RESEARCH ARTICLE

The Efficacy of Desmopressin and Imipramine in Retaining the Instilled Suspension in the Bladder

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Funding: School of Medicine, Tehran University of Medical Sciences, Tehran, Iran Grant number: 98-3-101-45499

Potential competing interests: No potential competing interests to declare.

Abstract

Objective: To assess the efficacy of desmopressin and imipramine in reducing urination frequency, and retaining the instilled suspension in the bladder without maintaining the mouse under anesthesia.

Method: Twenty-four mice were divided into four groups: (1) control, (2) four-hour water deprivation, (3) two µg/kg intraperitoneal desmopressin, and (4) two µg/kg intraperitoneal desmopressin plus 30 mg/kg imipramine gavage. Micturition frequency was recorded using voiding spot assay. Animals were catheterized and 50 µl of methylene blue was instilled into the bladder. The mice were then placed on a white paper to recover. Blue voiding was documented.

Results: Urination frequency was significantly lower in the desmopressin group. The water-deprived and desmopressin/imipramine-combination groups did not differ significantly compared to control group. All animals voided methylene blue upon recovery.

Conclusion: Desmopressin notably reduced urination frequency. In contrast, water deprivation did not do so. Desmopressin and imipramine failed to cause suspension retention in awake mice.

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Running title: Bladder suspension retention using drugs

Keywords: Desmopressin; imipramine; bladder cancer; animal model.

Introduction

Many urologic diseases and conditions are successfully modeled in mice, including genitourinary cancers[1]. Bladder cancer is the most common cancer of the genitourinary tract and various animal models have been developed to study this disease. One excellent form of animal cancer models is orthotopic xenograft which means the cancer cells develop the tumor in the bladder (orthotopic) and are derived from human origin, either human cell line or patient-derived (xenograft). Orthotopic models provide natural micro-environment of bladder tumor [2][3]. One method of developing these models is to catheterize female mice, irritate bladder epithelium using chemical or physical approaches, and instill cancer cells into the bladder space [4].

After cells are instilled, they need dwelling time to be implanted. Dwelling time depends on factors including; mouse strain, cell line, cell number, and volume of injection. Dwelling time varies from 45 min to 3 hours as reported in different studies. The chemical irritation also requires 15 to 20 minutes of dwelling time [5][6]. So an animal should be maintained under anesthesia for at least 90 minutes. Studies report that repeated administration of Ketamine/ Xylazine, as conventional anesthetic drugs in mice, increases the mortality rate by up to 50% [7].

We hypothesized that we could achieve the needed dwelling time without keeping animals under anesthesia if we reduce urine production and urination frequency. In this study, we used desmopressin acetate, arginine vasopressin analog, to assess the effect of desmopressin on the urination frequency and dwelling time in female BALB/c mice. We also studied the effect of adding imipramine, as a detrusor relaxant and bladder neck constrictor, in retaining the instilled suspension in the bladder.

Material & Methods

"Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed and the study is approved by the ethics committee of the children's medical center, Tehran, Iran. The study comprises 2 parts. In the first part named "Modified void spot assay", we assessed the desmopressin and imipramine effect on urination frequency on the first day. In the second part named "Methylene blue voiding proof", we studied the desmopressin and imipramine effect on dwelling time on the second day.

Animals and Grouping

We used 24 female BALB/c mice aged 6-8 weeks with average weight of 25 grams. Animals were kept in 12/12 hours' light-dark cycle and had free access to food and water until the study day. They were divided into four identical groups including control (group 1), water-deprived (group 2), desmopressin-treated (group 3), and desmopressin/imipramine combination-treated (group 4).

Drug administration and water deprivation

Two micrograms per kilogram (µg/kg) of Desmopressin (DDAVP®) ampule, 4µg per ml concentration, was injected into the peritoneal space of animals in groups 3 and 4, one hour before the examination. Mice in group 4 also received 30mg/kg imipramine through gavage administration. Groups 1 and 2 received intraperitoneal saline injection with same volume as injected desmopressin. Access to water was banned in the second group 4 hours before the examination.

Modified void spot assay study

This examination was performed in the specific low-noise room with the least human interaction to minimize stress and tension in animals. Glass cages were used covered with 13 separate, easily removable white bare papers. Each animal was placed in one cage. Every 15 minutes, the examiner assessed cages for new urination spots and removed one paper layer. Each new spot was recorded as one urination. The study was conducted for 3 hours from 13:30 to 16:30 resulting in 13 reports for each mouse.

Methylene Blue voiding proof test

To assess the efficacy of water deprivation, desmopressin, and imipramine in increasing dwelling time without anesthesia, mice bladders were filled with 50 microliters of 10-time diluted Methylene Blue solution. Animals were anesthetized via intraperitoneal injection of 80 mg/kg Ketamine (10%) and 10 mg/kg Xylazine (2%) cocktail. They were then gently placed on a heating pad in the dorsal recumbent position. Eyes were covered with ophthalmic ointment to avoid corneal damage. The pubic area was cleaned with pivodine-iodine solution. Mice were then catheterized using a 24-gauge intravenous catheter lubricated with lidocaine 2% gel. Methylene Blue was instilled intra-vesically. Animals were then immediately placed in separate cages on bare white paper under warmer.

Statistical analysis

Using the Kolmogorov-Smirnov test, we assessed if the variables are normally distributed. One-way Anova was applied to compare urination frequency count between the study groups. All analyses were done using GraphPad Prism version 9.3.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. P-value less than 0.05 was considered statistically significant.

Results

Modified void spot assay

The Sum of the micturition count of each group is illustrated in figure 1. Table 1 reports how many times each mouse urinated during examination period. Average urination frequency was significantly lower in desmopressin-administered mice compared to control group (5.2±1.2 vs 2.2±1.9 p-value=0.031). There was no significant difference between control and water deprived or imipramine+desmopressin groups (5.2±1.2 vs 4.2±1.7 p-value=0.74 and 3.2±1.9 p-value=0.21, respectively).

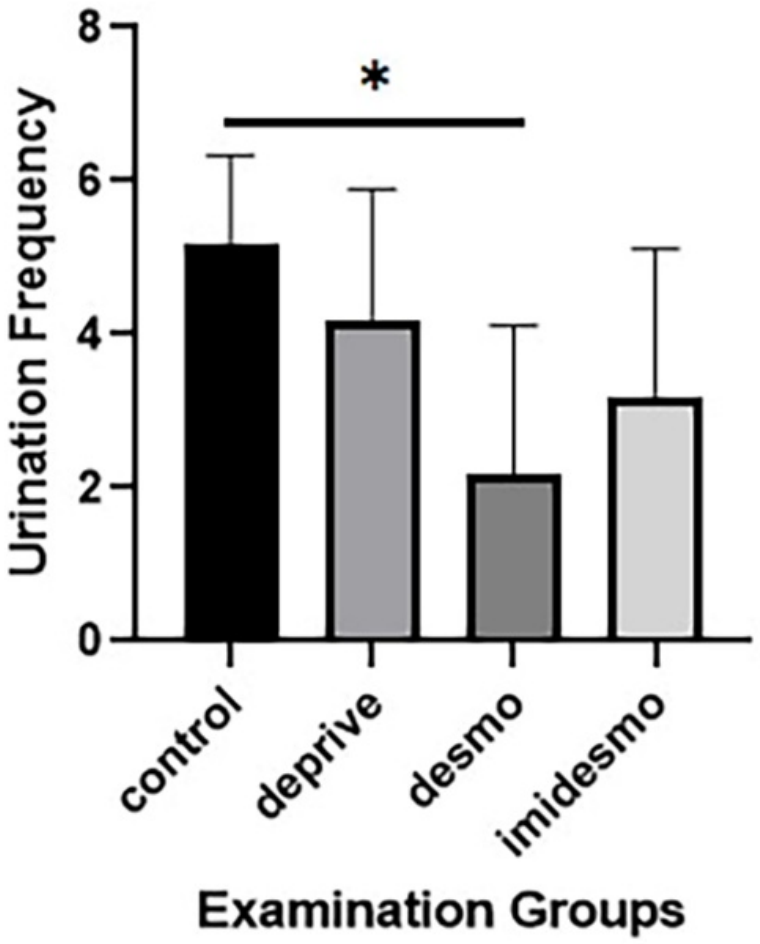

Figure 1. Comparison of urination frequency between study groups. “desmo: Desmopressin; imidesmo: imipramine + desmopressin” *: p-value < 0.05; desmopressin and control were significantly different.

Table 1. Urination frequency of each mouse in each group during 3 hours of examination

	Control	Deprived	Desmopressin	IMP+Des*
mouse 1	6	3	4	4
mouse 2	7	6	5	1
mouse 3	4	3	0	4
mouse 4	5	2	1	1
mouse 5	4	6	2	3
mouse 6	5	5	1	6

**: Imipramine + Desmopressin*

Methylene Blue voiding proof test

All animals, regardless of their groups, urinated immediately after anesthesia effects wore off. Voided methylene blue formed large blue spots on paper. Therefore, awake dwelling time was zero in all study groups.

Discussion

Orthotopic models in bladder cancer are advantageous since they recapitulate the natural microenvironment in which the tumor occurs [8]. Nevertheless, these models require dwelling time following instillation of tumor cell suspension, which necessitates long anesthesia period. Theoretically, if we reduce urine production and/or block bladder outflow, we could achieve the needed dwelling time without keeping the mouse under anesthesia. So, we used desmopressin, which is the synthetic analog of arginine vasopressin, known as anti-diuretic hormone to reduce urine production. Also, we used imipramine to block bladder outflow. In the present study we showed that desmopressin decreases urination frequency; however, does not affect the dwelling time following anesthesia and is not useful for animal models that require retaining of a suspension within the bladder. Adding imipramine does not affect awake dwelling time as well.

We administered desmopressin and imipramine at dosage of 2 µg/kg and 30 mg/kg, respectively, according to literature data [9][10]. Although anti tumorigenesis effects are mentioned for desmopressin, this effect is reported after repeated intravenous administration following tumor formation. Therefore, we presume that single dose administration of desmopressin prior to tumor cells instillation does not affect tumor formation.

Although desmopressin administration significantly reduced average urination frequency (5.2 to 2.2, p-value < 0.05), voiding of all the animals during methylene blue test revealed that desmopressin cannot be effectively used to induce tumor without keeping the mouse under anesthesia. We administered 30 mg/kg imipramine orally to relax detrusor and constrict bladder neck so as to prevent bladder emptying. However, all six animals who received desmopressin and imipramine and underwent methylene blue instillation, urinated upon waking up as well. We presume the higher urination frequency in group 4 for in comparison to group 3, is due to the water introduced during imipramine oral administration.

Water deprivation for 4 hours did not affect frequency significantly (5.2 to 4.2, p-value > 0.05), hence the author recommends ad libitum access to water until surgery time.

This study has some limitations. We did not quantify urine volume and voided methylene blue. Hence, the possibility that little amount of the instilled solution remains inside bladder cannot be ruled out. However, the chance of tumor formation decreases considerably. Also limited number of studied animals reduces the power of the study. Moreover, the injectable form of anti-cholinergic drugs was not available and imipramine was administered with water that consequently may increase urine volume and frequency. This is the first study that evaluates the use of desmopressin and imipramine in increasing dwelling time following bladder instillation of tumor cells. Future studies should focus on more potent agents as well injectable forms of medications that do not require fluid intake.

Conclusion

Intraperitoneal desmopressin injection at dosage of 2 µg/kg significantly reduces urination frequency in mice. In contrast, water deprivation for 4 hours does not affect frequency notably. In conclusion, desmopressin and anticholinergic drugs do not seem to be an effective way of inducing retention.

Statements and Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the “Guideline for the Care and Use of Laboratory Animals in Iran” and under approval of Research Ethics Committees of School of Medicine, Tehran University of Medical Sciences (Approval ID: IR.TUMS.MEDICINE.REC.1398.923). All animal experiments were conducted following the national guidelines and the relevant national laws on the protection of animals.

Availability of data and materials

All data are presented in the manuscript.

Competing interests

All authors declare that they do not have any competing interest.

Funding

School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Grant number: 98-3-101-45499

Authors' contributions

Masoud Bitaraf: Study design and performance, Writing and final revision of the manuscript

Saman Behboodi Tanourlouee: Study performance, Writing primary draft of the manuscript

Erfan Amini, Samad Muhammadnejad, and Masoumeh Majidi Zolbin: Consult on study design, Approval of the final manuscript

Ashkan Azimzadeh: Study performance

Abdol-Mohammad Kajbafzadeh: Study design, Project supervision, Approval of the final manuscript

Acknowledgements

Dr Negar Toroghi for guidance on drug dosage and administration.

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