Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol13Issue3abst043P.pdf

Development and validation of a *Rhizopusoryzae* infection model in the mouse

Mucormycosis,is a disease resulting in significant morbidity and mortality with *Rhizopusoryzae* being one of the most frequently isolated causative agents. The development of novel therapies for such diseases is often hampered by a lack of a simple animal model. The purpose of this study was to develop a murine model of mucormycosis that demonstrated a consistent and robust infection, allowing further study of disease progression and the evaluation of novel therapeutic candidates.

R. oryzae strains ATCC MYA-4621 and MYA-3791 were grown over 7 days on potato dextrose agar (PDA) slants at 37°C. Female Balb/c mice (14-22 g, n=8/group) were infected with either $5x10^4$ or $5x10^5$ cfu/mouse by intravenous injection. Animals were observed, and removed from study by euthanasia with CO_2 asphyxiation if there was deterioration in clinical condition. Tissues were analysed for fungal burden using methods from Rementeria*et al* (1). Once the optimum infecting dose was established, the model was validated with Liposomal Amphotericin B (LAmB) dissolved in DI water. This was administered daily by intraperitoneal injection at doses ranging from 0.16 – 40 mg/kg/day. Survival data was analysed using A Kaplan Meier survival plot and Kruskal-Wallis ANOVA followed by a Dunn's multiple comparison test were used for fungal burden analysis, with $P<0.05$ taken to be significant. Data were expressed as mean ± standard deviation.

In the absence of treatment, the median survival time was 1-2 days depending on the strain used for infection. Treatment with LAmB had a significant improvement on survival time at doses of 5mg/kg and above with the median survival time at 20 mg/kg being 7 and 4 days for MYA-4621 and MYA-3791 respectively. There was no additional benefit from increasing the dose to 40 mg/kg. Fungal burden was also significantly reduced in both strains in the kidney and liver when levels in LAmB treated animals were compared to vehicle treated control animals (Table1)

Table 1 : Fungal burden in mice infected with MYA4621 and MYA-3791 and treated with LAmB or vehicle. ** P<0.01, *** P<0.001, ****P<0.0001 versus vehicle control

	MYA-4621				MYA-3791			
	glucosamine/ kidney (μg/g)	Std Dev	glucosamine/ liver (μg/g)	Std Dev	glucosamine/ kidney (μg/g)	Std Dev	glucosamine/ liver (μg/g)	Std Dev
Vehicle	145.9	92	77	40.3	9.1	0	32.3	14.8
1.25	25.5**	44.4	11.7**	23.1	9.1	0	19.8	13
5	9.1****	0	2.7****	0	9.1	0	13.1	4.4
20	9.1****	0	2.7****	0	9.1	0	26.1	34.4
40	9.1****	0	2.7****	0	9.1	0	5.4***	4.4

This systemic infection model can now be used to characterize novel therapies for the treatment of mucormycosis.

Rementeria*et al*(1991). *Journ Med Vet Mycology.* **29**:15-23.