

Changes in neutrophil motility accompanying dapsone and rifampicin therapy

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Summary Dapsone has been shown to stimulate the motility of normal neutrophils *in vitro* and the neutrophils of patients with lepromatous and tuberculoid leprosy *in vivo*. It is suggested that dapsone possesses immunostimulatory activity.

Introduction

We have previously reported that dapsone therapy in patients with lepromatous leprosy was associated with progressive improvement of neutrophil migration and mitogen-induced lymphocyte proliferation (Anderson, Gatner, Imkamp & Kok, 1980).¹ These observations could have been due to a primary immunopharmacological action of the drug or alternatively to a secondary phenomenon due to the antimicrobial activity of the drug (as a result of decreased levels of bacteria-derived serum inhibitors of leucocyte functions). We have now investigated the *in vitro* effects of dapsone on the motility of normal neutrophils and the *in vivo* effects in patients with tuberculoid leprosy receiving dapsone only and rifampicin or dapsone only in a group of patients with lepromatous leprosy.

Patients and methods

PATIENTS

Twenty-four new untreated admissions (6LL, 6BL, 8BT and 4BB) were classified according to the clinical and histopathological criteria of Ridley and

Jopling (1966).² The 6LL and 6BL patients comprised the lepromatous leprosy group and the 8BT and 4BB patients formed the tuberculoid leprosy group.

EXPERIMENTAL PROTOCOL

Patients in the lepromatous leprosy group received dapsone only (DDS, 100 mg daily) for the first week of therapy after which therapy with rifampicin only for 6 weeks (600 mg daily) was commenced. Testing of neutrophil motility was performed prior to dapsone therapy, 2 h after the ingestion of a single 100 mg oral dose (to investigate possible immunopharmacological effects) and one week after the ingestion of 100 mg dapsone daily; testing was also performed after the 6-week period of rifampicin therapy. In patients with tuberculoid leprosy neutrophil motility was tested prior to dapsone therapy and 4 weeks after therapy with 100 mg dapsone daily.

STUDIES OF NEUTROPHIL MOTILITY

Neutrophils were obtained from heparinized venous blood (5 units heparin/ml) processed as previously described (Anderson *et al*, 1980)¹ and resuspended to a final concentration of 5×10^6 /ml. One leucoattractant was used, viz. endotoxin-activated autologous serum (EAS) prepared by activation of fresh serum with 100 μ g/ml of bacterial endotoxin (*E. coli*: 0127: B8 Difco, Detroit, Michigan, USA). Neutrophil motility was assessed as previously described (Anderson and van Rensburg, 1979).³ Chambers were incubated and the results expressed as the average number of cells reaching the lower surface of the 5 μ -pore size Millipore filter after 3 h incubation and expressed as an average for triplicate filters.

Dapsone powder for *in vitro* experiments was obtained from Lennon Laboratories (Pty) Ltd, Port Elizabeth, South Africa, and dissolved in HBSS. The concentration range investigated was 10^{-6} M– 10^{-2} M. In these experiments to assess the effects of dapsone on the motility of neutrophils from normal adult volunteers to autologous EAS an incubation period of 2 h was used. Dapsone was present with the neutrophils in the upper compartment of the chemotaxis chamber throughout the incubation period.

Results

In vitro effects of dapsone

Results on the migration of normal neutrophils are shown in Table 1. Dapsone at concentrations of $> 10^{-4}$ M caused stimulation of motility which was statistically significant at concentrations of 10^{-3} M, 2.5×10^{-3} M and 5×10^{-3} M.

Table 1. The effects of dapsone *in vitro* on the migration of normal neutrophils to autologous EAS

Dapsone concentration	Neutrophil migration to EAS	P value*
Control	169 ± 18 [†]	
1 × 10 ⁻³ Molar	243 ± 20	< 0.05
2.5 × 10 ⁻³ Molar	266 ± 21	< 0.05
5 × 10 ⁻³ Molar	259 ± 22	< 0.05

*Statistical analysis was performed by the Student's *t* test for paired means.

[†]Results as the mean value in cells/microscope high power field for six different individuals

In vivo effects of dapsone and rifampicin

Results in patients with lepromatous leprosy are shown in Table 2. Dapsone therapy was associated with significantly improved neutrophil motility to autologous EAS. Rifampicin therapy was associated with a slight decrease in neutrophil motility (Table 2).

Table 2. Effects of dapsone only and rifampicin only on neutrophil migration to autologous EAS in patients with lepromatous leprosy

Pre-therapy	Neutrophil migration to EAS		
	2 h after ingestion of 100 mg dapsone	After 1 week 100 mg dapsone daily	After 6 weeks' therapy with rifampicin only (600 mg daily)
28.5 ± 12.6*	57.4 ± 28.4 (< 0.05) [‡]	75.3 ± 20.4 [†] (< 0.05)	44.8 ± 15.9

*Results as the mean value with standard error as cells/high power field.

[†]Dapsone therapy stopped at this stage prior to commencement of rifampicine therapy.

[‡]P value calculated by the Wilcoxon Signed-Rank test.

In vivo effects of dapsone therapy

Results in patients with tuberculoid leprosy are shown in Table 3. Ingestion of dapsone caused a significant increase of neutrophil motility.

Table 3. Effects of Dapsone only on neutrophil migration to EAS in patients with tuberculoid leprosy

Neutrophil migration to EAS	
Prior to dapsone therapy	4 weeks after ingestion of 100 mg dapsone daily
109.2 ± 23.4*	200.0 ± 38.1 (< 0.05) [†]

*Results as mean cells/microscope high power field with standard error.

[†]P value calculated by the Wilcoxon Signed-Rank test.

Discussion

In this study dapsone at concentrations of $>10^{-4}$ M has been shown to stimulate neutrophil motility *in vitro*. These findings suggest that *in vivo* stimulation of leucocyte migration would require serum levels of dapsone of $> 25 \mu\text{g/ml}$ which indicates that dapsone stimulation would not be achieved following ingestion of 100 mg of dapsone. However we have previously reported for levamisole that concentrations of this agent required to stimulate neutrophil motility *in vivo* are considerably less than those required *in vitro* (Anderson, Oosthuizen, Theron & van Rensburg, 1979).⁴

In order to investigate the immunopharmacological activity of dapsone the effects of the drug on neutrophil motility were studied prior to and 2 h after the ingestion of a single 100 mg oral dose. Significant stimulation of neutrophil migration was observed which justifies our previous suggestion (Anderson *et al.* 1980)¹ that dapsone may possess immunostimulatory activity. It was not established if the dapsone-mediated changes in motility were cell or serum-related. Rifampicin therapy was associated with a slight decrease in neutrophil motility *in vivo*. Stendahl, Molin and Dahlgren (1970)⁵ have reported that dapsone *in vitro* had no effects on PMN migration to zymosan-activated serum which is not in agreement with this report. These differences are probably related to the low concentrations of dapsone investigated by Stendahl *et al.*⁵

These findings show that dapsone in addition to antimicrobial and suspected anti-inflammatory activity (Thomson and Souhami, 1975)⁶ possesses immunostimulatory properties. There is the suggestion that dapsone therapy may be involved in the pathogenesis of erythema nodosum leprosum (ENL) since dapsone causes increased migration of neutrophils and may therefore also increase the locomotion of mononuclear cells and macrophages. Further studies are in progress to investigate the mechanism of dapsone-mediated stimulation of neutrophil motility.

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