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Fenticonazole: A New Antifungal Imidazole Derivative In Vitro and In Vivo Antimycotic Activity

Fenticonazol: ein neuer Imidazol-Abkömmling. In vitro und in vivo antimykotischer Wirkung.

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Summary: The activity of α-(2,4-dichlorophenyl)-β, N-imidazolylethyl 4-phenylthiobenzyl ether nitrate (Fenticonazole) compared with those of reference standard drugs, was tested in vitro, in solid medium, against numerous fungi.

The antifungal activity spectrum is very large and Fenticonazole shows a good activity against dermatophytes.

The antifungal activity of Fenticonazole in experimental dermatomycosis by Microsporum canis in guinea pig was studied.

Fenticonazole, at different concentrations, shows to be a very active drug and its ED_{50} is about 0.5%.

Zusammenfassung: Die Wirksamkeit von α-(2,4-Dichlorophenyl)-β,N-imidazolylethyl-4-phenylthiobenzyl-aether-nitrat (Fenticonazol) wurde bei verschiedenen Pilzen mit der von bekannten Arzneimitteln verglichen und im Festmedium untersucht.

Das Spektrum der fungiziden Wirkung ist sehr breit: die Sensitivität von Fenticonazol war am auffälligsten bei Dermatophyten. Die fungizide Wirkung von Fenticonazol wurde bei der experimentellen Microsporum-canis-Dermatomykose des Meerschweinchens bestimmt. Fenticonazol weist eine hohe Aktivität aus und der ED_{50}-Wert entspricht einer Konzentration von ca. 0,5%.

Introduction

Among numerous new imidazole derivatives synthesized in our laboratories, the most active drug was α-(2,4-dichlorophenyl)-β, N-imidazolylethyl 4-phenylthiobenzyl ether nitrate, Fenticonazole, a drug with satisfactory antibacterial and notable antimycotic action (1, 2, 3).

The aim of this investigation is to verify the "in vitro" activity of Fenticonazole, compared to different reference compounds, against numerous fungi, using a solid medium, and to study the protective dose of Fenticonazole on an experimental infection induced by inoculation of Microsporum canis on the scarified skin of guinea pig.

Materials and Methods

Antifungal activity in vitro

The antifungal activity of Fenticonazole was compared with the activity of Miconazole, Clotrimazole and Econazole against a certain number of fungi.

Sabouraud dextrose agar (DIFCO) at pH 5,6 was used as test medium.

Drugs were dissolved in 10 ml of N-N-dimethylformamide and successive 2 fold dilutions were made in the same solvent.

0.2 ml of each predilution were added to the tubes containing 20 ml of melted Sabouraud dextrose agar and, after homogenization, poured into sterile Petri plates.

The concentrations ranged from 160 to 0.039 mcg/ml for all the tested drugs.

In preliminary tests it was observed that the amount of N-N dimethylformamide added to the test medium did not inhibit fungal growth.

Preparation of inoculum: Some days before the test, a standardized fragmented mycelial inoculum was prepared as described by Granade and Artis (4).

Several tufts of hyphae from a luxuriant agar culture growth were transferred into a flask containing 15 ml of Sabouraud liquid medium.

The flasks were incubated at 25°C and shaked once each day to have aeration and to prevent sporulation by keeping the culture submerged.

The nonsporulated mycelium was harvested, during the exponential phase of growth (5–10 days) by centrifugation at 9000 rpm for 15 min.

After the elimination of the supernatant, the fungal precipitate was suspended in sterile deionized water and washed twice by centrifugation in the same diluent and then suspended in 3–4 ml of Sabouraud liquid medium.

The fungal suspension was subsequently grinded with sterile ground-glass in order to break the aggregates.

The determination of a sample of the fragmented mycelial suspension density was effectued on the Coleman Junior II A spectrophotometer at $\lambda = 450$ nm. The rest of the mycelial suspension was opportunely diluted in broth in order to have a suspension containing 10^6–10^7 U. F. C./ml.

With a micropipette were inoculated 0.005 ml of mycelial suspension on the surface of the agar containing the concentrations of the different drugs and then the inoculum was scraped on the agar.

Subsequently the plates were incubated at 25°C for 10 days.

After this period, the minimum inhibitory concentration (MIC), the lowest drug concentration which prevented fungal growth, was evaluated.

The results of the antifungal activity of Fenticonazole and of the positive control drugs against various microorganisms are collected in the table 1.

Antifungal activity in vivo

In order to evaluate the protective dose of Fenticonazole, an experimental infection induced by Microsporum canis ATCC 11621 on the scarified skin of guinea pigs was effectued.

Albino Pirbright guinea pigs weighing about 300 g were used. The animals were prepared for infection by electrical clipping of their backs on which a depilatory paste was spread over 5×5 cm area.

Table 1
M. C. I. (mcg/ml) in solid medium of Fenticonazole in comparison with other commercial imidazoles against numerous fungi

Strains	Fentico-nazole	Micona-zole	Clotri-mazole	Econazole
Trichophyton mentagrophytes Me 3865	5	20	20	40
Trichophyton mentagrophytes 601	0.625	0.625	0.625	1.25
Trichophyton mentagrophytes 10148	1.25	20	1.25	1.25
Trichophyton mentagrophytes sp.	0.625	0.312	0.156	0.625
Trichophyton mentagrophytes 9533	0.312	0.156	0.039	0.156
Trichophyton mentagrophytes IMV 8628	0.625	0.312	0.019	0.156
Trichophyton mentagrophytes ATCC 9129	0.039	0.312	0.078	0.156
Trichophyton verrucosum sp.	0.625	5	0.625	1.25
Trichophyton verrucosum IMV 8991	0.078	0.312	0.625	0.156
Trichophyton rubrum 17910	10	20	1.25	5
Trichophyton tonsurans DC-LH 746	0.312	0.156	0.625	0.625
Trichophyton tonsurans IMV 9224	0.039	0.156	0.039	0.039
Trichophyton terrestre CDC-LH-709	1.25	10	0.625	0.312
Microsporum canis sp.	1.25	5	1.25	0.312
Microsporum canis ATCC 11621	1.25	2.5	0.625	0.312
Microsporum canis IMV 10241	0.625	0.625	0.078	0.039
Microsporum canis ISM 72/4	0.625	2.5	0.078	0.625
Microsporum canis IPC	0.625	1.25	0.312	0.625
Microsporum canis LU	2.5	2.5	0.312	0.625
Microsporum canis 8144	2.5	5	0.312	0.625
Microsporum canis 8082	0.625	2.5	0.312	0.625
Microsporum gypseum ATCC 14638	20	20	2.5	1.25
Microsporum gypseum IMV 20207	0.312	5	0.312	0.625
Microsporum fulvum IP 138	5	40	0.625	0.312
Microsporum cookei CDC 77082296	2.5	10	0.625	0.156
Epidermophyton floccosum ISM 71/81	1.25	2.5	1.25	0.312
Aspergillus niger ATCC 16404	160	160	160	80
Aspergillus fumigatus ISM 70/110	20	10	5	5
Penicillum chrysogenum ISM 71/89	2.5	1.25	2.5	0.312
Penicillum chrysogenum 152	2.5	2.5	5	0.625
Sporotricum schenckii sp.	5	20	10	20

After few minutes the paste was removed thus obtaining a completely smooth, depilated surface.

At least 4 h after depilation, the skin was scarified by cross-cutting with a lancet, and the area was infected with a piece of agar culture.

A 10 days culture of Microsporum canis on Sabouraud dextrose agar in 20×200 mm test-tube was sufficient for inoculating two animals.

Drugs: After three days when the infection had developed, local daily therapy for 7 days began by smearing the infected area with Fenticonazole, or with Miconazole suspended, at 3-2-1-0.5% in the following excipient:

Propylene glycol	5%
Hydrogenated Lanolin	1%
Almond oil	10%
Xalifin 15	15%
Cetyl alcohol	3%
Glyceryl monostearate	3%
Purified water to	100 g.

Treatment: The following groups of animal were formed (total = 90 guinea pigs):
a) Controls: infected and non treated;
b) Controls: infected and treated with excipient;
c) Fenticonazole: 0.5-1-2-3%;
d) Miconazole: 0.5-1-2-3%.

Some animals, in the period between the start of the infection and the beginning of the treatment, accidentally died and they were excluded from the statistical evaluation.

Observation and evaluation: The progress of the infection and the protective effect of Fenticonazole and of Miconazole were observed, respectively, midweek, by pulling out hair from the infected area and fixing on Sabouraud agar plate added with antibiotics in order to inhibit growth of bacterial colonies. Further samples were generally taken 3 and 5 days after the end of the treatment period, so as to ascertain whether there had been any relapses. The plates were immediately incubated for 5 days at 26°C and growth was then evaluated. Remembering that from each animal the hair were taken from three different points of the infected area, the score of the mycelial growth has been tabulated in conformity with following standard:

$4 = +++$ $3 = ++\pm; +\pm\pm$
$2 = ++-; +\pm-; \pm\pm\pm$ $1 = +--; \pm\pm-; \pm--$
$0 = ---$

where $+$ = large mycelium growth
$\pm$ = weak mycelium growth
$-$ = no growth

Statistical analysis: The differences among the various groups of animals were tested on the basis of the areas (Area A and area B).

The two areas were obtained as follows.
1) Area A relative to the period of treatment (relative to the scores 6/3; 8/5; 10/7) and representing the direct action on the infection.
2) Area B relative to the period following the treatment (relative to the scores 13/7 and 15/7) and representing roughly the length of action.

On these areas mean ± standard deviation ($\overline{X} \pm SD$) were calculated and the differences among treatments were tested by the analysis of the variance.

The difference of the scores among groups was tested using the "chi square" test.

PD_{50} of Fenticonazole and of Miconazole was evaluated according to Bliss (5).

All the statistical elaboration was done on UNIVAC 1100 with SPSS programs.

Results

Antifungal activity in vitro

The antifungal activities expressed in mcg/ml of Fenticonazole and reference standards are listed in Table 1. The results show that Fenticonazole indeed has a really good activity against numerous fungi, especially on the dermatophytes (6). Fenticonazole proves to be effective on Trichophyton and Microsporum species with values better or coinciding with those of the drugs used as reference standards. Fenticonazole displays also interesting activity against fungi responsible for deep infections, and such behaviour is similar to that of other imidazole derivatives. The results obtained in agar medium are, generally, comparable with those had in liquid medium (3, 6).

Table 2
Average values of the scores at different times of observation obtained with Fenticonazole and Miconazole cream at various concentrations in experimental infection with M. canis

		3/0*	6/3	8/5	10/7	13/7	15/7
Control not treated		4	3.56	3	3.44	3.78	2.89
Control placebo		4	3.44	2.44	3.22	3.11	3
Fenticonazole	0.5%	4	0.33	0.22	0.78	0.56	1.22
	1 %	3.89	0.33	0.33	1.78	1.56	1.78
	2 %	4	0	0	0	0.22	0.11
	3 %	3.78	0	0	0	0.25	0.5
Miconazole	0.5%	4	0.78	0.78	1.22	1.56	1.33
	1 %	4	0.11	0.11	0.55	1.56	0.78
	2 %	4	0	0	0.16	0.16	0.16
	3 %	4	0	0	0.11	1	0.78

*Numerator: days of infection
Denominator: days of treatment

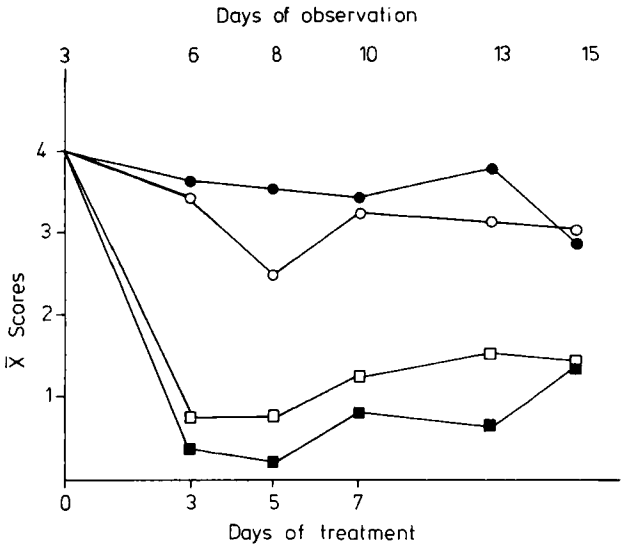
Table 3
Average values ± DS of areas A and B among different groups of treatment

	Doses	Groups	Area A	Area B
Controls		1	10.0 ± 1.80	6.6 ± 1.00
		2	9.1 ± 1.36	6.1 ± 1.45
Fenticonazole	0.5%	3	1.2 ± 1.36	2.0 ± 0.93
	1 %	4	2.4 ± 1.50	3.3 ± 2.50
	2 %	5	0 ± 0.00	0.3 ± 1.06
	3 %	6	0 ± 0.00	0.7 ± 0.89
Miconazole	0.5%	7	2.7 ± 2.73	2.8 ± 2.76
	1 %	8	0.7 ± 1.30	2.3 ± 2.12
	2 %	9	0.1 ± 0.41	0.4 ± 0.82
	3 %	10	0.1 ± 0.33	1.7 ± 1.20

Area A is relating to times 6/3; 8/5; 10/7
Area B is relating to times 13/7; 15/7

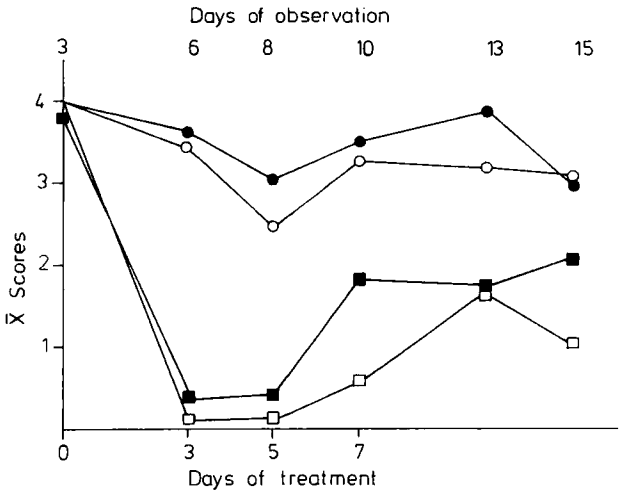
Antifungal activity in vivo

In the table 2 and graphs 1-4, are listed the average values of the scores at different times of observation in order to make known a brief and total account of the experience. It is evident that both drugs, at all concentrations, cause a drastic decrease of the infection; because that action is manifest just at the lower concentrations, it is not possible to show a real dose depending activity, even if the higher concentrations (2-3%) seem better than the lower concentrations (0.5-1%). The calculation of the areas (area A = activity, area B = lenght of activity) is synthesized in table 3 where it is evident that both drugs are very efficient at all the concentrations, with regard to the activity and to the lenght of activity. The differences of activity among the preparations had been overall analyzed on the basis of the areas A and B. There groups have been compared: animal controls and animals treated respectively with Fenticonazole and Miconazole, without to consider the concentrations of the drugs (pool). The



● Controls not treated
○ Controls treated with placebo
■ Fenticonazole 0.5%
□ Miconazole 0.5%

Graph. 1:
Average values of the scores, at different times of observation, of experimental infections on guinea pig by M. canis, treated with Fenticonazole and Miconazole cream at 0.5% concentration.

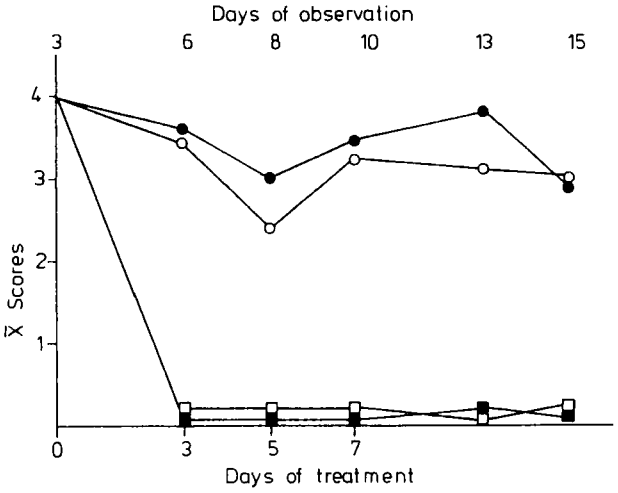


● Controls not treated
○ Controls treated with placebo
■ Fenticonazole 1%
□ Miconazole 1%

Graph. 2:
Average values of the scores, at different times of observation, of experimental infections on guinea pig by M. canis, treated with Fenticonazole and Miconazole cream at 1% concentration.

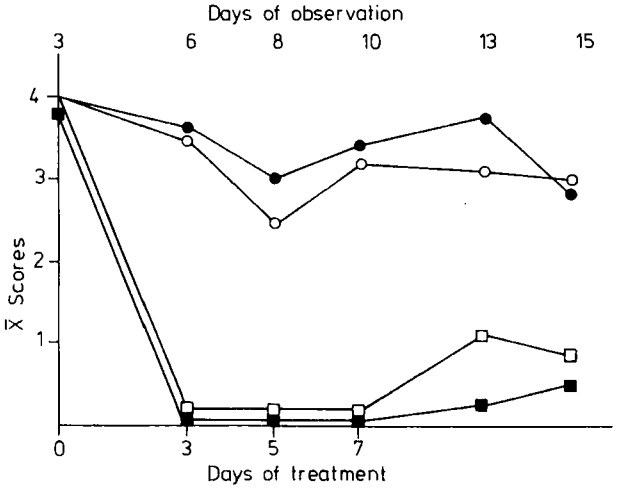
results are listed in table 4 where it is evident that the activity of Fenticonazole and of Miconazole are comparable but they do not differentiate between them (analysis of variance orthogonal comparison) wheter the activity (area A) or the lenght of action (area B). In order to understand the disposition of the scores, the frequencies of scores 0, 1, 2, 3, 4 relating to the pool of the groups, are listed in table 5. The left side of the table shows the scores relating to the period of treatment while the right side shows those relating to the period of observation. By analysis of the frequencies (X^2) it is evident that both drugs are active and that any difference between drugs can be seen (table 6).

With regard to the ED_{50}, evaluation we are refering to the frequency of score 0 in each groups (frequency of score 0 relating to total scores) during the period of the treatment. The results are listed in table 6 where we show that for each drug ED_{50} is approximately 0.5%.



● Controls not treated
○ Controls treated with placebo
■ Fenticonazole 2%
□ Miconazole 2%

Graph. 3:
Average values of the scores, at different times of observation, of experimental infections on guinea pig by M. canis, treated with Fenticonazole and Miconazole cream at 2% concentration.



● Controls not treated
○ Controls treated with placebo
■ Fenticonazole 3%
□ Miconazole 3%

Graph. 4:
Average values of the scores, at different times of observation, of experimental infections on guinea pig by M. canis, treated with Fenticonazole and Miconazole cream at 3% concentration.

Conclusions

In the test for the evaluation of the antifungal activity, in solid medium, Fenticonazole proves to be effective on a great number of fungi and, particularly, on dermatophytes with an activity better or similar to those of the reference compounds.

This activity emphasized the specificity of the product for a certain type of fungi responsable for many cutaneous infections in man. From an experimental infection induced in guinea pig by Microsporum canis, Fenticonazole topically administered at various doses, in comparison with an active standard, proves to be a very active drug with excellent healing capacity (7).

Both drugs (Fenticonazole and Miconazole) cause a drastic decrease of the infection also at the lower concentrations. Moreover in this experiment, by analysis of the frequencies, it is

Table 4
Average values ± DS of areas A and B in the pool of the groups of treatment

Groups	N° animals	Area A*	Area B*
Controls	18	9.5 ± 1.62	6.3 ± 1.24
Fenticonazole	31	0.9 ± 1.40	1.6 ± 1.90
Miconazole	33	1.0 ± 1.90	1.9 ± 2.07

*Analysis of variance orthogonal comparison
Controls versus treated $p < 0.05$
Fenticonazole versus Miconazole $p > 0.05$

Table 5
Frequencies of various scores relating to the periods of treatment and of observation

Times: 6/3 + 8/5 + 10/7							Times: 13/7 + 15/7						
Groups	Scores					Total	Groups	Scores					Total
	0	1	2	3	4			0	1	2	3	4	
Controls	0	4	3	26	21	54	Controls	1	4	3	7	21	36
Fenticonazole 0.5 - 3%	73	10	10	0	0	93	Fenticonazole 0.5 - 3%	27	24	5	3	3	62
Miconazole 0.5 - 3%	78	13	3	5	0	99	Miconazole 0.5 - 3%	29	18	13	3	3	66
	151	27	16	31	21	246		57	46	21	13	27	164
	Test X^2 $p < 0.05$ tab. 3 × 5							Test X^2 $p < 0.05$ tab. 3 × 5					

Table 6
Frequency of scores 0 relating to total scores in each group during the period of the treatment
Determination of ED_{50}.

Groups	Score 0 / total scores	%	ED_{50}* (approximate)
Control not treated	0/27	0	
Control placebo	0/27	0	
0.5%	17/24	71	0.416
1 %	11/24	46	
2 %	21/21	100	
3 %	24/24	100	
0.5%	14/27	52	0.468
1 %	21/27	78	
2 %	17/18	94	
3 %	26/27	96	

*According Bliss C
Quartier J. Pharm. Pharmac. 11, 192, 1938.

possible to see that both drugs are active and the results obtained show that PD_{50} is approximately 0.5%.

On the basis of the results obtained both vitro and vivo experimental models, Fenticonazole seems to be an interesting drug to test in patients affected by mycotic infections.

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