Comparative efficacy of fluconazole and liposomal amphotericin B in diabetic mice infected with an azole resistant or sensitive *Candida albicans*

Shirleen Simargi, Jon Olson, Jill Adler-Moore
California State Polytechnic University, Pomona, USA (Email: sisimargi@cpp.edu)

ABSTRACT

Objective: Diabetes compromises blood circulation and immune responses, as well as major metabolic pathways, which can lead to increased susceptibility to infection. In the present study, we examined this possibility using diabetic versus non-diabetic mice with systemic candidiasis caused by a *Candida albicans* azole sensitive(*C.alb-S*) or azole resistant(*C.alb-R*) strain. Infected mice were treated with fluconazole (Flu) or liposomal amphotericin B (AmBisome®, AmBi). Methods: ICR male mice (3-4 weeks old) were maintained on a high (60%) fat diet for 4 weeks. Diabetes was then chemically induced by one intraperitoneal nicotinamide (60 mg/kg) and streptozotocin (100 mg/kg) treatment; by day 7-14 mean blood glucose levels were ≥200 mg/dL Diabetic mice (n=9-14/gp) and non-diabetic mice (n=14-16/gp) were challenged intravenously (IV) with *C.alb-R* (ATCC 62342) (diabetic: 3.5x10ex6 cells/mouse; non-diabetic: 1.4x10ex6 cells/mouse) or *C.alb-S* (CP620) (diabetic: 1.5x10ex6 cells/mouse; non-diabetic: 6.9 x10ex6 cells/mouse) and 24h later, given 5 mg/kg AmBi or 5% dextrose in water (D5W) intravenously for 6 days or Flu 40 mg/kg PO 2X/day for 6 days. Tissues (kidneys, liver, spleen) were collected (n=3-6/group) on d4 (diabetic) and d7 (non-diabetic) post-challenge, homogenized and plated on Sabouraud's agar to determine fungal burden (CFU/g). Other mice (n=6-10/gp) were monitored for morbidity to d28. Results: With a *C.alb-R* infection, AmBi treated, non-diabetic and diabetic mice, had significantly better survival than Flu or D5W treated mice (AmBi 83-100% survival, Flu 0-30% survival, D5W (0-20% survival) (p≤0.01). Significant decreases in CFU/g kidneys were observed with AmBi treatment versus D5W or Flu in diabetic and non-diabetic mice (p≤ 0.04); no fungi were recovered in livers or spleens of AmBi treated mice (p≤ 0.04). In comparison, with *C.alb-S* infection, AmBi or Flu treated, non-diabetic mice had 100% and 80% survival, respectively, but in diabetic mice, survival was 100% for AmBi and only 16% for Flu even though this was a Flu sensitive strain (p=0.0021). D5W yielded 0% (non-diabetic) and 14% (diabetic) survival. The fungal burden in the livers and spleens was significantly lower with AmBi treatment versus Flu or D5W, with AmBi treatment reducing the liver and spleen fungal burdens to undetectable levels. In the kidneys, the fungal burden in non-diabetic mice was significantly lower for AmBi versus Flu or D5W, but there was no significant difference in kidney fungal burden in diabetic mice given AmBi or Flu. Conclusions: In non-diabetic mice, AmBi and Flu were effective in treating systemic candidiasis caused by a *C. albicans* azole sensitive strain, while AmBi was effective in treating non-diabetic and diabetic mice infected with a *C. albicans* azole sensitive or resistant strain. Notably, in diabetic mice challenged with a *C. albicans* azole sensitive strain, Flu was associated with poor survival and elevated tissue fungal burden, indicating that the diabetic condition enhanced susceptibility to systemic candidiasis whether it was caused by a *C. albicans* azole sensitive or resistant strain.

INTRODUCTION

Diabetes is a chronic metabolic disorder due to improper insulin utilization or insufficient insulin production by the pancreatic β-cells, which eventually leads to hyperglycemia and long-term complications[1]. Obesity is a strong risk factor of type 2 diabetes. The excess accumulation of fat contributes to chronic low-grade inflammation, which together with hyperglycemia compromise the blood circulation, immune responses[2,3], and metabolic pathways[4] leading to increased susceptibility to infection such as candidiasis[5].

Fluconazole (Flu), has been used as first line therapy for localized and systemic candidiasis[6], but the incidence of azole resistance is rising[7]. Unlike azoles, the incidence of resistance to polyene, broad spectrum, antifungal drugs, such as liposomal amphotericin B (AmBisome® [AmBi]) remains minimal. This study was done to compare the efficacy of Flu versus AmBi in diabetic vs. non-diabetic mice with systemic candidiasis when it is caused by a *C. albicans* azole sensitive(*C.alb-S*) or azole resistant(*C.alb-R*) strain.

RESULTS

Fig 1-2. AmB and Flu treatment against azole sensitive *C. albicans*

Fig 1a. Diabetic mice: survival

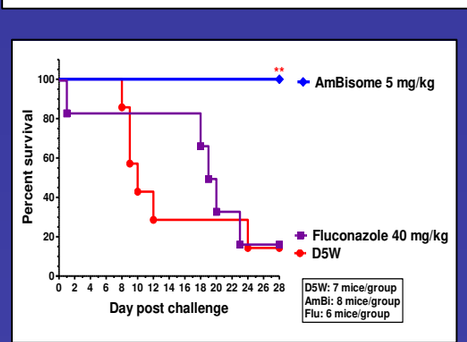

Fig 1a. In diabetic mice, AmBi yielded significantly higher survival (100%) vs. Flu (16%) and D5W (14%) (p=0.0021) even though this was a Flu sensitive strain (p=0.0021).

Fig 1b. Non-diabetic mice: survival

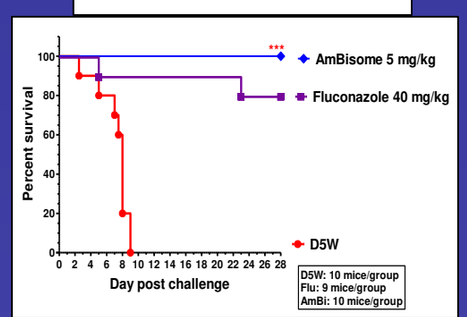

Fig 1b. AmBi or Flu treated non-diabetic mice had significantly higher survival 100% and 80%, respectively vs. D5W (0%) (p≤0.0003).

Fig 2. CFU data-kidneys

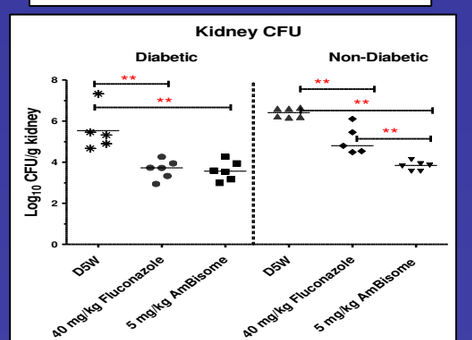

Fig 2. Flu and AmBi yielded significantly lower kidney fungal burden in diabetic and non-diabetic mice compared to D5W (p≤0.0080; p≤0.0080).

Fig 3-4. AmBi and Flu treatment against azole resistant *C. albicans*

Fig 3a. Diabetic mice: survival

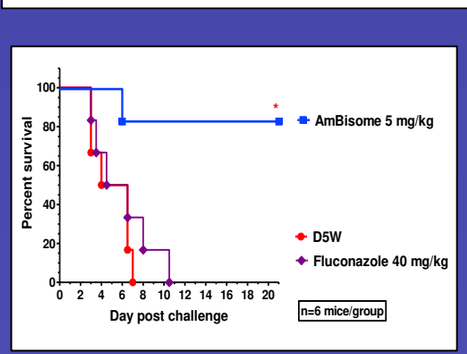

Fig3 a,b. *AmBi treated diabetic and non-diabetic mice had significantly higher survival (83-100%) vs. Flu (0-30%) and D5W (0-20%) (p≤0.01)

Fig 3b. Non-diabetic mice: survival

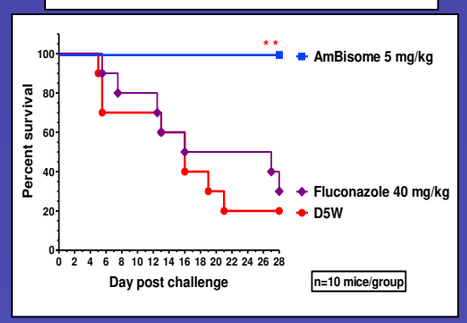

Fig 4. CFU data-kidneys

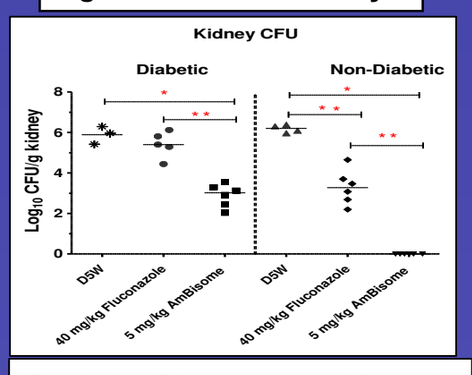

Fig4.*AmBi significantly decreased the kidney fungal burden in diabetic and non-diabetic mice vs Flu and D5W (p ≤0.02)

METHODS

- ICR male mice (3-4 weeks old) were induced by high fat diet (60% kCal/fat) for 3-4 weeks
- Diabetes induction: nicotinamide (60 mg/kg) and 15 minutes later streptozotocin (100 mg/kg) treatment.
- The weights and blood glucose levels were monitored 2x/week until the mean blood glucose levels were ≥200 mg/dL.
- Diabetic mice (n=9-14/gp) and non-diabetic mice (n=14-16/gp) were challenged intravenously (IV) with *C.alb-R* (ATCC 62342, Flu MIC>500ug/ml, AmBi 1.25ug/ml) (diabetic: $3.5x10^6$ cells/mouse; non-diabetic: $1.4x10^6$ cells/mouse) or *C.alb-S* (CP 620, Flu MIC=2ug/ml, AmBi=0.625ug/ml) (diabetic: $1.5x10^6$ cells/mouse; non-diabetic: $6.9x10^6$ cells/mouse).
- Treatments: 5 mg/kg AmBi or 5% dextrose in water (D5W), IV or Flu 40 mg/kg PO 2X/day for 6d.
- Tissues (kidneys, liver, spleen) were collected (n=3-6/group) on d4 (diabetic) and d7 (non-diabetic) post-challenge, to determine fungal burden (CFU/g). Other mice (n=6-10/gp) were monitored for morbidity and survival to d28.

CONCLUSIONS

- AmBisome was effective in treating systemic candidiasis in diabetic and non-diabetic mice caused by *C.alb-S or C.alb-R* strains based on increased survival and reduced fungal burden.
- Fluconazole was not effective for treating systemic candidiasis in either diabetic or non-diabetic mice when it was caused by *C.alb-R.*
- Fluconazole was effective in treating systemic candidiasis caused by *C.alb-S* in non-diabetic, but not diabetic mice.
- Since diabetes reduces the efficacy of Fluconazole but not AmBisome for treatment of systemic candidiasis, it is possible that this observation may be true for other fungal infections.

ACKNOWLEDGMENTS

Support Provided by research grants from:
GILEAD
NSF
CAL POLY POMONA