Intravesical Liposomal Tacrolimus Protects against Radiation Cystitis Induced by 3-Beam Targeted Bladder Radiation

Bharathi Raja Rajaganapathy,* Joseph J. Janicki,*,† Peter Levanovich, Pradeep Tyagi, Jason Hafron,‡ Michael B. Chancellor,†,§ Sarah Krueger and Brian Marples||

From the Departments of Urology and Radiation Oncology, Oakland University William Beaumont School of Medicine, Royal Oak, Michigan, and Lipella Pharmaceuticals and Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania

Abbreviations and Acronyms

3D = 3-dimensional

CT = computerized tomography

IMI = intermicturition interval

lipo-tacrolimus = liposomal formulation of tacrolimus

RC = radiation cystitis

RT = radiotherapy

SARRP = small animal radiation research platform

Accepted for publication March 24, 2015.

Study received approval from the Beaumont Health System Research Institute institutional animal care committee.

Supported by the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health Award R44DK102247 and an American Urological Association Research Scholars Fellowship (BRR).

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

* Equal study contribution.

† Financial interest and/or other relationship with Lipella Pharmaceuticals.

‡ Financial interest and/or other relationship with Myriad Genetics, Genomic Health and Bayer.

§ Correspondence: Department of Urology, Oakland University William Beaumont School of Medicine, 3811 West 13 Mile Rd., Royal Oak, Michigan (telephone: 248-551-8296; FAX: 248-551-0486; e-mail: chancellormb@gmail.com).

|| Financial interest and/or other relationship with *International Journal of Radiation Oncology Biology Physics*.

Purpose: We primarily determined whether the small animal radiation research platform could create a rat radiation cystitis model via targeted bladder irradiation (phase I). The response to treating early phase radiation cystitis in rats with transurethral catheter instillation of liposomal tacrolimus was also examined (phase II).

Materials and Methods: In phase I 16 adult female Sprague Dawley® rats were used. Metabolic urination patterns were analyzed before and after exposure to 20, 30 or 40 Gy radiation. In phase II irradiated rats were randomly assigned to receive a single instillation of saline or liposomal tacrolimus.

Results: The 40 Gy radiation dose induced statistically significant reductions in the intermicturition interval compared to the lower radiation doses. By approximately 20 minutes 40 Gy radiation caused a significant decrease in the mean intermicturition interval ($p < 0.0001$). Histological analysis revealed degenerative epithelial changes and urothelial swelling with evidence of pseudocarcinomatous epithelial hyperplasia. Therefore, 40 Gy were chosen for the phase II efficacy study. There was no measurable change in total voided urine volume after irradiation, or after liposomal tacrolimus or saline instillation. Liposomal tacrolimus significantly increased the post-irradiation intermicturition interval by approximately 30 minutes back to baseline ($p < 0.001$).

Conclusions: The radiation cystitis rat model showed a dose dependent decrease in the intermicturition interval without inducing short-term skin or gastrointestinal damage. This study demonstrates that liposomal tacrolimus may be a promising new intravesical therapy for the rare, serious condition of radiation cystitis.

Key Words: urinary bladder; cystitis; abnormalities, radiation-induced; tacrolimus; models, animal

RADIATION is often used as primary or adjuvant treatment to manage pelvic malignancies. The principal aim of RT is to deliver the highest possible dose of radiation to a targeted region while simultaneously sparing normal tissue. Nevertheless, in a case of pelvic irradiation a degree of healthy bladder tissue involvement is inevitable.[1]

RC, which can develop after the completion of pelvic radiotherapy, presents a range of clinical symptoms

0022-5347/15/1942-0578/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2015.03.108
Vol. 194, 578-584, August 2015
Printed in U.S.A.

for which there is no recommended standard treatment.[2] Pelvic irradiation may result in damage to multiple bladder cell types, including urothelial, neuronal, detrusor and vascular smooth muscle cells. RC can reduce bladder capacity and compliance. The extent of the injury can vary depending on several factors, of which all can severely degrade the quality of life of cancer survivors and require long-term followup and treatment.[3] These injuries can be challenging to the urologist, and are a source of substantial morbidity and sometimes mortality for patients.

The discovery and assessment of innovative therapies and treatment related toxicity in cancer survivorship research have relied heavily on small animals. The current irradiation technique typically used on animals involves simple single beam/single fraction delivery. This greatly differs from that of human treatment, which incorporates advanced 3D imaging, planning and computer controlled delivery. Furthermore, there is a lack of conformity between the uniform doses of radiation used on research animals and the nonuniform doses administered as standard clinical conformal RT.[4]

A proposed tool to overcome this disparity is an image guided small animal irradiator such as SARRP. In the research setting SARRP provides a sophisticated method of delivering radiation and it reconciles many concerns associated with standard preclinical radiation devices.[5] Current management methods vary by the degree of radiation cystitis. Acute RC is managed by anticholinergics and other agents for symptomatic relief. Chronic RC is treated systemically with little effect or with intravesically instilled agents that focus on sterilization, lavage and arrest of focal bleeding points. However, as doses escalate, intravesical treatments frequently result in increased toxicity.[6]

The calcineurin inhibitor tacrolimus hinders the production and release of pro-inflammatory cytokines in T cells. It serves as a potent immunosuppressant that improves barrier function of the skin and mucosa.[7] Systemic administration carries a high incidence of adverse events such as nephrotoxicity and hypertension, which increases morbidity due to a direct inhibitory effect on cell mediated immunity.[8,9] However, when used in site specific fashion to treat dermal inflammatory conditions in an ointment or lotion formulation, minimal adverse events develop.[10]

This knowledge has prompted studies of intravesical instillation of tacrolimus in the bladder as potential treatment for radiative hemorrhagic cystitis. However, the delivery of this pharmaceutical is limited due to its hydrophobic nature. When used with hydrophilic substances such as liposomes, tacrolimus becomes highly soluble.[11] A pharmacokinetic study revealed that liposomes used as a delivery vehicle increased endocytosis while decreasing systemic exposure and vehicle related toxicity compared to nonencapsulated tacrolimus in rats.[12,13] Lipo-tacrolimus also caused significantly less bladder inflammation in healthy rats than nonencapsulated tacrolimus.[12]

We hypothesized that SARRP would enable the development of a rodent model of acute RC. Using this model we further hypothesized that liposome encapsulated tacrolimus would be an effective treatment for SARRP induced RC in rats.

MATERIALS AND METHODS

Animals

We used 40 adult female Sprague Dawley rats (strain 400) at age 3 months that weighed a mean ± SD of 250 ± 50 gm. The protocol was approved by the institutional animal care committee at Beaumont Health System Research Institute, Royal Oak, Michigan. A total of 16 rats were used to develop a RC animal model (phase I). The rats were divided into 4 groups of 4 with each group receiving a different amount of radiation. One rat per group served as a control. Upon developing a suitable RC rat model we used 24 rats for the treatment efficacy study (phase II). Three rats served as controls and 21 received 40 Gy radiation. Of the irradiated rats 12 were treated with lipo-tacrolimus and 9 received saline.

Equipment

Metabolic Cages. The cages allowed free access to food and water, and separated urine from feces. On the day of a micturition study the rats were placed in cages at mid afternoon. All voiding measurements were made during a 12-hour overnight period.

Balances. An Ohaus Scout® Pro SP401 balance was placed below each metabolic cage with a urine collection container. These devices were interfaced with a computer that recorded the time and the mass of urine every 5 seconds.

SARRP. For SARRP irradiation (225 kVp, Xstrahl, Gulmay Medical, Suwanee, Georgia) and imaging, the rat was anesthetized with 1.5% to 2% isoflurane and transferred to a warmed stage for tail vein injection of CT contrast medium for bladder visualization. After injection the rat was positioned vertically on the SARRP. Anesthesia was maintained throughout imaging and irradiation. Contrast enhanced CT was performed using SARRP. The target of irradiation was set at the center of the bladder. Treatment planning software (3D Slicer, http://www.slicer.org) was used to determine the precise beam arrangement needed to limit toxicity and focus the total dose on the bladder alone (fig. 1). The rat recovered in a warming tank and was returned to general housing.

Lipo-Tacrolimus. A formulation of tacrolimus encapsulated in multilamellar sphingomyelin liposomes (Lipella Pharmaceuticals, Pittsburgh, Pennsylvania) was

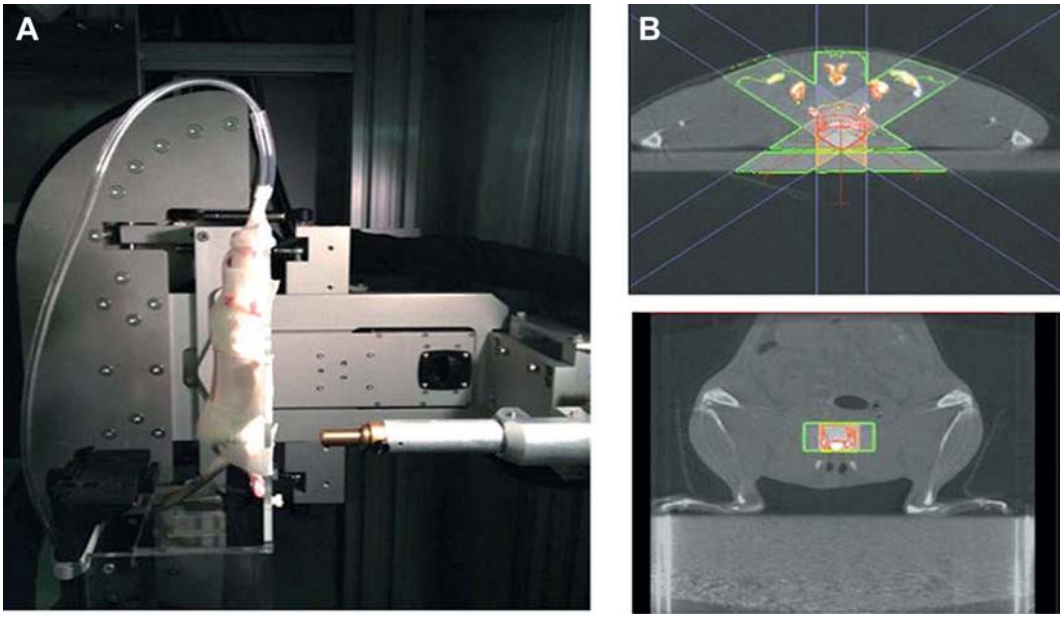

Figure 1. SARRP animal stage was modified to accommodate rats positioned vertically. SARRP x-ray unit in treatment position (*A*). SARRP CT with bladder targeting using treatment planning software (*B*). Red outline indicates volume that would receive total dose divided among 3 beams to spare skin and normal tissue.

prepared as 10% weight per weight using a previously described method.[13] For treatment 20 mg lipo-tacrolimus powder were suspended in 10 ml sterile saline. A 0.5 ml volume was used for instillation.

Phase I

After 1-week acclimation baseline micturition measurements were made twice weekly for 2 weeks. Each group received a unique dose of bladder targeted radiation via SARRP, including 0, 20, 30 or 40 Gy. After irradiation, the rat was returned to social general housing for a minimum of 24 hours before continuing metabolic cage measurements that were performed twice per week for a total of 6 weeks. The rat was placed in a housing unit between each measurement. Radiation doses and treatment plans were intended to produce acute effects in the bladder to develop a viable bladder model, enabling lipo-tacrolimus evaluation.

Histology. Six weeks after irradiation all rats were sacrificed. The bladder was harvested and cryopreserved with TFM™-5 Tissue Freezing Medium gel for histological analysis. The cryopreserved bladder was cut into 8 μm sections. Air-dried frozen sections were fixed in 10% buffered formalin for 20 minutes and stained with hematoxylin and eosin. Stained sections were sealed with Permount™ mounting medium using glass coverslips.

Analysis. IMI distributions were obtained from voiding studies by a program that iterated through the raw balance data to find the intervals between each micturition event. A micturition event was defined as a weight increase in excess of 150 mg during each 5-second period. For each group the IMIs before radiation were compared with values after radiation using the nonparametric Wilcoxon rank sums test due to small sample size.

Phase II

Tests for lipo-tacrolimus treatment efficacy were initiated after findings indicated that 40 Gy bladder irradiation caused a significant decrease in IMI. Initially, 4 baseline voiding studies per rat were performed for 2 weeks. Radiation (40 Gy) was administered to 21 rats. Four post-radiation voiding studies were completed during a 2-week period following the minimum 24-hour general housing rest period. A total of 12 rats received an intravesical instillation of lipo-tacrolimus and 9 received saline. Four measurements were made after instillation during a 2-week period.

Instillation. Each rat was anesthetized with 1.5% to 2% isoflurane and given an injection of buprenorphine that varied by body weight. A 0.5 ml volume of lipo-tacrolimus was instilled intravesically using a catheterized transurethral approach for 30 minutes. The rat was recovered from anesthesia and returned to general housing.

Analysis. The average IMI at each time point was used for analysis. One-way ANOVA was performed. Data were grouped based on time points (baseline, and after radiation and instillation) and treatment groups (saline and lipo-tacrolimus). Multiple logistic regression analysis was done with JMP® to identify confounding variables. Post hoc analysis was performed with the pairwise Student t-test. Time point and treatment groupings resulted in a total of 15 pairwise comparisons. Voiding consistency was analyzed by applying the pairwise t-test to the total volume of each measurement.

The chosen α level was 0.05. The Bonferroni correction was applied to establish significance for the pairwise t-tests.[14]

RESULTS

Radiation Tolerance

Contrast enhanced CT guided irradiation on SARRP was well tolerated by rats in each group. There was no mortality, weight loss, or significant damage to skin or gastrointestinal function. However, some hair loss was noted at higher doses.

Phase I

RC Rat Model. Baseline and post-radiation IMIs did not significantly differ in the 20 Gy group ($p = 0.69$). Baseline IMI signals in the remaining 2 dose groups (30 and 40 Gy, respectively) were not statistically distinguishable. The mean score of IMI rank sums for each 3-rat dose group was 127 and 131, respectively. Because this difference was relatively small, the null hypothesis (that the baseline radiation IMI signals of the 2 groups were the same) could not be rejected. The p value of the corresponding chi-square test was 0.67. The 30 and 40 Gy radiation doses caused a dose dependent change in IMI probability density. With each increased dose radiation appeared to affect the IMI signal by shifting probability density functions toward shorter IMI values. However, only the effect of the 40 Gy dose was strong enough to result in a statistically significant effect ($p = 0.0024$). Figure 2 shows relative differences between the groups before and after radiation at each radiation level.

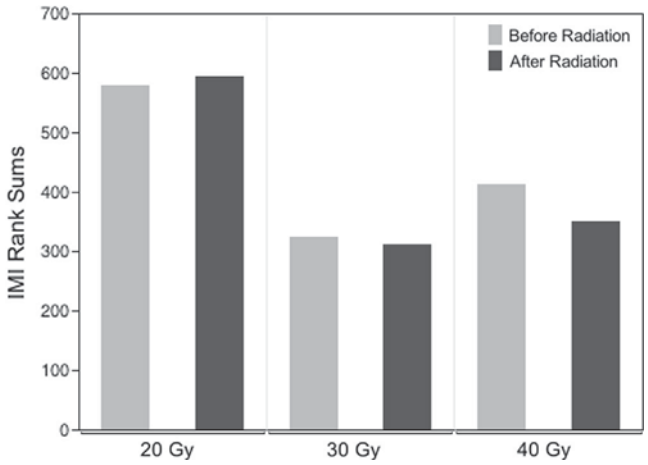

Figure 2. Relative IMI rank sum scores of bladders irradiated with 20, 30 and 40 Gy doses. Dose of 20 Gy did not result in significant change in IMI probability density. Compared to 30 Gy treatment with 40 Gy produced more significant IMI decrease ($p = 0.0024$).

Histology. Hematoxylin and eosin staining of the harvested bladder tissue from the different treatment groups was performed to assess histological changes induced by radiation (fig. 3). Bladders of the 20 Gy group demonstrated no inflammatory change (fig. 3, *B*). The 30 Gy group bladders had edematous changes in the lamina propria with accompanying infiltration of immune cells with ectatic blood vessels in the lamina propria and hyperplastic urothelium (fig. 3, *C*). Degenerative-type epithelial changes were seen in bladders harvested from the 40 Gy group, including cytoplasmic ballooning due to severe mucosal edema and small nests of urothelial cells in the lamina propria surrounding the blood vessels. Figure 3, *D* shows histological findings indicating pseudocarcinomatous urothelial hyperplasia of the bladder, a condition associated with RT. Figure 3, *A* shows histology of a control bladder not exposed to radiation for comparison.

Phase II

Voiding Consistency. Analysis was performed to determine whether the total amount of urine voided during measurements remained constant throughout the study. Multiple logistic regression analysis was used to separate the confounding factors (treatment and time point). The p value of these factors was 0.25 and 0.22, respectively, suggesting that volume did not change throughout the study due to either factor. Multiple pairs t-test analysis between each time point in each treatment group confirmed that mean total volume did not significantly differ throughout the study (see table).

Histology. The harvested bladder was stained with hematoxylin and eosin 6 weeks after irradiation and 2 weeks after treatment (fig. 4). In saline treated rats we found edematous changes accompanying inflammatory cell infiltration and hyperplastic urothelial changes (fig. 4, *A*). Lipo-tacrolimus treated rats showed minimal edematous changes (fig. 4, *B*).

Treatment Efficacy. One-way ANOVA of IMIs demonstrated a significant difference between time point groups ($p < 0.05$, see table). Multiple logistic regression analysis of IMIs revealed significant effects of treatment ($p = 0.03$) and time point ($p < 0.0001$) on IMI distribution.

There was no significant difference in baseline or post-radiation data between each of the treatment groups (eg saline vs lipo-tacrolimus). However, there was a significant decrease in IMI between baseline and post-radiation IMIs in each treatment

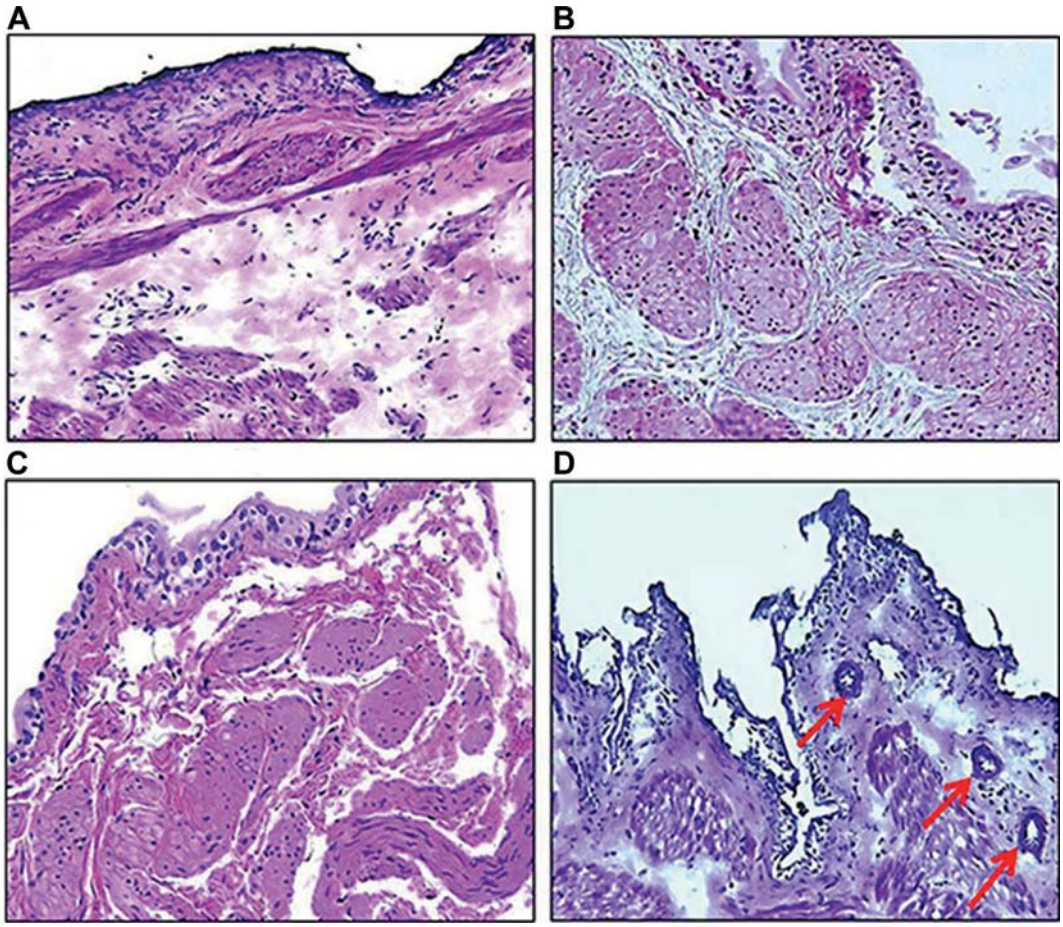

Figure 3. Harvested bladder tissue from treatment groups. Histology of control bladder not exposed to radiation (*A*). Rat bladder from group irradiated with 20 Gy demonstrated no inflammatory change (*B*). Bladder from group irradiated with 30 Gy shows edematous changes accompanying inflammatory cell infiltration, blood vessel dilatation and hyperplastic urothelium (*C*). Note degenerative-type epithelial changes in bladder from group irradiated with 40 Gy, including cytoplasmic ballooning and pseudocarcinomatous epithelial hyperplasia (arrows) (*D*). H&E, reduced from ×20.

group (eg saline/baseline vs saline/post-radiation). IMIs after radiation in each treatment group did not significantly differ. These comparisons support the notion that RT effects did not differ among the treatment groups.

Values in post-instillation controls did not significantly differ from values in the post-radiation or the baseline group. The lipo-tacrolimus group showed a significant increase in IMI from after radiation to after instillation ($p < 0.001$). Furthermore, there was no significant difference from baseline after lipo-tacrolimus treatment ($p = 0.019$). These results indicate that lipo-tacrolimus had a significant effect on increasing the IMI distribution back to baseline conditions but the effect of saline, if any, was less significant.

A comparison between saline and lipo-tacrolimus after instillation suggested that there was a significant difference between the effects of each treatment ($p = 0.0001$). Lipo-tacrolimus showed a greater treatment effect by increasing IMI back to baseline values.

DISCUSSION

Radiation treatment for pelvic malignancies is typically associated with radiation injury to the bladder, which can lead to RC. The precise mechanism of delivery to particular anatomical locations makes radiation treatment distinct from the systemic approach to chemotherapy. This precision substantially reduces damage to healthy cells in the body.[15] The practice still carries short-term and long-term risks. The extent of this damage varies and it may result in secondary cancers that are considered a tremendous concern in pediatric patients who receive RT.[16] The late sequelae of RT may take months or years to develop, including bothersome symptoms such as hematuria. Although no definitive treatment is currently available,

Effect of 40 Gy radiation and treatment on IMIs and volume in treatment groups before and after radiation, and after instillation

Treatment (time point)	Mean ± SD IMI (mins)	Mean ± SD Total Vol (ml)
Saline:		
Baseline	67.3 ± 8.4	5.5 ± 1.6
After radiation	46.2 ± 5.1	6.7 ± 1.2
After instillation	56.6 ± 5.9	7.2 ± 1.1
Lipo-tacrolimus:		
Baseline	64.5 ± 5.9	6.7 ± 1.7
After radiation	47.7 ± 4.2	7.6 ± 1.9
After instillation	76.8 ± 10.1	7.1 ± 1.0

various interventions are used today for RC and hemorrhagic cystitis.[17]

Guided by clinical considerations, this rodent model of RC was developed as a conduit between research and human treatment, which provided the opportunity to test innovative treatments. The model as well as the treatment was assessed by observing micturition pattern variances. As an indicator of bladder function, these changes in micturition intervals in irradiated rats were studied noninvasively by measuring changes in overnight voiding patterns. This method makes repeat measurements possible in the same animal, resulting in a decrease in the number of animals needed for therapeutic studies.

A dose dependent decrease in IMI was observed in the rat group irradiated with a 40 Gy dose. The change in IMI was moderate and insignificant at the 30 Gy dose but drastic and significant at 40 Gy relative to baseline. Degenerative-type changes in rat urothelium in the group irradiated with 40 Gy were consistent with decreased IMI in this group. The rat group irradiated with 20 Gy showed no significant change in IMI, consistent with the mild inflammatory changes on histology.

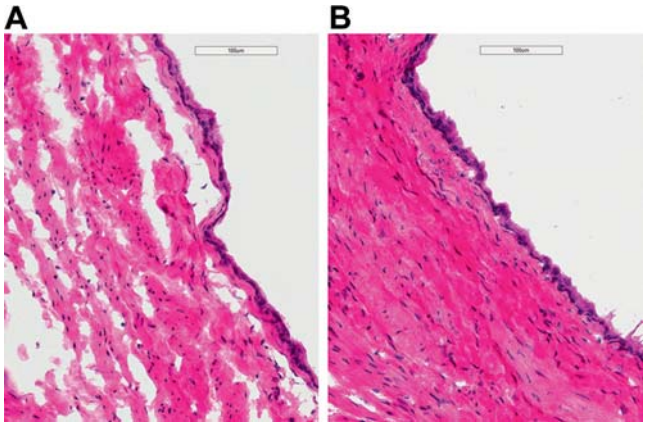

Figure 4. Harvested rat bladder 6 weeks after 40 Gy irradiation and 2 weeks after saline instillation shows edematous changes accompanying infiltration of inflammatory cells and hyperplastic urothelial changes (*A*). Bladder from group treated with lipo-tacrolimus shows minimal edematous change (*B*). H&E, reduced from ×20, scale bar indicates 100 μm.

The radiation dosing regimen used in the current study was greater than in previous studies in which radiation associated bladder toxicity was measured.[18] CT guided SARRP treatment limits radiation exposure to surrounding tissue and allowed us to test RC therapy. Future studies are planned to evaluate low dose, fractionated radiation delivery schemes and assess damage at longer time points.

Radiation induced vascular damage in the bladder is considered a major mechanism of RC pathogenesis and histological changes are considered time and radiation dose dependent.[19,20] These changes in the RC acute phase may appear 4 to 6 weeks after radiotherapy and are characterized by inflammatory changes in the bladder wall caused by the breakdown of the urothelial barrier.[20] Stromal and urothelial changes such as edema with acute inflammation, hemorrhage and vascular dilatation with fibrin thrombi, endothelial swelling and vessel wall thickening with light necrosis distinguish the acute from the chronic phase of RC. The localized urothelial damage seen at lower doses progresses to extensive denudation and mucosal ulceration at higher doses (fig. 3, *B* to *D*). The group irradiated with 40 Gy showed evidence of urothelial hyperplasia with nests and cords that extended into the lamina propria around ectatic blood vessels. This pseudoneoplastic condition, mimicking invasive urothelial carcinoma, is referred as pseudocarcinomatous urothelial hyperplasia (fig. 3, *D*). This histological feature serves as a signature of the pathological aberrations seen in the bladder after ischemia and chronic radiation.[21]

Our results suggest that using SARRP for targeted radiation delivery provides advanced irradiation, imaging and planning capabilities that are suitably downsized for small animal radiation research and appropriate for the development of a RC rat model. IMI results suggest that changes in IMI at different radiation doses measures a change in the voiding pattern and in bladder function.

Lipo-tacrolimus treatment had a significant effect on increasing the IMI distribution back to baseline conditions. It may be viewed as a potential drug candidate for RC treatment. Histology results provide strong evidence that lipo-tacrolimus helped heal the cystitis induced via RT. The immunosuppressant property of tacrolimus likely improves the barrier function of the urothelium by disrupting inflammatory signaling events mediated by calcineurin. Lipo-tacrolimus also causes acute arteriole vasoconstriction, which may suppress hemorrhagic cystitis symptoms.

CONCLUSIONS

In settings that attempt to model clinical RT for pelvic cancer 3-beam SARRP radiation treatment is an effective method of inducing RC. The RC rat model demonstrated a dose dependent decrease in IMI without inducing short-term skin or gastrointestinal damage. This study also shows that liposomal tacrolimus may be a promising new intravesical therapy for acute RC.

ACKNOWLEDGMENTS

Bernadette Zwaans, Laura Lamb and Barb Pruetz assisted with tissue samples and histology.

REFERENCES

1. Rajaganapathy BR, Jayabalan N, Tyagi P et al: Advances in therapeutic development for radiation cystitis. Lower Urinary Tract Symptoms 2014; **6:** 1.
2. Veerasarn V, Boonnuch W and Kakanaporn C: A phase II study to evaluate WF10 in patients with late hemorrhagic radiation cystitis and proctitis. Gynecol Oncol 2006; **100:** 179.
3. Echols RM, Tosiello RL, Haverstock DC et al: Demographic, clinical, and treatment parameters influencing the outcome of acute cystitis. Clin Infect Dis 1999; **29:** 113.
4. Wong J, Armour E, Kazanzides P et al: High-resolution, small animal radiation research platform with x-ray tomographic guidance capabilities. Int J Radiat Oncol Biol Phys 2008; **71:** 1591.
5. Dilworth JT, Krueger SA, Wilson GD et al: Preclinical models for translational research should maintain pace with modern clinical practice. Int J Radiat Oncol Biol Phys 2014; **88:** 540.
6. Smit SG and Heyns CF: Management of radiation cystitis. Nat Rev Urol 2010; **7:** 206.
7. Rico MJ and Lawrence I: Tacrolimus ointment for the treatment of atopic dermatitis: clinical and pharmacologic effects. Allergy Asthma Proc 2002; **23:** 191.
8. Naesens M, Kuypers DR and Sarwal M: Calcineurin inhibitor nephrotoxicity. Clin J Am Soc Nephrol 2009; **4:** 481.
9. Akar Y, Yucel G, Durukan A et al: Systemic toxicity of tacrolimus given by various routes and the response to dose reduction. Clin Experiment Ophthalmol 2005; **33:** 53.
10. Ebert AK, Rosch WH and Vogt T: Safety and tolerability of adjuvant topical tacrolimus treatment in boys with lichen sclerosus: a prospective phase 2 study. Eur Urol 2008; **54:** 932.
11. Patel P, Patel H, Panchal S et al: Formulation strategies for drug delivery of tacrolimus: an overview. Int J Pharm Investig 2012; **2:** 169.
12. Nirmal J, Tyagi P, Chancellor MB et al: Development of potential orphan drug therapy of intravesical liposomal tacrolimus for hemorrhagic cystitis due to increased local drug exposure. J Urol 2013; **189:** 1553.
13. Chuang YC, Tyagi P, Huang HY et al: Intravesical immune suppression by liposomal tacrolimus in cyclophosphamide-induced inflammatory cystitis. Neurourol Urodyn 2011; **30:** 421.
14. Dunn OJ: Multiple comparisons among means. J Am Stat Assoc 1961; **56:** 52.
15. Rossi P: CancerQuest: Benefits of RT. Available at http://www.cancerquest.org/radiation-benefits.html. Accessed October 2014.
16. Brenner DJ: Induced second cancers after prostate-cancer radiotherapy: no cause for concern? Int J Radiat Oncol Biol Phys 2006; **65:** 637.
17. Manikandan R, Kumar S and Dorairajan LN: Hemorrhagic cystitis: a challenge to the urologist. Indian J Urol 2010; **26:** 159.
18. Kanai AJ, Zeidel ML, Lavelle JP et al: Manganese superoxide dismutase gene therapy protects against irradiation-induced cystitis. Am J Physiol Renal Physiol 2002; **283:** F1304.
19. Stewart FA: Mechanism of bladder damage and repair after treatment with radiation and cytostatic drugs. Br J Cancer, suppl., 1986; **7:** 280.
20. Soler R, Vianello A, Fullhase C et al: Vascular therapy for radiation cystitis. Neurourol Urodyn 2011; **30:** 428.
21. Lane Z and Epstein JI: Pseudocarcinomatous epithelial hyperplasia in the bladder unassociated with prior irradiation or chemotherapy. Am J Surg Pathol 2008; **32:** 92.