Mononuclear leucocyte function in patients with lichen planus and cutaneous lupus erythematosus during chemotherapy with clofazimine

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Summary

Mitogen-induced transformation and the production of reactive oxidants by mononuclear leucocytes (MNLs) from patients with chronic dermatological disorders were investigated in vitro before and during the administration of the antimycobacterial/immunosuppressive agent clofazimine (200 mg 3 times weekly). Seven patients, 4 with lichen planus and 3 with cutaneous lupus erythematosus, were included in the study. Clofazimine administration did not influence the mitogen-induced proliferative responses of patients’ MNLs. However, chemotherapy with this drug stimulated the production of reactive oxidants by MNLs. Since reactive oxidants are immunosuppressive it is possible that these effects may be involved in the pharmacotherapeutic activity of clofazimine.

The phenazine derivative clofazimine (3-(p-chloro-anilino)-10-(p-chlorophenyl)-2,10-dihydro-2-(isopropyliminophenazine)) is an antileprosy drug that, because of its immunosuppressive effects, has also been used successfully in various chronic dermatological disorders including discoid and subacute lupus erythematosus (LE). The inhibitory effect of clofazimine on mitogen-stimulated mononuclear leucocyte (MNL) transformation used to evaluate cell-mediated immunity has been demonstrated both in vitro and in vivo in normal volunteers and in patients with lepromatous leprosy. In addition, clofazimine stimulates the production of reactive oxidants by polymorphonuclear and MNLs in vitro. There have been no reported investigations into the in vivo effect of clofazimine on the generation of these agents.

A study, planned as a pilot trial, investigated the effect of clofazimine administration on cell-mediated immunity and on the production of reactive oxidants by MNLs ex vivo.

Patients and methods

Patients

The patients were all black South Africans and were seen at the Dermatology Outpatient Clinic of Ga-Rankuwa Hospital, Pretoria. Four had lichen planus (LP) and 3 LE (2 subacute and 1 discoid). The average age in the LP group was 22.8 years (range 18-27 years) and in the LE group 33.7 years (range 33-34 years). The male : female ratios were 1:3 for patients with LP and 1:2 for patients with LE respectively.

All patients received clofazimine 200 mg 3 times weekly; administration was supervised by nurses. Compliance was checked by qualitative determination of clofazimine serum levels by thin-layer chromatography according to the method of Lanyi and Dubois.

Laboratory investigations

The following investigations were carried out before treatment and after 1 and 3 weeks of clofazimine therapy. Results were expressed as mean values (±SEM) for each series of experiments. Statistical analyses were performed by the Mann-Whitney U-test. MNLs were separated from heparinised venous blood by Ficoll-Hypaque density gradient centrifugation.

Effects of clofazimine treatment on MNL transformation. The ex vivo effects of clofazimine administration on spontaneous and on stimulated MNL transformation in response to T-cell mitogen phytohaemagglutinin (PHA) (Wellcome Reagents, Beckenham, Kent, UK) at final concentrations of 2.5 μg/ml and 5 μg/ml were investigated according to the uptake of radiolabelled thymidine by MNLs, as previously described. The results are expressed as radioactive counts/min (cpm).

Effects of clofazimine treatment on production of reactive oxidants by MNLs. To determine the ex vivo effects of clofazimine on the production of reactive oxidants by MNLs, the luminol-enhanced chemiluminescence technique was used. Spontaneous and PHA-stimulated chemiluminescence of 1 x 10^6 MNLs in a reaction volume of 0.4 ml integrated over 5 seconds were measured in an LKB Wallac 1251 luminometer (Turku, Finland) after the addition of 1 x 10^6 luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Sigma Chemical Co., St Louis, Mo., USA). The highest count (in mVs) for each specimen was evaluated.

Results

Patients

Good clinical responses were obtained in 2 patients with LP and in 2 with subacute LE. One of the LP patients showed fair improvement and 1 LP and 1 discoid LE patient did not improve at all during the treatment regimen. No significant side-effects were noticed.

Effects of clofazimine treatment on MNL transformations

The results of spontaneous and PHA-stimulated (2.5 μg/ml) MNL transformation before and during treatment with clofazimine are shown in Table I. A slight decrease in reactivity to
Effects of clofazimine treatment on production of reactive oxidants by MNLs

A marked increase in the spontaneous production of reactive oxidants was observed after 3 weeks of treatment. However, due to the small number of cases this difference is not statistically significant. The PHA-stimulated chemiluminescence of the patients' MNLs basically followed the same course as the spontaneous generation of reactive oxidants. Comparison of the results at 3 weeks with those obtained before treatment showed a significant increase \( P < 0.025 \) (Fig. 1).

![Graph showing production of reactive oxidants by MNLs](image)

**Fig. 1.** Production of reactive oxidants by \( 1 \times 10^6 \) MNLs of patients with LP and cutaneous LE as measured by luminol-enhanced chemiluminescence in the absence and presence of PHA before and during treatment with clofazimine (results are expressed as mVs ± SEM).

**Discussion**

The results of this study, albeit in a small group of patients, demonstrate that clofazimine chemotherapy is accompanied by increased production of reactive oxidants by PHA-activated MNLs. These oxidants, which probably originate from monocytes in the MNL populations, are antiproliferative and immunosuppressive. In \textit{vivo} studies have implicated stimulation of the generation of reactive oxidants as a possible mode of action for the antirheumatic drugs D-penicillamine and benoxaprofen, as well as for the immunosuppressive effect of clofazimine. However, we were unable to demonstrate significantly decreased mitogen-activated proliferative responses of patients' MNLs during treatment. This probably reflects our inability to recreate the \textit{in vitro} chemotherapeutic situation in \textit{vivo}. However, during intense inflammatory reactions, such as occur in patients with leprosy during type III (immune complex) and type IV (cell-mediated) reactions, the mononuclear phagocyte/lymphocyte ratio is higher than we achieved in our \textit{in vitro} experimental system. Consequently the level of oxidative damage inflicted on lymphocytes by clofazimine-exposed phagocytes is probably greater. In view of the small number of patients no attempt was made to correlate clinical responses to the stimulation of the production of reactive oxidants by MNLs statistically. However, on an individual basis such a correlation seemed to exist, a finding which suggests that further research in this direction is merited.

In conclusion, the results presented indicate that the immunosuppressive properties of clofazimine may be related to the stimulation of the production of reactive oxidants by MNLs. Further investigations are needed to confirm and extend these findings.

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**REFERENCES**