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Hseih J[1], Ahn J[2], Ou R[3], Zimmern P[1]

1. *UT Southwestern Medical Center,* ***2.*** *Department of Chemistry, University of Texas-Dallas, Richardson, TX 75083,* ***3.*** *Dept. of Urology, Guangzhou First Municipal People's Hospital*

RECOVERY OF R11, A NOVEL BLADDER-SPECIFIC CELL PERMEABLE PEPTIDE, IN THE RABBIT BLADDER AND ANTIBIOTIC EFFICACY OF R11-TAGGED TO CIPROFLOXACIN IN VITRO

Hypothesis / aims of study

Prior studies in a mouse model demonstrated that a new cell permeable peptide, R11, is bladder specific and can be retrieved in the bladder wall after intra-vesical instillation. This unique tissue specificity makes R11 a suitable candidate for intra-vesical antibiotic delivery for recurrent or relapsing bacterial cystitis. The rabbit bladder model is commonly used for pre-clinical studies on bacterial cystitis because the bladder surface glycosaminoglycan layer (GAG) composition is very similar to humans (ref 1). Therefore, we tested the uptake efficiency of R11 administered intra-vesically in the rabbit model recovery and evaluated the efficacy of R11-conjugated to an established fluoroquinolone antibiotic (Ciprofloxacin) in vitro.

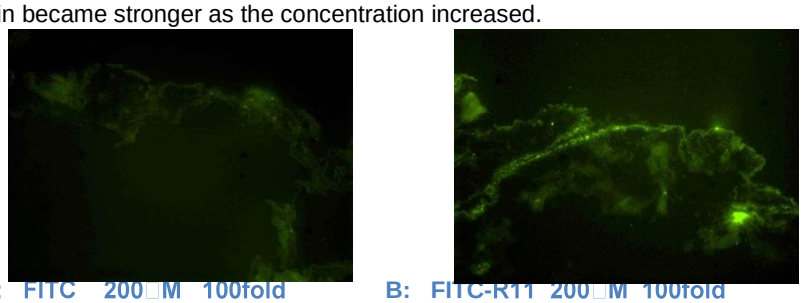
Study design, materials and methods

Following approval by Institution Animal Care and Usage Committee, an in vivo model (New Zealand White rabbits) was employed. Synthetic R11 tagged with fluorescein isothiocyanate (FITC) was instilled in the bladder at a concentration of 200 micromoles. Controls received FITC alone, without R-11 conjugation. Following 30 minutes of instillation, animals were sacrificed 3 hours later and bladders were harvested to measure R11 uptake and for tissue localization using OCT frozen section.

Since E.Coli is responsible for approximately 90% of urinary tract infection, we used E.Coli for the proof of principle to test the activity of conjugated Ciprofloxacin in medium against non-conjugated Ciprofloxacin. Antibiotic activity was carried out using disk diffusion method in triplicate; the diameter of each paper disc was 0.8 cm. Means of disk diffusion were read after overnight incubation at 37°c.

Results

In Fig. 1, the control group (A) that received FITC only exhibited a diffuse pattern, considered as background or autofluorescence. In contrast, the group (B) with FITC-R11 exhibited a strong fluorescence activity in the transitional cell layers; and the fluorescence intensity was dose-dependent based on fluorometry. Clearly, R11 was found in the bladder wall after intra-vesical delivery and this unique property makes it a suitable candidate for intra-vesical drug delivery. As shown in Table 1, antibiotic activity of R11-Ciprofloxacin was weaker than Ciprofloxacin alone at different concentrations. The antibiotic activity of R11-Ciprofloxacin became stronger as the concentration increased.



A: FITC 200□M 100fold B: FITC-R11 200□M 100fold

Figure 1 Uptake of FITC-R11 in bladder tissue after bladder instillation. Twenty-four hours after instillation, tissue localization of R11 was confirmed by microscope. A: R11-FITC; B: FITC.

Table 1: Comparative antibiotic activity of Ciprofloxacin and R11-Ciprofloxacin using E. Coli and escalating antibiotic concentration dosing. All measures were done in triplicate.

	100 □g	10 □g	5 □g
Ciprofloxacin	ND	2.0±0.1 cm	1.8±0.2 cm
R11-Ciprofloxacin	2.0±0.1 cm	1.0±0.2 cm	0.8±0.1 cm

ND: not determined

Interpretation of results

Following initial successful experiments in the mouse model, we confirmed the bladder-specific uptake of R11 after intra-vesical instillation in the rabbit model. Furthermore, we tested the potential of R11 as a drug delivery vehicle by coupling it with a known antibiotic commonly used in the treatment of bacterial cystitis in humans. We observed a decrease in antibiotic activity between the plain form and the tagged form, suggesting that an equivalent dosage of conjugated drug will need to be calculated for in vivo studies.

Concluding message

Considering the high affinity and rapid uptake of R 11 into the superficial layers of the rabbit bladder, we have established that R11 can travel through the GAG layer and therefore represents a promising vector for in vivo drug delivery in the bladder wall.

With the intent of delivering antibiotic in situ to improve the difficult treatment of recurrent bacterial cystitis, we tested the antibiotic efficacy of R11 tagged to Ciprofloxacin and found it weaker than the original compound, suggesting the need for dose adjustment in future in vivo testing.

References

1. Arch. Biochem. Biophys. 375:270-277

Specify source of funding or grant	**The Cain Foundation**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Review Board and Animal Care and Usage Committee at UT Southwestern Medical Center**