CLOFAZIMINE POTENTIATES THE SYNTHESIS OF PROSTAGLANDIN E2 BY HUMAN POLYMORPHONUCLEAR LEUCOCYTES *IN VITRO*

Sir,

In addition to its value as an anti-microbial agent, clofazimine (Lamprene, B663) in high dosage also possesses properties which are of established value in the treatment of erythema nodosum leprosum (ENL)[1–3] and possibly also in reversal (upgrading) reactions.[4,5] The exact mechanisms of clofazimine-mediated anti-inflammatory and immunosuppressive activity are unknown. We have suggested previously that they may be related to the pro-oxidative properties of the drug,[6] ie, the ability of clofazimine to stimulate polymorphonuclear leucocyte (PMNL) and macrophage membrane associated oxidative metabolism.[6–8] In this preliminary report the effects of clofazimine on both the spontaneous and leucoattractant-stimulated synthesis of the anti-inflammatory[9,10] immunosuppressive[11] prostaglandin (PG), PG E2, by human PMNL *in vitro* are described.

PMNL-enriched suspensions were prepared as previously described[5] and resuspended to a concentration of 1×10^7/ml in Hanks' balanced salt solution (HBSS). The synthetic chemotactic tripeptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Miles Laboratories Inc, Elkhart, Indiana, USA) at a final pre-determined concentration of 0·1 μM was used as the stimulant of

PG E2 synthesis. Clofazimine was completely solubilized using the following procedure: 10 mg of the drug was dissolved in 0·1 ml of 100% glacial acetic acid and 0·3 ml of 100% dimethyl sulphoxide (DMSO) and brought to 1 ml with 0·6 ml of distilled H_2O. The drug was diluted in distilled H_2O to a concentration of 200 μg/ml followed by addition of 0·1 ml of 1N NaOH, filtration through a 0·2 μm pore size micropore filter and $\frac{1}{10}$ dilution in HBSS to give a stock solution of 20 μg/ml which was brought to pH 7 and centrifuged in a microfuge at 12,000 rpm for 3 min. Solvent controls without clofazimine were identically processed. No residual particulate material relative to control systems could be detected in the clofazimine stock solution by a laser nephelometric procedure. The effects of clofazimine on the spontaneous and stimulated synthesis of PG E2 by human PMNL were measured at final drug concentrations of 1, 2·5, 5 and 10 μg/ml. Reaction mixtures contained 2×10^6 PMNL in a final volume of 1 ml HBSS. After incubation at 37°C/30 min, 1 ml of ice-cold HBSS was added to each tube and the tubes transferred to an ice-bath. After removal of PMNL by centrifugation the supernatants were assayed for PG E2 using a competitive binding radioimmunoassay (RIA) system (New England Nuclear Corp, Boston, Mass, USA). Results are shown in Table 1 and are expressed as p grams PG E2/10^6 PMNL/30 min.

Table 1

Clofazimine concentration	Spontaneous synthesis of PG E2 by PMNL	FMLP-stimulated synthesis of PG E2
Control (no Clofazimine)	5·2 ± 2·1†	13·0 ± 2·1
1 μg/ml Clofazimine	6·0 ± 2·8	25·0 ± 5·6*
2·5 μg/ml Clofazimine	8·6 ± 3·2	52·3 ± 6·5*
5 μg/ml Clofazimine	14·0 ± 3·5*	76·7 ± 6·7*
10 μg/ml Clofazimine	25·1 ± 5·0*	81·3 ± 6·3*

* $P < 0{\cdot}005$ by comparison with the corresponding control (without clofazimine) systems.

† Results as the mean value in p grams PG E2/10^6 PMNL ± SEM of 6 experiments.

Clofazimine at all concentrations tested increased the spontaneous and especially the FMLP-stimulated synthesis of PG E2 by PMNL. Ingestion of 200 mg of clofazimine daily gives peak serum levels of 0·7–1 μg/ml and probably higher tissue concentrations[12] indicating that these effects may be operative *in vivo*. Solvent control systems which were included for each clofazimine concentration did not affect PG E2 synthesis. Likewise clofazimine *per se* did not interfere with the RIA for PG E2 as shown by the inclusion of control systems containing clofazimine only (no PMNL or FMLP).

PG E2 inhibits T-lymphocyte proliferation and antibody production.[11] Likewise pharmacological amounts of PG E2 or its poorly metabolizable analogues relieve nephritis and adjuvant arthritis in animals and eliminate immune complex arthritis.[9, 10] Potentiation of PG E2 production by PMNL, monocytes and macrophages in response to pro-inflammatory stimuli such as leucoattractants and antigens, if operative *in vivo*, is a likely mechanism of clofazimine-mediated anti-inflammatory and immunosuppressive activity.

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