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Positive Interaction of Nikkomycins and Azoles against *Candida albicans* In Vitro and In Vivo

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Nikkomycins X and Z (NZ), competitive inhibitors of fungal chitin synthetase, were combined with azoles in a series of in vitro checkerboard assays to test for synergism against *Candida* spp. All combinations of nikkomycins and azoles tested resulted in marked synergistic activity against an isolate of *Candida albicans*, with fractional inhibitory concentration indices ranging from 0.016 to 0.28. No synergistic effect was demonstrable with isolates of *C. tropicalis*, *C. parapsilosis*, or *C. krusei*, though results for the latter two were suggestive of an additive effect. In survival models of mice infected intravenously with *C. albicans*, NZ administered singly in doses ranging from 5 to 50 mg/kg of body weight twice a day was able to delay the onset of mortality but showed no dose-response effect. The combination of NZ and the azole R 3783 administered orally in a ratio of 8:1 to 40:1 or greater (wt/wt) enhanced survival better than did the drugs given individually, but this effect was less evident for combinations involving fluconazole. In short-term organ load assays with outbred mice infected intravenously with *C. albicans*, high ratios of NZ to R 3783 reduced the CFU per gram in kidneys more significantly than did the drugs individually. Statistically significant reductions were not seen for short-term fungal burden assays using combinations of NZ and fluconazole in outbred mice or in inbred mice more susceptible to candidiasis. In a model of rat vaginal candidiasis, the combination of NZ and R 3783 administered either orally or vaginally was more effective than the drugs used singly. Thus, under certain conditions, combination therapy with nikkomycin and select azoles may offer promise for an increased therapeutic effect in candidiasis.

With the increase in the number of immunocompromised patients in the last decade, there has been a concomitant increase in the numbers of life-threatening mycoses (17, 20). Unfortunately, antifungal drugs released for clinical use over the last decade have not offered substantive improvements in efficacy over amphotericin B. Recently, several new compounds active against the cell walls of fungi have been evaluated. These compounds include derivatives of echinocandin B, which inhibit beta-glucan synthesis (6, 22), and the nikkomycins, which inhibit chitin synthesis (4). While these drugs show promise, their spectrum of activity appears to be limited in comparison to those of the azoles and amphotericin B (6, 12). Previous data obtained with nikkomycin used as therapy in a mouse model of candidiasis showed only modest efficacy (1).

One approach to increasing the therapeutic index of anti-infective drugs is to test them in combinations for synergistic interactions. While there have been a number of reports concerning positive interactions of antifungal drugs or antifungal and antibacterial drugs, few combinations have shown substantial improvements over the effects of the single drugs; many have shown antagonism (2, 3, 5, 13, 16, 19, 23).

Because of previous reports that azole compounds interfered with the normal synthesis of chitin in the cell wall of *Candida albicans* (9, 18, 21) and the recent report describing synergy between the nikkomycin complex and three azoles, it seemed appropriate to try various combinations of azoles and purified nikkomycins X (NX) and Z (NZ) against this yeast. This report describes the results of in vitro tests and subsequent therapy experiments with mouse models of candidiasis.

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MATERIALS AND METHODS

Drugs. NX and NZ, clotrimazole, and the triazole R 3783 were received as powders from Bayer AG (Wuppertal, Germany); fluconazole was received as a powder from Pfizer, Inc. (Groton, Conn.); miconazole and itraconazole were received as powders from Janssen Pharmaceutica (Piscataway, N.J.); and ketoconazole was purchased as Nizoral (200-mg tablets). For use in susceptibility tests, the nikkomycins and fluconazole were dissolved directly into the test medium, ketoconazole was dissolved in 0.2 N HCl, and all other azoles were dissolved in 95% ethanol prior to dilution in the test medium. For administration to animals, the nikkomycins and fluconazole were prepared as solutions in 0.1% agar (Difco, Detroit, Mich.) with 0.5% glucose. R 3783 was prepared as a suspension in the same material. The concentrations of nikkomycins and azoles were adjusted for oral delivery in 0.1-ml volumes. Azoles and nikkomycins were administered as combinations in 0.1-ml volumes in agar only.

Organisms and conditions of culture. All strains used in this study, *C. albicans* B311 (originally obtained from H. Hasenclever, National Institutes of Health) and H12 (Bayer AG), *C. tropicalis* 1525 and 53 (obtained from J. Domer, Tulane School of Medicine, New Orleans, La.), *C. parapsilosis* CDC-60 (J. Domer), and *C. krusei* 351 (J. Domer), except H12, were stored at −70°C until needed, and inocula were propagated in broth containing 2% glucose and 1% yeast extract (Difco, Detroit, Mich.) at 37°C overnight. The *C. albicans* H12 was propagated on Kimmig agar at 28°C overnight.

Animals. CFW female mice (average weight, 20 to 24 g) (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) and DBA/2N male mice (average weight, 20 to 22 g) (Simonsen Laboratories, Gilroy, Calif.) were used for organ load and survival experiments, while male CF1 mice (aver-

age weight, 20 to 22 g) (F. Winkelmann, Bersuchstierzucht GmbH and Co. KG, Borchen, Germany) were used for the drug-combination survival experiments.

In vitro testing. All tests were performed with broth by using microtiter plates. Isolates were tested in yeast-nitrogen base with glucose and 0.15% asparagine at room temperature at a final inoculum concentration of 10^4 CFU/ml. MICs were read at 48 h and defined as the lowest concentration showing the complete inhibition of growth. For wells with combinations of drugs, endpoints were determined on a row-by-row basis. The fractional inhibitory concentration (FIC) indices were calculated as described by Hallander et al. (7).

Experimental design for in vivo testing. The therapeutic efficacies of the compounds, as indicated by their effects on the survival of infected mice and on the number of viable fungi in the target organs, were examined.

(i) Survival experiments. All survival experiments were designed so that a course of acute infection could be monitored, with deaths generally starting within 5 to 12 days of infection. The purpose of this approach was to provide a rigorous test of the antifungal agent, with the outcome largely determined before an active immune response could influence the results. Infection was by the intravenous route, and therapy was begun at 24 h after infection. NZ was given twice a day and azoles were given once daily, starting at 24 h after infection for various periods as indicated in the figures. All animals were held at least 28 days and monitored daily for mortalities. There were 15 to 20 mice per group in the treatment groups and 15 to 80 mice in the control groups.

(ii) Short-term organ load experiments. Experiments to determine the fungal burden in the kidneys (organ load) of infected and control animals were conducted as previously described (10), except that azoles were administered once daily only and NZ was given twice daily.

Statistical analyses. Results from short-term organ loads were analyzed by Duncan's multiple range test, with significance determined at the $P = 0.05$ level. Survival data were analyzed in a pairwise fashion by the log-rank test, with significance determined at the $P = 0.05$ level. All analyses were performed with Systat software (Evanston, Ill.).

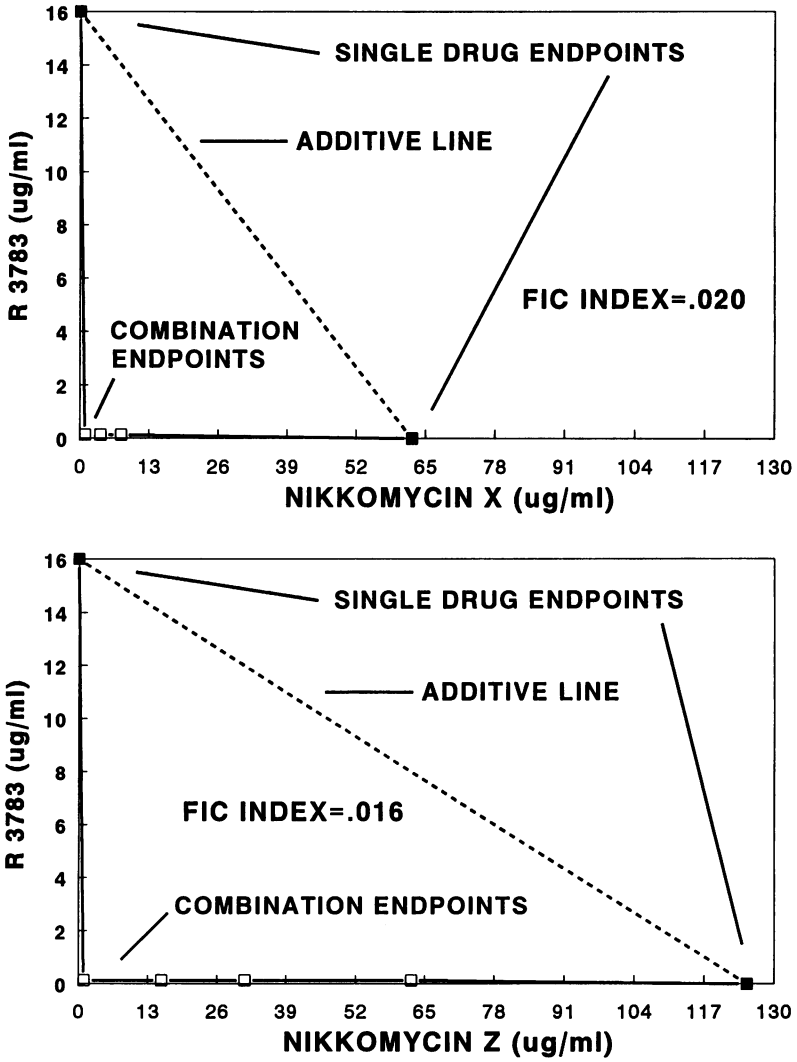

FIG. 1. Results of 96-well checkerboard synergy study with R 3783 and either NZ (top) or NX (bottom) against *C. albicans* B311 infection.

TABLE 1. FIC indices for combinations of azoles and NX and NZ against *C. albicans*

Azole	MIC of azole (μg/ml)	FIC value[a]	
		NX	NZ
Bifonazole	8	0.190	0.190
Clotrimazole	2	0.076	0.023
Fluconazole	>64	0.281	0.188
Itraconazole	>64	0.133	0.008
Ketoconazole	64	0.063	0.031
Miconazole	4	0.047	0.047
R 3783	16	0.020	0.016

[a] FIC value of ≤0.5 indicates synergy.

TABLE 2. Susceptibility results for combinations of R 3783 and NZ against non-*C. albicans* isolates of *Candida*

Isolate	MIC of azole (μg/ml)	MIC of NZ (μg/ml)	FIC value[a]
C. krusei	4	>500	0.750
C. parapsilosis	4	>500	0.625
C. tropicalis 1525	1	>500	2
C. tropicalis 53	1	>500	1

[a] FIC value of ≤0.5 indicates synergy.

RESULTS

MIC checkerboard testing. NX and NZ were tested in combination with several azoles by the checkerboard method against *C. albicans* B311. NZ and the azole R 3783 were tested against single isolates of *C. parapsilosis* and *C. krusei* and two isolates of *C. tropicalis*. As can be seen from the representative isobologram in Fig. 1 and the summary of all results from Table 1, all of the combinations resulted in pronounced synergy versus *C. albicans*, with FIC indices (7) ranging from 0.016 to 0.28 (synergy, ≤0.5). In most cases, MICs were lower with NX than with NZ, resulting in slightly higher FIC values for the NX combinations. In contrast, no synergy was demonstrable with the non-*C. albicans* isolates, likely because of the high resistance of these isolates to NZ alone (Table 2). However, the data suggest an additive effect in the case of *C. parapsilosis* and *C. krusei*. Also, because no endpoint was reached with NZ, the FIC indices represent maximum values but in no case could they be less than 0.5. The combinations tested against the various isolates resulted in trailing endpoints similar to those seen with the azoles alone.

In vivo experiments. In an initial experiment, it was determined that NZ used alone in a systemic candidiasis survival experiment did result in a delay in the onset of mortalities (Fig. 2). However, with three-drug concentrations ranging from 5 to 50 mg/kg of body weight, no dose-response effect was seen, and the results were statistically inferior to those obtained with 10 mg of R 3783 per kg.

Using combinations of NZ and azoles, two experiments were conducted with a survival model of systemic candidiasis by using 0.5 mg of either R 3783 or fluconazole per kg of body weight in combination with 10 mg of NZ per kg. At these concentrations, R 3783 used alone had little effect on survival, while the combination of NZ and R 3783 increased survival to a statistically significant level in comparison to NZ alone (Fig. 3). In an experiment with a lower infecting dose, 0.5 mg of fluconazole per kg and 10 mg of NZ per kg had a statistically significant effect on survival under these conditions, while no additional benefit was seen with the combination of fluconazole and NZ (Fig. 4). An additional experiment in which the concentrations of both NZ and R 3783 were increased (with a final ratio of 8.3:1) again showed statistically significant interaction between the two compounds (Fig. 5).

In an experiment to determine the effects of the combination of NZ and R 3783 on short-term organ loads, four different groups with drug concentrations spanning ratios from 2.5:1 to 40:1 were compared with controls and single-drug treatment groups. The results, shown in Table 3, demonstrated that kidney organ loads in groups treated with a 10:1 ratio of NZ to azole were not statistically different from those in animals treated with the same concentration of

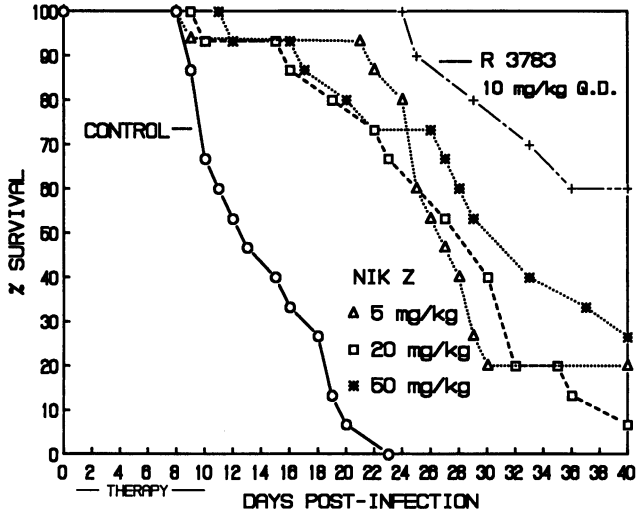

FIG. 2. Survival study of DBA/2N mice infected intravenously with 3.1×10^4 CFU of *C. albicans* B311 and treated orally with NZ or R 3783. The period of treatment is indicated under the *x* axis. Q.D., once daily.

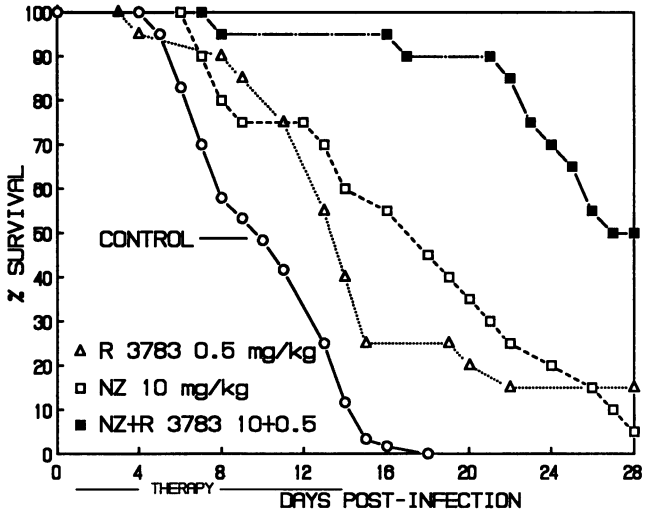

FIG. 3. Survival study of CF1 mice infected intravenously with 5×10^5 CFU of *C. albicans* H12 and treated orally with R 3783, NZ, the combination of these drugs, or an agar vehicle. The period of treatment is indicated under the *x* axis.

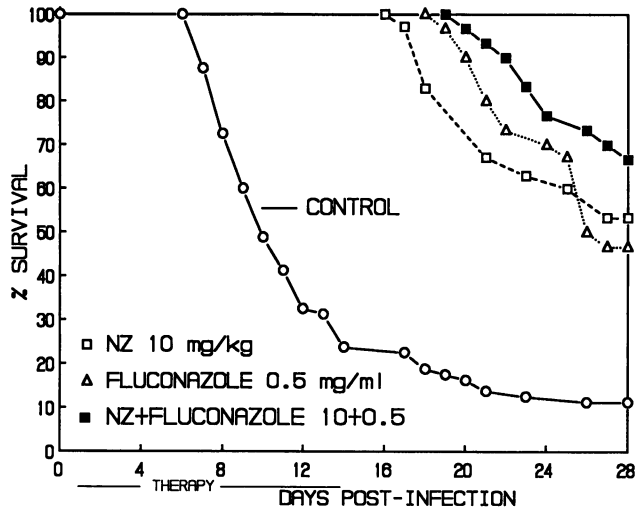

FIG. 4. Survival study of CF1 mice infected intravenously with 2×10^5 CFU of *C. albicans* H12 and treated orally with fluconazole, NZ, the combination of these drugs, or an agar vehicle. The period of treatment is indicated under the *x* axis.

azole alone. Only the group treated with a 40:1 ratio had results statistically different from those of the groups treated with a single drug at the same concentrations of NZ and azole, respectively. In an experiment using a 40:1 ratio of NZ to fluconazole and a similar infection inoculum, however, the combination did not result in a statistical improvement over that seen with fluconazole alone (Table 4). Similarly, with inbred DBA/2N mice, a strain more susceptible to candidiasis (10), and 40:1 and 100:1 ratios of NZ to fluconazole, the combinations did not result in a statistical improvement over that obtained with fluconazole alone (Tables 5 and 6).

Lastly, a model of vaginal candidiasis in rats was used to evaluate the combination of NZ and R 3783 in comparison to the latter compound and fluconazole used alone. The combination was administered orally in one group and was used to irrigate the vaginas of a second group, while the azoles used singly were given orally. The results, presented in Table 7, suggest that the combination given by either route was more effective than both azoles given singly. The combination was also more rapidly active, with the oral administration resulting in the only negative culture at 24 h posttherapy, while 50% were culture negative at 72 h.

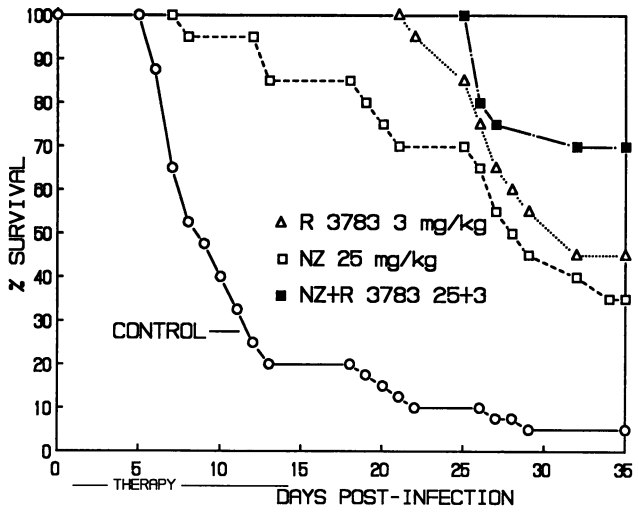

FIG. 5. Survival study of mice infected intravenously with 5×10^5 CFU of *C. albicans* H12 and treated orally with R 3783, NZ, the combination of these drugs, or an agar vehicle. The period of treatment is indicated under the *x* axis.

TABLE 3. Short-term organ loads in kidneys of mice infected with *C. albicans* B311[a]

Treatment group	Dose (mg/kg)	Mean log CFU ± SEM	Statistical group(s)[b]
Control	None	4.56 ± 0.26	A
NZ	5	4.31 ± 0.25	A
NZ	20	4.15 ± 0.21	A
NZ + R 3783	5 + 0.5	4.19 ± 0.32	A
R 3783	0.5	4.02 ± 0.15	A, B
R 3783	2	3.67 ± 0.18	B, C
NZ + R 3783	20 + 0.5	3.39 ± 0.31	C
NZ + R 3783	20 + 2	3.31 ± 0.10	C
NZ + R 378	5 + 2	3.08 ± 0.09	C

[a] Animals were infected with 50,000 CFU of *C. albicans* B311.
[b] Groups with the same letter are not statistically different.

DISCUSSION

The results of the in vitro tests described herein indicate that combinations of azoles and nikkomycins can result in consistent and substantial reductions in the MICs for *C. albicans*. Although there have been numerous reports for various combinations of azoles, polyenes, and 5-fluorocytosine, improvements in susceptibility were variable in nature and rarely resulted in more than a fourfold decrease in the MIC from that seen with individual drugs (3, 5, 16, 23).

While the mechanism by which the combination of nikkomycin and azole can increase susceptibility is unknown, it has been reported previously that several agents that interfere with normal cytoplasmic membrane synthesis or architecture can cause aberrant synthesis of chitin (8, 9, 18, 21). With the azoles and inhibitors of beta-glucan synthesis, i.e., papulacandin B or cilofungin, the effect is demonstrable at subinhibitory concentrations of these agents. Given that the active form of chitin synthetase is embedded in the cytoplasmic membrane, it is a reasonable assumption that changes in the normal composition of the membrane might affect the activity of this enzyme. Milewski et al. (15) postulate that subinhibitory concentrations of azoles may preferentially affect the ergosterol content of chitosomes, leading to a reduced capability of the cell to synthesize chitin. Reasons for the lack of activity against other species of *Candida* have

TABLE 4. Short-term organ loads in kidneys of outbred mice infected with *C. albicans* B311[a]

Treatment group	Dose (mg/kg)	Mean log CFU ± SEM	Statistical group[b]
Control	None	4.24 ± 0.14	A
NZ	20	4.16 ± 0.23	A
NZ + fluconazole	20 + 0.5	3.97 ± 0.32	A
Fluconazole	0.5	3.63 ± 0.07	B
Fluconazole	25	3.27 + 0.05	B

[a] Animals were infected with 52,000 CFU of *C. albicans* B311.
[b] Groups with the same letter are not statistically different.

TABLE 5. Short-term organ loads in kidneys of inbred mice infected with *C. albicans* B311[a]

Treatment group	Dose (mg/kg)	Mean log CFU ± SEM	Statistical group[b]
Control	None	6.53 ± 0.20	A
NZ	20	5.90 ± 0.10	B
Fluconazole	0.5	5.01 ± 0.15	C
NZ + fluconazole	20 + 0.5	4.78 ± 0.12	C
NZ + fluconazole	25	4.20 + 0.09	D

[a] Animals were infected with 26,000 CFU of *C. albicans* B311.
[b] Groups with the same letter are not statistically different.

not been determined but may involve reduced transport of NZ intracellularly (24).

While the criteria for determining synergy in in vitro tests are well established, no such guidelines exist for in vivo experimentation. In general, the term implies a combination effect greater than the sum of the effects of the individual drugs (14). The term has been applied to combinations of two active agents or in situations where an active agent was used with a second agent that, by itself, was inactive against the target organism but which in combination caused an increase in efficacy. An example of the latter situation would be the combination of amphotericin B with rifampin, which, in certain situations, has been shown to be synergistic in vitro and in animal models of mycoses (13, 19). In this case, rifampin has no activity alone but potentiates the activity of the antifungal agent, amphotericin B.

Despite the dramatic increases in susceptibility obtained with the nikkomycins and azoles, the in vivo results did not match the findings of the in vitro tests; the effect was restricted to certain azoles and to certain ratios of nikkomycin to azole. Given that the in vitro results show a 10-fold difference in the FIC values for R 3783 and fluconazole, it is not surprising that the in vivo data were more favorable for combinations of R 3783 and NZ. These results may, in part, be explainable by the different pharmacokinetic behavior of these compounds in the hosts or even within the target organs. As mentioned, aberrant chitin synthesis in azole-treated fungal cells was found to be most pronounced in cells treated within a narrow range of concentrations (9, 18). Thus, there may be a narrow tolerance with respect to dosing for reproducing this phenomenon in vivo. If, indeed, precise ratios and absolute concentrations of each component are required for increased inhibition of the yeast cells, it is also likely that the conditions would be different for lipophilic azoles like R 3783 and hydrophilic compounds like fluconazole, compounds which show differences in pharmacokinetic behavior and which are metabolized differently. Thus, it follows that before this concept could be applied in clinical medicine, additional preclinical work would have to be performed to better evaluate the critical requirements for each mycotic syndrome or combination of specific agents. Alternatively, evaluation of these combinations in superficial infections in which set concentrations could be controlled through localized applications may provide useful information to validate this approach in non-life-threatening situations.

TABLE 6. Short-term organ loads in kidneys of inbred mice infected with *C. albicans* B311[a]

Treatment group	Dose (mg/kg)	Mean log CFU ± SEM	Statistical group[b]
Control	None	5.90 ± 0.14	A
Fluconazole	0.2	5.21 ± 0.14	B
NZ	20	5.19 ± 0.13	B
NZ + fluconazole	20 + 0.2	4.86 ± 0.19	B
Fluconazole	10	3.92 ± 0.09	C

[a] Animals were infected with 26,000 CFU of *C. albicans* B311.
[b] Groups with the same letter are not statistically different.

TABLE 7. Semiquantitative culture results in a rat model of vaginal candidiasis

Treatment group	Dose (mg/kg)	No. of cultures with indicated result[a] (n = 4)											
		24 h posttherapy						72 h posttherapy					
		−	±	1+	2+	3+	4+	−	±	1+	2+	3+	4+
Control	None						4						4
R 3783	5			2	1		1	1	1	2			
Fluconazole	5		1	2	1				3	1			
NZ	20		1				3	1					3
NZ + R 3783	5 + 20	1		1	1		1	2	1		1		
Topical NZ + R 3783	3 + 15		1	3				2	1		1		

[a] −, no colonies; ±, 1 to 5 colonies; 1+ to 3+, between 5 and 200 colonies; 4+, ≥200 colonies.

REFERENCES

1. **Becker, J. M., S. Marcus, J. Tallock, D. Miller, E. Krainer, R. K. Khare, and F. Naider.** 1988. Use of the chitin-synthesis inhibitor nikkomycin to treat disseminated candidiasis in mice. J. Infect. Dis. **157:**212–214. (Letter.)
2. **Brajtburg, J., D. Kobayashi, G. Medoff, and G. S. Kobayashi.** 1982. Antifungal action of amphotericin B in combination with other polyene or imidazole antibiotics. J. Infect. Dis. **146:**138–146.
3. **Corrado, M. L., M. Kramer, M. Cummings, and R. H. Eng.** 1982. Susceptibility of dematiaceous fungi to amphotericin B, miconazole, ketoconazole, flucytosine and rifampin alone and in combination. Sabouraudia **20:**109–113.
4. **Dahn, U., H. Hagenmaier, H. Hohne, W. A. Konig, G. Wolf, and H. Zahner.** 1976. Nikkomycin, ein neuer Hemmstoff der Chitinsynthese bei Pilzen. Arch. Microbiol. **107:**143–160.
5. **Dupont, B., and E. Drouhet.** 1979. In vitro synergy and antagonism of antifungal agents against yeast-like fungi. Postgrad. Med. J. **55:**683–686.
6. **Gordee, R. S., D. J. Zeckner, L. C. Howard, W. E. Alborn, Jr., and M. Debono.** 1988. Anti-Candida activity and toxicology of LY121019, a novel semisynthetic polypeptide antifungal antibiotic. Ann. N.Y. Acad. Sci. **544:**294–309.
7. **Hallander, H. O., K. Dornbusch, L. Gezelius, K. Jacobson, and I. Karlsson.** 1982. Synergism between aminoglycosides and cephalosporins with antipseudomonal activity: interaction index and killing curve method. Antimicrob. Agents Chemother. **22:**743–752.
8. **Hector, R. F., and P. C. Braun.** 1986. Synergistic action of nikkomycins X and Z with papulacandin B on whole cells and regenerating protoplasts of *Candida albicans*. Antimicrob. Agents Chemother. **29:**389–394.
9. **Hector, R. F., and P. C. Braun.** 1987. The effects of bifonazole on chitin synthesis in *Candida albicans*, p. 369–382. *In* R. Fromtling (ed.), Recent trends in the discovery, development and evaluation of antifungal agents. J. R. Prous Science Publishers, Barcelona, Spain.
10. **Hector, R. F., and E. Yee.** 1990. Evaluation of Bay R 3783 in rodent models of superficial and systemic candidiasis, meningeal cryptococcosis, and pulmonary aspergillosis. Antimicrob. Agents Chemother. **34:**448–454.
11. **Hector, R. F., E. Yee, and M. S. Collins.** 1990. Use of DBA/2N mice in models of systemic candidiasis and pulmonary and

systemic aspergillosis. Infect. Immun. **58:**1476–1478.
12. **Hector, R. F., B. L. Zimmer, and D. Pappagianis.** 1990. Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. Antimicrob. Agents Chemother. **34:**587–593.
13. **Hughes, C. E., C. Harris, J. A. Moody, L. R. Peterson, and D. N. Gerding.** 1984. In vitro activities of amphotericin B in combination with four antifungal agents and rifampin against *Aspergillus* spp. Antimicrob. Agents Chemother. **25:**560–562.
14. **Jawetz, E.** 1967. Combined antibiotic action: some definitions and correlations between laboratory and clinical results. Antimicrob. Agents Chemother. **6:**203–209.
15. **Milewski, S., F. Mignini, and E. Borowski.** 1991. Synergistic action of nikkomycin X/Z with azole antifungals on *Candida albicans*. J. Gen. Microbiol. **137:**2155–2161.
16. **Montgomerie, J. Z., J. E. Edwards, Jr., and L. B. Guze.** 1975. Synergism of amphotericin B and 5-fluorocytosine for *Candida* species. J. Infect. Dis. **132:**82–86.
17. **Perfect, J. F., W. W. Pickard, D. L. Hunt, B. Palmer, and W. A. Schell.** 1991. The use of amphotericin B in nosocomial fungal infection. Rev. Infect. Dis. **13:**474–479.
18. **Pfaller, M. A., J. Riley, and T. Koerner.** 1990. Effects of terconazole and other azole antifungal agents on the sterol and carbohydrate composition of *Candida albicans*. Diagn. Microbiol. Infect. Dis. **13:**31–35.
19. **Polak, A., H. J. Scholer, and M. Wall.** 1982. Combination therapy of experimental candidiasis, cryptococcosis and aspergillosis in mice. Chemotherapy **28:**461–479.
20. **Saral, R.** 1991. *Candida* and *Aspergillus* infections in immunocompromised patients: an overview. Rev. Infect. Dis. **13:**487–492.
21. **Surarit, R., and M. G. Shepherd.** 1987. The effects of azole and polyene antifungals on the plasma membrane enzymes of *Candida albicans*. J. Med. Vet. Mycol. **25:**403–413.
22. **Taft, C. S., and C. P. Selitrennikoff.** 1990. Cilofungin inhibition of (1-3)-beta-glucan synthase: the lipophilic side chain is essential for inhibition of enzyme activity. J. Antibiot. **43:**433–437.
23. **Uno, J., M. L. Shigematsu, and T. Arai.** 1983. Novel synergism of two antifungal agents, copiamycin and imidazole. Antimicrob. Agents Chemother. **24:**552–559.
24. **Yadan, J.-C., M. Gonneau, P. Sarthou, and F. Le Goffic.** 1984. Sensitivity to nikkomycin Z in *Candida albicans*: role of peptide permeases. J. Bacteriol. **160:**884–888.