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# Inhibition of lymphocyte transformation by mepacrine and chloroquine

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Mepacrine inhibited the uptake and the incorporation of leucine, thymidine and uridine into acid-insoluble material in human lymphocytes stimulated by phytohaemagglutinin (PHA) in vitro. The IC50 for each uptake was of the order of 2 μM. Mepacrine was inhibitory if applied at any time up to 48 h after PHA. The inhibition differed from that produced by colchicine and prostaglandin $E_1$. The dose-response curve was steep, nearly all incorporation being inhibited by 2 × IC50. Chloroquine also had a steep dose-response curve, was about one-fifth as potent as mepacrine and was maximally effective in the first 6 h after PHA.

Lymphocytes in tissue culture exposed to plant mitogens such as phytohaemagglutinin (PHA) transform in 3-5 days into lymphoblasts. In the process the cells take up sugars, amino acids and pyrimidines and incorporate them into carbohydrates, proteins and nucleic acids (Stjernholm & Falor 1970). Transformation is inhibited by a variety of drugs, including corticosteroids, colchicine, some prostaglandins, some inhibitors of prostaglandin synthesis, and chloroquine (Hurvitz & Hirschhorn 1965; Berenbaum et al 1976; Panush 1976; Gery & Eidinger 1977; Goodwin et al 1977; Gordon et al 1979).

Mepacrine, which differs from chloroquine only in having an acridine nucleus instead of a quinoline, is strongly concentrated by leucocytes (Shannon et al 1944). Like chloroquine, it is bound to nucleic acids (Irvin & Irvin 1954; Krey & Hahn 1974) and inhibits DNA and RNA polymerases (Cohen & Yielding 1965; O'Brien et al 1966). Also like chloroquine it is concentrated in sub-ceullar components, probably lysosomes (Allison & Young 1964) and some of its actions may be attributed to inhibition of release of lysosomal enzymes. The experiments reported here are directed to establishing whether mepacrine inhibits transformation, whether mepacrine and chloroquine act in the same way, and whether their actions differ from those of other inhibitors.

## MATERIALS AND METHODS

### *Preparation of lymphocytes*

Peripheral venous blood (50 to 100 ml) was collected from healthy volunteers into siliconized glass bottles containing preservative-free heparin to a final concentration of 20 $uml^{-1}$. After addition of an equal volume of RPMI 1640 medium (media production, Wellcome Research Labs) supplemented with penicillin (200 $uml^{-1}$) and streptomycin (100 $uml^{-1}$) the blood was layered in 12 ml aliquots onto 9 ml Ficoll/Plaque (5·7 g Ficoll 400 and 9·0 g Diatrizoate sodium per 100 ml solution, Pharmacia) in sterile, plastic, disposable Universal containers (Sterilin). The containers were centrifuged at 400 *g* for 40 min at room temperature (20 °C). The cells at the interface were removed, washed three times with RPMI 1640 medium at room temperature and finally resuspended at a concentration of 1·5 × $10^6$ cells $ml^{-1}$ in RPMI 1640 made to contain 10% human AB serum previously inactivated at 56 °C for 1 h and filtered through a 0·22 μm Millex-GS filter (Millipore). Cell viability was assessed by measuring the exclusion of 0·5% trypan blue (Pappenheimer 1917) after 15 min exposure at 37 °C. Viability was always greater than 98%.

### *Cell cultures*

Aliquots (100 μl) of cell suspension were distributed into wells of sterile, disposable, flat bottom microtitre plates (Microtest II, Falcon, No. 3040). Drugs and phytohaemagglutinin (PHA, Wellcome) were added with a randomized allocation of treatments. The plates were covered with a lid (Falcon, No. 3041) and incubated for various times at 37 °C in 5% $CO_2$ in humidified air. The cultures were pulsed with 1·0 μCi [$^3$H]uridine (sp. act. 45 Ci $mmol^{-1}$, Radiochemical Centre, Amersham) after 20 h or with 1·0 μCi [$^3$H]leucine (sp. act. 30 Ci $mmol^{-1}$, Radiochemical Centre, Amersham) or 0·4 μCi [$^3$H]thymidine ([$^3$H]TdR) (sp. act. 26 Ci $mmol^{-1}$, Radiochemical Centre, Amersham) after 68 h. Incubation was con-

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tinued for another 4 h. The samples were aspirated onto glass fibre discs with a cell harvester (Ilacon) and washed with 0·9% NaCl (saline). [$^3$H]uridine incorporation into RNA was determined by washing with 2·5% perchloric acid, and [$^3$H]thymidine incorporation into DNA and [$^3$H]leucine incorporation into protein by washing with 5% trichloracetic acid. The discs were finally cleared with methanol. The dried discs were transferred to scintillation vials containing 5 ml scintillation fluid (Aquasol, New England Nuclear) and counted in a liquid scintillation counter (Beckman, LS-200B).

*Drugs*

All drugs were made up in RPMI 1640 medium containing 10% human AB serum. Stock solutions (400 $\mu$M) of freshly prepared chloroquine diphosphate (Sigma Chemical Co.), colchicine, 3-isobutyl-1-methyl-xanthine (IBMX) (Koch-Light Laboratories Ltd), (−)-isoprenaline hydrochloride (Sigma Chemical Co.), DL-mepacrine hydrochloride (K & K Laboratories Inc.), and prostaglandin $E_1$ ($PGE_1$) (Sigma Chemical Co.) were serially diluted to the appropriate concentrations and added to the cell cultures in 50 $\mu$l aliquots. $PGE_1$ was initially dissolved in dimethyl sulphoxide (DMSO, BDH, reagent grade) and serially diluted with RPMI 1640 medium with 10% human AB serum to give a final concentration of 0·5% DMSO in the cell cultures. This concentration of DMSO did not affect lymphocyte transformation or cell viability. PHA (6, 8 or 20 $\mu$g ml$^{-1}$) was added in RPMI 1640 medium containing AB serum (50 $\mu$l to each well).

## RESULTS

*Effects of mepacrine on PHA-stimulated [$^3$H]-thymidine incorporation*

Mepacrine inhibited PHA-induced synthesis of protein, RNA and DNA, as measured by incorporation of specific radiotracers into acid-insoluble material. The dose-response curves for each inhibition showed a steep drop to complete or nearly complete inhibition. Fig. 1 shows curves obtained against [$^3$H]thymidine incorporation. IC50 values have been estimated by linear interpolation on the log dose-response plot. Values in nearly all experiments lay in the range 1–3 $\mu$M (Table 1). Differences in IC50 for protein, RNA and DNA syntheses are insignificant.

Mepacrine was equally effective against DNA synthesis if it was added up to 24–30 h after PHA and still had a significant effect after 48 h (Fig. 2). The processes initiated by DNA do not require the

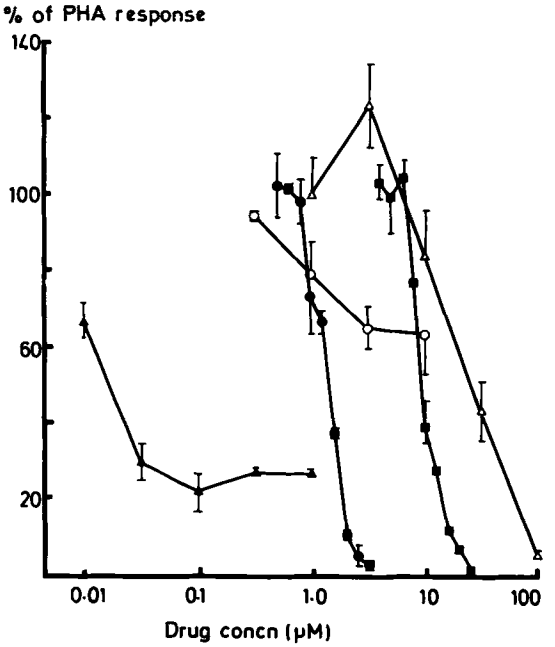


FIG. 1. Inhibition of PHA-induced 72 h thymidine incorporation in human lymphocytes by mepacrine (●), chloroquine (■), colchicine (▲), prostaglandin $E_1$ (○), and isoprenaline (△). Each point is the mean of 4 replicate samples and the vertical bars indicate s.e. mean.

presence of PHA in the medium for more than the first hour or so (Hadden et al 1970; Younkin 1972), so this finding suggested that mepacrine was not acting by blocking PHA receptors. More direct experimental evidence was given by the results shown in Table 2. Treatments were applied to lymphocytes in two phases, one lasting 1, 2 or 24 h, after which the cells were washed, resuspended in fresh medium and distributed in micro-titre plates for a second treatment lasting 72 h. Exposure to PHA for 1 or 2 h followed by 72 h in PHA-free medium was sufficient

Table 1. Inhibition of uptake of leucine, uridine and thymidine into human lymphocytes.

| Marker | IC50 μM: mean ± s.e. (No. of observations) [$^3$H]Leucine | [$^3$H]Uridine | [$^3$H]Thymidine |
|---|---|---|---|
| Duration | 72 h | 24 h | 72 h |
| Mepacrine | 2·25 ± 0·24(4) | 1·68 ± 0·17(6) | 1·56 ± 0·30(8) |
| Chloroquine | 7·70 ± 2·80(2) | 24·93 ± 5·75(4) | 9·92 ± 0·54(6) |
| Ratio of IC50 | 3·4 | 14·8 | 6·4 |

Statistical significance of differences: The six experiments on mepacrine and uridine incorporation were done on the same batches of cells as six of the eight experiments on mepacrine and thymidine incorporation. In each experiment the IC50 for thymidine was less than for uridine; the mean difference was 0·48 μM, with s.e. 0·11, $t = 4·36$ and $P < 0·01$. Mepacrine and chloroquine were both tested on thymidine and uridine incorporation with four batches of cells. In each experiment mepacrine was more potent than chloroquine: for thymidine the mean difference was 9·40 μM (s.e. 0·64, $t = 14·71$, $P < 0·001$), and for uridine the mean difference was 23·45 μM (s.e. 5·83, $t = 4·02$, $P < 0·03$).

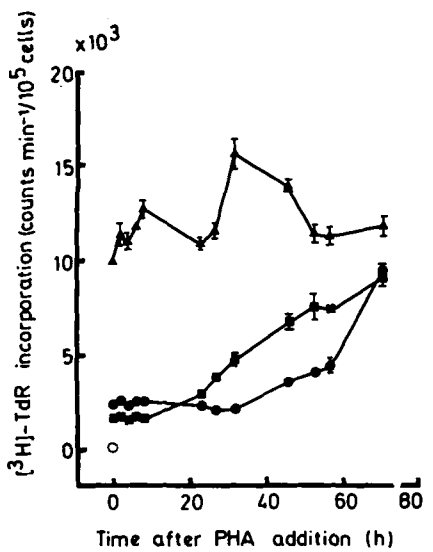


FIG. 2. Effect of mepacrine (1·6 μM) (●) and chloroquine (20 μM) (■) at various times after PHA (2 μg ml⁻¹) on the 72 h thymidine incorporation in human lymphocytes. Each point is the mean of 4 replicate samples and the vertical bars indicate s.e. mean. (▲), no drug; (○), no drug, no PHA. Where no error bars are shown s.e. <500 count min⁻¹ per 10⁵ cells.

to initiate transformation and indeed had a marginally greater effect than exposure to PHA for the whole 72 h period. Double exposure to PHA had still less effect. The inhibitory effect of excess mitogen is well known (Dent 1971; McClain & Edelman 1976) and this observation was not unexpected. Mepacrine given in the second period was equally effective as an inhibitor whether PHA was given only before the mepacrine, or only with the mepacrine, or both. On the other hand, exposure of cells to mepacrine, for a single hour, followed by washing did not impair their sensitivity to PHA applied simultaneously or subsequently. These results taken in conjunction with those shown in Fig. 2 show that mepacrine does not block the receptors to PHA, but acts at some later process in the sequence of events leading to increased DNA synthesis. Some inhibition could be produced by mepacrine in the period before PHA, provided that the period lasted for 2 h or longer. As mepacrine is concentrated by leucocytes as a two-phase process, with a slow component having a half time about 40 min (Trist 1979) this inhibition can be attributed to the accumulation of sufficient intracellular mepacrine for some to persist after exposure to PHA.

Table 2. Influence of time of adding reagents on [³H]thymidine uptake of lymphocytes.

Data shown as a percentage of the uptake after PHA in standard conditions

| Main treatment (for 72 h) | None | PHA (5 μg ml⁻¹) | Mepacrine (3·2 μM) | PHA (5 μg ml⁻¹) + Mepacrine (3·2 μM) |
|---|---|---|---|---|
| | | m±s.e., $n = 4$ | | |
| Pre-treatment: | | | | |
| None | 2·8±0·8 | 100·0*±5·2 | 0·8±0·4 | 2·0±0·5 |
| PHA 5 μg ml⁻¹ | 115·2±10·8 | 83·5±12·8 | 1·4±0·5 | 5·1±2·9 |
| 1 or 2 h | | | | |
| Mepacrine : 1 h (3·2 μM) | 2·0±1·5 | 107·6±9·6 | 1·2±0·5 | 1·2±0·7 |
| : 2 h | 1·5±0·4 | 64·5±25·4 | 0·3±0·2 | 0·4±0·3 |
| : 3 h | 6·8±6·3 | 43·6±4·9 | 1·4±0·3 | 0·5±0·3 |
| PHA + Mepacrine | | | | |
| : 1 h | 100·4±16·7 | 80·8±11·2 | 3·1±1·5 | 3·2±1·6 |
| : 2 h | 47·7±4·2 | 103·8±8·0 | 0·9±0·4 | 0·4±0·2 |

* 100% = 71557 counts min⁻¹ per 10⁵ lymphocytes.

Lymphocytes were treated in two phases: a pre-treatment with either medium alone, PHA (5 μg ml⁻¹), mepacrine (3·2 μM) or PHA (5 μg ml⁻¹) plus mepacrine (3·2 μM) for 1, 2 or 24 h, after which the cells were washed and resuspended in either medium alone, PHA (5 μg ml⁻¹), mepacrine (3·2 μM) or PHA (5 μg ml⁻¹) plus mepacrine (3·2 μM). The [³H]thymidine incorporation was measured 72 h after the cells were washed.

The process or processes blocked by mepacrine are not confined to the period 48–72 h, in which DNA and protein synthesis predominates. RNA synthesis, measured by [³H]uridine uptake over the first 24 h after PHA was equally effectively blocked by mepacrine, suggesting that the drug either acted on at least two processes or affected some basic metabolic activity necessary for both processes.

*Effect of mepacrine in the presence of 3-isobutyl-1-methyl-xanthine (IBMX).*

Lymphocyte proliferation is modulated by cyclic nucleotide levels, and many inhibitors of transformation (e.g. catecholamines and prostaglandins) are thought to act by raising cyclic AMP (cAMP) (Strom et al 1977). To ascertain whether mepacrine might also be acting through a cAMP mechansim the effect of mepacrine was measured on lymphocytes exposed to IBMX. The cAMP phosphodiesterase inhibitor IBMX at 100 μM, a concentration producing a near maximal effect, reduced the incorporation of [³H]thymidine after PHA to 34 and 41% of the control uptake in two experiments. The residual uptake in the presence of IBMX was inhibited by mepacrine, with unaltered potency (IC50 1·4 μM and 1·8 μM without IBMX; 1·4 μM and 2·1 μM with IBMX). It therefore appeared that mepacrine was not acting by increasing cell cAMP. Further evidence was obtained by direct measurement of lymphocyte cAMP levels. Mepacrine (up to 100 μM) did not alter the resting cAMP content of lymphocytes after 15 min incubation (A. Johnson, personal communication).

*Comparison with chloroquine and other inhibitors of lymphocyte transformations*

Chloroquine inhibited PHA-induced incorporation of thymidine as expected (Hurvitz & Hirschhorn 1965). The log dose-response curve was as steep as with mepacrine but chloroquine was less potent (Table 1). Administered some hours after PHA the activity of chloroquine diminished earlier than that of mepacrine in equipotent concentrations (Fig. 2). A similar time course has been observed by Gery & Eidinger (1977). As an inhibitor of uridine incorporation chloroquine was unusually variable, as the large standard error in Table 1 indicates, but the mean relative potencies of mepacrine and chloroquine as inhibitors of protein and nucleic acid synthesis did not differ significantly.

Several other compounds were compared as inhibitors of lymphocyte transformation. The dose-response curves differed considerably (Fig. 1), none showing the steep progress from IC50 to IC100 consistently found with chloroquine and mepacrine. Isoprenaline came nearest, but differed in showing in four experiments modest stimulation of thymidine incorporation with sub-inhibitory concentrations. Colchicine was a much more potent inhibitor but about a fifth to a third of the incorporation in each sample was insensitive to concentrations up to 100 × IC50. A similar incomplete inhibition by high concentrations, against stimulation by concanavalin A is shown by Wang et al (1975). Prostaglandin $E_1$ gave a rather flat dose-response curve over the whole range of its activity.

## DISCUSSION

The results show that mepacrine inhibits PHA-induced lymphocyte transformation. This is not surprising as mepacrine shares many properties in common with the structurally similar antimalarial, chloroquine, which is a known inhibitor of lymphocyte transformation (Hurvitz & Hirschhorn 1965).

Although mepacrine and chloroquine have similar-shaped dose response curves, which were unlike those of colchicine and $PGE_1$, mepacrine does appear to act differently to chloroquine. The loss of inhibitory activity of chloroquine when added some 6 to 8 h after PHA has been previously described (Hurvitz & Hirschhorn 1965; Gery & Eidinger 1977). It is within these first few hours of transformation that RNA synthesis is initiated (Darzynkiewicz et al 1965). Hurvitz & Hirschhorn (1965) speculated that chloroquine acts on RNA synthesis by inhibiting the release, from the lysosome, of essential RNAses required for derepression of the cell. The fact that actinomycin, an inhibitor of ribosomal RNA synthesis, has a similar time effect curve to chloroquine (Fig. 3 of Kay et al 1969) and that the present study has shown chloroquine inhibits the incorporation of [$^3$H]uridine within the first 24 h (Table 1) both support an involvement of chloroquine with RNA metabolism. As described, inhibition by mepacrine is not confined to the first 6 h of transformation but is still present during the PHA-independent stage of transformation (Younkin 1972). Similar IC50 values for both [$^3$H]uridine and [$^3$H]thymidine incorporation suggest that in part mepacrine acts like chloroquine, but it also may be supposed to act on other processes during transformation or to act on some basic cellular mechanism required for many processes, e.g. inhibition of RNA and DNA polymerase (O'Brien et al 1966), inhibition of glycolysis (Bowman et al 1961; Fraser & Kermack 1957), inhibition of ATP-requiring membrane pumps (Trist 1979). Alternatively the difference in action between mepacrine and chloroquine might be due to their distribution within the cell. For example both drugs have been shown to bind to isolated DNA but there is some doubt as to whether chloroquine does so within the cell (Hurvitz & Hirschhorn 1965). However, mepacrine can be seen to bind avidly to the nuclei of viable leucocytes in culture (N. G. Read, personal communication). Experiments are needed to distinguish between these possibilities.

The results of this study provide a comparison between mepacrine and chloroquine as inhibitors of lymphocyte transformation. Both drugs are highly concentrated by white cells (Shannon et al 1944; Allison & Young 1964). Other bases have been shown to be taken up by leucocytes in culture (Marks & Medzihradsky 1974), and the effects of such compounds on lymphocyte transformation also require investigation.

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