THE ANTIFUNGAL ACTIVITY OF DERMOSTATIN

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(Received for publication April 30, 1971)

Dermostatin is a polyene antibiotic which possesses a typical spectrum of antimicrobial activity. *In vivo*, by parenteral or oral administration, dermostatin was less effective than amphotericin B against *Candida albicans*. The *in vivo* activity of dermostatin was comparable to amphotericin B against *Cryptococcus neoformans* and *Blastomyces dermatitidis*; amphotericin B was more effective than dermostatin against *Histoplasma capsulatum*. Further studies are needed to determine if dermostatin could be useful in the treatment of cryptococcosis or blastomycosis in man.

Dermostatin, produced by *Streptomyces viridogriseus*, was reported to possess antifungal properties[1]. The original structural studies placed dermostatin in the pentaene class of polyene antibiotics; however, the structure has recently been revised, and dermostatin is now classified as a hexane[2,3]. Dermostatin was reported inhibitory in a range of 0.15~20 μg/ml against clinical isolates of fungi responsible for deep-seated mycoses[4]. A 0.5% formulation of dermostatin was reported an effective topical treatment in 66 cases of human dermatophytosis[5]. The *in vitro* anti-*Candida* activity of this antibiotic was reversed by an equal molar concentration of cholesterol[6]. In addition, leakage of phosphates, carbohydrates, and amino acids from yeast cells caused by dermostatin was nullified with cholesterol. The mode of action of dermostatin appears quite similar to that of other polyenes that interact with sterols in cell membranes.

The purpose of this study is to evaluate the *in vitro* activity of dermostatin against pathogenic microorganisms and to investigate its effectiveness against experimental infections of *Candida albicans*, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Histoplasma capsulatum*.

Materials and Methods

1. *In vitro* studies. The preparation of inocula and procedures for carrying out the broth dilution and disc plate tests using SABOURAUD's broth and MUELLER-HINTON agar, respectively, are the same as those reported previously[7,8]. For the agar dilution technique, serial Log_2 dilutions of antibiotics were made in melted SABOURAUD's agar and poured into Petri plates. Each plate containing antibiotic, as wellas control plates, was inoculated

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by discreetly spotting 0.05 ml aliquots of a standardized suspension of each of three organisms onto the agar surface. The Petri plates were incubated at 37°C for 4 days.

2. *In vivo* studies. The procedures for infecting and administering dermostatin and amphotericin B to pre-X-irradiated mice are the same as those used previously[9]. The antibiotics were administered intraperitoneally in all cases except where noted. The antibiotics were administered 0 and 2 hours postinfection to mice receiving two treatments, and 0, 2, 24, and 26 hours postinfection to mice receiving four treatments. All surviving animals were given an arbitrary average survival time equivalent to that of the last day of each experiment. The degree of significance for dermostatin treatments, as compared to untreated controls, was calculated by *t* test and P values were determined. An indication of virulence of the infecting inocula was obtained by a virulence titration and calculation of the number of LD_{50}'s[10].

Results and Discussion

The fungistatic and fungicidal concentrations of dermostatin ranged from 0.78~3.12 μg/ml for three isolates of *C. albicans* and 5~>10 μg/ml for three isolates of dermatophytes (Table 1). Dermostatin was inhibitory to dimorphic pathogenic fungi in the yeast phase. Three isolates of *H. capsulatum* were less susceptible than three isolates each of *C. neoformans* or *B. dermatitidis* (Table 2). Amphotericin B was more active against *H. capsulatum* and approximately equal to dermostatin against *B. dermatitidis* and one isolate of *C. neoformans*. When a 30 μg disc of dermostatin was compared with cephalothin, a clinically useful antibacterial antibiotic, only slight activity was detected (Table 3).

Dermostatin treatments of 3.12, 1.56, and 0.78 mg/kg significantly extended the average survival time of mice infected with *C. albicans* A26 (Table 4). Amphotericin B showed greater activity than dermostatin at all concentrations evaluated. An LD_{50} of 32 mg/kg for dermostatin compared with an LD_0>50 mg/kg for amphotericin B indicated that dermostatin is more toxic to mice than amphotericin B. Dermostatin was inactive by oral administration compared to amphotericin B (Table 5). Doses of 3.12 mg/kg of dermostatin were significantly effective against *C. neoformans* WS34

Table 1. *In vitro* activity of dermostatin against *Candida albicans* and dermatophytes

Organism	MIC (μg/ml)*	MLC (μg/ml)**
Candida albicans A50	0.78	3.12
Candida albicans A26	1.56	1.56
Candida albicans A25	0.78	3.12
Microsporum gypseum 350	5.0	>10
Trichophyton rubrum 67-643	5.0	10
Trichophyton mentagrophytes 6	5.0	5.0

* The minimal inhibitory concentration (MIC) was determined by the broth dilution method after 48 hours incubation at 30°C.

** The minimal lethal concentration (MLC) for *C. albicans* was determined three days after a transfer was made from a 48 hours MIC endpoint to antibiotic-free medium. The MLC for dermatophytes was determined eight days after a transfer was made from the 48 hours MIC endpoint.

Table 2. Comparison of the *in vitro* antifungal activity of dermostatin with amphotericin B

Organisms	MIC (μg/ml)*	
	Dermostatin	Amphotericin B
Histoplasma capsulatum 26	2.5	0.5
H. capsulatum 4-1372	2.5	0.5
H. capsulatum 67-905	2.5	0.5
Cryptococcus neoformans 67-1162	0.625	0.5
C. neoformans 66-856	1.25	0.5
C. neoformans WS 34	1.25	0.5
Blastomyces dermatitidis 6059	0.625	0.5
B. dermatitidis 34	0.625	0.5
B. dermatitidis 66	0.625	0.5

* Minimal inhibitory concentration was determined after four days incubation at 37°C by the agar dilution test.

Table 3. Antibacterial activity of dermostatin *in vitro* by BAUER-KIRBY method using cephalothin as positive control.

Organism	Zone of inhibition (mm)	
	Dermostatin (30 μg/disc)	Cephalothin (30 μg/disc)
Staphylococcus aureus H33	9	43
S. aureus H400	9	34
S. aureus 3074	8	32
Streptococcus sp. (Group D) 238B	8	18
Streptococcus sp. (Group D) 9960	< 6	20
Streptococcus sp. (Group D) 55992	< 6	16
Escherichia coli EC34	< 6	21
E. coli EC35	< 6	19
E. coli EC38	< 6	24
Proteus mirabilis PR3	< 6	23
P. mirabilis PR4	< 6	29
P. mirabilis PR5	< 6	27

Table 4. Comparison of the *in vivo* activity of dermostatin and amphotericin B against *Candida albicans* A26.

Antibiotic	Dose (mg/kg×4)	Average survival time (days ±SE)* during 8 days	Per cent extention of survival above untreated controls**
Dermostatin	6.25	2.2±0.2	0
	3.12	6.5±1.0	117 (P=0.01)
	1.56	4.5±0.7	50 (P=0.025)
	0.78	3.8±0.5	27 (P=0.05)
Amphotericin B	6.25	>8.0±0.0	167 (P≤0.0005)
	3.12	>8.0±0.0	167 (P≤0.0005)
	1.56	>8.0±0.0	167 (P≤0.0005)
	0.78	>8.0±0.0	167 (P≤0.0005)

* Untreated mice infected with 1.5×10^6 cells had an average survival time of 3.0±0.3 days. The infective challenge was equivalent to 5.6 times 1 LD_{50}.
** The LD_{50} for uninfected mice receiving dermostatin was 32 mg/kg×4 doses, whereas the LD_0 for amphotericin B was >50 mg/kg×4 doses.

Table 5. A comparison of the *in vivo* activity of dermostatin and amphotericin B by oral administration against *Candida albicans* A26

Antibiotic	Dose (mg/kg×2)	Average survival time (days ±SE)* during 8 days	Per cent extension of survival beyond untreated controls
Dermostatin	12.5	3.7±0.5	22 (P>0.1)
	6.25	3.8±0.8	26 (P>0.1)
	3.12	3.2±0.2	7 (P>0.1)
Amphotericin B	12.5	7.0±1.0	134 (P<0.0005)
	6.25	>8.0±0.0	168 (P<0.0005)
	3.12	7.8±0.2	160 (P<0.0005)

* Untreated mice infected with 1.5×10^6 cells had an average survival time of 3.0±0.2 days. The infective challenge was equivalent to 17.8 times 1 LD_{50}.

Table 6. Comparison of the *in vivo* activity of dermostatin and amphotericin B against *Cryptococcus neoformans* WS34

Antibiotic	Dose (mg/kg×2)	Average survival time (days ±SE)* during 10 days	Per cent extension of survival above untreated controls
Dermostatin	6.25	7.2±0.9	47 (P=0.01)
	3.12	8.2±0.2	67 (P<0.0005)
	1.56	6.2±0.6	27 (P=0.1)
	0.78	6.7±0.6	37 (P=0.025)
Amphotericin B	6.25	9.0±0.6	84 (P<0.0005)
	3.12	7.7±0.7	57 (P=0.005)
	1.56	6.2±0.5	27 (P=0.05)
	0.78	6.7±0.5	37 (P=0.01)

* Untreated mice infected with 1.5×10^6 cells had an average survival time of 4.9±0.5 days. The infective challenge was equivalent to 48.9 times 1 LD_{50}.

and compared favorably with amphotericin B (Table 6). All dose levels of dermostatin and amphotericin B were significantly effective in extending the survival time of mice infected with *B. dermatitidis* 6059 (Table 7). Dermostatin was effective only at doses of 6.25 mg/kg in extending the survival time of *H. capsulatum* 26 infected mice, while amphotericin B showed greater effectiveness at all doses administered (Table 8).

Dermostatin exhibited a spectrum of antimicrobial activity typical of other polyene antibiotics[11]. This antibiotic showed the greatest *in vitro* activity against *Candida* and yeast phase dimorphic fungi. The low *in vivo* activity against *C.*

Table 7. A comparison of the *in vivo* activity of dermostatin and amphotericin B against *Blastomyces dermatitidis* 6059

Antibiotic	Dose (mg/kg×4)	Average survival time (days ±SE)* during 11 days	Per cent extension of survival beyond untreated controls
Dermostatin	6.25	>11.0±0.0	116 (P<0.0005)
	3.12	9.5±0.5	86 (P<0.0005)
	1.56	7.5±0.5	47 (P=0.005)
	0.78	6.0±0.0	18 (P=0.005)
Amphotericin B	6.25	10.8±0.2	112 (P<0.0005)
	3.12	9.8±0.5	92 (P<0.0005)
	1.56	7.5±0.2	47 (P<0.0005)
	0.78	6.8±0.5	33 (P=0.0005)

* Untreated mice infected with 1×10^5 cells had an average survival time of 5.1±0.2 days. The infective challenge was equivalent to 56 times 1 LD_{50}.

Table 8. A comparison of the *in vivo* activity of dermostatin and amphotericin B against *Histoplasma capsulatum* 26

Antibiotic	Dose (mg/kg×4)	Average survival time (days ±SE)* during 11 days	Per cent extension of survival beyond untreated controls
Dermostatin	6.25	7.7±0.2	20 (P<0.0005)
	3.12	6.8±0.4	6 (P>0.1)
	1.56	6.5±0.3	2 (P>0.1)
	0.78	6.7±0.2	5 (P>0.1)
Amphotericin B	6.25	>11.0±0.0	72 (P<0.0005)
	3.12	10.3±0.3	61 (P<0.0005)
	1.56	8.8±0.7	38 (P=0.01)
	0.78	8.3±0.4	30 (P<0.0005)

* Untreated mice infected with 5×10^6 cells had an average survival time of 6.4±0.2 days. The infective challenge was equivalent to 10 times 1 LD_{50}.

albicans by oral administration indicates that dermostatin, like other polyenes, is poorly absorbed from the gastrointestinal tract. Interestingly, amphotericin B showed high *in vivo* activity by oral administration in mice but is known to be poorly absorbed by man[12]. Dermostatin appears to be less effective than amphotericin B against *C. albicans* and *H. capsulatum*. However, the *in vivo* activity of both polyene antibiotics was comparable against *C. neoformans* and *B. dermatitidis*. The *in vitro* and *in vivo* activity of dermostatin against both of these fungal pathogens is indeed interesting. Determination of whether or not dermostatin offers any advantage over amphotericin B for the chemotherapy of human deep-seated mycotic infections requires further investigation.

Acknowledgement

The excellent technical assistance of Albert Black and Melvin Johnson is appreciated.

References

1) Thirumalachar, M. J. & S. K. Menon: Dermostatin, a new antifungal antibiotic. Hindustan Antibiot. Bull. 4 : 106~108, 1962
2) Narasimhachari, N. & M. B. Swami: Chemistry of dermostatin. II. A new pentaene structure. Chemotherapy (Basel) 13 : 181~187, 1968
3) Narasimhachari, N. & M. B. Swami: Dermostatin: a revised hexaene structure. J. Antibiotics 23 : 566, 1970
4) Padhye, A. A.; B. B. Gokhale & M. J. Thirumalachar: Studies on some cases of deep mycoses with special reference to the *in vitro* activity of some new antifungal antibiotics. Hindustan Antibiot. Bull. 5 : 74~78, 1963
5) Gokhale, B. B.; M. V. Joglekar, A. A. Padhye & M. J. Thirumalachar: Dermostatin in the treatment of trichophytosis. Hindustan Antibiot. Bull. 4 : 127~129, 1962
6) Ramachandran, S. & R. S. Sukapure: Studies on the mode of action of antibiotics. III. Dermostatin. Hindustan Antibiot. Bull. 5 : 104~106, 1963
7) Gordee, R. S. & T. R. Matthews: Evaluation of the *in vitro* and *in vivo* antifungal activity of pyrrolnitrin. Antimicr. Agents & Chemoth. -1967 : 378~387, 1968
8) Sherris, J. C.; A. L. Reshad & G. A. Lighthart: Laboratory determination of antibiotic susceptibility to ampicillin and cephalothin. Ann. N. Y. Acad Sci. 145 : 248~267, 1967

9) GORDEE, R. S. & T. R. MATTHEWS: Evaluation of systemic antifungal agents in X-irradiated mice. Appl. Microbiol. 20 : 624～629, 1970
10) REED, L. J. & H. MUENCH: A simple method for estimating 50 percent endpoints. Amer. J. Hyg. 27 : 493～497, 1938
11) PANSY, F. E.; H. BASCH, W. P. JAMBOR, G. MAESTRONE, R. SEMAR & R. DONOVICK: Hamycin: *in vitro* and *in vivo* studies. Antimicr. Agents & Chemoth. -1966 : 399～404, 1967
12) LOURIA, D. B.: Some aspects of the absorption, distribution, and excretion of amphotericin B in man. Antibiot. Med. & Clin. Ther. 5 : 295～301, 1958