Combination Therapy in Treatment of Experimental Pulmonary Aspergillosis: Synergistic Interaction between an Antifungal Triazole and an Echinocandin

Vidmantas Petraitis,1 Ruta Petraitiene,1 Alia A. Sarafandi,1 Amy M. Kelaher,1 Caron A. Lyman,1 Heather E. Casler,1 Tin Sein,1 Andreas H. Groll,1 John Bacher,2 Nilo A. Avila,3 and Thomas J. Walsh1
1Immunocompromised Host Section, Pediatric Oncology Branch, National Cancer Institute, 2Surgery Service, Veterinary Resources Program, Office of Research Services, and 3Department of Radiology, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland

Invasive pulmonary aspergillosis is an important cause of morbidity and mortality in immunocompromised patients. Simultaneous inhibition of fungal cell-wall and cell-membrane biosynthesis may result in synergistic interaction against Aspergillus fumigatus. We studied the antifungal activity of micafungin, a new echinocandin, in combination with ravuconazole, a second-generation triazole, against experimental invasive pulmonary aspergillosis in persistently neutropenic rabbits. This combination led to significant reductions in mortality (P ≤ .001), residual fungal burden (P ≤ .05), and serum galactomannan antigenemia (P ≤ .01), compared with either agent alone. Combination therapy also resulted in reduction (P ≤ .05) of organism-mediated pulmonary injury and of pulmonary infiltrates detected by thoracic computed tomography (P ≤ .001). No toxicity was observed with the echinocandin-triazole combination. An MTT hyphal damage assay demonstrated significant in vitro synergistic interaction between the antifungal triazole and the echinocandin. The combination of an antifungal triazole and echinocandin may represent a new strategy for treatment of invasive pulmonary aspergillosis.

Invasive pulmonary aspergillosis is an important cause of morbidity and mortality in patients with cancer, transplant recipients, and other individuals with immuno-deficiencies [1–6]. Despite the use of amphotericin B and its lipid formulations, itraconazole, and voriconazole, mortality attributable to aspergillosis remains high [7].

The echinocandins are a novel class of semisynthetic lipopeptide antifungal compounds that inhibit synthesis of 1,3-β-D-glucan, a key component of the cell wall of most pathogenic fungi. Inhibition of 1,3-β-D-glucan synthesis results in disrupted fungal cell-wall structure, cell damage, and cell injury or death [8, 9]. Echinocandins may cause cell injury or death in regions of hyphal growth, particularly at branch points and advancing tips; however, other cells of the hyphal structure may remain viable and be recovered from tissue [10, 11]. This selective effect of echinocandins contrasts with its effect on Candida species, which may be completely eradicated from tissue by this type of antifungal agent [11]. Micafungin (FK463) is a novel water-soluble, parenterally administered investigational echinocandin lipopeptide that has been well studied in laboratory animals and humans [11–14].

The antifungal triazoles inhibit fungal cell-membrane biosynthesis through inhibition of ergosterol formation at the level of lanosterol C14-demethylase [15]. Ravuconazole is a novel antifungal triazole that has potent
and relatively broad-spectrum in vitro antifungal activity against medically important yeast and filamentous fungi [16–22].

We hypothesized that simultaneous inhibition of the biosynthesis of key components of the fungal cell wall and cell membrane by a combination of an echinocandin and an antifungal triazole may result in a synergistic interaction in vitro and in vivo. We therefore examined the potential therapeutic utility of a combination of micafungin and ravuconazole in the treatment of experimental invasive pulmonary aspergillosis in persistently neutropenic rabbits.

**MATERIALS AND METHODS**

**Organisms.** National Institutes of Health isolate *Aspergillus fumigatus* 4215 (ATCC MYA-1163), obtained from a patient with a fatal case of pulmonary aspergillosis, was used in the experiments. The organisms were subcultured from a frozen isolate (stored at −70°C) onto Sabouraud dextrose slants (BBL) and incubated for 24 h at 37°C. The slants were then allowed to grow at room temperature for an additional 5 days before conidia were harvested.

MICs against *A. fumigatus* were determined according to National Committee for Clinical Laboratory Standards M38-A microdilution methods [23, 24]. The MIC of ravuconazole for *A. fumigatus* was 1.0 µg/mL in RPMI 1640 medium. For micafungin, the minimum effective concentration (defined as the lowest concentration that caused the formation of microcolonies of *A. fumigatus*) was 0.25 µg/mL in RPMI 1640 medium (BioWhittaker).

**MTT hyphal damage assays.** A rapid colorimetric assay using tetrazolium salt (MTT) [25] was used to evaluate the effects of micafungin and ravuconazole on damage to the hyphae of *A. fumigatus*. The yellow MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma) is cleaved by dehydrogenases of metabolically active fungi, reducing it to its purple MTT-formazan derivative, which is then measured spectrophotometrically after alcohol extraction from the hyphae. Hyphal damage was assessed at 24 h after exposure to micafungin alone, ravuconazole alone, or a combination of micafungin and ravuconazole at concentrations ranging from 0.5 to 25 µg/mL for micafungin and from 0.5 to 12.5 µg/mL for ravuconazole; results were reported as the mean percentage of hyphal damage for each concentration. Absorbance readings were made on a microplate spectrophotometer (Multiscan MMC/340; Titertek) at 570 and 690 nm. Hyphal damage was calculated by comparing the absorption of aliquots extracted from drug-treated organisms with that of aliquots from untreated control organisms, using the following equation (where A is absorbance): percentage of hyphal damage = [1 − (A with drug/A without drug)] × 100.

**Animals.** Healthy female New Zealand White rabbits (Hazelton Research Products) weighing 2.6–3.5 kg at the time of endobronchial inoculation were used in all experiments. National Institutes of Health guidelines for animal care, in fulfillment of the guidelines of the National Research Council [26], were followed. Rabbits were individually housed and maintained with water and standard rabbit feed ad libitum. A total of 36 rabbits were used in all experiments. Vascular access was established in each rabbit by the surgical placement of a silastic tunneled central venous catheter, as described elsewhere [27]. The silastic catheter permitted nontraumatic venous access for administration of parenteral agents and for repeated blood sampling for study of biochemical and hematological parameters, plasma pharmacokinetics, and serum galactomannan.

**Inoculation.** Pulmonary aspergillosis was established as described elsewhere [28]. Conidia were harvested under a laminar airflow hood with a solution of 10 mL of 0.025% Tween 20 (Fisher Scientific) in normal saline (NS; Quality Biological), transferred to a 50-mL conical tube, washed, and counted with a hemacytometer. The concentration was adjusted to give each rabbit a predetermined inoculum of 1.0 × 10³–1.25 × 10⁵ conidia of *A. fumigatus* in a volume of 250–350 µL.

Inoculation was performed on day 2 of the experiments and while the rabbits were under general anesthesia. Each rabbit was anesthetized with 0.5–0.6 mL of a 2:1 mixture (vol:vol) of intravenously administered ketamine (100 mg/mL) and xylazine (20 mg/mL). The *A. fumigatus* inoculum was then administered intratracheally via a Flagg O straight-blade laryngoscope (Welch Allyn) with a tuberculin syringe attached to a 5.25-inch 16-gauge Teflon catheter (Becton Dickinson Infusion Therapy Systems).

**Immunosuppression and maintenance of neutropenia.** Profound and persistent neutropenia (granulocyte concentration of <100 granulocytes/µL) and thrombocytopenia (platelet count of 30,000–50,000 platelets/µL) was achieved by an initial course of 525 mg/m² cytarabine administered for 5 consecutive days beginning with day 1 of the experiment. A maintenance dose of 484 mg/m² cytarabine was administered for 4 additional days, on days 8, 9, 12, and 13 of the experiment, to maintain persistent neutropenia. Methylprednisolone at a dose of 5 mg/kg of body weight/day was administered on days 1 and 2 of chemotherapy (study days 1 and 2).

Ceftazidime (75 mg/kg given intravenously twice daily), gentamicin (5 mg/kg given intravenously every other day), and vancomycin (15 mg/kg given intravenously daily) were administered from study day 4 (day 4 of immunosuppression) until study completion to prevent opportunistic bacterial infections during neutropenia. To prevent antibiotic-associated diarrhea due to Clostridium difficile, all rabbits continuously received 50 mg of vancomycin per liter of drinking water.

**Antifungal compounds and treatment regimens.** Rabbits...
were grouped to receive micafungin, 1 mg/kg intravenously (MICA1); ravuconazole, 2.5 mg/kg intravenously (RAV2.5); or a combination of MICA1 and RAV2.5 (MICA + RAV) for treatment of established invasive pulmonary aspergillosis or no drug (untreated controls). Doses were selected for which the most data were available from clinical trials. Higher doses may have more antifungal activity but may not permit testing the hypothesis of possible synergistic interaction. Micafungin was provided as a sterile powder (50 mg/vial) and was reconstituted with sterile NS to achieve a 1-mg/mL concentration. Ravuconazole was provided as a BMS-379224 dilyne salt and dissolved, according to the recommendations of the manufacturer, in 5% dextrose in water (D5W) injection solution (Abbott Laboratories) to produce a 37.5-mg/mL stock solution, which was reconstituted with D5W to a 9.375-mg/mL solution. The reconstituted ravuconazole was administered at room temperature as a slow intravenous bolus over the course of 1 min. Antifungal therapy was administered for 12 days starting 24 h after endotracheal inoculation of A. fumigatus conidia.

Outcome variables. The following panel of outcome variables was used to assess antifungal efficacy: residual fungal burden (log cfu/g), survival, pulmonary infarct score, lung weight, computed tomography (CT) scores, and serum galactomannan index (GMI). Pulmonary infarct score, lung weight, and CT scores were measures of organism-mediated pulmonary injury.

Fungal cultures. Lung tissue from each rabbit was sampled and cultured by a standard excision of tissue from each lobe. Each fragment was weighed individually, placed in a sterile polyethylene bag (Tekmar), and homogenized with sterile saline for 30 s (Stomacher 80; Tekmar) [29]. Lung homogenate dilutions (10^-1 and 10^-2) were prepared in sterile NS. Aliquots (100 μL) from homogenates and homogenate dilutions were plated onto Sabouraud glucose agar plates and incubated at 37°C for the first 24 h and then held at room temperature for another 24 h. The number of colony-forming units of A. fumigatus was counted and recorded for each lobe, and the number of colony-forming units per gram was calculated. A finding of 1 colony of A. fumigatus was considered to be a positive result.

Pulmonary lesion scores and lung weights. The entire heart-lung block was carefully resected at autopsy. The lungs were weighed and inspected by at least 2 observers who were blinded to the treatment group and who recorded the number of hemorrhagic infarct lesions (if any) in each individual lobe. The number of A. fumigatus-positive lobes was calculated, and the mean number of positive lobes per rabbit was calculated for each treatment group.

Survival. The survival time in days after inoculation was recorded for each rabbit in each group. Surviving rabbits were euthanized by sodium pentobarbital anesthesia on day 13 after inoculation, 24 h after the last dose of study drug.

Histopathology. Pulmonary lesions were excised and fixed in 10% neutral buffered formalin. Paraffin-embedded tissue sections were then sectioned and stained with either periodic acid–Schiff or Grocott-Gomori methenamine silver stain.

CT. Serial CT scans of the lungs were performed during all experiments to monitor the effects of antifungal therapy on organism-mediated pulmonary injury during the course of infection. In brief, rabbits were sedated with ketamine and xylazine and then placed prone, head first, on the scanning couch. CT was performed with an ultrafast electron beam CT scanner (HiSpeed CT/i, model CE 0459; GE Medical Systems), as described elsewhere [30]. Using the high-resolution, table-incremented, volume-acquisition mode, 3 mm–thick ultrafast CT scans were performed every 4 s. A small scan circle and a 9.6 cm–diameter reconstruction circle with a matrix of 512 x 512 pixels were used, which resulted in a pixel size of <1 mm. Scan parameters were 80 kV and 120 mA, and scan duration was 0.8 s. In virtually all cases, 30 slices were sufficient to scan the entire thorax of the rabbit. Images were photographed using lung windows with a level of ~600 Hounsfield units and a width of 1800 Hounsfield units.

CT images were evaluated after the completion of all experiments. The pulmonary infiltrate score for each animal was evaluated by the same investigator, who was blinded to the treatment groups. According to the image analysis protocol, the right and left lungs were each divided into 3 lobes (upper, middle, and lower), and each lobe was assessed to determine a pulmonary infiltrate score. The accessory lobe in the rabbit is designated as the “left middle lobe.” A mean CT pulmonary infiltrate score was established by evaluating the infiltrates in each lobe. The pulmonary infiltrates score for each lobe was 0, 0.5, 1, 1.5, 2, 2.5, or 3 (where 0 represents no infiltrates in that lobe and 3 represents infiltrates occupying the entire lobe) and was evaluated on every day on which the lungs were scanned. CT scans were performed on days 1, 4, 6, 8, and 11 of treatment. The pulmonary infiltrate score for each rabbit for that day was calculated by adding the scores of all 6 lobes. The mean CT pulmonary infiltrate score for that day represents the mean score of all rabbits in each treatment group.

Galactomannan assay. Blood from each rabbit infected with A. fumigatus was collected every other day for determination of serum galactomannan concentrations. Serum galactomannan concentrations were determined by the Platelia Aspergillus EIA (Genetic Systems/Sanofi Diagnostics Pasteur) 1-stage immunoenzymatic sandwich microparticle assay method, which was performed according to the manufacturer’s directions. The assay uses rat monoclonal antibody EB-A2, which is directed against Aspergillus galactomannan [31]. The monoclonal antibody is used to sensitize the wells of the microplate
and to bind to the antigen. Peroxidase-linked rat monoclonal antibody is used as the detector antibody. The optical absorbances of specimens and controls were determined by a microplate spectrophotometer equipped with 450- and 620-nm filters (TiterTec Multiscan MMC/340).

ELA data were expressed as serum GMIs plotted over time. The GMI for each test serum is equal to the optical density of a sample divided by the optical density of a threshold serum sample provided with the test kit. Serial serum galactomannan levels were plotted over the course of time of administration of antifungal compound and time of initiation of study drug treatment.

**Pharmacokinetic studies.** The plasma pharmacokinetics of ravuconazole and micafungin were investigated in 5–8 infected animals; minimal plasma sampling was used in all cohorts. Time points for sampling were determined by inspection of full plasma concentration profiles obtained in healthy rabbits after administration of similar doses [32, 33]. Plasma samples were obtained on day 5 of antifungal therapy. Blood samples were drawn immediately in heparinized syringes after antifungal agents were administered and then at 0.15, 2, 6, 12, and 24 h after administration of study drugs. Plasma was immediately separated by centrifugation and stored at -70°C until assays were done.

Concentrations of ravuconazole in plasma were determined after protein precipitation with acetonitrile (1:2, vol:vol) by reversed-phase high-performance liquid chromatography. The mobile phase consisted of acetonitrile:water (58:42, vol:vol). Separation was achieved using a C-18 YMC ODS-AQ column (150 × 4.6-mm internal diameter and 5-μm particle size; YMC). Ravuconazole was detected by UV detection at 284 nm. Quantitation was based on the peak height concentration response of the reference standard, BMS-207147. Ten-point standard curves (range of concentration, 0.05–20 μg/mL) were linear, with r² > 0.996. The lower limit of quantitation was 0.1 μg/mL in plasma. Accuracies were within 12%, and intra- and interday variability (precision) was <10%.

Micafungin was extracted from acidified heparinized plasma by liquid:liquid extraction with acetonitrile-based organic solvents and diluted with phosphate buffer. Concentrations of micafungin were determined by reversed-phase high-performance liquid chromatography. The mobile phase consisted of 20 mM KH₂PO₄:acetonitrile (59:41, vol:vol) delivered at 1 mL/min. Samples were maintained in the autosampler at room temperature in amber glass vials. The injection volume was 75 μL of micafungin eluted at 10.3–13.8 min, using a 5-μm TSK-GEL silica-based analytical column (ODS80TM 150 × 4.6 mm; Tosohas) maintained at 50°C in conjunction with a precolumn filter containing a 5-μm insert; fluorometric detection (273 nm excitation and 464 nm emission) was used to measure concentrations. Quantitation was based on the peak height of micafungin and the nonweighted concentration response of the external calibration standard. Eight- to 10-point standard curves were linear, with r² > 0.987. The lower limit of quantitation was 0.1 μg/mL in plasma. Accuracies were within 1.7%–12.8%, and intra- and interday variability (precision) ranged from 1.2% to 6.8%.

**Pharmacokinetic data analysis and toxicity studies.** Pharmacokinetic parameters for ravuconazole and micafungin were determined by model-independent analysis. The following pharmacokinetic parameters were determined by standard equations [34]: maximum concentrations in plasma; concentrations at 24 h after administration of antifungal agents; the area under the plasma concentration–time curve from 0 to 24 h (AUC₀₋₂₄), calculated by trapezoidal estimation; plasma clearance; apparent volume of distribution at steady state; and plasma half-life. Chemical determinations of potassium, aspartate aminotransferase, alanine aminotransferase, creatinine, urea nitrogen, and total bilirubin concentrations in serum were performed on the penultimate sample drawn from each rabbit.

**Statistical analysis.** Comparisons between groups were performed by the Kruskal-Wallis test (nonparametric analysis of variance) or the Mann-Whitney U test, as appropriate. The central hypothesis of this analysis is based on the response resulting from combination treatment, in comparison with that resulting from administration of micafungin alone, ravuconazole alone, or no treatment (untreated controls). A 2-tailed P value of <.05 was considered to be statistically significant. Survival was plotted by Kaplan-Meier analysis. Differences in the

![Figure 1](https://example.com/f1.png) Synergistic hyphal damage to *Aspergillus fumigatus* resulting from treatment with micafungin (MICA), ravuconazole (RAV), or a combination of both (MICA + RAV). In vitro MTT assays demonstrated significant \( P < .001 \), by analysis of variance) concentration-dependent synergistic interactions at concentrations ≤5 μg/mL for micafungin and ≤2.5 μg/mL for ravuconazole. Data are mean values for each treatment group.
survival rates in treatment groups and among untreated controls were analyzed by log-rank test. Values are expressed as mean ± SEM.

RESULTS

In Vitro Studies
MTT hyphal damage assays found that micafungin alone, ravuconazole alone, and the micafungin-ravuconazole combination each had a concentration-dependent effect on A. fumigatus hyphal damage. An additive or synergistic effect on hyphal damage was observed with the echinocandin-triazole combination. The combination of 1 µg/mL of micafungin and 0.5 µg/mL of ravuconazole resulted in significantly greater hyphal damage than was observed with either drug alone (47.3% ± 1.7% for the combination vs. 1.5% ± 0.3% for micafungin alone and 18.0% ± 5.0% for ravuconazole alone; P < .001). Similarly, all combinations of the 2 drugs at lower concentrations (≤5 µg/mL) resulted in significantly greater hyphal damage than did either of the drugs alone (P ≤ .03; figure 1).

In Vivo Therapeutic Studies

Residual fungal burden. There was a significant reduction in the residual fungal burden of A. fumigatus in lung tissue from rabbits that received the micafungin-ravuconazole combination, compared with the effect of micafungin alone, ravuconazole alone, or no treatment (P < .01, P < .05, and P < .01, respectively). There were no significant differences among animals receiving micafungin alone or ravuconazole alone and untreated controls in clearance of A. fumigatus from lung tissue (figure 2).

Survival. Survival was significantly improved among the rabbits treated with the echinocandin-triazole combination. Nine (75%) of 12 rabbits (P < .001) that received the combi-
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Figure 3. Effects of micafungin and ravuconazole in vivo on hyphal damage of Aspergillus fumigatus. A, Organisms from untreated control rabbits demonstrated dichotomously branching septate hyphae (original magnification, ×630). B, Organisms from the lung tissue of a rabbit treated with the echinocandin micafungin demonstrate increased fragmentation of hyphal elements and spherical chlamydoconidial structures (original magnification, ×630; insert, ×1000). C, A. fumigatus organisms from the lung tissue of a rabbit treated with the antifungal triazole ravuconazole reveal disintegration and disruption of hyphal structures (original magnification, ×630; insert, ×1000). D, The combined morphologic effects of echinocandins and triazoles are each apparent in the hyphal elements of organisms from rabbits treated with both compounds (original magnification, ×630). The spherical chlamydoconidial structures (D, left insert) are evidence of the effect of echinocandins observed in B, and the focal hyphal disintegration and disruption (D, right insert) are compatible with the effects of triazoles that also are evident in C. Scale bar, 20 μm.

Organism-mediated pulmonary injury. Rabbits treated with combination therapy also had significant reductions in organism-mediated pulmonary injury, as measured by total lung weight and pulmonary infarct score (figure 2). The mean lung weights for combination-treated rabbits were significantly reduced, compared with those of micafungin- or ravuconazole-treated rabbits, and none of 8 untreated controls (figure 2).

Histopathology. The histopathological features of aspergillosis were studied in the lungs of rabbits in all treatment groups (figure 3). Organisms from untreated control rabbits demonstrated typical dichotomously branching septate hyphae of A. fumigatus (figure 3A). In figure 3B, organisms from the lung tissue of a rabbit that received MICA1 had increased fragmentation of hyphal elements and spherical chlamydoconidial structures. As depicted in figure 3C, there is a focal disintegration and disruption of hyphal structures in tissue from a rabbit that received triazoles. This pattern of damage was especially apparent transseptally over several contiguous fungal

nation survived through the entire study, compared with none of 8 micafungin-treated rabbits, 2 (25%) of 8 ravuconazole-treated rabbits, and none of 8 untreated controls (figure 2).
Figure 4. Mean pulmonary infiltrate scores determined by image analysis of serial computed tomographic scans of untreated controls and rabbits treated with micafungin (1 mg/kg/day; MICA1), ravuconazole (2.5 mg/kg/day; RAV2.5), or a combination (MICA + RAV). Animals that received the combination demonstrated significant resolution of pulmonary infiltrates, compared with untreated controls ($P < .001$, by analysis of variance).

Ultrafast CT scans. Ultrafast CT scans demonstrated significant resolution of pulmonary infiltrates in rabbits that received combination treatment, which was consistent with the reduction in organism-mediated pulmonary injury ($P < .001$; figure 4). During the first 4 days of treatment, there was an increase in pulmonary infiltrates in all treatment groups. After day 4 of treatment, there was a steady decrease in pulmonary infiltrates among the animals receiving combination therapy. In contrast, progressive increases in pulmonary infiltrates were observed in the lungs of animals from all other groups.

Serum galactomannan. We also investigated the expression of galactomannan, as a surrogate marker of therapeutic response, in serum samples from rabbits with pulmonary aspergillosis (figure 5). Serial serum samples from all treatment groups and untreated controls showed progressive galactomannan antigenemia through day 8 of treatment. However, the levels of galactomannan were significantly lower on day 8 in combination-treated rabbits, compared with rabbits that received single agents and untreated controls ($P < .01$).

Pharmacokinetics and safety. The plasma concentration-versus-time profiles of ravuconazole and micafungin observed after administration of either compound alone and both in combination are depicted in figure 6. With the exception of an increase in the mean peak plasma concentration of ravuconazole (from $2.38 \pm 0.20 \mu g/mL$ for ravuconazole alone to $3.39 \pm 0.92 \mu g/mL$ for the racuvoconazole-micafungin combination; $P = .0127$), coadministration of micafungin (1 mg/kg) did not change the plasma pharmacokinetic parameters of ravuconazole (2.5 mg/kg). Coadministration of racuvoconazole resulted in a decrease in the mean AUC$_{0-24}$ of micafungin to 72% (from $28.5 \pm 2.34 \mu g/mL$ for micafungin alone to $20.7 \pm 1.9 \mu g/mL$ for the micafungin-ravuconazole combination), in association with lower mean peak plasma concentrations (from $21.9 \pm 2.05$ to $8.6 \pm 0.78 \mu g/mL$; $P = .0016$), faster clearance (from $0.036 \pm 0.00$ to $0.049 \pm 0.00 \mu g/mL/h$; $P = .0295$), and lower mean trough plasma concentrations at the end of the 24-h dosing interval (from $0.23 \pm 0.04$ to $0.12 \pm 0.03 \mu g/mL$). No differences were detected in the plasma concentrations of potassium, creatinine, urea nitrogen, hepatic transaminases, or bilirubin among rabbits treated with the echinocandin-triazole combination and untreated control rabbits.

DISCUSSION

The echinocandin-triazole combination demonstrated significantly enhanced efficacy, compared with either drug alone, in persistently neutropenic rabbits with invasive pulmonary aspergillosis, as measured by reduction of residual fungal burden (log cfu/g), improved survival, reduced galactomannan antigenemia, and decreased pulmonary injury (lung weights, pulmonary infarct scores, and CT scan image score), all of which were super-

Figure 5. Expression of galactomannan in persistently neutropenic rabbits with pulmonary aspergillosis that were treated with micafungin (1 mg/kg/day; MICA1), ravuconazole (2.5 mg/kg/day; RAV2.5), or a combination (MICA + RAV). Animals that received combination treatment had significantly lower levels of galactomannan antigenemia ($P < .01$).
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Figure 6. Plasma concentration profiles of ravuconazole (RAV) vs. a combination of ravuconazole and micafungin (MICA + RAV; A) and micafungin (MICA) vs. the combination (B) after multiple intravenous daily doses. All values are mean ± SEM.

rior to the results of treatment with single agents and of no treatment. The echinocandin-triazole combination also exerted a potent in vitro synergistic interaction in causing hyphal damage of A. fumigatus, which is consistent with the pulmonary injury findings. To our knowledge, this is the first study to demonstrate that the combination of echinocandins and triazoles has a significant impact on survival, quantitative culture results, galactomannan antigenemia, and markers of organism-mediated pulmonary injury, compared with either compound alone.

This synergistic interaction between the echinocandin and the triazole is most likely due to simultaneous inhibition of biosynthesis of 1,3-β-D-glucan in the fungal cell wall and ergosterol in the cell membrane. These apparently independent mechanisms of action likely would lead to an additive or synergistic interaction against filamentous fungi. This additive-to-synergistic interaction stands in contrast to the potential antagonism observed with azoles and polyenes [35–37]. Antifungal azoles deplete the fungal cell membrane of ergosterol and thereby diminish the principal biochemical target of amphotericin B. Both azoles and polyenes ultimately interact at the level of ergosterol and thus have the potential for antagonism [38].

The antifungal effect observed with micafungin and ravuconazole is likely applicable to other members of the classes of echinocandins and triazoles. For example, Perea et al. [39] reported in vitro synergistic interactions between caspofungin and voriconazole. Manavathu et al. [40] reported in vitro synergistic interactions between amphotericin B and caspofungin, as well as between itraconazole and caspofungin, whereas the effect of combinations of amphotericin B and itraconazole was additive. Kirkpatrick et al. [41] described in vivo synergy between voriconazole and caspofungin in reducing positive cultures in experimental disseminated aspergillosis in guinea pigs. The safety profile of the echinocandin-triazole combination in our studies appears to be favorable, with no evidence of hepatic dysfunction or transaminase elevation. Both compounds were administered parenterally without adverse infusion-related reactions.

A pharmacokinetic interaction was observed between the triazole and the echinocandin in this study. The trend of the interaction resulted in slightly increased ravuconazole exposure and decreased echinocandin exposure. Although these compounds do not share metabolic pathways, there may be interaction at the level of inducible transport proteins that could be modulated during combination therapy. These data underscore the importance of conducting formal pharmacokinetic studies in patients to fully understand the plasma profiles of both compounds during combination therapy before conducting randomized trials.

Synergistic interaction may also occur between echinocandins and polyenes. Arikian et al. [42] and Douglas et al. [43] reported synergy in studies with caspofungin and amphotericin B. Kohno et al. [44] reported enhanced effects of a combination of micafungin with amphotericin B in a murine model of invasive pulmonary aspergillosis. The use of other combinations to treat experimental aspergillosis also has been studied, including rifampin with amphotericin B, 5-fluorocytosine with amphotericin B, and azoles with amphotericin B [45–49]. Each of these combinations has limitations of toxicity or paucity of a combination effect.

Combinations of antifungal agents, in comparison with single agents, may confer the benefit of increasing efficacy, sparing toxicity, or both. An analysis of the collective efficacy and safety data from our study and of those from other laboratory studies indicates that carefully conducted phase 1–2 clinical trials of safety, tolerance, and pharmacokinetics are warranted to begin exploring the therapeutic potential and safety profile of the echinocandin-triazole combination in treatment of invasive aspergillosis.
References


