphate broth, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine and incubated in Falcon 75-cm^2 tissue culture flasks at 37° in a humidified atmosphere of 95% air/5% CO_2. After 90 min, media were gently aspirated and the attached cells washed twice with medium. Fresh medium was added back to the flasks and the cultures grown until almost confluent. Secondary cultures were established by trypsinization and passage at a ratio of 1:4. Cells from various passages were frozen in liquid nitrogen in a biological freezing unit (Union Carbide) at 2×10^6–4×10^6 cells per 1-ml vial in medium containing 30% foetal calf serum and 10% dimethyl sulphoxide. On thawing each vial was used to establish one 75-cm^2 flask.

Determination of cell count and growth rate. Approximately 3×10^4–5×10^4 cells/ml medium were seeded into Linbro 24-well plates and incubated at 37° in a humidified atmosphere of 95% air/5% CO_2. Medium was replaced with fresh medium containing 20% foetal calf serum and methanol or the compound under investigation. Cell numbers were determined in replicate wells on various days during culture growth. Cell cultures were detached following two washes in CMFS by incubation with 0.1% (w/v) trypsin in CMFS containing 0.08% EDTA. Older cultures (>7 days post-seeding) were dispersed with Hank's balanced salt solution containing 0.25% (w/v) pancreatin and 0.05% (w/v) bacterial collagenase. This solution was required to release cells enmeshed in extracellular matrix. Cells dispersed with a Pasteur pipette were diluted in Isoton II and counted using a Coulter counter (Coulter Electronics, Luton, U.K.).

The growth rate of the cell cultures, in the presence of each concentration of compound, was measured from the gradient of its log cell vs time graph and the ED_{50} value for each compound (the concentration of compound required to produce 50% inhibition of the control cell growth rate) was determined from a graph of cell culture growth rate vs compound concentration.

Determination of cell growth rate by [^{3}H]*thymidine incorporation.* Cell cultures were grown in the presence of compound under investigation as described earlier. After 3 days the cultures were pulsed with 1 μCi/ml [^{3}H]thymidine for 3 hr at 37°.

Plates were placed on ice and the cell sheets washed twice (200 μl) with ice-cold CMFS and fixed with 1 ml of 10% trichloroacetic acid containing 1 mM thymidine for 15 min. The trichloracetic acid precipitates were washed twice (200 μl) with ethanol

and dissolved in 0.1 N NaOH (200 μl) overnight at room temperature. Samples were neutralised with 0.1 N HCl, added to PCS, and radioactivity assayed by liquid scintillation counting (efficiency for ^3H 30–32%).

A plot of log incorporated cpm vs incubation period was linear during the 3-hr period with a gradient equivalent to the cell growth rate. The ED_{50} value for individual compounds was calculated from a graph of cell culture growth rate vs compound concentration.

ED_{50} values of compounds determined by cell counting or [^{3}H]thymidine incorporation methods correlated closely ($r = 0.98$).

Binding of compounds to serum proteins. Equilibrium dialysis was used to determine protein-bound and free levels of ICI 55897, ICI 115432 and clofibric acid in growth media and in media supplemented with bovine serum albumin. Compounds, dissolved in methanol, were spiked with 0.1 μCi/ml of the corresponding ^{14}C-labelled compound and combined with media containing 20% foetal calf serum as previously described. Solutions were dialysed against serum-free medium using rotating Dianorm equilibrium dialysis cells (3.5 hr, 12.5 rpm, 37°). The dialysis membrane (Spectrapor 2, mol. wt cut-off 12,000) was soaked overnight in distilled water. On attaining equilibrium, samples of media from both sides of the dialysis membrane were diluted in water, combined with PCS and radioactivity assayed by liquid scintillation counting (efficiency for ^{14}C 65%).

RESULTS

Neonatal rat heart cell cultures

Primary cultures derived from neonatal rat hearts consisted of several morphologically distinct cell types. Cultures were completely devoid of beating cardiomyocytes but contained a few 'fibroblast-like' and polygonal cells. The majority of cells were extremely large, quite refractive to light, extensively spread and had obvious filamentous cytoplasm. Secondary cultures established from primary cultures following trypsinization retained similar morphological cell types until the fourth passage. Further passages resulted in the disappearance of the large refractive cells which were replaced by a more homogeneous population of polygonal cells. These cells, which we have designated RHMC, have been passaged more than 40 times without further morphological changes. Cultures of RHMC cells have a mean doubling time of 48 hr and secrete an insoluble extracellular matrix which becomes very extensive at 14 days post-seeding. Analysis of the matrix by the enzymic digestion methods described by Jones *et al.* [10] showed it to have a similar composition to that produced by rat smooth muscle cell cultures.

Effect of ICI 55897 on cellular macromolecular biosynthesis and proliferation

Concentrations of ICI 55897 greater than 50 μM caused a dose-dependent decrease in the amount of matrix protein synthesised by RHMC cell cultures. Decreased matrix production correlated with a reduction in the cell growth rate. The effect of ICI 55897 and other agents on cell proliferation was determined either by counting total cell numbers or by measuring the incorporation of [^{3}H]thymidine into a trichloroacetic acid insoluble cell fraction. Both methods produced similar results.

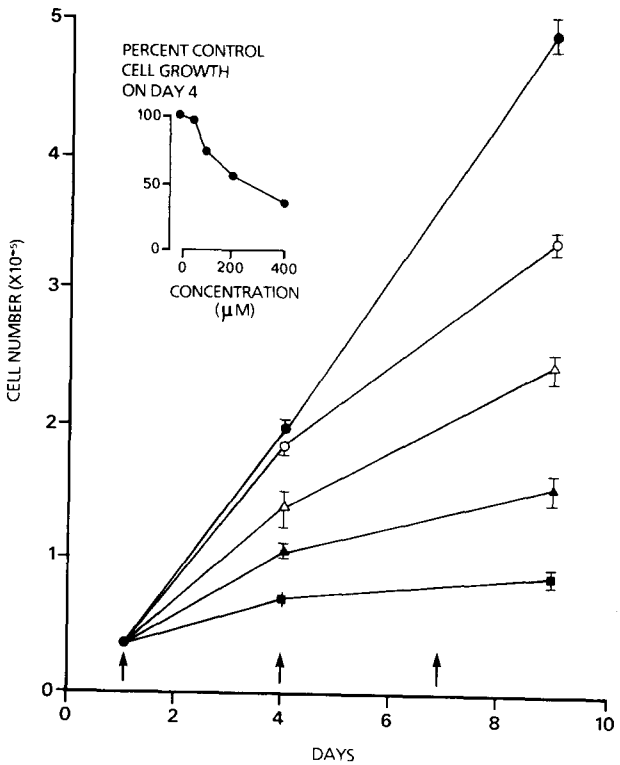

Fig. 2. Effect of various concentrations of ICI 55897 on the growth of RHMC cell cultures. Cells were seeded into multiwell plates at 3.0×10^4 cells per well. Cultures were grown in the absence (●) or presence of 50 μM (○), 100 μM (△), 200 μM (▲) and 400 μM (■) ICI 55897. Arrows indicate changes of media and compound. Values show the mean cell counts ± S.D. of four separate cultures. The inset shows the concentration-dependent decrease in cell number on day 4 calculated as per cent of the control cell number.

Inhibition of RHMC cell culture growth by ICI 55897 occurred in a dose-dependent manner as shown in Fig. 2. The ED_{50} concentration calculated as the mean and S.D. from six separate experiments was 231 ± 24 μM in the presence of 20% foetal calf serum.

The anti-proliferative effect of ICI 55897 on RHMC cell growth was reversible (Fig. 3). Cells were exposed to 330 μM ICI 55897, a near maximal inhibitory concentration, for up to 5 days. When washed with compound-free medium, the cells resumed growth at a rate similar to untreated cell cultures. However, the maximum cell density which the cultures attained was less than that of control cell cultures. Although the reason for this observation is unknown it may indicate that a cell selection has occurred. Interestingly cells cultured for several days in the presence of cytostatic concentrations of ICI 55897, when examined by light microscopy, appeared to have increased in size and possessed larger nuclei relative to controls.

The reversible effect of ICI 55897 on RHMC cell culture growth suggests that the compound is cyto-

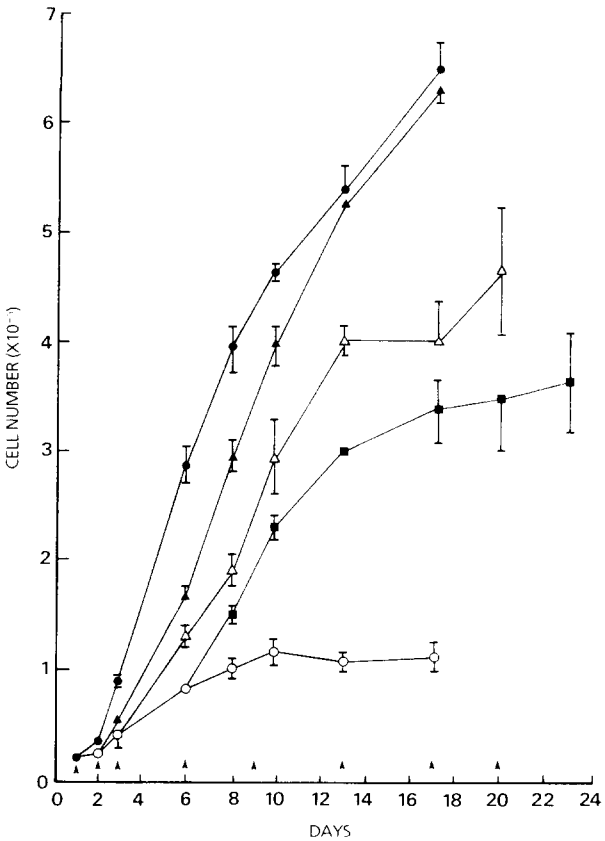

Fig. 3. Reversible effect of ICI 55897 on the growth of RHMC cell cultures. Cells were seeded into multiwell plates at 3.0×10^4 cells per well. One day later medium on all cultures was changed. Control cultures (●) received medium plus vehicle alone (1% methanol) and all other cultures received medium containing 330 μM ICI 55897. Cell cultures exposed to ICI 55897 for 1 (▲), 2 (△) or (■) 5 days were washed 5 times with medium containing 20% foetal calf serum at 37° and incubation continued with compound-free medium. Another series of cell cultures was continually exposed to ICI 55897 (○). Arrows indicate changes of media and compound. Values show the mean cell counts ± S.D. of four separate cultures.

static rather than cytotoxic. Lactate dehydrogenase (LDH) levels in media taken from RHMC cell cultures exposed to 500 μM ICI 55897 for 3 days did not significantly differ from LDH levels in control cell culture media. Vital staining (trypan blue) of both compound-treated and control cultures showed the cells to be >98% viable. The effect of ICI 55897 on RHMC cell culture growth, therefore, is not due to a loss of cell viability.

Inhibition of cell growth by ICI 55897 is not confined to RHMC cell cultures. Cultures of human embryonic lung fibroblasts, human endothelial cord cells, rabbit synovial fibroblasts and P388D$_1$ mouse macrophage-like cells were sensitive to growth inhibition by ICI 55897.

Effect of serum albumin on the anti-proliferative effects of ICI 55897 and related compounds

Binding of ICI 55897 to serum albumin in the culture medium may reduce the level of free compound causing an underestimate of its potency. An experiment was carried out in which growth media containing 20% FCS was supplemented with increasing concentrations of bovine serum albumin. The free concentration of ICI 55897 in these media was determined by equilibrium dialysis after spiking the media with ^{14}C-labelled ICI 55897. As shown in Fig. 4 the inhibition of RHMC culture growth by ICI 55897 can be overcome by increasing the albumin concentration, demonstrating that the anti-proliferative effect of ICI 55897 depends on the concentration of unbound compound (Fig. 4 inset).

The other oxyalkanoic acids, clofibric acid and ICI 115432, also inhibited the growth of RHMC cell cultures. Their rank order of potency, ICI 115432 > ICI 55897 > clofibric acid, was related to their log P values, which measure their lipophilicity (Table 1). The log P related increase in anti-proliferative potency is most striking when the ED_{50} concentrations for free compound rather than total compound are compared. This reflects the affinity of the compounds for albumin as lipophilicity increases.

We also tested the *R* and *S* enantiomers of both ICI 115432 and the related compound ICI 107030

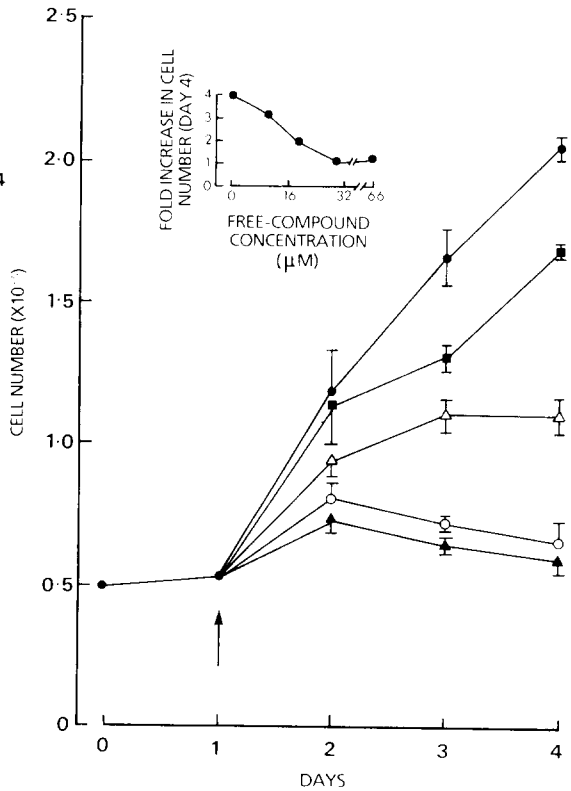

Fig. 4. Effect of serum albumin on the anti-proliferative property of ICI 55897. Cells were seeded into multiwell plates at 5.0×10^4 cells per well. One day later the media were changed. Cell cultures were grown in the absence (●) or presence of 492 μM ICI 55897 (■, △, ○, ▲). Media containing 20% foetal calf serum was supplemented with 0 (○), 0.5 (▲), 1 (△) and 2% (w/v) (●, ■) bovine serum albumin. Values show the mean cell counts ± S.D. from three separate cultures. The inset shows the effect of the free concentration of ICI 55897 on cell growth, over a 3-day period. The free concentrations of ICI 55897 in growth media at 37° were 9.8, 18.6, 29.5 and 65.6 μM in media supplemented with 2, 1, 0.5 and 0% bovine serum albumin respectively.

Table 1. Anti-proliferative potency and lipophilicity of oxyalkanoic acids

Compound	log P	ED_{50} (μM)	ED_{50} free (μM)
Clofibric acid	3.08	1850	1438
ICI 55897	4.30	231	26
ICI 115432	5.85	120	5

The concentration of compound causing 50% inhibition of the RHMC cell culture growth rate (ED_{50}) was determined by cell counting as previously described. ED_{50} free is the free compound concentration required to inhibit the cell culture growth rate by 50%. The free concentrations of compounds, in media supplemented with 20% foetal calf serum, were determined by equilibrium dialysis of medium containing a range of compound concentrations and spiked with the respective ^{14}C-labelled compounds. Graphs of free-compound concentration vs total compound in the media were drawn and used to find the ED_{50} free value corresponding to the ED_{50} value for total compound. Log P values were measured for the neutral species in octanol–water by the method described by Kaufman *et al.* [25].

in order to gain insight into the possibility of a stereoselective anti-proliferative effect. The *R* and *S* enantiomers of ICI 115432 and ICI 107030 were shown to be >90 and >98% optically pure respectively [9]. This means that there is no more than 5 and 1% of the less predominant enantiomers of ICI 115432 and ICI 107030 respectively. As shown by the ED_{50} values (Table 2) both the racemic mixtures and enantiomers of these compounds are potent inhibitors of RHMC cell culture growth. However, since only a small difference in potency was found between the respective *R* and *S* enantiomers of ICI 115432 and ICI 107030 it is unlikely that these oxyalkanoic acids inhibit cell growth by a mechanism involving interaction with a stereoselective receptor. From their known optical purity, if a stereoselective mechanism was responsible for cell growth inhibition, we would have expected at least 20- and 100-fold differences in potency between the *R* and *S* enantiomers of ICI 115432 and ICI 107030 respectively.

Anti-proliferative effect of anti-inflammatory agents

We have assessed the effect of several anti-inflammatory agents on the growth rate of RHMC cell cultures, measured by the incorporation of [^{3}H]thymidine into a trichloroacetic acid insoluble cell fraction as shown in Fig. 5. ICI 55897 is included for comparison. All compounds tested inhibited the growth rate of RHMC cell cultures in a dose-dependent manner. The rank order of potency causing inhibition of cell culture growth was as follows with ED_{50} concentrations shown in parentheses: benoxaprofen (160 μM) > phenylbutazone (195 μM) > ICI 55897 (230 μM) = indomethacin (230 μM) > flurbiprofen (540 μM) = naproxen (550 μM) > ibuprofen (750 μM) = ketoprofen (760 μM) > aspirin (1500 μM).

DISCUSSION

Acidic non-steroidal anti-inflammatory agents have been shown to reversibly inhibit the proliferation of hepatoma and human fibroblast cell cultures [11, 12]. The rank order of potency inhibiting cell growth was similar to their anti-inflammatory activity and ability to inhibit prostaglandin biosynthesis [11]. Our data also demonstrate that acidic non-steroidal anti-inflammatory agents inhibit the proliferation of a mammalian cell culture in a dose-dependent fashion. Since the concentrations of the agents required to inhibit cell growth greatly exceeds those required to inhibit prostaglandin biosynthesis it is unlikely that the cytostatic action of these drugs is due to inhibition of prostaglandin biosynthesis. The same conclusions were proposed by Mello *et al.* [12] after failing to reverse the cytostatic action of indomethacin on hepatoma cells by the addition of exogenous prostaglandins.

The mechanism responsible for the anti-proliferative action of the non-steroidal anti-inflammatory agents remains unknown although, since their action is reversible, it is not a cytotoxic effect. Indomethacin arrests cell growth in the G1 phase of the cell cycle [13, 14], a process which is accompanied by specific changes in the transport of several amino acids [15].

It is not clear whether the non-steroidal anti-inflammatory agents and the oxyalkanoic acids described here inhibit mammalian cell growth by a common mechanism. Several observations indicate different mechanisms.

The cytostatic activities of the non-steroidal anti-inflammatory agents parallel their anti-inflammatory activities [12, 16]. However, the oxyalkanoic acids, although possessing little or no activity in classical models of acute inflammation, are cytostatic. Also, whereas non-steroidal anti-inflammatory agents arrest the growth of HeLa cells [17], we found the growth of HeLa cells to be refractory to inhibition by the oxyalkanoic acids (unpublished observations).

A common feature of both the non-steroidal

Table 2. Anti-proliferative potency of ICI 107030 and ICI 115432 and their respective *R* and *S* enantiomers

	ED_{50} (μM)		
Compound	Racemic mixture	*R* enantiomer	*S* enantiomer
107030	130	200	120
115432	120	205	110

ED_{50} values were determined by cell counting as previously described. The enantiomers of ICI 107030 were *R*(−) and *S*(+) and >98% optically pure. The enantiomers of ICI 115432 were *R*(+) and *S*(−) and >90% optically pure.

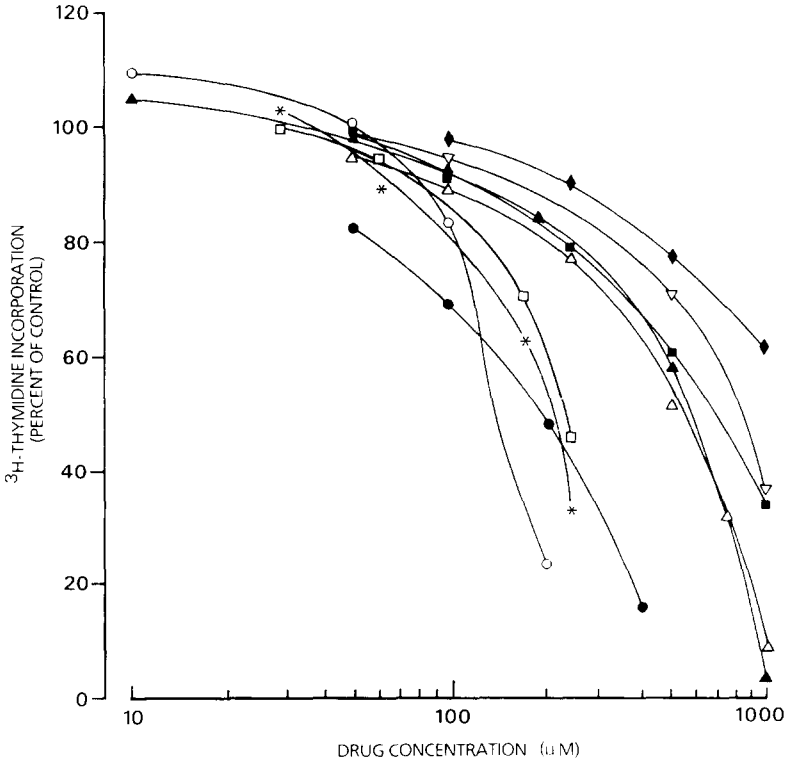

Fig. 5. [^{3}H]Thymidine incorporation into trichloracetic acid insoluble fraction of RHMC cell cultures grown in the presence of different concentrations of anti-inflammatory agents. Benoxaprofen (○), phenylbutazone (*), indomethacin (□), flurbiprofen (▲), naproxen (△), ibuprofen (■), ketoprofen (▽) and aspirin (◆). ICI 55897 is shown for comparison (●). Measurements were made on the fourth day of culture growth after 3 days exposure to compound. Values show the means of four separate cultures and are percentages of the mean values for control cultures.

anti-inflammatory agents [16] and the oxyalkanoic acids is that their cytostatic potency increases with their lipophilicity. Our observations suggest that the oxyalkanoic acids interact with a lipophilic cell target site since: (1) an increase in the log P value of the compound was associated with increased anti-proliferative potency, and (2) similar anti-proliferative potencies of the respective *R* and *S* enantiomers of ICI 115432 and ICI 107030 suggest that their anti-proliferative effect is not stereoselective but more in keeping with a non-specific interaction such as with the cytoplasmic and/or mitochondrial membranes. We have evidence (S. J. Foster and M. E. McCormick, to be submitted for publication) suggesting that the oxyalkanoic acids uncouple oxidative phosphorylation, implicating the mitochondrial membrane as a possible site of action. Whitehouse [18–20] has also shown that a wide range of anti-inflammatory and hypocholesterolemic agents uncouple oxidative phosphorylation.

At the pharmacological level, inhibition of prostaglandin biosynthesis is currently the accepted mode of action of the non-steroidal anti-inflammatory agents although many reports indicate additional mechanisms. It is possible that these agents have several mechanisms which contribute to their efficacy. Whereas inhibition of prostaglandin biosynthesis is responsible for their analgesic, anti-pyretic and acute anti-inflammatory actions other properties may influence some chronic aspects of inflammation. For example, it is unlikely that, with the exception of aspirin, those non-steroidal anti-inflammatory agents shown in Fig. 5 achieve therapeutic plasma concentrations sufficient to produce an anti-proliferative effect *in vivo* since: (1) a high degree of plasma albumin binding will reduce the effective free-drug level [21], (2) their *in vivo* anti-inflammatory effects occur at peak plasma levels far below their *in vitro* anti-proliferative concentrations; and (3) high concentrations of these agents are poorly tolerated because of their ulcerative potential.

Several new agents having novel pharmacological activity profiles may be useful in treating chronic inflammatory diseases. Benoxaprofen is only a moderate inhibitor of prostaglandin biosynthesis and it is not possible to explain its anti-inflammatory effect by this action alone [22]. We have shown, Fig. 5, that benoxaprofen has an anti-proliferative effect in cell cultures whilst others have shown it to be anti-chemotactic towards monocytes [23]. These additional properties may be partly responsible for its clinical efficacy.

ICI 55897 possesses minimal activity in standard acute anti-inflammatory and analgesic tests [6]. It caused no detectable inhibition of prostaglandin biosynthesis in rheumatoid synovial cell cultures at concentrations as high as 500 μM whereas indomethacin totally inhibited prostaglandin production at 14 μM [24]. Clearly, the anti-arthritic effect of ICI 55897 evident in the clinic [7, 8] cannot be explained on

the basis of classical anti-inflammatory properties. The anti-proliferative effect which we have observed for ICI 55897 may, in part, be responsible for its anti-arthritic properties. In 133 patients with rheumatoid arthritis taking a single daily dose of 200 mg ICI 55897, the steady-state plasma levels ranged from 390 to 692 μM with a mean of 548 μM (H. Adam *et al.*, personal communication). These plasma levels correspond to free-drug levels of 2.3–5.3 μM which we have shown can cause as much as a 20% decrease in the growth rate of RHMC cell cultures.

Goldberg *et al.* [1, 3] have shown that pharmacological concentrations of sodium aurothiomalate inhibit the proliferation of cell cultures derived from patients with rheumatoid arthritis. Similar findings have been reported for D-penicillamine, a disease-modifying anti-rheumatic agent, albeit at higher than normal pharmacological concentrations [2]. It is tempting to speculate that the disease-modifying anti-rheumatic agents such as ICI 55897, gold and D-penicillamine share a common effect responsible for their therapeutic efficacy in rheumatoid arthritis, which may be to slow down or arrest proliferation of cells in the rheumatoid pannus tissue.

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