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Original article

Serial retrograde instillations of sustained release formulation of mitomycin C to the upper urinary tract of the Yorkshire swine using a thermosensitive polymer: Safety and feasibility

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Abstract

Background: MitoGel is a novel drug formulation intended for the treatment of upper tract urothelial cancer with proven feasibility and safety in an animal model.

Objective: To evaluate the feasibility, safety, toxicokinetics, and histologic changes associated with serial retrograde MitoGel instillations to the upper urinary tract in a swine model.

Design, setting, and participants: Overall, 27 Yorkshire swine underwent 6 once-weekly unilateral retrograde instillations of MitoGel. Doses of 14, 28, or 56-mg mitomycin C (respective concentrations of 2, 4, and 8 mg/ml with 9 animals per group) were evaluated. Additionally, 6 animals received sterile water as a procedure control, and 9 received gel alone (without mitomycin C), as a vehicle control.

Outcome measurements and statistical analysis: Blood and urine samples were collected for determination of MMC toxicokinetics and for hematology, biochemistry, coagulation, and urinalysis throughout the study. Two-thirds of the cohort were euthanized 24 hours after final instillation, and one-third was euthanized 1 month after final instillation. Necropsy was performed to evaluate the histologic effects of treatments.

Results and limitations: All animals received all 6 doses of agents per protocol. No mortality, clinical adverse events, or meaningful changes in hematology, chemistry, coagulation, or urinalysis were attributable to MitoGel, RTGel alone, or water instillations. Peak plasma levels of MMC were 2 orders of magnitude less than known toxicity thresholds. MitoGel-related dose-dependent microscopic findings were seen in the treated kidneys and ureters, but were of limited severity, lacked associated clinical adverse findings, and decreased over time.

Conclusions: Serial retrograde instillations of MitoGel to the pyelocaliceal system were technically feasible, and produced no observable adverse clinical, laboratory, or histologic effects.

Keywords: Carcinoma; Transitional cell; Mitomycin; Hydrogel; Models; Animal; Swine

1. Introduction

Retrospective studies have demonstrated that in select cases of low-grade upper tract urothelial cancer (UTUC), renal-sparing management provides cancer-specific outcomes equivalent to radical nephroureterectomy [1,2]. Given the known effect of radical nephroureterectomy on

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renal functional outcomes [3], and the known association between surgically induced chronic kidney disease and survival [4], renal-sparing management of low-grade UTUC provides superior renal functional outcomes while maintaining adequate cancer control in appropriately selected patients. However, renal-sparing management carries with it numerous technical challenges. The difficult to access and delicate nature of the upper urinary tract can prevent accurate disease evaluation, risk stratification, and endoscopic tumor ablation, often necessitating repeated interventions under general anesthesia. In addition, topical agents, who have been shown to reduce the risk of recurrence in the bladder, remain within the upper urinary tract for a very brief period of time, owing to ureteral peristalsis and the propensity of the upper tract to rapidly drain liquid agents. As such, despite the potential benefits represented by renal-sparing management, these various challenges remain obstacles to its use, and help to explain the significant number of patients who are treated with extirpative nephroureterectomy for even low-risk forms of UTUC [5,6].

MitoGel is a novel drug, composed of a mixture of aqueous polymers with reverse-thermal gelation properties (RTGel) and mitomycin C (MMC). When cold, MitoGel is a liquid and on warming to body temperature, its viscosity increases rapidly to that of a thick gel. Initial assessment of a comparable formulation in patients with low-grade bladder cancer supports a chemoablative effect on these tumors [7]. Given these properties, there is an interest in using MitoGel as a treatment for UTUC, where it could be instilled into the upper tract as liquid, gelatinize, and then slowly dissolve with the production of urine. This dissolution process would result in a sustained release of MMC into the upper urinary tract over a few hours. Extended exposure of the urothelium to MMC was previously shown to be an important parameter in its antitumor activity in both in vitro and in vivo settings [8,9]. Although antegrade administrations of MitoGel have been evaluated in a preclinical model [10], the safety and feasibility of retrograde administrations of MitoGel is not fully established. As such, we sought to evaluate feasibility and safety of retrograde MitoGel use in a large animal model.

2. Materials and methods

2.1. Study design

We used adolescent female Yorkshire swine as our animal model given the noted similarities between human and swine urinary tract size, anatomy, and urodynamics [11]. Only female pigs were used owing to the fact that male pig urethral anatomy precludes cystoscopic or catheterized access to the bladder. Pigs ranged in age from 17 to 22 weeks (weight 62–80 kg). We used a total of 42 animals with a 5-group study design (Table 1). Animals were assigned to treatment group using a simple randomization procedure. A total of 6 animals were randomized to receive sterile water for injection (WFI) instillations as a procedure control group. Overall, 9 animals were randomized to receive instillations with RTGel alone as a vehicle control group. Finally, 27 total animals were randomized to receive instillations with 1 of 3 doses of MitoGel (containing 14, 28, or 56-mg MMC at respective concentrations of 2, 4, and 8 mg/ml; 9 animals per group). Doses were determined based on the maximal feasible concentration of MitoGel (8 mg/ml) owing to the solubility limitation of MMC, and the previously assessed safe volume for retrograde instillation to the swine pelvicalyceal system (approximately 7 ml). Throughout the study, all animals were observed for morbidity, mortality, and injury at least twice daily, and a more detailed clinical examination of each animal was performed after each instillation. Blood (complete blood count, biochemistry, and coagulation panel) and urine samples (urinalysis) were collected from all animals weekly during the treatment period and before necropsy.

Table 1
Treatment group assignments.

Group number	Treatment[a]	Treatment concentration (mg/ml)	Total MMC dose (mg)	Number of animals[b]
1	Sterile water	0	0	6
2	MitoGel 14 mg	2	14	9
3	MitoGel 28 mg	4	28	9
4	MitoGel 56 mg	8	56	9
5	RTGel	0	0	9

[a]Doses were administered on days 1, 8, 15, 22, 29, and 36.
[b]Four (group 1) or 6 animals (groups 2–5) were euthanized 24 hours after the final dose; the remaining animals were euthanized 1 month after the final dose.

The study was conducted at MPI Research (Matawan, MI), according to the United States Department of Agriculture's Animal Welfare Act (9 CFR Parts 1, 2, and 3) and the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, DC, 2011.

2.2. Study agent instillation procedure

Under cystoscopic and fluoroscopic guidance, the left ureteral orifice was cannulated with a guidewire and 7 Fr open-ended ureteral catheter with a glued Luer-lock connector. A retrograde pyelogram was performed to confirm appropriate position of the catheter. Next, a specialized injector device (Fig. 1) was used to instill 7 ml of the study agent over a period of 40 to 70 seconds. After instillation, the ureteral catheter was removed, and the animal was left undisturbed for a minimum of 5 minutes after instillation. The animal was then allowed to recover from the anesthesia. The right ureter and pelvicalyceal system was left untreated and served as an experimental within subject control.