mycoses 31 (5) 259–267 · accepted/angenommen: April 24, 1988 · © Grosse Verlag Berlin 1988

In Vitro Effect of Terbinafine on Human Leukocyte Chemotaxis and Chemiluminescence

In vitro-Effekt von Terbinafin auf die Chemotaxis und Chemilumineszenz von Leukozyten des Menschen

M. Schaude, W. Mlineritsch and B. Mandak
Sandoz-Forschungsinstitut, Wien, Österreich

Schlüsselwörter: *Candida albicans* – polymorphonuclear leukocytes – chemotaxis – chemiluminescence – terbinafine
Key words: *Candida albicans* – polymorphkernige Leukozyten – Chemotaxis – Chemilumineszenz – Terbinafin

Summary: The in vitro effect of terbinafine, a broad spectrum antifungal agent on human peripheral blood polymorphonuclear leucocyte (PMNL) functions was studied in comparison to the known inhibitor cytochalasin B (CYTB). At concentrations above and below therapeutically achieveable plasma levels, terbinafine had no effect on either viability, chemotaxis or chemiluminescence of PMNL whether following simultaneous addition of the drug or prior treatment of the PMNL cells with drug. CYTB caused a statistically significant decrease in motility and chemiluminescence response of PMNL. The present in vitro studies demonstrate no adverse immunotoxic effects of terbinafine on major neutrophil functions even at concentrations several-fold higher than those generally found in plasma of humans receiving oral treatment.

Zusammenfassung: Terbinafin, ein Breitband-Antimykotikum, wurde hinsichtlich seines in vitro-Effektes auf verschiedene Funktionen peripherer, polymorphkerniger Leukozyten (PMNL) des Menschen im Vergleich zu Cytochalasin B (CYTB), einem bekannten Hemmstoff, untersucht. Bei Konzentrationen, die höher bzw. niedriger waren als die therapeutisch erreichbaren Plasmaspiegel, hatte Terbinafin unabhängig davon, ob die Substanz gleichzeitig zugegeben wurde oder ob die PMNL mit der Substanz vorbehandelt wurden, weder einen Effekt auf die Vitalität noch auf die Chemotaxis oder Chemilumineszenz von PMNL. CYTB führte zu einer statistisch signifikanten Abnahme der Motilität und Chemilumineszenz von PMNL. Die vorliegenden In vitro-Untersuchungen zeigen, daß Terbinafin selbst bei Konzentrationen, welche um ein Vielfaches höher waren als die üblicherweise im Plasma des Menschen nach oraler Therapie bestimmten, keinen immunotoxischen Effekt auf die wichtigsten Funktionen von Neutrophilen hat.

Introduction

Neutrophil polymorphonuclear leucocytes are one of the major cell types involved in resistance to the early stages of fungal infection (4, 25, 27, 33, 36) and are known to be of great importance especially in immunocompromised patients (10, 31). Depletion of neutrophil numbers, or defects in their function, is often an important predisposing factor for development of serious mycotic infections, requiring treatment with antifungal agents (8). Since many of the newer

antifungal drugs exert fungistatic rather than fungicidal activity in vitro at therapeutic concentrations (20), these drugs presumably work under in vivo conditions in collaboration with natural defence mechanisms such as polymorphonuclear leukocytes. Since drug-induced modifications of PMNL cell function could interfere with the cellular immune response of the body (14, 34, 35, 37) the rational for development of new antifungal therapy must consider not only classical notions, such as the sensitivity of the microorganisms, the spectrum covered, or a higher dose-efficiency, but also the possible immunomodulatory influence. In recent years a number of reports have examined the effects of antimicrobial agents on certain function of PMNL including chemotaxis (7, 14, 18, 29, 32), uptake and killing (11, 26, 28), lymphocyte blastogenesis (22) and certain metabolic activities as measured by the chemiluminescence (CL) response (1, 2, 7, 19, 29, 35), and it has become increasingly apparent that some antimicrobial agents may adversely affect immune cells and abrogation of host defence might occur during chemotherapy.

A prompt response of PMNL to migrate to and accumulate at sites of fungal invasion is central to effective host defence against infection. The chemotactic attraction of these cells from the circulating blood is brought about by a series of effector substances which emanate from the invaded tissues (18). A further important activity of phagocytes is their ability to respond to appropriate stimuli by activation of the respiratory burst, production of reactive intermediates such as superoxide anion, hydrogen peroxide, hydroxyl radicals and singlet oxygen, and the stimulation of the hexose monophosphate shunt (19). This activation which is an essential step in host defence against invading microorganisms is accompanied by light emission or CL by the PMNL (3, 15, 23).

The close correspondence observed between the CL emitted and the microbicidal activity of the phagocyte (5, 9, 15, 24, 40) has enabled luminometry to be used as a sensitive and rapid test to detect cell-drug interactions (19).

The present study was undertaken to investigate the in vitro effect of terbinafine, a new broad spectrum antifungal agent of the allylamine class (38), for oral administration at concentrations ranging from below to several fold above those therapeutically achieveable in comparison to the known inhibitor CYTB (6, 13) on human PMNL chemotaxis (CT) and phagocytosis-associated respiratory burst as measured by CL.

Material and Methods

Reagents

The test media used throughout these experiments were medium 199 (Gibco 400–1200) and RPMI 1640 (Gibco 0741800) buffered with 20 mM N-2-hydroxyethyl piperazine-N'-ethanesulfonic acid (HEPES, Serva 25245) supplemented with 5% pooled human serum. 7-dimethylamino-naphtalene-1,2-dicarbonic-acidhydracide (DMNH, Boehringer Mannheim 66433) a highly efficient CL oxidant indicator (16) was maintained as a stock solution of 10 mM in dimethylsulfoxide (DMSO, Merck 2950) and diluted with RPMI immediately before use. CYTB (Sigma C-6762) was dissolved in DMSO to form a stock solution of 1 mg ml^{-1}, stored at $-20°C$ and subsequently diluted in RPMI without serum. Urovist 65% and Urovison 58% were purchased from Schering AG, FRG. N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Sigma F-3506) was dissolved in DMSO at a stock concentration of 1 mM, stored at $-20°C$ and subsequently diluted in RPMI without serum. Bovine serum albumine (BSA, A-3912), Ficoll type 400 (F-4375) and agarose (A-6013) were obtained from Sigma.

Terbinafine

Terbinafine (SF 86-327, batch 86-913) was synthesized at the Sandoz Forschungsinsitut, Vienna (38). For each experiment the anti-

fungal was freshly prepated as stock solution at 500 mg l^{-1} in 5% DMSO/RPMI. Further dilution was made with the test media.

Candida albicans

Candida albicans ATCC 2655 was grown to the stationary-phase in Sabouraud-glucose broth (Merck 8339) by incubation for 24 h at 30°C in an orbital shaker set at 150 rev. min^{-1}. Under these culture conditions *C. albicans* grew as an essentially pure yeast-form population and viability was assessed by means of a methylene blue dye exclusion technique and in all cases was found to be >98%. The fungi were harvested by centrifugation at 1600 × x for 10 min and washed twice in RPMI without serum. For opsonization the cell pellet was resuspended in RPMI to give a final cell count at 2×10^7 colony forming units (CFU) ml^{-1} at least 30 min before use.

Preparation of PMNL and serum

Human PMNL were fractionated and purified from aliquots of 50 ml freshly drawn, heparinized (10 IU ml^{-1}) whole venous blood of normal healthy volunteers using a modification of the one-step Hypaque-Ficoll density gradient technique described by Ferrante & Thong (17). 50 ml of the separation medium were made up by mixing 4 g Ficoll, 8.42 g Urovist, 8.86 g Urovison and water. The pH was adjusted to 7.4 with NaOH and the medium strilized by filtration through a 0.45 µm filter. The density of the medium was 1.1125 g ml^{-1} at 20°C. Sterile, disposable 50 ml clear-plastic tubes (Falcon 2070) were used for centricugation. Twenty-five milliliters of blood were carefully layered on to 15 ml of the gradient and spun at 800 × g in swing-out buckets at room temperature for 40 min. The PMNL band which separates as a single layer within the Hypaque-Ficoll was collected, washed twice with RPMI and resuspended in RPMI to the appropriate working concentrations. Cell suspensions thus prepared contained greater than 98% PMNL and more than 95% of cells were viable after isolation as assessed by exclusion of trypan blue. Pooled non-inactivated normal human serum (PHS) was prepared from 5 donors and stored in 1 ml aliquots at −20°C before use.

PMNL pretreatment

Aliquots of 1 ml of PMNL suspension (10^7 cells ml^{-1}) were placed into plastic tubes (Falcon 2070) and preincubated with 10 µl of terbinafine at the final concentration of 1.25, 2.5, 5, 10 mg l^{-1}, CYTB at 1.25 mg l^{-1} or the diluent control under slight agitation in a waterbath at 37°C. After centrifugation for 10 min at 400 × g, the cell pellet was resuspended either in a small portion of the supernatant to give a final concentration of 5×10^7 cells ml^{-1} for the CT assay or in RPMI at 2×10^6 cells ml^{-1} for the CL assay.

Chemiluminescence assay

CL was measured in a Biolumat LB 9505 six-channel photoanalyzer (Berthold, Wildbad, FRG) using a DMNH – enhanced system (16, 23). For the evaluation of CL in PMNL cells, the reaction mixture consisted of 200 µl pretreated or untreated PMNL suspension (2×10^6 cells ml^{-1}), 100 µl of a known concentration of terbinafine (1.25, 2.5, 5,10 mg l^{-1}), CYTB (2.5 mg l^{-1}) or the diluent control, and 25 µl of diluted DMNH (2.5×10^{-5} M) in disposable 12 × 47 mm Lumocuvettes (3M Medical Products Div., St. Paul, Minn.). The CL reaction was induced by addition of 200 µl RPMI containing 2×10^7 CFU ml^{-1} opsonized *C. albicans* in suspension to yield a yeast to PMNL ratio of 10:1. During each experimental assay, the CL response (intensity of emitted light in counts per minute, cpm) was measured for each of the six channels every 20 s for 20 min after stimulation with yeast at a chamber temperature of 37°C. The Biolumat was interfaced with an Apple II computer and Spirit-80 printer for data analysis and print-

ing. In all experiments reagents and vials were dark-adapted before use. Three parameters were used to analyse the CL response: peak intensity of the emitted light, time to peak response, and area under the intensity-time curve.

Chemotaxis assay

PMNL chemotaxis was performed by the under-agarose technique (18, 30, 39) with slight modifications. 5 ml agarose medium consisted of 1 ml 3.75% agarose solution, 0.5 µl 10 × medium 199, 0.05 ml of 15 mM HEPES, 0.05 g BSA, 50 µl of a known concentration of terbinafine (1.25, 2.5, 5, 10 mg l^{-1}), CYTB (1.25 mg l^{-1}) or the diluent control. The pH of the medium was adjusted to 7.4 before addition of the agarose and the medium filled up with water to 5 ml. Five milliliter aliquots of this agarose solution were transferred to each 60 × 15 mm tissue culture dish (Falcon 3002) and allowed to harden. This dishes were then transferred to the refrigerator for 60 min to facilitate cutting of the wells. Six series of three wells 2.4 mm in diameter and spaced 2.4 mm apart were cut in each plate using a plexiglas template and stainless steel punch (30). The agarose plugs were removed using a hypodermic needle. Five µl of cell suspensions (5×10^7 cells ml^{-1}) pretreated with drugs were added to the six middle wells in the agarose plate with the corresponding concentration of drugs. The inner wells were filled with 5 µl of RPMI without serum, and the outer wells were filled with 5 µl of chemotactically active FMLP solution (2.5×10^{-7} M). The plates were incubated at 37 °C in a humidified atmosphere. After incubation for 1.5 h, the cells were permanently fixed by addition of 3 ml methanol overnight at 4 °C and 10% formalin for 30 min. After fixation the gel was removed intact and the plates stained with 30% Giemsa for 5 min and air dried. The linear distances migrated by the 10 fastest moving cells toward chemotactically active material as compared to the negative control (RPMI) were quantified by measurement of the greatly enlarged projection (E. Biegler, Digital viewer). Chemotactic responsiveness is described as percent of control migration, chemotactic ratio (A/B) or as chemotactic differential (A − B), where A and B represent the migration distances toward the attractant well and the control well respectively.

Statistics

Each test was run on blood obtained from ten different donors on different days for each concentration of terbinafine and CYTB. Results are expressed as means ± SEM. An overall assessment of differences between various dose means of the test compounds and the control was performed by a one way analysis of variance followed by a multiple T-test. The level of significance was 95%.

Results

In preliminary experiments it was found that the solubilizing agent DMSO at the highest concentration used in these studies (0.05%) did not have any significant impact on CL response or motility of human PMNL cells. None of the test drugs had any effect on PMNL viability at the concentrations tested after exposure for periods up to 30 min as measured by trypan blue exclusion. The effects of terbinafine and CYTB on neutrophil CT was expressed by the chemotactic ratio, chemotactic differential or percent of control migration are summarized in Table 1 and represented graphically in Figure 1. When PMNL cells were preincubated with 1.25, 2.5, 5, 10 mg l^{-1} of terbinafine for 30 min and placed in an agarose gel containing drug concentrations corresponding to that in the medium used for preincubation, none of the individual doses of terbinafine resulted in a mean directional migration toward FMLP which was significantly different from that of control cells that had been preincubated with

Table 1: Chemotaxis towards FMLP (2.5×10^{-7} M) of human PMNL from 10 healthy subjects after preincubation of cells in vitro with different concentrations of terbinafine, CYTB, or the diluent control. After 30 min the PMNL were concentrated (5×10^7 cells ml^{-1}), placed in agarose gels containing the corresponding concentration of the test drugs and incubated for 1.5 h at 37 °C. The data represent the mean ± SEM of 10 experiments, each with 6 determinations.

Drug	Concentration ($mg\ l^{-1}$)	Mean ± SEM (n = 10) chemotactic ratio	chemotactic differential	migration (% control)
Control		3.07 ± 0.16	1.35 ● 0.08	100.00 ± 0.00
Cytochalasin B	1.25	5.06 ± 0.52*	0.54 ± 0.08*	31.28 ± 4.92*
Terbinafine	1.25	3.08 ± 0.13	1.37 ± 0.07	100.87 ± 0.97
Terbinafine	2.5	3.01 ± 0.14	1.34 ± 0.07	99.72 ± 1.25
Terbinafine	5.0	3.04 ± 0.13	1.34 ± 0.07	99.36 ± 1.13
Terbinafine	10.0	3.01 ± 0.12	1.32 ± 0.06	98.37 ● 1.53

* Statistically significant difference to the diluent control at the 95% level.

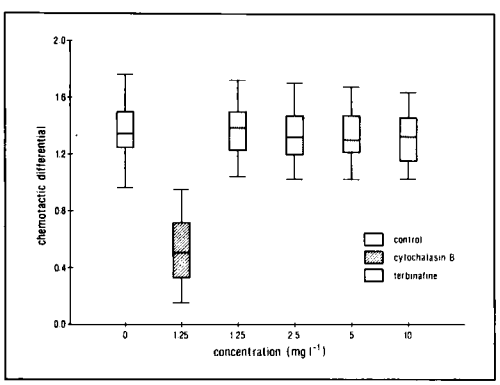

Fig. 1: Chemotaxis toward FMLP (2.5×10^{-7} M) of human PMNL from 10 healthy subjects after preincubation of cells in vitro with different concentrations of terbinafine, CYTB, or the diluent control as expressed by the chemotactic differential. After 30 min the PMNL were concentrated (5×10^7 cells ml^{-1}), placed in agarose gels containing the corresponding concentrations of the test drugs and incubated for 1.5 h at 37 °C. Boxes represent the median and quartiles from 10 experiments and the range of the values (* statistically significant difference to the diluent control at the 95% level).

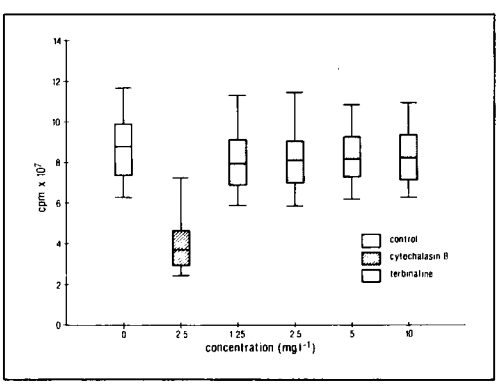

Fig. 2: Peak chemiluminescence response of human PMNL from 10 healthy subjects under the presence of different concentrations of terbinafine, CYTB, or the diluent control mixed in 100 µl aliquots with 200 µl of PMNL suspension (2×10^6 cells ml^{-1}), 200 µl of *C. albicans* (2×10^7 CFU ml^{-1}) and 25 µl DMNH (2×10^{-5} M) in the reaction tubes. Boxes represent the median and quartiles of 10 experiments and the range of the values (* statistically significant difference to the diluent control at the 95% level).

the solvent system without the drug. In contrast CYTB at the concentration of 2.5 $mg\ l^{-1}$ strongly reduced the chemotactic migration to 31.28% of the control. The decreased CT of PMNL to FMLP caused by CYTB did not appear to the result of cytotoxicity since cell viability remained >95% as determined by trypan blue exclu-

Table 2: Chemiluminescence response of human PMNL from 10 healthy subjects under the presence of different concentrations of terbinafine, CYTB, or the diluent control mixed in 100 µl aliquots with 200 µl of the PMNL suspension (2×10^6 cells ml^{-1}), 200 µl of *C. albicans* (2×10^7 CFU ml^{-1}) and 25 µl DMNH (2×10^{-5} M) in the test tubes. The data represent the mean ± SEM of 10 experiments.

Drug	Concentration (mg l^{-1})	Mean ± SEM (n = 10) peak intensity (×10^7 cpm)	area under the curve (×10^7 cpm)	time to peak (min)
Control		8.77 ± 0.53	12.29 ± 0.98	10.18 ± 0.29
Cytochalasin B	2.5	3.95 ± 0.45*	4.17 ± 0.56*	12.53 ± 0.38*
Terbinafine	1.25	8.10 ± 0.53	10.76 ± 0.94	10.68 ± 0.30
Terbinafine	2.5	8.22 ± 0.54	11.09 ± 0.96	10.58 ± 0.33
Terbinafine	5.0	8.29 ± 0.45	11.22 ± 0.92	10.06 ± 0.30
Terbinafine	10.0	8.27 ± 0.47	11.05 ± 0.90	10.02 ± 0.43

* Statistically significant difference to the diluent control at the 95% level.

Table 3: Chemiluminescence response of human PMNL from 10 healthy subjects after preincubation of cells in vitro with different concentrations of terbinafine, CYTB, or the diluent control. After 30 min 200 µl of the PMNL suspension (2×10^6 ml^{-1}), were added to a mixture of 200 µl of *C. albicans* (2×10^7 CFU ml^{-1}) and 25 µl DMNH (2×10^{-5} M) with the corresponding drug concentrations and the CL reaction started. The data represent the mean ± SEM of 10 experiments.

Drug	Concentration (mg l^{-1})	Mean ± SEM (n = 10) peak intensity (×10^7 cpm)	area under the curve (×10^7 cpm)	time to peak (min)
Control		8.24 ± 0.58	11.09 ± 1.15	8.81 ± 0.33
Cytochalasin B	2.5	3.36 ± 0.67*	3.29 ± 0.73*	12.82 ± 0.32*
Terbinafine	1.25	7.40 ± 0.75	9.89 ± 1.29	9.69 ± 0.42
Terbinafine	2.5	7.62 ± 0.76	10.20 ± 1.33	9.45 ± 0.41
Terbinafine	5.0	7.52 ± 0.57	10.09 ± 1.27	9.15 ± 0.35
Terbinafine	10.0	7.59 ± 0.63	10.11 ± 1.29	9.19 ± 0.43

* Statistically significant difference to the diluent control at the 95% level.

sion with PMNL incubated for up to 30 min with this drug. The effects of various concentrations of terbinafine and CYTB on neutrophil oxidative metabolism are expressed in terms of peak-, time to peak and area under the curve CL response. Terbinafine at concentrations of 1.25, 2.5, 5 or 10 mg l^{-1} did not cause any significant effect on the CL response of neutrophils to opsonized *C. albicans* cells, either immediately after addition (Table 2, Figure 2) of the drug to the reaction mixture or following additional pretreatment of the PMNL cells with the corresponding drug concentrations for a period of 30 min (Table 3, Figure 3). The addition of CYTB at a

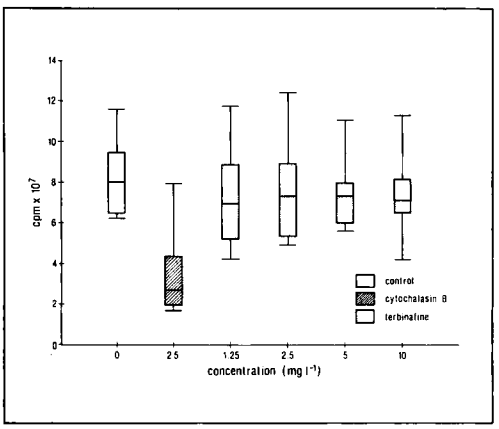

Fig. 3: Peak chemiluminescence response of human PMNL from 10 healthy subjects after preincubation of cells in vitro with different concentration of terbinafine, CYTB, or the diluent control. After 30 min 200 µl of the PMNL suspensions (2×10^6 cells ml^{-1}) were added to a mixture of 200 µl of *C. albicans* (2×10^7 CFU ml^{-1}) and 25 µl DMNH (2×10^{-5} M) with the corresponding drug concentrations and the CL reaction started. Boxes represent the median and quartiles of 10 experiments and the range of the values (* statistically significant difference to the diluent control at the 95% level).

concentration of 1.25 mg l^{-1} to the CL reaction mixture resulted in statistically significant reduction in terms of peak-, time to peak- and area under the curve CL response in comparison to untreated control cells (Table 2, Figure 2). Following preincubation of PMNL cells for a period of 30 min with CYTB at a concentration of 1.25 mg l^{-1}, a comparable marked inhibition of the CL response was observed but not further increase of this effect (Table 3, Figure 3).

Preliminary experiments indicated that removal of unbound drug by washing and resuspending cells resulted in a return to the level of CL activity in control cells never exposed to CYTB.

The observed suppression of CL was not due to nonspecific quench of CL by the drug, since we found no effect on CL when the drug was added to the PMNL immediately prior to initiation of CL by addition of fungi.

Discussion

Chemotherapy, a necessity for controlling and alleviating fungal infections, may itself compromise the immune-system of patients. Since PMNL participate in anti-microbial activity during chemotherapy (4, 27, 33, 36), adverse effects of drugs on the activity of these cells would be undesirable. In this paper a possible action of terbinafine, a new broad spectrum antimycotic has been investigated on at least two phases of PMNL phagocytosis – CT and oxidative metabolism at concentrations ranging from below to several-fold those occuring during the course of oral treatment. A number of antifungal drugs of different chemical classes have been shown to adversely affect neutrophil migration (14, 29, 32). There has been no study investigating the comparative effects of allylamine derivatives on CT so far. The data presented demonstrate that terbinafine at concentrations up to 10 mg l^{-1}, which is about 5 times the peak concentration found in plasma of patients after oral treatment (21), had no significant effect on CT of human PMNL cells using FMLP as the source of chemoattractant, whereas CYTB exerted a strong inhibition at 1.25 mg l^{-1} under the same test conditions. Additional preincubation of PMNL cells for 30 min did not alter the response to these drugs. In agreement with our own experience Becker et al. (6), Davis et al. (13) and Rank et al. (32) have demonstrated for CYTB and the polyene antifungal amphotericin B that such drug-induced impairment of PMNL motility not necessarily implies an irreversible toxicity of the drug. PMNL cells treated with CYTB or amphotericin B for 30 min could be washed with subsequent reversal of inhibition.

A DMNH-enhanced CL assay was used in this study to evaluate the effect of terbinafine on the phagocytic and killing activity of PMNL cells. It has been demonstrated that CL is linearly related to the microbicidal activity (5, 9, 15, 24, 40) of these cells and a reduction or increase in CL response of phygocytic cells may indicate either inhibi-

tion or enhancement of the cellular immune response (1, 2). Because the assay is extremely sensitive and easy to perform, the CL reaction has been used several times in screening for antifungal drug-acquired defects of PMNL cells (2, 7, 29, 35). We used DMNH instead of luminol as a luminescence indicator since it proved more effective than luminol in measuring CL of PMNL and permits the research of cells in small numbers (16). Terbinafine at concentrations up to 10 mg l^{-1} did not cause any significant effect on CL response. These findings for terbinafine are consistent with previous reports by Abruzzo et al. (2) concerning the observation that there is no inhibition of neutrophil function. However, Abruzzo et al. demonstrated a significant decrease in time to peak response with terbinafine at concentrations from 5 to 25 mg l^{-1} using mouse spleen cells, which consisted of 6% neutrophils, and zymosan as target particle for the phagocytosis assay. This decrease in time to peak which was not evident in our investigations, was explained to be a prestimulation of cells during the preincubation period. In our investigations we used a pure human neutrophil suspension and *Candida* yeast cells as target particle for phagocytosis. The suitability of the DMNH-enhanced CL reaction used in this study for detection of drug-acquired defects of PMNL cells has been proved by the established inhibitor CYTB (13) which exerted a statistically significant decrease of CL response under the above test conditions. In conclusion this study revealed no effects of terbinafine on major functions of phagocytic cells such as chemotaxis and oxidative metabolism at concentrations similar to those attainable in clinical practice. Further research should clarify the in vivo relevance of these data.

References

1. Abruzzo, G. K., D. M. Giltinan, T. P. Capizzi and R. A. Fromtling (1986): Influence of six antifungal agents on the chemiluminescence response of mouse spleen cells. Antimicrob. Agents Chemother. 29, 602–607.
2. Abruzzo, G. K., R. A. Fromtling, T. A. Turnbull and D. M. Giltinan (1987): Effects of bifonazole, fluconazole, itraconazole, and terbinafine on the chemiluminescence response of immune cells. J. Antimicrob. Chemother. 20, 61–68.
3. Allen, R. C. and L. D. Loose (1976): Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem. Biophys. Res. Commun. 69, 245–252.
4. Baccarini, M., E. Blasi, P. Pucetti and F. Bistoni (1983): Phagocytic killing of *Candida albicans* by different murine effector cells. Sabouraudia 21, 271–286.
5. Ballart, I. J., M. E. Estevez, R. A. Diez and L. Sen (1987): Comparison of *Candida* killing activity measured by chemiluminescence and cytomorphological methods in human phagocytes. J. Immunol. Methods 97, 263–268.
6. Becker, E. L., A. T. Davis, R. D. Estensen and P. G. Quie (1972): Cytochalasin B. IV. Inhibition and stimulation of chemotaxis of rabbit and human polymorphonuclear leukocytes. J. Immunol. 108, 396–402.
7. Björksten, B., C. Ray and P. G. Quie (1976): Inhibition of human neutrophil chemotaxis and chemiluminescence by amphotericin B. Infect. Immun. 14, 315–317.
8. Boden, G. P. (1975): Infections in cancer patients. Dancer Treat. Rev. 2, 89–128.
9. Brummer, E., A. M. Sugar and D. A. Stevens (1985): Enhanced oxidative burst in immunologically activated but not elicited polymorphonuclear leukocytes correlates with fungicidal activity. Infect. Immun. 49, 396–401.
10. Carpentier, F. M., T. E. Kiehn and D. Armstrong (1981): Fungemia in the immuncompromised host; changing pattern, antigenemia, high mortality. Am. J. Med. 71, 363–370.
11. Chan, C. K. and E. Balish (1978): Inhibition of granulocyte phagocytosis of *Candida albicans* by amphotericin B. Can. J. Microbiol. 24, 363–364.
12. Cohen, M. S., P. S. Shirley and L. R. Chatelet (1983): Further evaluation of luminol-enhanced luminescence in the diagnosis of disorders of leukocyte oxidative metabolism: role of myeloperoxidase. Clin. Chem. 29, 513–515.
13. Davis, A. T., R. Estensen and P. G. Quie (1971): Cytochalasin B III. Inhibition of human polymorphonuclear leukocyte phagocytosis. Proc. Soc. Exp. Biol. Med. 137, 161–164.
14. Davies, R. R. and F. Zaini (1985): Antifungal drugs affecting the chemotaxis of polymorphonuclear neutrophils. J. Med. Vet. Mycol. 23, 119–123.
15. Eschenbach, C. (1982): Luminol-Chemilumineszenz phagocytierender neutrophiler Granulocyten. Klin. Wochenschr. 60, 357–364.
16. Eschenbach, C. und U. Adrian (1985): DMNH – Ein neuer kompetenter Indikator für die Messung der Chemilumineszenz von neutrophilen Granulocyten und Monocyten. Klin. Wochenschr. 63, 1218–1225.
17. Ferrante, A. and Y. H. Thong (1980): Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human blood by the hypaque-ficoll method. J. Immunol. Methods 36, 109–117.

18. Forsgren, A. and D. Schmeling (1977): Effect of antibiotics on chemotaxis of human leukocytes. Antimicrob. Agents Chemother. 11, 580–584.
19. Fromtling, R. A. and G. K. Abruzzo (1985): Chemoluminescence as a tool for the evaluation of antimicrobial agents: a review. Methods Find. Clin. Pharmacol. 7, 493–500.
20. Fromtling, R. A. (1984): Imidazoles as medically important antifungal agents: an overview. Drugs Today 20, 325–349.
21. Ganzinger, U., A. Stephen, G. Hitzenberger, R. Baumgartner, M. Madoerin, P. H. Mekler, B. P. Richardson, S. Brüggemann, W. Suter, R. Racine, P. Donatsch, F. Schatz and H. Haberl (1983): SF 86-327: Evaluation of toxicity in laboratory animals, tolerance and pharmacokinetics after oral application to man In: Proceedings of the 13th International Congress of Chemotherapy, Vienna, K. H. Spitzy and K. Karrer (Eds.), H. Eggermann, part 116, PS 4.8/4–13.
22. Gergely, P., K. Nekam, I. Lang, L. Kalmar, R. Gonzales-Gabello and A. Perl (1984): Ketoconazole in vitro inhibits mitogen blastogenesis, antibody-dependent cellular cytotoxicity, natural killer activity and random migration of human leukocytes. Immunopharmacology 7, 167–170.
23. Giltinan, D. M., T. P. Capizzi, G. K. Abruzzo and R. A. Fromtling (1986): Design and analysis considerations in evaluating the chemiluminescence response of mouse spleen cells. J. Clin. Microbiol. 23, 531–535.
24. Grebner, J. V., E. L. Mills, B. H. Gray and P. G. Quie (1977): Comparison of phagocytic and chemiluminescence response of human polymorphonuclear neutrophils. J. Lab. Clin. Med. 89, 153–159.
25. Hurtrel, B., P. H. Lagrange and J. C. Michel (1980): Systemic candidiasis in mice. II. Main role of polymorphonuclear leukocytes in resistance to infection. Ann. Immunol. (Paris) 131, 105–118.
26. Johnson, E. M., D. W. Warnock, M. D. Richardson and C. J. Douglas (1986): In-vitro effect of itraconazole, ketoconazole and amphotericin B on the phagocytic and candidacidal function of human neutrophils. J. Antimicrob. Chemother. 18, 83–91.
27. Lehrer, R. I. and M. J. Cline (1969): Interaction of *Candida albicans* with human leukocytes and serum. J. Bacteriol. 98, 996–1004.
28. Le Moli, S., R. Seminara, R. D'Amelio, O. Pontesilli and R. Nisini (1983): In vitro effect of ketoconazole on human neutrophil chemotaxis and *Candida albicans* killing. Boll. Ist. Sieroter. Milan. 62, 531–534.
29. Marmer, D. J., B. T. Fields, G. L. France and R. W. Steele (1981): Ketoconazole, amphotericin B, and amphotericin B methyl ester: comparative in vitro and in vivo toxicological effects on neutrophil function. Antimicrob. Agents Chemother. 20, 660–665.
30. Nelson, R. D., P. G. Quie and R. L. Simmons (1975): Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J. Immunol. 115, 1650–1656.
31. Paleske, A. v., U. Müllerleile, V. Gressler, M. Garbrecht and D. K. Hossfeld (1987): Incidence and treatment of fungal infections in neutropenic patients. Hämatol. Bluttransfus. 30, 545–546.
32. Rank, E. L., R. L. Hopfer, R. P. Williams (1981): Amphotericin B causes a decrease in human leukocyte migration. J. Antimicrob. Chemother. 8, 497–500.
33. Rogers, T. J. and E. Balish (1980): Immunity to *Candida albicans*. Microbiol. Rev. 44, 660–682.
34. Rowan-Kelly, B., A. Ferrante and Y. H. Thong (1984): Modification of polymorphonuclear leucocyte function by imidazoles. Int. J. Immunopharmacol. 6, 389–393.
35. Sacchi, F., A. Fietta, P. Mangiarotti, V. De Rose, C. Grassi (1983): Interference between neutrophils and antimycotic agents. In: Proceedings of the 13th International Congress of Chemotherapy, Vienna, K. H. Spitzy and K. Karrer (Eds.), H. Eggermann, part 61, SE 4.8/2–11.
36. Segal, A. W. (1981): The antimicrobial role of the neutrophil leukocyte. J. Infect. 3, 3–17.
37. Siegel, J. P. and J. S. Remington (1982): Effect of antimicrobial agents on chemiluminescence of human polymorphonuclear leukocytes in response to phagocytosis. J. Antimicrob. Chemother. 10, 505–515.
38. Stütz, A. (1987): Allylamine derivatives – a new class of active substances in antifungal chemotherapy. Angew. Chem. Int. Ed. Engl. 26, 320–328.
39. Thong, Y. H., B. S. Douglas and A. Ferrante (1978): Abnormal neutrophil chemotaxis in a syndrome of unusual facies, proportionate small stature and sensorineural deafness-mutism. Acta Paediatr. Scand. 67, 383–387.
40. Welch, W. D., C. W. Graham, J. Zaccari and L. D. Thrupp (1980): Analysis and comparison of the luminol-dependent chemiluminescence response of alveolar macrophages and neutrophils. J. Reticuloendothel. Soc. 28, 275–283.

Correspondence:
Dr. M. Schaude, Sandoz-Forschungsinstitut,
Brunner Str. 59, A-1235 Wien.