Efficacy of oral BWC0977 against drug resistant *Escherichia coli* ST131-H30 in normal and diabetic murine model of ascending UTI infection

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INTRODUCTION

Escherichia coli ST131-H30 (or similar clonal groups) is the most widely circulating multiple antibiotic-resistant Gram-negative pathogen that causes urinary tract infections (UTI) among hospital patients, especially those with diabetes. Advances in diagnosis, treatment, and clinical management of antibiotic resistant infections, including complicated UTI, have been seriously hindered by the lack of an understanding of host-specific nature of the organism and absence of novel therapeutic molecules (small molecules, mono clonal antibodies, vaccines etc.). Much of the academic research in these arenas are not translated into product development stages due lack of availability of proper expertise in handling animal models for testing upcoming therapeutic compounds and a lack of support to develop new antimicrobial agents.

To address these needs, a mouse model of urinary tract infection by multidrug resistant ESBL *E. coli* ST131 H30Rx strain MVAST072 (fluoroquinolone resistant, produces type 1 fimbriae, isolated from a chronic cystitis case) was developed (M072; obtained from Dr Evgeni Sokurenko; Univ. of Washington).

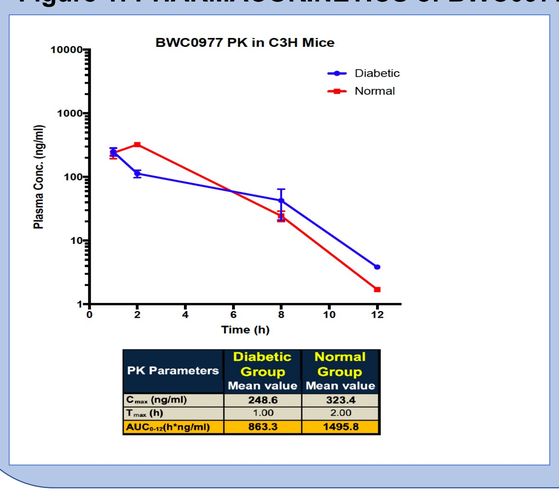
OBJECTIVES

Assess PK, tolerability and efficacy of BWC0977 in the optimized ascending UTI model in normal and streptozotocin (STZ) -induced diabetic mice using *E. coli* ST131 H30R strain M072. Towards this aim, multiple parameters were monitored:

- blood glucose levels to evaluate diabetic induction
- Establish the tolerability of BWC0977 and study its PK
- Determine minimum inhibitory concentration (MIC) of BWC0977 and other standard antibiotics against multidrug resistant ESBL *E. coli* ST131 H30Rx strain MVAST072
- Monitor bacterial burden in urine for 5 days post-infection (PI)
- Evaluate bacterial burden in the bladder and kidney on days 2 and 5 PI
- Monitor clinical observations (weight, general health) during the study
- Quantify the efficacy of the BWC0977 administered through oral gavage (100 or 200 mg/kg; b.i.d. for 3 days)

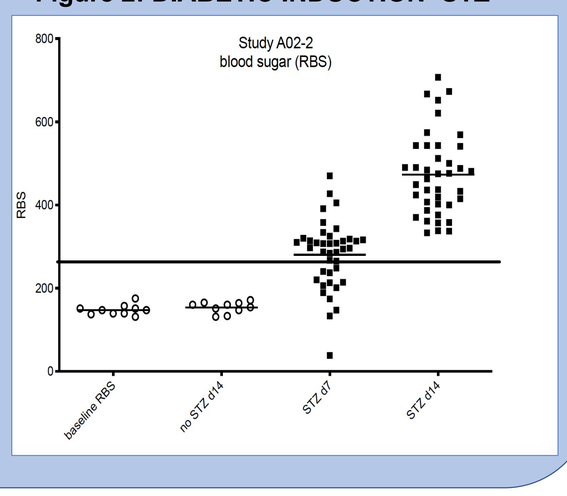
RESULTS

Figure 1: PHARMACOKINETICS of BWC0977



PK Parameters	Diabetic Group Mean value	Normal Group Mean value
C_{max} (ng/ml)	248.6	323.4
T_{max} (h)	1.00	2.00
AUC_{0-12}(h*ng/ml)	863.3	1495.8

Figure 2: DIABETIC INDUCTION -STZ



1. Pharmacokinetics (PK)- BWC0977 (100mg/kg) or vehicle (0.25% CMC, 0.1% Tween 80) administered by oral gavage, then followed up for PK sampling (Figure 1)
2. The second cohort was treated with BWC0977 twice daily for three days to assess tolerability
3. Animals were followed for weight loss and clinical signs (Figure 3)
4. Serum PK results supported use of the selected gavage doses for efficacy testing

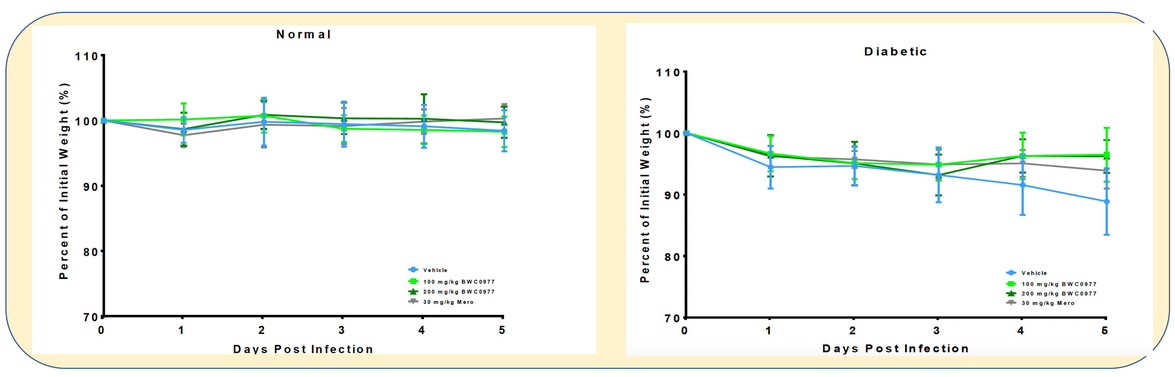
In vivo study design

Grouping-Mice (Table 1)
⬇
- / + STZ treatment
4 daily doses of 65mg/kg after a 4h fast (Figure 2)
⬇
Normal and Diabetic mice
PK, tolerability (100mg/kg)
⬇
Efficacy
⬇
Transurethral Inoculation
1E7 CFU *E. coli* M072
⬇
d2 PI-Treatment
Oral gavage
BWC0977 (100mg/kg, 200mg/kg) or 30mg/kg, SC-meropenem
d1-5 PI urine collected
⬇
Termination
Tissues / urine samples for estimating bacterial burden by qPCR
General health monitored over the course of the study

Table 1: Dosing groups for in vivo efficacy

Group	Treatment	Dosage	Sacrifice Day	Group size
1	Diabetic-Vehicle	N/A	5	10
2	Diabetic-BWC0977	100mg/kg	5	10
3	Diabetic-BWC0977	200mg/kg	5	10
4	Diabetic-Meropenem	30mg/kg	5	10
5	Normal-Vehicle	NA	5	10
6	Normal-BWC0977	100mg/kg	5	10
7	Normal-BWC0977	200mg/kg	5	10
8	Normal-Meropenem	30mg/kg	5	10
	Total	-	-	80

Figure 3: Monitoring Weight loss in M072 infected diabetic and normal C3H/HeN mice



Significant weight loss was observed in drug-treated, diabetic mice but the animals rebounded by day 3 at these doses. BWC0977 was tolerated well with no observable irreversible adverse signs in any treated mice

RESULTS

BWC0977- *In vitro* activity

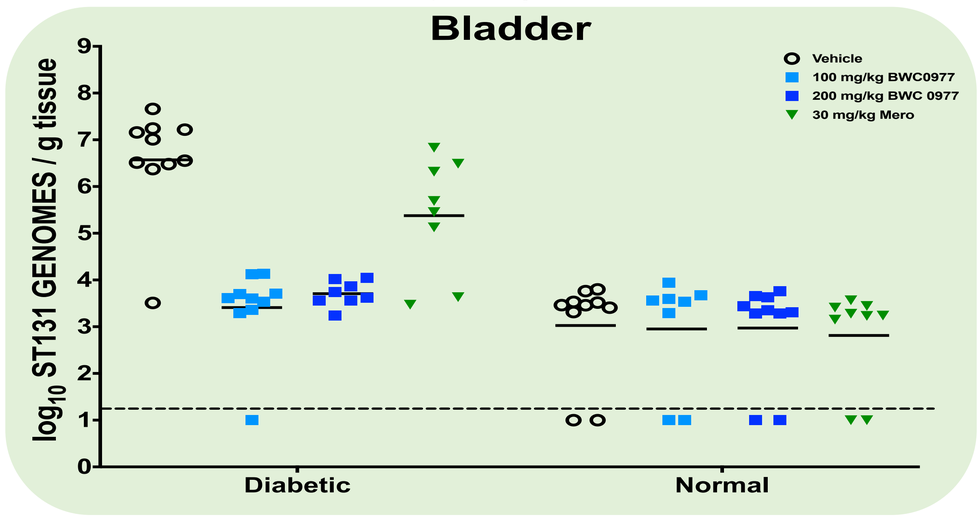
Table 2 – MICs of BWC0977 against *E.coli* strains

	MIC (µg/ml)								
E.coli	Ciprofloxacin	Levofloxacin	Doxycycline	Nitrofurantoin	TMP/SMX	Meropenem	Cefpodoxime	Polymixin B	BWC0977
ATCC 25922	< 0.03	< 0.03	1	16	< 0.06	≤ 0.125	1	0.5	0.125
ST131 M072	>16	16	16	16	>32	< 0.125	>32	0.5	0.125

BWC0977- *In vivo* efficacy - murine model of ascending UTI

Figure 4a and 4b: Comparison of M072 bladder and kidney burdens in C3H/HeN mice treated with BWC0977, meropenem or vehicle

4a



4b

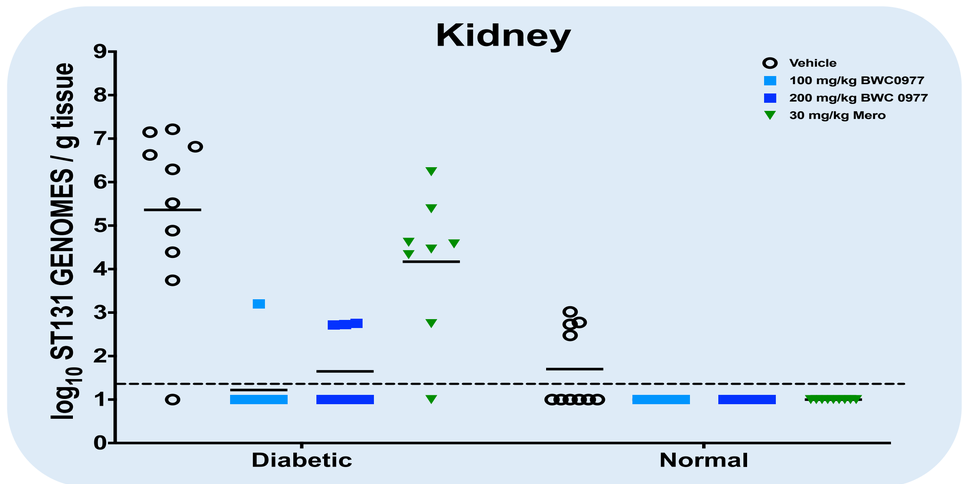
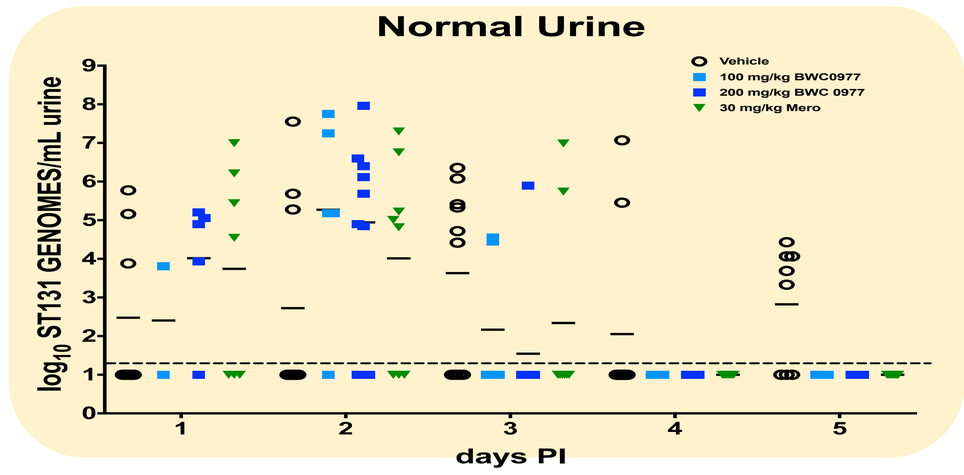
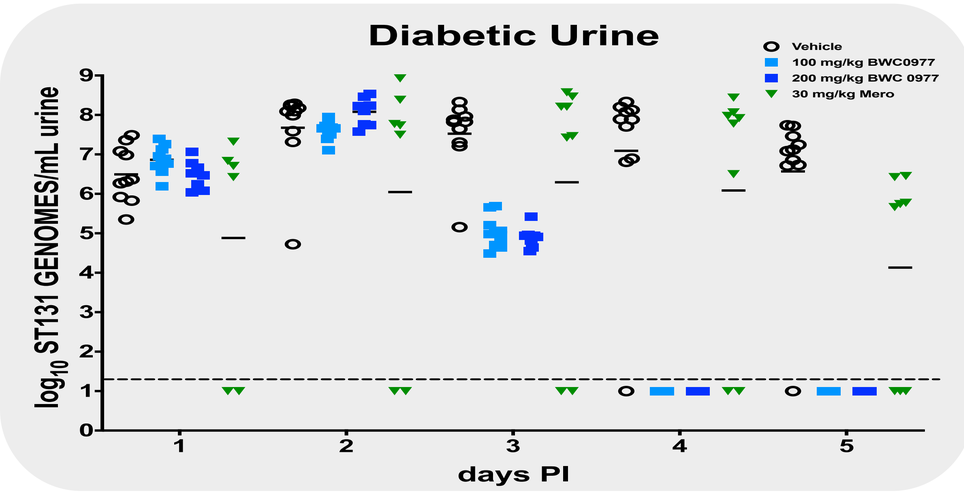


Figure 5a and 5b: M072 bacteriuria in diabetic and normal C3H/HeN mice treated with BWC0977, meropenem or vehicle

5a



5b



CONCLUSIONS

- BWC0977 (100 or 200mg/kg) delivered twice daily for 3 days by gavage starting 2d after TU challenge in both diabetic and normal C3H/HeN mice produced significant impact on bacteriuria and tissue burden and were better than meropenem intervention control.BWC0977 successfully eradicated detectable bacteria (qPCR) in the urine of diabetic animals
- This notable efficacy outcome support additional testing of BWC0977 against other UTI organisms and be further evaluated as a novel intervention for UTI caused by MDR *E. coli.*

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