A novel rabbit model for the evaluation of biomaterial associated urinary tract infection

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Objectives: *It was the objective of this study to establish an animal model which simulates the conditions of a biomaterial associated bacterial urinary tract infection.*
Methods: *The curled portion of polyurethane double pig-tail ureteric stents, pre-coated with P. aeruginosa, were inserted transurethrally into the bladder in eight rabbits. Eight control animals received sterile stent material. Microbiology studies of the stent, bladder tissue, and urine, as well as bladder histopathology were evaluated.*

Results: *P. aeruginosa was recovered from all stent, bladder, and urine specimens in the P. aeruginosa pre-coated stent group, and no P. aeruginosa was present in any of the control specimens ($p=0.0002$). The controls only developed minimal bladder inflammation, whereas the bladders of the P. aeruginosa pre-coated stent group were significantly more inflamed ($p \leq 0.01$).*
Conclusions: *This rabbit model was easy to manipulate, low in maintenance requirements, and had pathophysiologically distinct end points, suitable for the assessment of biomaterial associated urinary tract infections.*

Key Words: urinary tract infection, biomaterial, animal model

Introduction

Approximately 40% of all hospital acquired infections are related to the urinary tract,[1] and urinary tract infections (UTI) are among the most common factors leading to life threatening gram-negative sepsis.[2] Even with the use of closed sterile urinary drainage systems, the risk of urinary catheter associated UTI's remains at a relatively high rate of 5% per day of catheterization.[3] Research leading to the development of effective therapy for biomaterial associated UTI is thus of obvious importance. In order to facilitate investigation, it was the objective of this project to create a physiologically relevant and technically simple animal model for the evaluation of biomaterial associated UTI's.

In designing this animal model, several attributes were desired. The animal model should ideally be easy to manipulate, low in maintenance requirements, and have pathophysiologically distinct end points.

Material and methods

Experimental design

New Zealand male white rabbits between 3.5 kg and 4.0 kg were used. The curled portions of polyurethane double pig-tail ureteric stents (Cook Urological®, 6F 26 cm Spiral Tip Stent, trimmed to 7 cm in length) were found to be suitable for atraumatic transurethral insertion into the rabbit bladders, with excellent retention against the expelling forces of micturition. Stent portions were incubated for 48 hours in a nutrient broth culture containing approximately 10^{11} CFU/ml of *P. aeruginosa*. This inoculation process yielded

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between 10^5 and 10^6 CFU/cm^2 of *P. aeruginosa* on the stent material surface Figure 1. Stent portions pre-coated with *P. aeruginosa* were inserted into the bladders of eight rabbits which served as the experimental group, and sterile stent portions were inserted into an additional eight animals serving as controls.

Stent material insertion

The animals were sedated with a subcutaneous injection of a ketamine/xylazine/atropine mixture. General anesthesia was then induced and maintained using halothane/nitrous oxide/oxygen administered by mask. Using aseptic technique, a 7 French multipurpose catheter (Cordis Corporation, Miami, FL, USA) was introduced into the bladder transurethrally. During the insertion of the multipurpose catheter, the curved tip of the catheter was directed ventrally in order to avoid unwanted entry into the prostatic utricle, which is located a short distance distal to the bladder neck with the opening positioned dorsally. Once the tip of the multipurpose catheter was confirmed to be correctly positioned in the bladder lumen by observing an efflux of urine, it was then exchanged over a Teflon coated guide wire (Cook Urological®, 0.038 In. diameter, 145 cm, flexible curved tip). The stent portion and the pusher (referred to as "positioner" on the kit) were then sequentially placed over the guide wire, and the stent portion was positioned into the bladder lumen in its entirety using standard stent insertion technique. This procedure took an average of 5 to 10 minutes for each animal.

Retrieval and processing of specimens

The animals were sacrificed 10 days after the stent insertion by an intravenous barbiturate overdose. The bladders were exposed using a midline laparotomy incision.

For microbiology testing, the following specimens were obtained: 1) a bladder urine aspirate, 2) an approximately 4 mm^3 piece of bladder tissue including approximately 2 x 2 mm^2 of mucosal surface, and 3) the stent material. The stent material and bladder tissue were first agitated and sonicated in 5 ml of phosphate buffered saline (50 mM, pH 7.2) using a method recently tested by our laboratory and confirmed to retrieve >99.9% of viable bacteria. All specimens were then serially diluted in phosphate buffered saline, and plated onto trypticase soy agar. The plates were incubated for 48 hours at 37°C.

For histopathologic examination, whole bladders were removed and fixed in 10% buffered formalin. The specimens were paraffin embedded, sectioned at 5 μ thickness, and stained with hematoxylin and eosin (H&E). In an attempt to obtain sections representative of the overall changes in the bladders, circumferential coronal sections were taken from at least five evenly spaced levels in each bladder specimen. The histologic sections were evaluated by a single pathologist in a blinded fashion. The inflammatory response was graded from 0 to 3 for each of three cell types: neutrophilic infiltrate (also known as polymorphonuclear leukocyte, or PMN), lymphocytic infiltrate, and eosinophilic infiltrate. Inflammation of the bladder mucosa and detrusor were evaluated separately. The severity of the inflammatory response was graded using the grading scale as shown in Table 1 and Figure 2.

In the evaluation of the neutrophilic infiltrate, it was taken into account that the rabbit neutrophils stain red with eosin, in contradistinction to human neutrophils.[4,5] In other words, not all red inflammatory cells were eosinophils in the rabbit. Thus the distinction between rabbit neutrophils and

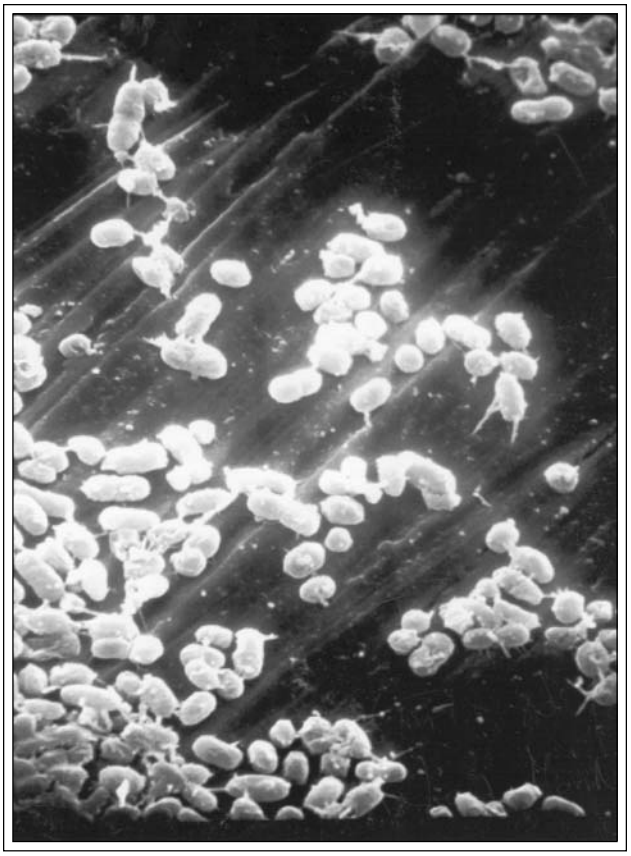

Figure 1. Scanning electron microscopy (Magnification = 5000x) demonstrating P. aeruginosa bacterial biofilm on a biomaterial surface (polyurethane).

TABLE 1. **Histologic criteria for grading of bladder inflammation**

Grade	Histologic criterion
0	No inflammation
1	Occasional patchy inflammatory infiltrates, no more than 3 foci around the luminal circumference
2	Subconfluent inflammation around luminal circumference, more than 3 foci but not involving the entire circumference
3	All of the luminal circumference involved with inflammatory changes

eosinophils were made by taking several criteria into account, including nuclear morphology, size of the cytoplasmic granules, and differences in the coloration of the granules.[4,5]

Statistical analyses

For the parametric microbiology results, the non-paired Student's t-test would normally be applicable. However, one of the data sets (the control group) has

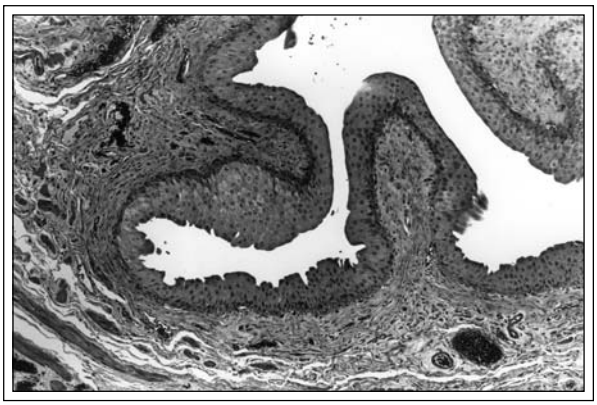

Figure 2a. No inflammation (grade 0) is present.

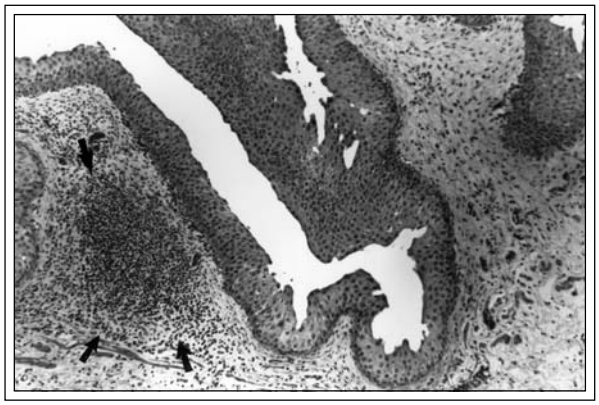

Figure 2b. A single focus of inflammatory infiltrate (grade 1) is present. The arrows point to the location of the inflammatory focus.

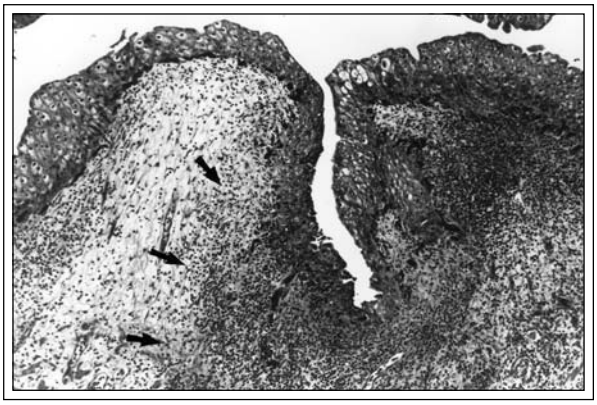

Figure 2c. Subconfluent inflammation (grade 2) involves the entire area to the right of the arrows, representing approximately 2/3 of the bladder mucosa depicted.

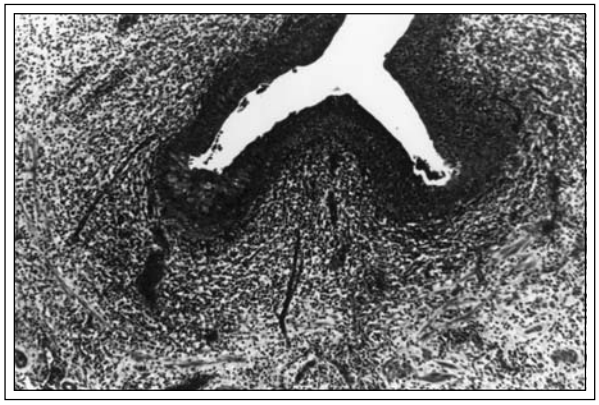

Figure 2d. Confluent inflammation (grade 3) involves the entire luminal circumference.

Figure 2 a,b,c,d. Coronal histologic sections of rabbit bladders stained with hematoxylin and eosin (H&E) are depicted, illustrating inflammatory changes at the bladder mucosa. The magnification was identical (10x) for all four photomicrographs; the differences in the mucosal contour were attributable to varying degrees of tissue edema.

TABLE 2. **Summary of the microbiology and histopathology data**

Rabbit	Biomaterial inserted	*P. aeruginosa*			Mucosal inflammation			Detrusor inflammation		
		Biomaterial (CFU/stent)	Bladder (CFU.5mm3)	Urine (CFU/ml)	PMN	Lymph.	Eosin.	PMN	Lymph.	Eosin.
1	*P. aeruginosa*	4.17E+06	1.17E+03	8.80E+06	3	3	0	1	0	0
2	*P. aeruginosa*	4.34E+07	6.65E+02	1.67E+05	3	2	0	2	1	0
3	*P. aeruginosa*	3.40E+08	3.00E+03	1.17E+05	3	2	0	1	0	0
4	*P. aeruginosa*	3.30E+06	1.67E+02	1.00E+05	3	1	0	1	0	0
5	*P. aeruginosa*	1.38E+08	1.92E+05	7.17E+09	2	1	0	1	1	0
6	*P. aeruginosa*	8.60E+08	2.09E+03	1.17E+02	2	1	0	1	0	0
7	*P. aeruginosa*	2.33E+08	1.17E+04	1.67E+06	2	0	0	1	0	0
8	*P. aeruginosa*	7.70E+07	2.34E+04	1.06E+06	1	1	0	2	1	0
9	Sterile	0.00E+00	0.00E+00	0.00E+00	1	1	0	0	0	0
10	Sterile	0.00E+00	0.00E+00	0.00E+00	1	0	0	0	0	0
11	Sterile	0.00E+00	0.00E+00	0.00E+00	0	0	0	0	0	0
12	Sterile	0.00E+00	0.00E+00	0.00E+00	0	0	0	0	0	0
13	Sterile	0.00E+00	0.00E+00	0.00E+00	0	0	0	0	0	0
14	Sterile	0.00E+00	0.00E+00	0.00E+00	0	0	0	0	0	0
15	Sterile	0.00E+00	0.00E+00	0.00E+00	0	0	0	0	0	0
16	Sterile	0.00E+00	0.00E+00	0.00E+00	0	0	0	0	0	0

The microbiology and histopathology data of the experimental (P.aeruginosa coated biomaterial inserted, n=8) and the control (sterile biomaterial inserted, n=8) animals are summarized. (CFU=Colony Forming Units, PMN=Neutrophilic (Polymorphonuclear) infiltrate, Lymph.=Lymphocytic infiltrate, Eosin.=Eosiinophilic infiltrate)

a variance of 0, rendering the Student's t-test not applicable. In consultation with a statistician, the Mann-Whitney rank sum test was used instead for the analysis of these data. Non-parametric grading data were analyzed using the Fisher's exact test. Statistical significance was defined as $p<0.05$.

Results

Microbiology

P. aeruginosa was recovered from the stent material, bladder tissue, and urine specimens from all eight animals which received stent material pre-coated with *P. aeruginosa* Table 2. In contrast, no *P. aeruginosa* was found on any of the specimens from the eight control animals Table 2. The difference was found to be statistically significant by the Mann-Whitney rank sum test ($p=0.0002$). There was no evidence of other organism contaminating the specimens.

Histopathology

A significantly higher proportion of the experimental animals (with stents pre-coated with *P. aeruginosa*) exhibited detectable inflammatory changes in the bladder (Grades 1, 2, or 3) compared to the control animals Table 2. This difference in inflammation between the two groups was present in both the bladder mucosa and the detrusor, and was statistically significant for the grades established for neutrophils ($p=0.007$ for mucosa, $p=0.0002$ for detrusor) and lymphocytic infiltrates ($p=0.010$ for mucosa, $p=0.007$ for detrusor). Neutrophilic infiltrates were generally more pronounced than lymphocytic infiltrates, and no eosinophilic infiltrates were noted in any of the specimens; this pattern was consistent with an acute inflammatory reaction. The mucosal inflammation was generally more pronounced than the inflammatory response seen in the detrusor. No other significant histopathologic anomalies were noted.

Discussion

In the treatment of urinary tract infections associated with biomaterials, interactions among the host, the bacterial biofilm, the biomaterial surface and the antimicrobial agent form a complex, dynamic system.[6-8] Many animal models have been used for studying UTI's,[9-12] but few of them have focused on the investigation of biomaterial associated UTI's. A rabbit model previously used by our laboratory simulated

catheter-associated UTI with the use of indwelling urethral catheters.[13] This model generated meaningful results, but was found to be highly labor intensive. To maintain the indwelling urethral catheter, continual physical restraint of the animal was required. The high phosphate content in the rabbit urine tended to occlude the catheters, and continuous intravenous hydration was necessary in order to keep the urine sufficiently dilute. Furthermore, the indwelling urethral catheter provided potential access for ascending organisms, and infections by organisms other than the one tested may confound the experimental results. Thus we explored the possibility of creating an alternative model for the evaluation of biomaterial associated UTI's.

In designing this animal model, it was our goal that the model should have the following attributes: 1) the animal species should be relatively easy to care for and to manipulate, and the administration of drugs by all commonly used routes should be possible, 2) the insertion of the biomaterial should be minimally invasive, and the biomaterial should be retained internally, so that the burden of animal care would be reduced, and the risk of unwanted ascending infections could be minimized, 3) the biomaterial and the insertion procedure should induce little or no inflammatory changes, whereas consistent tissue infection and inflammation should be induced by the addition of the pathogenic organism associated with the biomaterial.

Our choice of the rabbit was prompted by the balance between its relatively small size and its suitability for manipulations. The small animal size simplifies the housing and daily care requirements, and minimizes the associated costs. In spite of its small overall size, however, transurethral manipulations can be accomplished with ease using materials designed for humans. With the use of suitable atraumatic restraints, drug administration by the oral, subcutaneous, intramuscular, intraperitoneal, and intravenous routes can all be accomplished without the use of sedation or anesthesia. Where necessary, induction and maintenance of general anesthesia could also be achieved safely with only basic anesthetic equipment. Furthermore, both venous and arterial accesses can be achieved reliably via the ear vessels in experienced hands. Thus the rabbit appears to possess the desired attributes in regards to animal care, manipulation, and drug administration for the purposes of this animal model.

In the search for a suitable combination of biomaterial and insertion method, several options were considered and tested. Suprapubic insertions, either by open surgery or by percutaneous methods, were found to be excessively traumatic to the bladder tissue. Therefore we focused our efforts on transurethral insertion methods. Latex sheets (2.5 cm x 2.5 cm) rolled up on a urethral catheter could be successfully inserted into the female rabbit bladder transurethrally, but a large proportion of them were expelled by the animals upon voiding. Straight portions of stents or catheters were tried, but expulsion of the biomaterial by the animals was once again a problem. It was eventually identified that transurethral insertion of the curled portion of double pig-tail ureteric stents in male rabbits enabled us to achieve the objectives of atraumatic insertion, internalization of biomaterial, and reliable retention of the biomaterial.

If an animal model were to be useful for investigating biomaterial associated UTI's, the combination of the biomaterial and the pathogenic organism must be able to induce tissue infection, as evidenced by significant tissue inflammation. On the other hand, the insertion procedure by itself should induce little or no tissue inflammation. These important characteristics were confirmed in this rabbit model. Insertion of the biomaterial pre-coated with *P. aeruginosa* into the rabbit bladder resulted in the recovery of viable *P. aeruginosa* from the biomaterial, bladder urine, as well as the bladder tissue 10 days later. In seven out of eight of these animals, the inflammation was also found to be moderate or severe in nature (grade 2 or 3). Of the eight control animals, only two were found to have inflammatory changes, and the inflammation was mild in both bladders (grade 1). The mild inflammatory response noted in the control bladders suggests that the insertion procedure and the indwelling biomaterial only caused a limited degree of mechanical irritation. In contrast, animals which received biomaterial pre-coated with *P. aeruginosa* developed an inflammatory response significantly greater than what was induced by the biomaterial alone. Thus we concluded that the combination of the biomaterial and pathogenic organism was able to cause local tissue infection, and tissue inflammation could be used as an indicator of the severity of the tissue infection in this rabbit model.

In the microbiology specimens analyzed in these experiments, there was no evidence of any other contaminating bacterial organisms aside from the intention inoculation with *P. aeruginosa*. The strict observation to aseptic technique in the animal procedures without the use of prophylactic antibiotics, and limiting the handling of the *P. aeruginosa* inoculated stent material to the animals receiving the inoculated material, appeared to be effective in avoiding contamination by other unwanted organisms. If observed, however, other unwanted bacterial organisms can significantly alter the interaction among the host,

biomaterial, pathogenic organism and antimicrobial agents being investigated. The results should accordingly be interpreted with caution.

There were several potential pitfalls in this model which deserve further discussion. We found that the female rabbit urethra could be reliably catheterized simply by directing the tip of the catheter towards the ventral aspect of the vagina, as long as the rabbit was awake. If the female rabbit was under anesthesia, however, it was our experience that catheterization of the urethra was exceedingly difficult, presumably due to anatomical alterations caused by the relaxation of the pelvic floor. Thus we recommend that the rabbit UTI model as described should only be applied to the male rabbit. If the reader wishes to use female rabbits, consideration should be given to modifying the procedure to avoid having to catheterize the animals under anesthesia.

Compared to female rabbits, straight forward identification of the male urethral meatus was an advantage, but the capacious prostatic utricle of the male rabbit could cause difficulties. We initially used straight catheters for gaining access into the bladder transurethrally. The stent material positioned in this fashion was recovered either from the prostatic utricle, or the insertion procedure perforated the prostatic utricle and the stent material was retrieved from the peritoneal cavity. These problems were overcome by using the multipurpose catheter, which has a curved tip. By directing the curved tip ventrally, unwanted entry into the prostatic utricle could be avoided.

In the evaluation of the inflammatory infiltrate, the microscopist should be aware that the neutrophils in the rabbit stain a brilliant red with eosin.[4,5] This unusual feature can easily mislead the unfamiliar observer, resulting in the misinterpretation of the rabbit neutrophils as eosinophils. With attention to the distinct differences in the nuclear morphology, size of the cytoplasmic granules, and the granule coloration, however, definite distinction between rabbit neutrophils and eosinophils can be consistently achieved.[4,5,14] Furthermore, it has been firmly established that the function of the red staining rabbit neutrophils closely correspond with the human neutrophils.[5] Thus the significance of the neutrophil changes in this rabbit model can be interpreted according to the guidelines in the human literature.

Lastly, while the internalized biomaterial renders this animal model much less labor intensive as compared to models with indwelling urethral catheters,[13] it lacks continuity to the external environment, and may not fully simulate the pathophysiology of biomaterial infections associated with an externalized portion such as urethral catheters or suprapubic catheters. Because of these differences, this model is likely best used for comparing the efficacy of various treatments for UTI's associated with internalized biomaterials, or for comparing different biomaterials in their propensity for leading to biomaterial associated UTI's given standardized bacterial inoculums.

Conclusions

This novel rabbit model for the evaluation of biomaterial associated urinary tract infection fulfilled our designing goals of being easy to manipulate, low in maintenance requirements, and having pathophysiologically distinct end points. We are hopeful that this animal model will provide a reliable method for comparing the efficacy of antimicrobial agents or coatings, in the treatment or prevention of urinary tract infections. It would in turn help in advancing our understanding of biomaterial associated urinary tract infections, and in the development of effective therapy. □

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