Heterotopic Urinary Bladder with a Communicating Reservoir

Ryoichi Oyasu, David J. Manning, Michio Matsumoto, and Martin L. Hopp

Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611

SUMMARY

A heterotopic bladder attached to an Ommaya reservoir was transplanted to the back of a syngeneic rat. Following transplantation, the mucosa underwent ischemic necrosis. However, it was rapidly reepithelialized in 2 weeks as a result of spreading of the epithelium originating from the ureterotrigonal region. By light microscopy the regenerated epithelium was indistinguishable from the normal transitional epithelium, and the cannula connecting the reservoir to the bladder remained patent throughout the observation period of 22 weeks. One bladder, when examined at 22 weeks, contained a transitional cell papilloma with squamous metaplasia. Although further study is needed, this model is potentially useful in studies related to bladder carcinogenesis.

INTRODUCTION

The best way to assess the carcinogenicity of a chemical is to apply the test compound directly to the target organ or tissue. In the study of the urinary bladder system, the following 2 techniques are being commonly used. The 1st technique is by way of inserting a paraffin or cholesterol pellet containing the test compound into the bladder lumen, and the 2nd technique involves instilling a carcinogen solution or suspension directly into the lumen through the urethra. Either method is not satisfactory because it is frequently accompanied by stone formation, nonspecific irritation, infection, and/or loss of test compound by micturition. Furthermore, positive results cannot necessarily be attributed to the test compound since one of our recent studies (M. L. Hopp, M. Matsumoto, C. Lee, and R. Oyasu, unpublished data) as well as that by Hsu et al. (4) has shown that the carcinogen instilled into the bladder lumen is quickly absorbed and excreted from the kidney as its metabolites (vesicohepatic renal circulation). Thus, as much as 90% of [S-3H]AAF instilled into the bladder lumen will be absorbed within 3 hr, and approximately 25% of the dose will be excreted from the kidney during the same period. Therefore, carcinogenicity demonstrated by local application of carcinogen may not be attributed to that compound but rather to its metabolite(s) excreted in the urine after hepatic metabolism. It is clear, therefore, that an ideal working model for the urothelial tumor research should meet the following requirements: (a) the bladder to be tested should be lined by a transitional epithelium; (b) it should be free from infection or calculus formation; (c) it retains the instilled carcinogen for a reasonable period of time; (d) a carcinogen can be instilled into the lumen easily and repeatedly; and (e) most importantly, the bladder epithelium should not be exposed to the metabolites of the test compound. We believe that we have made a working model which reasonably well satisfies such criteria. Briefly, it is a bladder transplanted into the gluteal muscle and connected to an Ommaya reservoir implanted into the s.c. tissue of the back of a syngeneic rat.

MATERIALS AND METHODS

General

Young male ACI/Cox rats (Laboratory Supply Co., Indianapolis, Ind.) weighing 130 to 200 g were used as both donors and recipients. A group of 3 to 5 bladder recipient rats at a time were sacrificed 24 hr, 3 days, 1, 2, 4, 8, 14, 20, and 22 weeks after transplantation for histological examination of the bladder. A group of 2 additional animals were used at 2, 4, 8, and 22 weeks following bladder transplantation for an absorption study of the labeled carcinogen injected into the reservoir. One was sacrificed at 24 hr and the other at 72 hr following injection of the labeled compound.

Transplantation of Bladder

Preparation of Reservoir and Surgical Instruments. An Ommaya side-inlet flushing reservoir (850-1274; Heyer-Schulte Corporation, Goleta, Calif.) with an attached connector bent 90° in the middle was used. The distal end of the connector was cut to 3 mm, and the sharp edge was rounded by using a nail file and oilstone to minimize irritation to the bladder mucosa. The connector was rinsed in running hot tap water and then with 70% ethanol. It was then inserted into the reservoir and positioned such that the distal end pointed to the right and about 30° downward when placed on the back of the recipient animal. The entire unit was washed with mild soap, rinsed with hot running tap water, and autoclaved together with surgical instruments.

Preparation of Animals. Animals received 2 i.m. injections of ketamine hydrochloride (Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, N. Y.), 7.5 and 5.0 mg/100 g body weight with a 15-min interval to provide adequate analgesia. The donor and recipient animals were placed on a clean workboard side by side, with the donor supine and the recipient prone. The operative fields were clipped with an electric hair clipper and washed 2 times with 70% ethanol.

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2 To whom requests for reprints should be addressed.
3 Predoctoral fellow; USPHS Grant GM 00131.
4 The abbreviation used is: AAF, 2-acetylaminofluorene.

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Surgical Procedure. A Y-shaped midline incision was made on the back of the recipient rat and extended over the right gluteal muscle. A triangular piece of the muscle was removed from the most proximal end to make a room for a bladder transplant. The overlying flap of fascia, however, was left intact.

At this time, a midline incision was made on the abdomen of the donor. The bladder was exposed and trimmed of the prostate gland, seminal vesicle, and perivesical fat. The bladder was cut off at the neck and was placed in a Petri dish containing sterile 0.9% NaCl solution. It was washed twice by instilling 0.9% NaCl solution by syringe in order to remove a gelatinous coagulant and parasites (Tri-chosphomoides crassicauda) which are sometimes found in these animals. The reservoir was filled with 0.9% NaCl solution. The donor bladder was then placed on the free end of the connector and tied on with 4-0 silk suture. The dome of the reservoir was pressed several times to assess its function. The bladder-reservoir unit was placed in such a way that the dome was under the upper part of the Y and the bladder was within the groove created within the gluteal muscle. The unit was affixed tight to the tissue by several sutures applied to the reservoir and the connector. The fascia flap was then tied over the transplant using 0-6 silk sutures. The incision of skin was closed with 2-0 silk sutures and coated with petroleum jelly.

Postoperative Follow-up. The animals were observed daily. One week after the operation, the dermal sutures were removed, and the function of the reservoir was checked for the 1st time by compressing the dome of the reservoir lightly several times. Patency of the connector was confirmed by palpating the tension of the transplanted bladder.

Injection procedure. Two weeks after transplantation, the reservoirs received 3 weekly injections of sterile 0.9% NaCl solution. The skin over the reservoir was shaved and cleaned with 70% ethanol. The animal was held tight with a gloved hand and the function of the reservoir was tested. A sterile tuberculin syringe with a 25-gauge needle was inserted into the reservoir. Then another syringe containing 0.9% NaCl solution was also inserted into the reservoir. Fluid was aspirated into the 1st syringe and 0.9% NaCl solution was injected from the 2nd syringe. Both were removed and the reservoir was flushed several times. Usually about 0.2 ml was aspirated and the same amount or slightly more was injected. This kept the pressure within the system balanced with slight dilation of the bladder. The color of the aspirate was recorded.

Microbiology

Sterility of the bladder content was examined regularly. The 1st sample for microbiology was obtained 2 weeks posttransplant, and thereafter aspirate was submitted for microbiology once a week and finally at termination of the experiment. The aspirate was added to a thioglycolate tube, and after 48 hr a Gram stain was made. During the initial period, regardless of the result obtained from thioglycolate culture, the fluid was subcultured using blood agar and McConkey agar plates.

Removal and Examination of the Reservoir-Bladder Units

The animals were killed with an ether overdose, and their weight was recorded. The reservoir-bladder unit was exposed by a midline incision (Fig. 1). Degree of fibrosis was recorded, and evidence of infection was sought. Function of the reservoir was again checked, and the content of the reservoir was aspirated for final microbiological examination. The reservoir-bladder unit was removed. The bladder was then carefully separated from the connector, opened along its long axis, stretched on a piece of cardboard with pins, and inspected for parasites and ova under a dissecting microscope. The gross appearance of the mucosa was inspected. The bladder of the recipient was removed and inspected likewise under a dissecting microscope.

Histological Examination

Following 48-hr fixation in 10% neutral formalin, the bladder was cut into 3 pieces along the long axis and all were submitted for microscopic examination. Sections were taken from 3 different levels and stained with hematoxylin and eosin. The recipient’s own bladder was not studied microscopically.

Absorption of [9-14C]AAF from the Transplant Bladder Lumen

One hundred µCi of [9-14C]AAF (13.15 µCi/mmmole; International Chemical and Nuclear Corp., Irvine, Calif.) were dissolved with 0.1 ml 95% ethanol. Then, 5 ml of sterile 0.9% NaCl solution were added. Each animal reservoir received an 0.15-ml injection of suspension containing 3 µCi or 0.051 ng of AAF. A 0.05-ml aliquot of the suspension from the same tuberculin syringe was used to establish the amount of radioactivity injected. After injection, animals were kept individually in stainless steel metabolism cages (ACME Metal Products, Inc., Chicago, Ill.) for collection of feces and urines. Commercial rat chow and tap water were given ad libitum. After completion of the collection period, the animals were killed with an overdose of ether. Samples were obtained from arterial blood, urine, feces (including colonic content), reservoir fluid, and fluid contained within the transplant, liver, spleen, and kidney. The cage and funnel were rinsed with 1.0 M sodium acetate buffer (pH 6.0), and the total volume was measured. Two aliquots of 0.1 ml were counted using a Packard Model 3385 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

After removal of the reservoir-bladder unit, the connector was clamped, and the radioactivity of the fluid contained in the reservoir and bladder was measured separately. For the liver and other organs several small pieces were removed from different portions, weighed, digested by Soluene 100 (Packard Instrument Co.), and counted. Feces, including the content of the colon, were mixed using a mortar and pestle to ensure homogeneous mixture. Three samples were obtained for counting.

Blood was obtained by cardiac puncture into a tube containing heparin. After centrifugation, 0.1 ml of plasma was
RESULTS

General

Except for 2 animals, all animals maintained the original weight or gained weight.

Out of 38 bladder recipients, study was successfully carried out for completion in 35 rats. Complication occurred in 6 animals. Of these 6, 1 scheduled to be sacrificed at 8 weeks ripped the surgical wound open at 1 week. Surgical repair of the dehisced wound was unsuccessful, and the animal was killed and excluded from the study. Focal infection of the suture line apparently due to biting occurred in 2 animals several days before the scheduled time of killing at 2 weeks. Spontaneous drainage occurred in one of these rats, surgical drainage was induced in the other, and both were allowed to live for completion. Antibiotics were not used. Culture of the aspirate at termination of the scheduled experiment grew out α-hemolytic streptococcus in 1 animal and was negative in the other. These animals were included in our study. Dysfunction of the reservoir-bladder unit occurred in 3 animals. In 1, this was due to leakage of the injected 0.9% NaCl solution, occurring at Day 24. In 2 others, occlusion somewhere in the bladder-reservoir unit occurred at Days 22 and 42. After several attempts at restoring communication by repeated pumping of the reservoir, both animals were sacrificed. At autopsy the tip of the connector in both animals was found to be partially buried within the mucosa, thus creating a ball-valve effect. These 3 animals were excluded from the results. One of the rats that had a functional reservoir-bladder unit was scheduled to receive [9-14C]AAF at 2 weeks. Because of a technical failure, the radioactive material could not be injected into the reservoir and, therefore, the rat was removed from the study.

Pathology

Gross Examination. Brown fluid was present around the reservoir for the 1st 3 days at exploration of the s.c. tissue of the operative field. Loose fibrous connective tissue began to encase the reservoir and bladder at 1 week. The connective tissue response became more dense by 2 weeks, and the reservoir surface was entirely covered by a thin film of collagen fibers. Likewise, the surface of the bladder became completely covered by a thin layer of loose fibrous connective tissue enforced by the fascia on the surface side and the gluteal muscle in the remaining portion. By 8 weeks, the bladder could be separated from the muscle only with difficulty, but anatomically it was distinct. There was no further progression in degree of perivesical fibrosis and, if any, regression was observed.

The function of the unit was tested again after the entire unit was removed from the body. The decreased expansion bulge that was noted in some animals after 3 weeks following transplantation appeared to be due to rigidity of the bladder wall secondary to perivesical fibrosis.

The content of the bladder was initially brown serous fluid but became amber clear in all cases after 2 weeks.

After being separated from the connector, the bladder was opened along the long axis. Under a dissecting microscope, focal hyperemia was commonly observed in the mucosa for the 1st 2 weeks following transplantation. Thereafter, the mucosa was smooth and, in some areas, opaque due to mild fibrosis of the lamina propria. In 1 bladder removed after 22 weeks, a papillary lesion was observed at the trigone adjacent to the tip of the cannula. No parasites, ova, or calculi were found.

Microscopic Examination. At 24 hr following transplantation the epithelial cells became completely necrotic and detached off the edematous lamina propria, except in isolated areas where basal cells remained intact (Fig. 2). The tunica muscularis and adventitia appeared normal. At 72 hr, the mucosa was completely denuded of the epithelial layer, and the lamina propria showed coagulation necrosis (Fig. 3), which in 2 of 4 animals extended to involve part of the tunica muscularis. A small number of mononuclears, including mast cells, were found in the lamina propria. At the bladder neck where a silk suture had been applied, an active granulation tissue proliferated. Despite complete necrosis of the transitional epithelium of the bladder mucosa, the ureteral epithelium remained viable and appeared to extend along the bladder mucosa.

At 1 week following transplantation, about 70% of the mucosal surface area was reepithelialized in 3 of 5 bladders, the regeneration apparently starting at the bladder neck-trigone area (Fig. 4). In the remaining 2, epithelialization was limited to the neck area. The lamina propria still remained necrotic in some areas, although the tunica muscularis appeared viable. At the trigone and bladder neck area, active granulation tissue was present and involved the entire thickness.

At 2 weeks, the entire mucosa had been reepithelialized by a 2- to 4-cell-thick epithelium. In many areas, the epithelium was attenuated and only 2 cells could be recognized. In the area where the epithelium consisted of 3 to 4 cells, superficial cell differentiation could be demonstrated (Fig. 5). The lamina propria showed residual granulation tissue near and at the trigone, and in the remainder of the bladder, focal fibrosis was evident in the lamina propria and occasionally also in the superficial part of the tunica muscularis. In 3 of 4 animals, a few neutrophils and mononuclear cells were found in the lamina propria and tunica muscularis.

Between 4 and 8 weeks posttransplant, the histology of the bladder became stabilized. The entire mucosa was covered by a transitional cell epithelium, 3 to 4 cells thick, showing surface cell differentiation (Fig. 6). Mild fibrosis was observed in the lamina propria of 6 of 8 bladders (Fig. 7). Chronic inflammation was observed at the trigonal area in all animals. Here, an inflammatory polyp was found in 1 animal and papillary hyperplasia made up of 4 to 5 cells was observed at the trigone in 3 animals (Fig. 8).

An additional 4 animals were sacrificed, 1 at 14 and 20 weeks and 2 at 22 weeks. Except at the trigonal region, the mucosa was composed of well-differentiated transitional
epithelial cells, 3 cells thick. Mild fibrosis of the lamina propria was observed in 3 of 4 bladders. Focal papillary hyperplasia of trigone was noted in 1 bladder. A papillary growth which was demonstrated in a bladder removed after 22 weeks proved to be a papillary transitional cell tumor showing focal squamous differentiation. There was no evidence of invasion of the underlying tissue.

**Microbiology**

Once a week and at the completion of the scheduled study, the reservoir aspirate was obtained for culture study. Cultures were negative in all cases, except 2. One positive culture occurred in a rat that was sacrificed at 1 week. The animal was grossly free of infection and the urine was amber clear. Another positive culture occurred in an animal that was killed at 2 weeks. This animal had had a grossly infected suture line over the transplanted bladder for several days prior to scheduled killing.

**Absorption of [9-^14C]AAF**

The results are shown in Table 1. A substantial amount of radioactivity was lost from the bladder-reservoir unit within 24 hr and almost all within 72 hr. Although the number of animals studied was small and variation among animals was great, 12 to 49% of radioactivity was recovered in the urine, and 5 to 67% was excreted in the feces and colonic content. The only other large source of radioactivity was the liver, where approximately 2% of the dose was found. Although not shown in Table 1, there was no significant difference in the concentration of radioactivity between the reservoir and the bladder at both 24 and 72 hr following injection.

**DISCUSSION**

This study was initiated to find a working model suitable for testing the effect of locally applied carcinogen. Such a model should meet several criteria as listed in the beginning. In particular, the bladder should be diverted from the urinary system so that the secondary effect of the metabolites of the test compound that may be excreted in urine following absorption from the bladder wall can be eliminated. In addition, the test model must have an easy access to repeated instillations of the test compound without the risk of infection or calculus formation.

In the search for such a model, 2 attempts to transplant bladder tissue previously were reported. Yalciner and Friedell (7) injected a piece of bladder tissue by a trocar, and Roberts et al. (6) injected pieces of bladder tissue and air. Both groups produced cystic structures which ranged from microscopic size (7) to 11 mm (6) in the maximum dimension and which were lined by a transitional epithelium indistinguishable in the ultrastructure from the normal (6). The time required for completion of epithelialization ranged from 1 to 2 weeks, depending upon the size of the cyst. The wall of some cysts contained pieces of smooth muscle.

Our model differed in several points from those of the previously mentioned authors. The first difference was that...
our transplant had a structure identical to that of a normal bladder and, therefore, its physiological characteristics would be closer to normal. With our model, one can easily and repeatedly introduce carcinogen and other substances into the bladder lumen without traumatizing the bladder wall provided a 25- or 27-gauge needle is used for punctures. By compression of the dome of the reservoir several times, the material injected into the reservoir can be evenly mixed within the bladder-reservoir unit. When tested at 24 and 72 hr after injection of [9-14C]AAF, there was no significant difference in concentration of the carcinogen remaining within the lumen between the heterotopic bladder and the reservoir. A substantial amount of radioactivity disappeared within 24 hr following injection. We have not tested the absorption pattern of carcinogen instilled into the homotopic bladder in rats. One of our recent studies (unpublished data) using rabbits, however, indicates that AAF instilled into the bladder can be quickly absorbed and about one-fourth of the dose may be excreted from the kidney in 3 hr. Bryan and Morris also observed that the total radioactivity of a tryptophan metabolite was lost from the bladder urine within 24 hr (2). Absorption of chemical through the bladder wall is regarded as a passive process and not uniform to all substances. On the other hand, bladder mucosa is resistant to permeation of electrolytes (3). Whether our heterotopic bladder possesses such a resistance to electrolytes is unknown. Also unknown at this time is whether molecules of various types including carcinogens can pass from the blood into the heterotopic bladder lumen.

The second difference was that we can easily follow the morphological changes occurring in the heterotopic bladder mucosa by aspirating the reservoir content and submitting the fluid for cytological examination.

The third interesting observation we made was that none of the heterotopic bladders bore parasites and/or ova when examined 3 days following transplantation. We did observe degenerated ova in 1 bladder 24 hr after transplantation. Apparently, initial ischemia that caused necrosis of the mucosa also had a deleterious effect on the parasites and ova resulting in their total eradication.

Several problems were observed during the current study. The most common problem was the infection of the surgical field secondary to biting of the suture line by animals. Petroleum jelly used over the suture was not totally effective in preventing biting. Recently, we were told that painting collodione on the suture line following surgery is now prevent this complication by keeping the connector slightly longer.

A 3rd undesirable complication was frequent papillary hyperplasia in the trigonal mucosa. It was observed in 4 of 12 bladders after 4 weeks of heterotopic transplantation. In 1 bladder 22 weeks posttransplant, a papilloma was found in the trigone and near the cannula tip. It was a well-differentiated papillary tumor with partial squamous differentiation. Apparently, the mucosa at this location was more susceptible to proliferative changes than the remaining part. Whether these changes are secondary to irritation induced by the cannula tip is unknown. The close geographical association of the site of hyperplasia with the cannula tip suggests the presence of cause-and-effect relationship. The possibility that the cannula tip may be contaminated by potential carcinogen when smoothed with oilstone cannot be ruled out. We are currently using cardboard as a filling agent. Another factor we must take into consideration is the fact that ACI rats may be genetically susceptible to bladder tumorigenesis. According to the report of Maekawa and Odashima (5), bladder tumors, mostly papillomas, were observed in a relatively high frequency (3.6% for male, 4.8% for female) in a study conducted using a large number of aged ACI/N rats. Testing our model using other strains of rats is indicated to determine the incidence of hyperplasia and papilloma.

Despite some complications observed in this series of experiments, we believe the heterotopic bladder described here is a potentially useful model for various studies related to bladder carcinogenesis. The model is particularly suitable for testing the direct effect of a given compound as well as testing the effect of a very unstable reactive metabolite of a carcinogen. It also could be useful in studying the role of urine or urinary constituents on the carcinogenesis of a test compound. Although our experience with the heterotopic bladders is still limited, we believe that a great majority of them can remain functional for many weeks provided the aforementioned complications are eliminated or reduced by the appropriate measures described.

REFERENCES

Fig. 1. A reservoir-bladder unit transplanted to the back of a recipient rat, 2 weeks posttransplant. A blue dye has been injected into the reservoir to demonstrate patency of the unit. The bladder is buried within the right gluteal muscle at arrow.

Fig. 2. Heterotopic bladder, 24 hr posttransplant. Only basal cells remain. Bottom, a portion of the tunica muscularis. H & E, x 148.
Fig. 3. Heterotopic bladder, 72 hr posttransplant. The epithelial cells have been totally lost. The lamina propria is edematous and necrotic. H & E, × 148.

Fig. 4. Heterotopic bladder, 1 week posttransplant. Regeneration of the mucosal lining has started at the bladder neck-trigone area and spread laterally. H & E, × 148.

Fig. 5. Heterotopic bladder, 2 weeks posttransplant. The entire mucosa has been reepithelialized by 3- to 4-cell thick epithelium. Superficial cell differentiation has taken place. H & E, × 148.

Fig. 6. Heterotopic bladder, 8 weeks posttransplant. The mucosa is covered by a 3-cell thick transitional cell epithelium. The lamina propria and tunica muscularis are normal. H & E, × 148.

Fig. 7. Heterotopic bladder, 8 weeks posttransplant. The epithelium is 3 cells thick. The lamina propria is moderately fibrotic. H & E, × 148.

Fig. 8. Heterotopic bladder, 4 weeks posttransplant. A focal papillary hyperplasia is found in the trigonal area. H & E, × 60.
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