

Evaluation of Antifungal Treatment in a Neutropenic Mouse Model of Scedosporiosis

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ABSTRACT

Objectives: Scedosporiosis is a rare fungal infection with high mortality rates. Because clinical trials are hard to conduct, we developed a murine model for evaluating the efficacy of currently used antifungals in treating scedosporiosis.

Methods: MIC of isavuconazole (ISA), posaconazole (POSA), voriconazole (VORI), and micafungin (MICA) were determined against 9 clinical isolates of *Scedosporium apiospermum*, *S. boydii* and *Lomentospora prolificans* using the CLSI M38 method. ICR mice were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3, and +8 relative to intratracheal infection with 3.0×10^7 cells of *S. apiospermum*. For survival studies, treatment with placebo (vehicle control), ISA (110 mg/kg, tid, po), POSA (30 mg/kg, tid, po), VORI (40 mg/kg, qd, po), MICA (3 or 10 mg/kg, qd, ip) or a combination of MICA (10 mg/kg) + ISA (110 mg/kg) began 16 h post infection and continued for 7 days. For tissue fungal burden studies, dosing began 8 h post infection and continued for 3 days. Mice were sacrificed on day +4. Survival and tissue fungal burden (by qPCR) served as efficacy endpoints.

Results: *S. apiospermum* was the most susceptible to all 4 antifungals with MICA MIC of 0.25 µg/mL and azole MICs of 1 µg/mL. *S. boydii* was also susceptible to MICA (0.125 - 0.5 µg/mL) but with variable susceptibility to azoles (1-16 µg/mL). In contrast, *L. prolificans* strains were resistant (MICA MIC 2-4 µg/mL and azole MIC >16 µg/mL). *S. apiospermum* DI16-478 was used to test *in vivo* efficacy. Only MICA (10 mg/kg) treatment prolonged survival of mice (n=10) vs. placebo (median survival time = 8 days for MICA vs. 5 for placebo, p<0.03 by Log Rank) and reduced fungal burden in lungs (primary target organ), brains and kidneys (p<0.02, by Wilcoxon Rank Sum). None of the azoles prolonged survival despite the significant reduction in the lung fungal burden (p<0.002), possibly due to lack of reduction of fungal burden in kidneys and brains. MICA+ISA did not enhance survival nor reduce tissue fungal burden vs. placebo.

Conclusion: Despite the *in vitro* activity of tested antifungals, only MICA demonstrated modest efficacy in mice infected with *S. apiospermum*. A combination of MICA+ISA was ineffective in this model. Continued investigations of other drug combinations to treat scedosporiosis are needed.

INTRODUCTION

- Scedosporiosis comprises a wide range of clinical diseases ranging from localized to disseminated infections in both immunocompromised and immunocompetent hosts.
- Mortality rate can be as high as 80%, with *Scedosporium apiospermum* being the leading cause of eumycetoma in North America and Western countries (1).
- Due to the rarity of the disease, clinical trials for scedosporiosis are problematic and optimal antifungal therapy remain to be unclear.
- Here we assessed the *in vitro* and *in vivo* activity of MICA, ISA, POSA, and VORI against agents of scedosporiosis.

METHODS

- The *in vitro* susceptibility of MICA, ISA, POSA, or VORI was evaluated using the Clinical Laboratory and Standards Institute (CLSI) M38-A2 method.
- ICR mice were immunosuppressed by cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3, and +8 relative to infection (3).
- Immunosuppressed mice were intratracheally infected with *S. apiospermum* DI16-478, a susceptible strain to all tested antifungal agents.
- For survival studies, treatment with oral ISA (tid), POSA (tid) or VORI (qd), or intraperitoneal MICA (qd) started 16 h post infection and continued through day +7.
- For tissue fungal burden, treatment with either drug started 8 h post infection and continued through day +3. Mice were sacrificed on day +4 and fungal burden in target organs was determined by qPCR (4).
- Statistical analysis was carried out by the Log Rank Sum test for the survival studies and the non-parametric Wilcoxon Rank Sum test for the tissue fungal burden. P values of <0.05 being significant.

SUMMARY/CONCLUSIONS

- Despite the *in vitro* activity of all the tested antifungal against *S. apiospermum*, there was minimal benefit in treating neutropenic mice with any of the antifungal drugs in a monotherapy regimen.
- MICA at 10 mg/kg (qd) demonstrated the only modest activity of any given monotherapy.
- Despite of the modest, or lack of, enhanced survival of MICA- or ISA-treated mice, respectively, both drugs consistently reduced the tissue fungal burden in all target organs when compared to placebo-treated mice.
- Although it is currently unknown why the reduced fungal burden with MICA- or ISA-treated mice did not translate into strong survival benefit versus placebo-treated mice, mycotoxin(s) might be a suspected in killing infected mice.
- VORI or POSA had less of an effect on clearing the infection in infected organs.
- Combination therapy of MICA+ISA did not enhance survival time or reduce fungal burden in target organs when compared to placebo-treated mice.
- Continued investigation into other treatment modalities and the role of toxin in the pathogenesis of scedosporiosis are warranted.

REFERENCES

1. Francis and Walsh. Oncology 1992;6:133-44.

ACKNOWLEDGEMENTS

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RESULTS

Table 1: MIC of the four antifungal agents tested against *Scedosporium* species in µg/mL.

Isolate	MICA	ISA	POSA	VORI
<i>S. apiospermum</i> DI16-476	0.25	4	1	0.5
<i>S. apiospermum</i> DI16-477	0.25	1	0.5	Not determined
<i>S. apiospermum</i> DI16-478	0.5	1	0.5	1
<i>S. boydii</i> DI16-479	0.5	2	0.25	1
<i>S. boydii</i> DI16-480	0.5	8	2	1
<i>S. boydii</i> DI16-481	0.125	16	4	1
<i>S. prolificans</i> DI16-482	4	>16	>16	>16
<i>S. prolificans</i> DI16-483	2	>16	>16	>16
<i>S. prolificans</i> DI16-484	8	>16	>16	>16

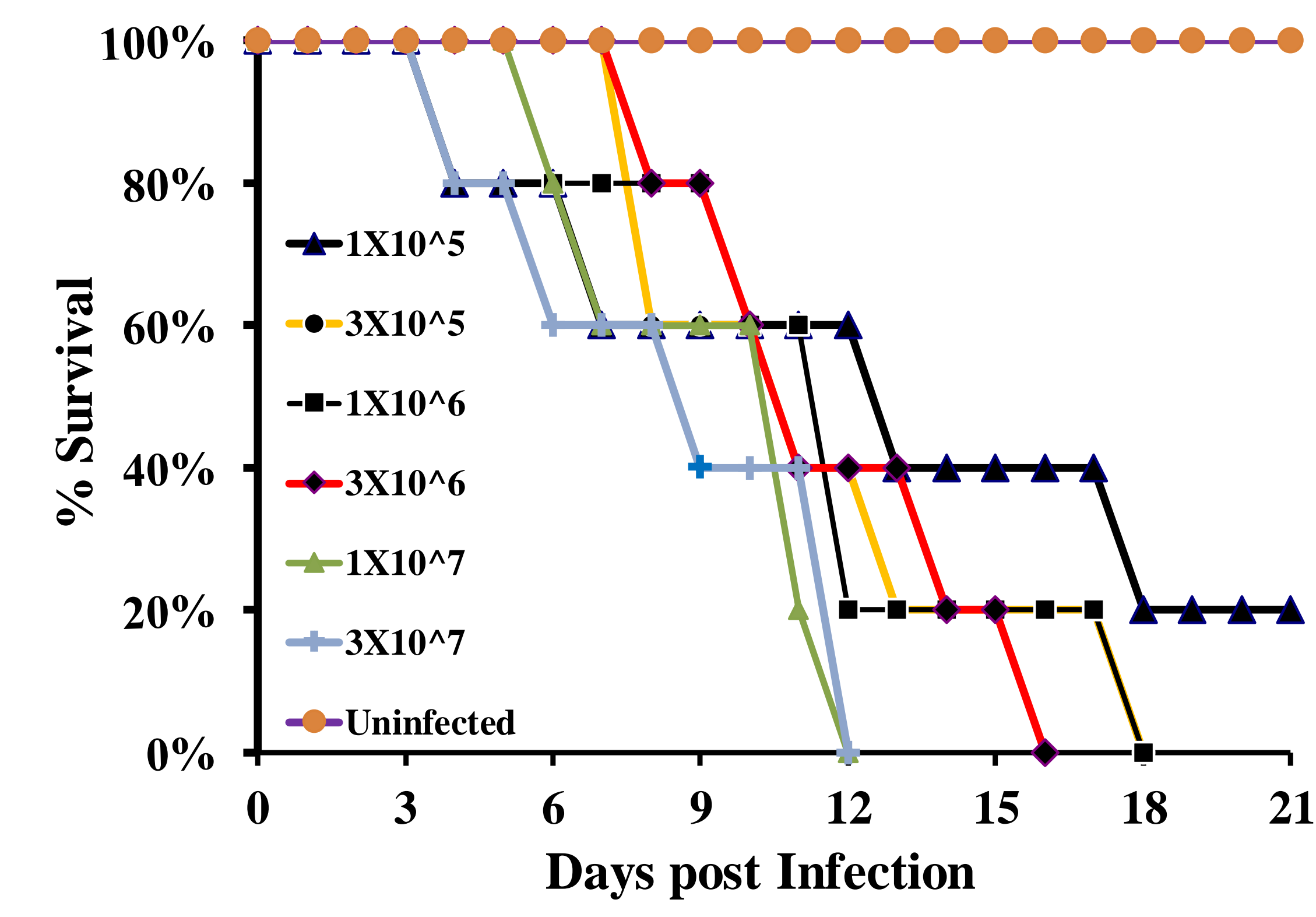


Figure 1. Development of a mouse model of pulmonary scedosporiosis. Five neutropenic mice (cyclophosphamide 200 mg/kg + cortisone acetate 500 mg/kg given on Days -2, +3, and +8) per group were infected by intratracheal instillation with different inocula of *S. apiospermum* DI16-478. Survival of mice served as an endpoint.

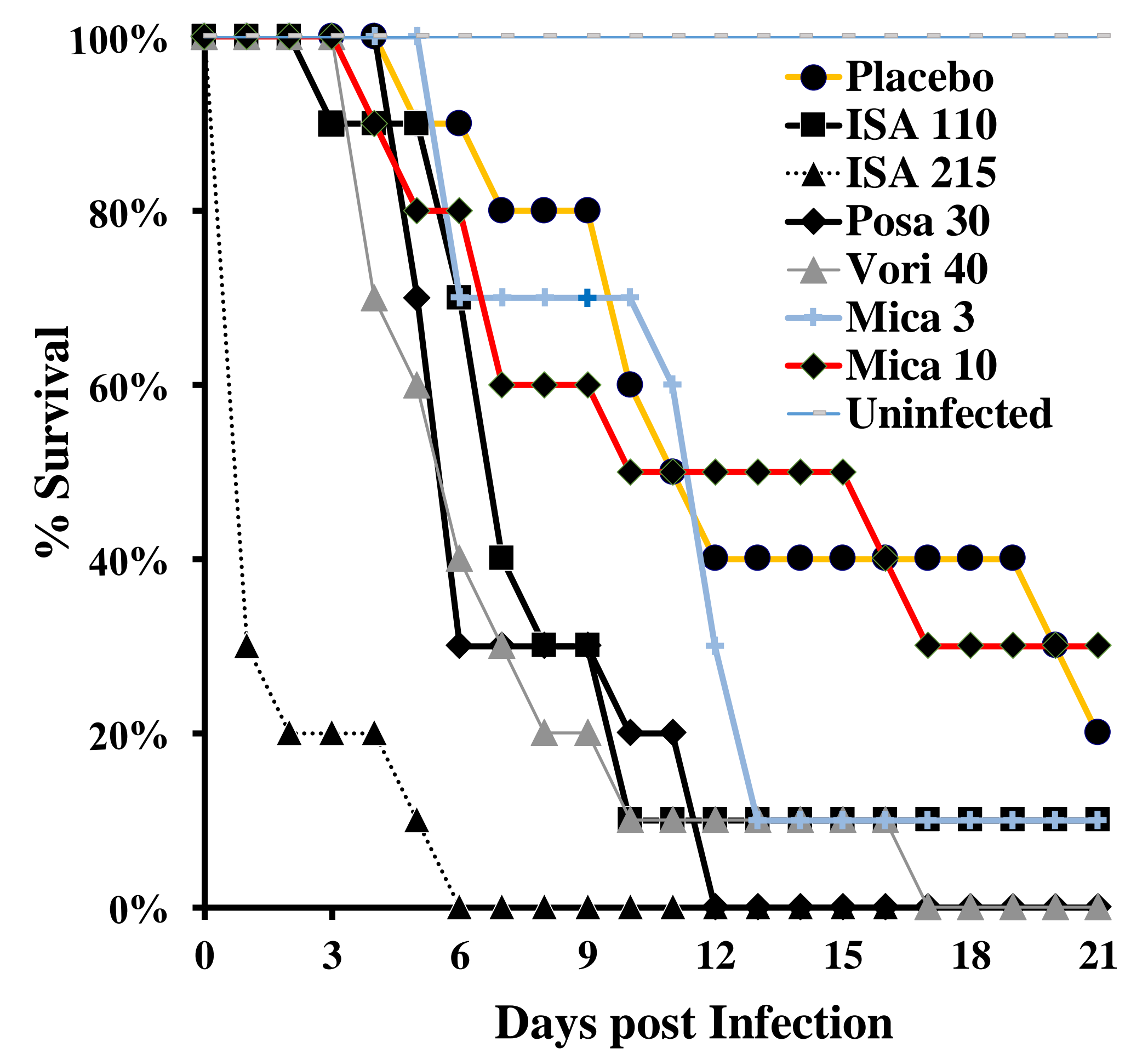


Figure 2. Effect of monotherapy on the survival of mice with scedosporiosis. Neutropenic mice (10/group) were infected by intratracheal instillation with *S. apiospermum* DI16-478 (3.5×10^7 spores). Treatment with each antifungal drug started 16 h post infection and continued for 7 days.

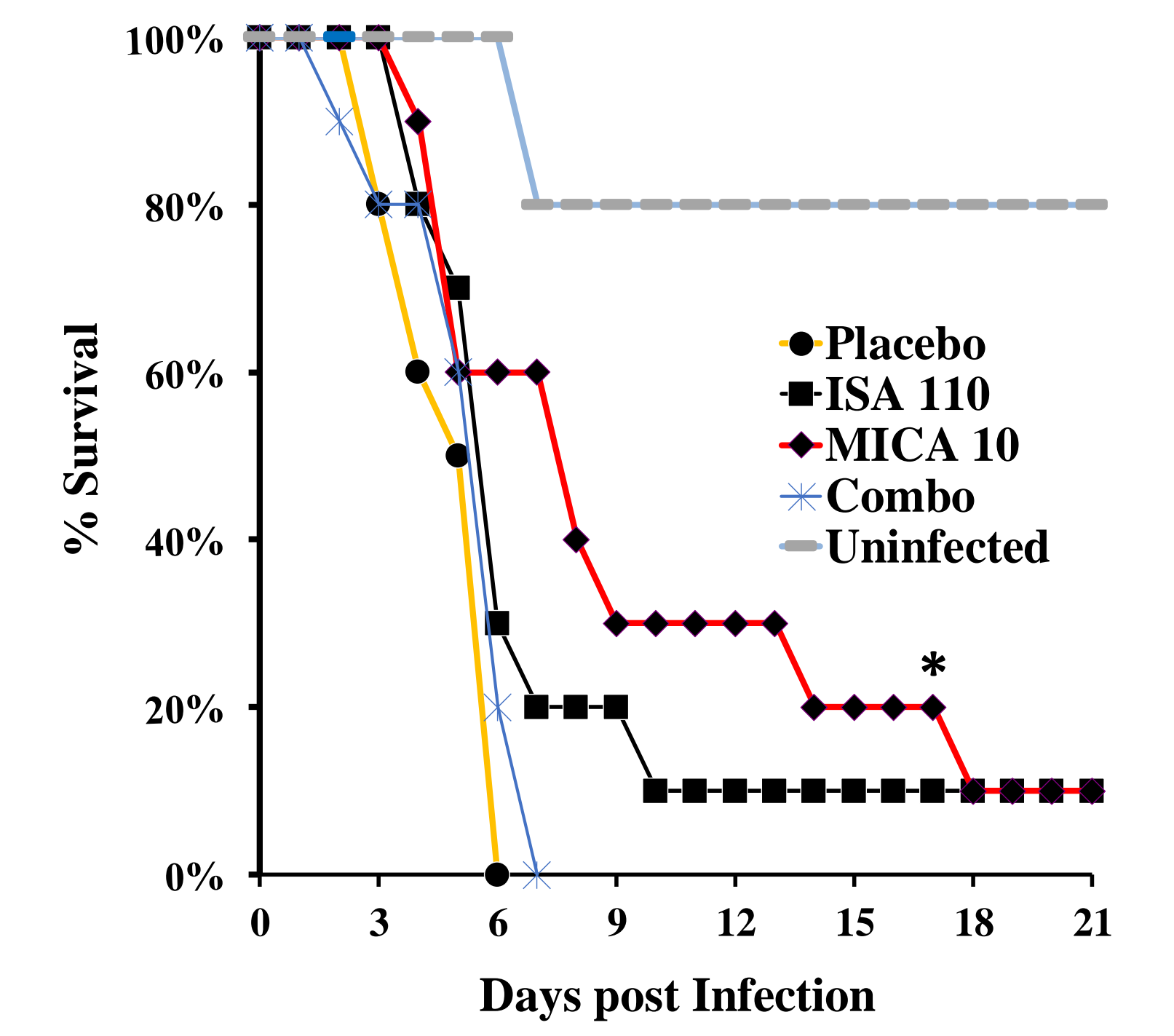


Figure 3. Effect of combination therapy on the survival of mice with scedosporiosis. Neutropenic mice (10/group) were infected by intratracheal instillation with *S. apiospermum* DI16-478 (3.5×10^7 spores). Treatment with each MICA, ISA or both started 16 h post infection and continued for 7 days. *P<0.03 vs. either placebo- or combination treated mice by Log Rank test.

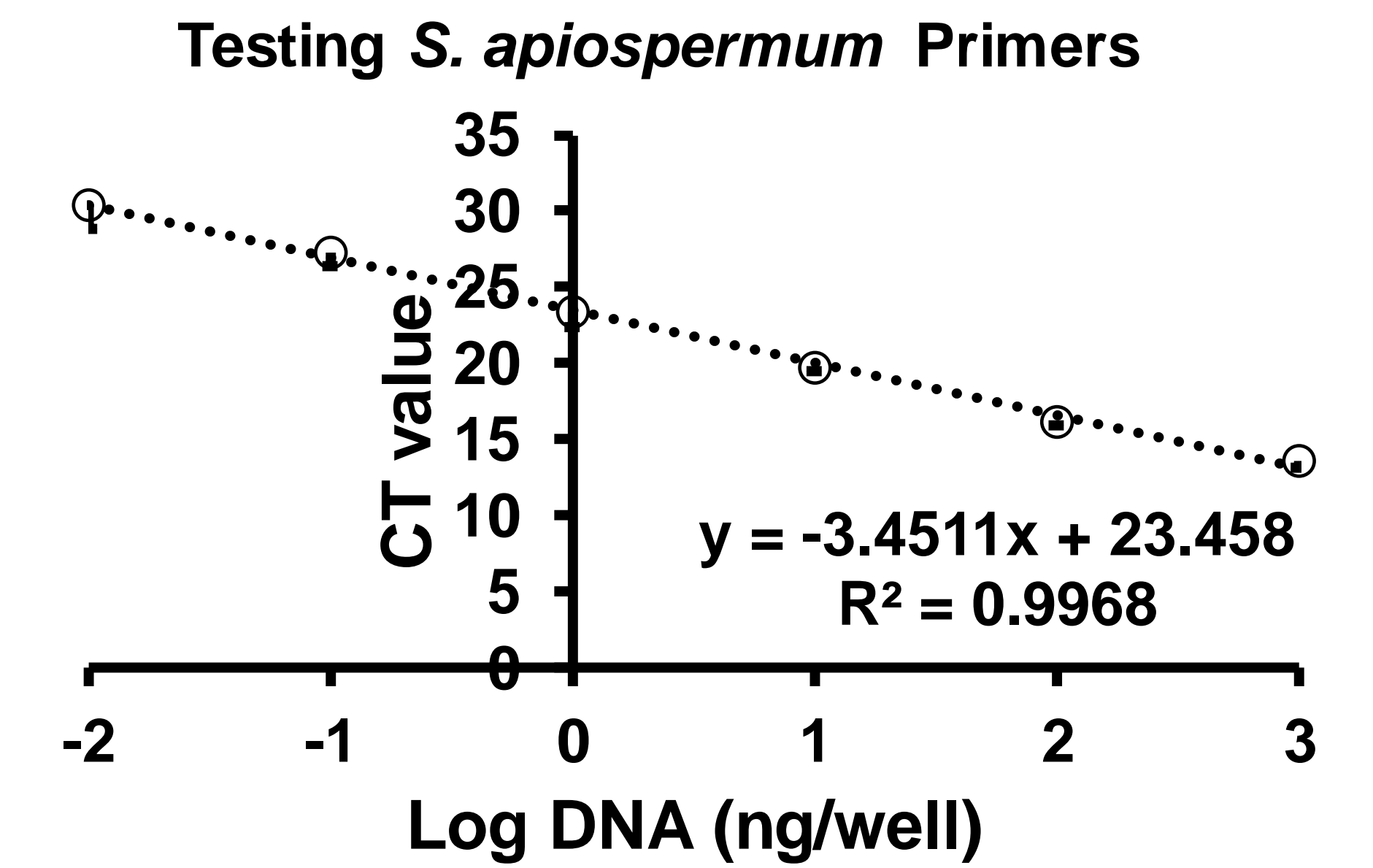


Figure 4. qPCR of *S. apiospermum* DNA in spiked lungs. A linear relationship between fungal actin DNA concentration spiked in lungs and the CT value detected with qPCR indicated the validity of the actin primers used (F primer; CCCTTGACTT-TGAGCAGGAG; R primer CTCAAGACCGAGGACAGAGG). Notice the R² value of 0.9968.

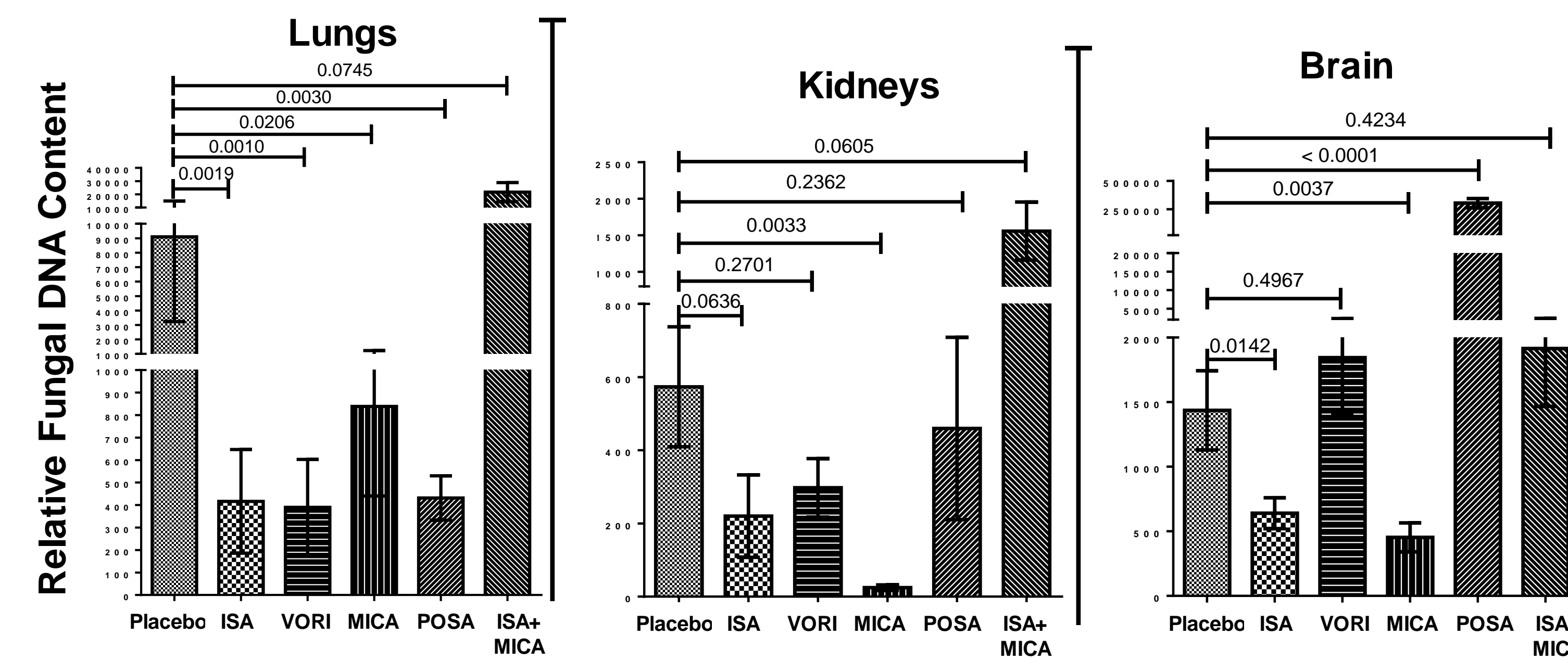


Figure 5. Determination of tissue fungal burden using qPCR. Neutropenic mice (n= 10/group) were infected intratracheally with *S. apiospermum* DI16-478 (1.7×10^7 spores). Treatment with each MICA, ISA or both started 8 h post infection and continued for 3 days. Mice were sacrificed on day 4 post infection. Lungs, brains and kidneys were harvest, homogenized and the DNA extracted to determine the tissue fungal burden by qPCR. Broken y-axis indicate artificial scaling. Statistical analysis was conducted by the Wilcoxon Rank Sum test for non-parametric comparisons with P<0.05 considered significant.