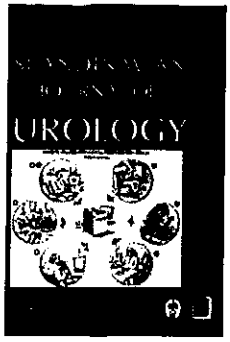
学位論文

Hyperthermic Therapy Using Warm Sterile Water Enhances Cytocidal Effects on Bladder Cancer Cells

香川大学大学院医学系研究科

医学専攻

松岡祐貴



Scandinavian Journal of Urology

ISSN: 2168-1805 (Print) 2168-1813 (Online) Journal homepage: https://www.tandfonline.com/loi/isju20

Hyperthermic therapy using warm sterile water enhances cytocidal effects on bladder cancer cells

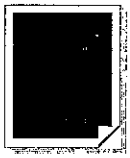
Yuki Matsuoka, Rikiya Taoka, Zhang Xia, Mikio Sugimoto & Yoshiyuki Kakehi

To cite this article: Yuki Matsuoka, Rikiya Taoka, Zhang Xia, Mikio Sugimoto & Yoshiyuki Kakehi (2020): Hyperthermic therapy using warm sterile water enhances cytocidal effects on bladder cancer cells, Scandinavian Journal of Urology, DOI: 10.1080/21681805.2019.1708967

To link to this article: https://doi.org/10.1080/21681805.2019.1708967

Published online: 13 Jan 2020.

SCANDINAVIAN JOURNAL OF UROLOGY
https://doi.org/10.1080/21681805.2019.1708967



Taylor & Francis
Taylor & Francis Group

ARTICLE

Hyperthermic therapy using warm sterile water enhances cytocidal effects on bladder cancer cells

Yuki Matsuoka, Rikiya Taoka, Zhang Xia, Mikio Sugimoto and Yoshiyuki Kakehi

Faculty of Medicine, Department of Urology, Kagawa University, Kagawa, Japan

ABSTRACT

Objectives: To investigate whether warm sterile water enhances the cytocidal effect of hypotonic shock on bladder cancer cells that show resistance to sterile water.

Methods: Four bladder cancer cell lines of varying grades (T24, RT4, J82, and RT112) were exposed to sterile water, and morphological changes were closely observed under microscopy. Changes in cell membrane integrity and cell viability after water exposure were measured to determine the effects of water-induced hypotonic shock. Additionally, the effects of warm sterile water were analyzed.

Results: T24, RT4, and J82 cells started swelling immediately upon exposure to water, followed by rupture within five minutes. RT112 cells demonstrated limited hypotonic swelling with significantly less cell rupture after 10 min. The percentages of viable cells at 10 min were 1.6 ± 0.8%, 3.5 ± 3.5%, 5.0 ± 3.2%, and 22.0 ± 10.3% for T24, RT4, J82, and RT112, respectively. The percentage of viable cells with 48 °C sterile water at one minute was 0% for RT112 cells.

Conclusions: These findings support the efficacy of sterile water against bladder cancer cells and reveal that warm sterile water enhances the cytocidal effects of hypotonic shock, potentially avoiding the need for radical surgery.

ARTICLE HISTORY
Received 11 May 2019
Revised 29 November 2019
Accepted 20 December 2019

KEYWORD
S: Bladder cancer; hypotonic shock; cytocidal; hyperthermic therapy

Introduction

Bladder cancer is the ninth most common cancer in the world, with 390,000 new cases diagnosed and 150,000 deaths each year [1]. Nearly three out of four patients diagnosed have non-muscle-invasive bladder cancer (NMIBC). During the course of NMIBC, as many as 50% of cases will recur, and in 9% of them, the cancer will invade the *muscularis propria* [2]. Radical cystectomy is the main-stayof therapy for muscle-invasive bladder cancer (MIBC). Despite well-performed surgery, cure rates with surgery alone are 66–80% [3,4]. Because of frequent recurrence and progression, bladder cancer is not only an epidemiological but also an economic problem. Therefore, preventing progression to MIBC is critical in the management of NMIBC.

Treatment of NMIBC includes transurethral bladder tumor resection (TURBT) with the use of adjuvant intravesical treatments. Adjuvant treatment after TURBT is generally determined by categorization of patients into risk groups that combine pathological and clinical features [5]. The standard method of adjuvant treatment is intravesical therapy using chemotherapeutic agents such as mitomycin C (MMC) and Bacillus Calmette-Guérin (BCG) vaccine. However, such intravesical therapies have some drawbacks. Although there is clear evidence for intravesical MMC chemotherapy in patients with low- and intermediate-risk tumors [6], patients with high-risk tumors do not appear to achieve the same benefit [7]. In addition, in all single instillation studies, the instillation was administered within 24 h. To maximize the efficacy of single instillation, it is important to devise flexible practices that allow the instillation to be given as soon as possible after TURBT. As severe complications have been reported in patients with drug extravasation [8]. European Association of Urology guidelines state that safety measures should be maintained [9]. BCG is superior to intravesical MMC chemotherapy in terms of recurrence, accounting for a 32% reduction in recurrence rates with BCG compared to that with MMC. Despite being an effective drug, 20–40% of patients undergoing BCG treatment experience recurrence [10], and around 20% of these patients progress to a muscle-invasive state [11]. Additionally, intolerance rates to BCG are as high as 70% [12]. Therefore, a new intravesical therapy with high effectiveness and low toxicity is required.

Prior research studies in colorectal and hepatocellular carcinoma have demonstrated results in regard to the cytolytic efficacy of intraperitoneal lavage with sterile water [13,14]. Our previous study was specifically intended to study the time-course of events in bladder cancer cells after exposure to sterile water. The effect of exposure to sterile water is time-dependent, with increased tumor cell lysis observed with prolonged exposure. Although these findings suggest that sterile water may be a new agent of intravesical therapy, bladder cancer cell lines differ in their sensitivity to hypotonic shock with sterile water [15]. Therefore, increased

CONTACT Rikiya Taoka rikiya@med.kagawa-u.ac.jp Faculty of Medicine, Department of Urology, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

analysis of the cytocidal effects of hypotonic shock are needed for successful clinical translation.

Hyperthermia is thought to have several beneficial effects on the treatment of bladder cancer. An additive anti-tumor effect of hyperthermia with select cytostatic drugs has been reported [16]. The aim of present study was to investigate the effect of hypotonic shock with warm sterile water on bladder cancer cells.

Material and methods

Cell culture and preparation

Human bladder cancer cell lines T24, RT4, J82, and RT112 were obtained from the American Type Culture Collection. We selected two low-grade cell lines (RT4 and RT112) and two high-grade cell lines (T24 and J82) for this study. These cell lines were maintained as adherent cells at 37 °C, 5% CO_2 in a humidified atmosphere in RPMI-1640 (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA), HEPES solution (Sigma) and penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA, USA).

Observation of morphological changes in bladder cancer cells after exposure to sterile water

After culturing bladder cancer cells in T75 flasks, the medium was completely removed from the flasks and sterile water was added. The flasks were mounted on the stage of a KEYENCE BZ-9000 All-in-One Fluorescence Microscope (Osaka, Japan), and serial changes in bladder cancer cells were recorded at 0, 1, 3, 5, and 10 min.

Evaluation of cell viability

Cells were plated in 96-well plates at 5×10^3 cells/well. After 24 h of incubation, these cells were exposed to 37–48 °C sterile water for 1, 3, 5, or 10 min after which the water was exchanged for medium. In the control group, the medium was renewed at the same time. After 24 h, cell viability was assessed by pulsing the cells for two hours with dimethyl thiazolyl diphenyl tetrazolium (MTT) salt (5 mg/mL in PBS), followed by solubilization of formazan crystals in 100 µL of lysis buffer containing 20% sodium dodecyl sulfate and 50% dimethylformamide. Color development was quantified by measuring the optical density at 570 nm. All measurements were repeated in quadruplicate, and the results are shown as mean ± SEM.

Evaluation of intact cell membranes

Bladder cancer cells were detached from culture flasks by a trypsin-EDTA solution and centrifuged. The pellets were suspended in 5 mL medium, and the cell suspension was divided into five tubes. After centrifugation, the pelleted cells were re-suspended in sterile water and incubated for 1, 3, 5, or 10 min. The suspending solution was displaced into LUNA-II™ (Logos Biosystems, Gyeonggi-do, South Korea) and the percentage of intact cell membranes in bladder cancer cells was determined by the trypan blue-exclusion method. All analyses were performed in triplicate, and the results are shown as mean ± SEM.

Results

Morphological changes were observed in all four bladder cancer cell lines after exposure to 37 °C sterile water (Figure 1). T24, RT4, and J82 cells started swelling immediately upon exposure to sterile water and ruptured within five minutes. RT112 cells demonstrated limited hypotonic swelling with few cell ruptures within 10 min.

The viability of bladder cancer cells was observed after exposure to 37 °C sterile water by MTT assay (Figure 2). The viability of all four bladder cancer cell lines decreased after exposure to sterile water. The percentages of viable cells at 10 min were 6.6 ± 0.8%, 0.8 ± 0.4%, 3.6 ± 0.3%, and 20.2 ± 0.2% for T24, RT4, J82, and RT112 cells, respectively.

In the trypan blue-exclusion assay (Figure 3), live cells with intact cell membranes were not stained. We determined the percentage of live cells with intact cell membranes by counting non-stained cells after exposure to sterile water. T24, RT4, and J82 cells exhibited similar decreases in cell viability at 10 min. The percentages of viable cells at 10 min were 1.6 ± 0.8%, 3.5 ± 3.5% and 5.0 ± 3.2% for T24, RT4, and J82 cells, respectively. In contrast, that of RT112 cells was 22.0 ± 10.3%.

Additionally, we evaluated the cell viability of RT112 cells after exposure to 40 °C, 44 °C, and 48 °C sterile water by MTT assay (Figure 4). The percentage of live cells decreased as the temperature increased. The percentage of viable RT112 cells treated with 48 °C sterile water at one minute was 0%, although viability was not reduced after exposure to 48 °C medium.

Discussion

We revealed the cytocidal effect of warm sterile water on bladder cancer cells that are resistant to 37 °C sterile water. Hyperthermic intravesical therapy using sterile water may thus be a feasible treatment option in NMIBC patients, potentially avoiding the need for radical surgery.

A common practice among oncologic surgeons is to irrigate the surgical field after surgical extirpation with the goal of potentially diluting and lysing viable tumor cells that might be free floating. Iitaka et al [17] investigated the role and mechanism of sterile water peritoneal lavage in three human gastric cancer cell lines. In their study, they found a rapid increase in cell volume followed by cell rupture after the cells were exposed to sterile water. Our previous study revealed the cytocidal effects of sterile water on bladder cancer cells [15]. However, prior bladder cancer research has demonstrated conflicting evidence regarding the role of intravesical therapy with sterile water. Sakai et al. [18] investigated whether irrigation with a large amount of sterile water prevented recurrence of superficial bladder cancer in the clinical setting. There were no significant differences in the

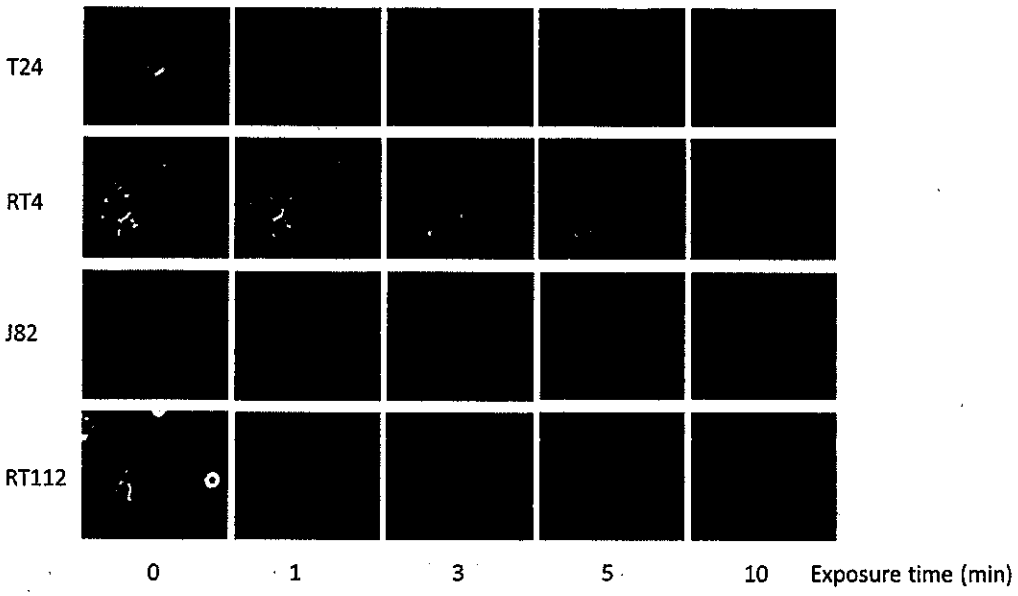


Figure 1. Representative images of T24, RT4, J82, and RT112 cells before and after exposure to 37 °C sterile water. T24, RT4, and J82 cells started swelling immediately upon exposure to sterile water and ruptured within five minutes. RT112 cells demonstrated limited hypotonic swelling with few cell ruptures within 10 min.

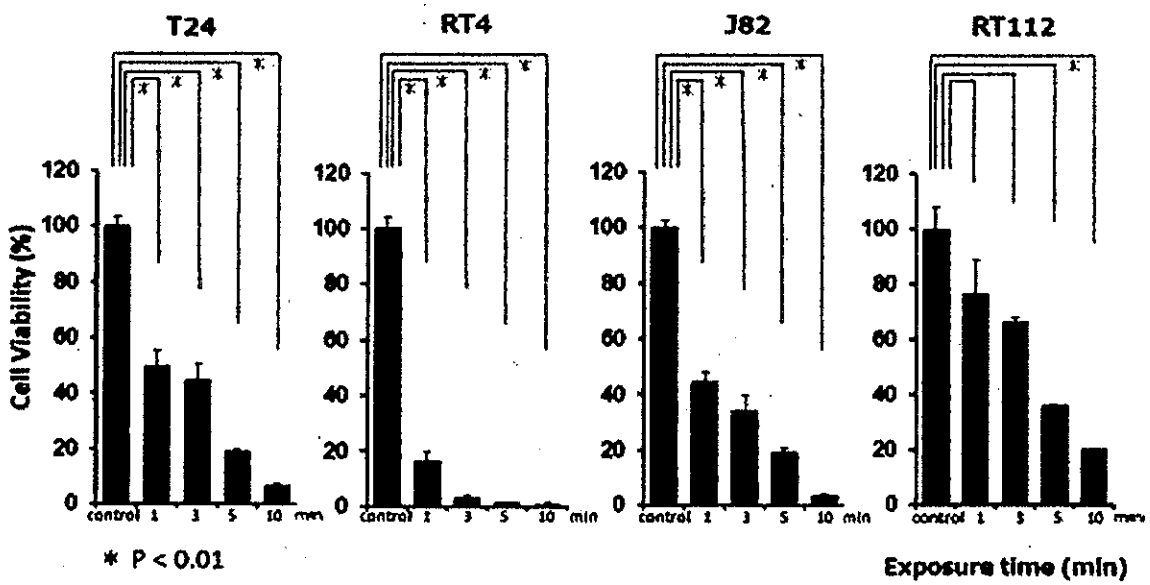


Figure 2. Cell viability of bladder cancer cells after exposure to 37 °C sterile water. The viability of all four bladder cancer cell lines started to decrease after exposure to sterile water.

one- and two-year recurrence-free rates between patients undergoing sterile water irrigation and control patients. We hypothesize that this may be because bladder cancer cells showing resistance to sterile water were present. In our previous study, the observed cytocidal effect was different for each bladder cancer cell line. Thus, simply irrigating with sterile water may not be effective for successful clinical translation.

Hyperthermia is not a novel bladder cancer treatment. In 1992 Matzkin et al. [19] performed an *in vitro* study in which hyperthermia was used to treat superficial bladder cancer cells. They concluded that the cytocidal effects of hyperthermia on bladder cancer cells were variable and limited. Furthermore, they concluded that experiments using a combination of hyperthermia and intravesical anti-cancer agents were needed. Since then, the effects of hyperthermia and intravesical chemotherapy have been reported in several clinical studies [20,21] for the treatment of superficial bladder cancer. Hyperthermia increases the efficacy of treatment with several cytotoxic agents [22]. The aim of present study was therefore to investigate whether hyperthermia enhances the effect of sterile water on bladder cancer cells.

First, we confirmed the cytocidal effect of hypotonic shock induced by 37 °C sterile water on bladder cancer cells. All four bladder cancer cell lines showed cell swelling followed by cell rupture after exposure to sterile water. T24, RT4, and J82 cells started swelling immediately upon exposure to sterile water and ruptured within five minutes. RT112 cells demonstrated limited hypotonic swelling with limited cell ruptures. MTT and trypan blue-exclusion assays showed similar results. The duration of exposure to sterile water required to lyse T24, RT4, and J82 cells was within 10 min. However,

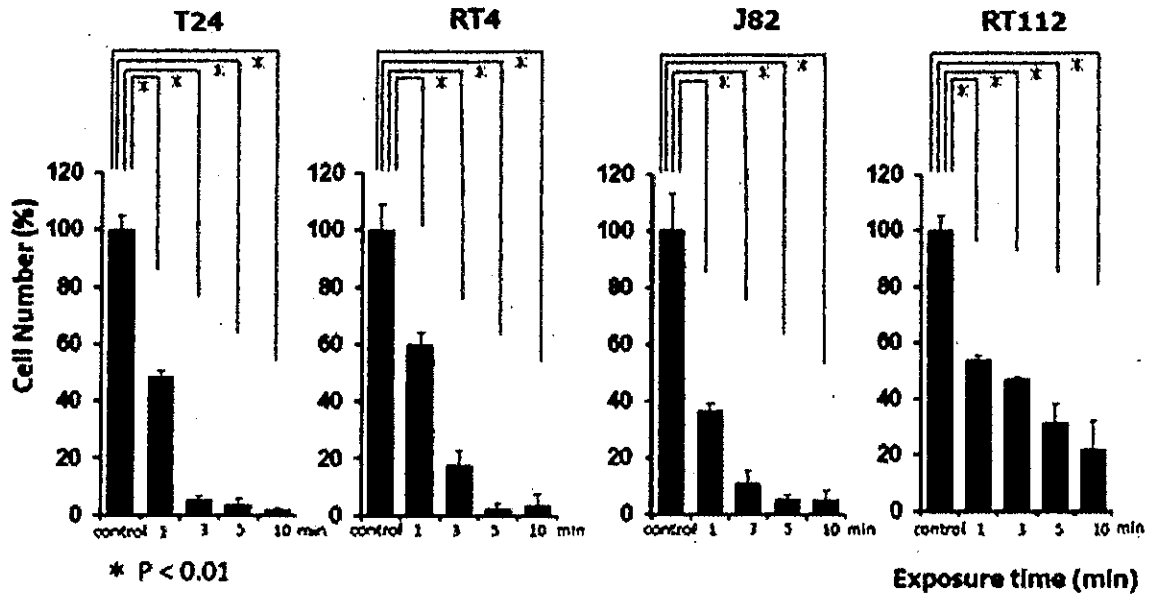


Figure 3. Intact cell membranes of bladder cancer cells after exposure to 37 °C sterile water. The number of intact membranes of T24, RT4, and J82 cells decreased more rapidly upon exposure to sterile water than that of RT112 cells.

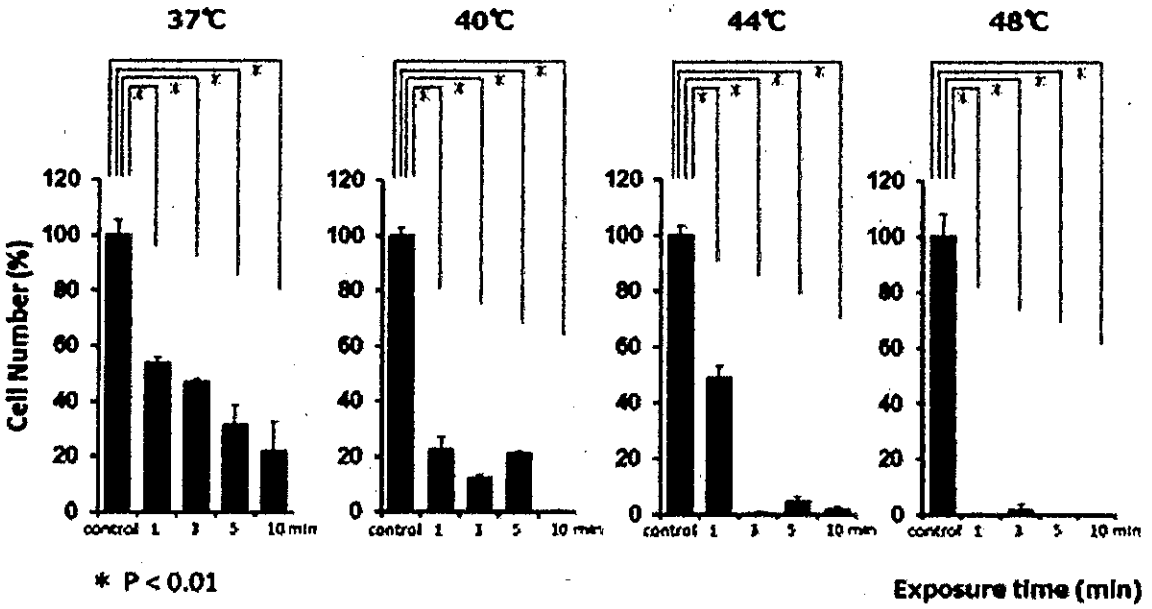


Figure 4. Intact cell membranes of RT112 cells after exposure to warm sterile water. The percentages of live cells decreased as the temperature increased.

RT112 cells required more than 10 min. T24, RT4, and J82 cells thus appeared to be sensitive to sterile water. In contrast, RT112 cells may require a longer duration of exposure to sterile water to observe cytocidal effects. The high sensitivity of T24 cells is contrary to the finding of the previous study [15]. The reason for this discrepancy is unknown, but the states of cell lines may be different. Potentially, the strength of the cytoskeleton or cell membrane or the expression of chloride channels may differ among bladder cancer cell lines of different grades, as seen in other malignancies [23]. Sterile water monotherapy may therefore be insufficient for RT112 cells.

Secondly, we investigated the effect of hypotonic shock with warm sterile water on bladder cancer cells. We evaluated the cell viability of RT112 cells after exposure to 40 °C, 44 °C, and 48 °C sterile water. The percentage of live cells decreased as the temperature increased. Damage to all four bladder cancer cell lines was evident at one minute after exposure to 48 °C sterile water. RT112 cells demonstrated hypotonic swelling immediately, with many cell ruptures within 10 min. This may be because the movement of water molecules is proportional to the temperature.

The results of our present study provide experimental evidence that warm sterile water has hypotonic shock-induced cytocidal effects on cultured bladder cancer cells showing resistance to 37 °C sterile water. The biggest potential use for this treatment is early post-operative instillation, as floating tumor cells exhibit the greatest contact area with sterile water. We believe that single bladder irrigation directly after TURBT is the most efficient method. Regarding the toxicity of

sterile water, a prior research study reported that bladder irrigation with sterile water resulted in fewer adverse effects than intravesical MMC [24]. However, while higher temperatures may be more beneficial, they may also irreversibly damage the normal urothelium and bladder wall. No patients had CTCAE grade IV or V adverse events in a cohort study with hyperthermic intravesical chemotherapy [25]. In addition, sterile water is readily available and relatively inexpensive. Hyperthermic therapy using warm sterile water for bladder cancer may represent a new method with high effectiveness and low toxicity. In order to introduce this procedure to the clinic, we are planning to conduct a pilot study of single bladder irrigation with warm sterile water for 30 min after TURBT.

The major limitation of the present study is that our findings must be interpreted in the context of the study design: our data were generated from cultured bladder cancer cell lines of different grades in an *in vitro* setting. In the clinical setting, inherent characteristics of patients' cancer cells and immunology likely play significant roles in the survival of cancer cells, among other processes. Even with these caveats, the advantage of this approach with its low cost and low toxicity has the potential to lead to breakthrough treatment for patients with NMIBC.

Conclusions

Warm sterile water enhances the cytocidal effects of hypotonic shock. These findings suggest that hyperthermic intravesical therapy with sterile water may represent a new adjuvant therapy after TURBT with high effectiveness and low toxicity and may be a feasible option for treatment of NMIBC patients, potentially avoiding the need for radical surgery.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol. 2017;71:96–108.

[2] Liedberg F, Hagberg O, Holmang S, et al. Local recurrence and progression of non-muscle-invasive bladder cancer in Sweden: a population-based follow-up study. Scand J Urol. 2015;49:290–295.

[3] Zehnder P, Studer UE, Skinner EC, et al. Unaltered oncological outcomes of radical cystectomy with extended lymphadenectomy over three decades. BJU Int. 2013;112:E51–E58.

[4] Mitra AP, Quinn DI, Dorff TB, et al. Factors influencing post-recurrence survival in bladder cancer following radical cystectomy. BJU Int. 2012;109:846–854.

[5] Burger M, Catto JW, Dalbagni G, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol. 2013;63:234–241.

[6] Perlis N, Zlotta AR, Beyene J, et al. Immediate post-transurethral resection of bladder tumor intravesical chemotherapy prevents non-muscle-invasive bladder cancer recurrences: an updated meta-analysis on 2548 patients and quality-of-evidence review. Eur Urol. 2013;64:421–430.

[7] Sylvester RJ, Oosterlinck W, Holmang S, et al. Systematic review and individual patient data meta-analysis of randomized trials comparing a single immediate instillation of chemotherapy after transurethral resection with transurethral resection alone in patients with stage pTa-pT1 urothelial carcinoma of the bladder: which patients benefit from the instillation? Eur Urol. 2016;69:231–244.

[8] Oddens JR, van der Meijden AP, Sylvester R, et al. One immediate postoperative instillation of chemotherapy in low risk Ta, T1 bladder cancer patients. Is it always safe? Eur Urol. 2004;46:336–338.

[9] Guidelines on Non-muscle-invasive Bladder Cancer [Internet]. The Netherlands: European Association of Urology (EAU); 2019. Available from: https://uroweb.org/guideline/non-muscle-invasive-bladder-cancer/

[10] Catalona WJ, Hudson MA, Gillen DP, et al. Risks and benefits of repeated courses of intravesical bacillus Calmette-Guerin therapy for superficial bladder cancer. J Urol. 1987;137:220–224.

[11] Sanli O, Dobruch J, Knowles MA, et al. Bladder cancer. Nat Rev Dis Primers. 2017;3:17022.

[12] Brausi M, Oddens J, Sylvester R, et al. Side effects of Bacillus Calmette-Guerin (BCG) in the treatment of intermediate- and high-risk Ta, T1 papillary carcinoma of the bladder: results of the EORTC genito-urinary cancers group randomized phase 3 study comparing one-third dose with full dose and 1 year with 3 years of maintenance BCG. Eur Urol. 2014;65:69–76.

[13] Ito F, Camoriano M, Seshadri M, et al. Water: a simple solution for tumor spillage. Ann Surg Oncol. 2011;18:2357–2363.

[14] Lin CH, Hsieh HF, Yu JC, et al. Peritoneal lavage with distilled water during liver resection in patients with spontaneously ruptured hepatocellular carcinomas. J Surg Oncol. 2006;94:255–256.

[15] Taoka R, Williams SB, Ho PL, et al. In-vitro cytocidal effect of water on bladder cancer cells: the potential role for intraperitoneal lavage during radical cystectomy. Can Urol Assoc J. 2015;9:109–113.

[16] Hahn GM. Potential for therapy of drugs and hyperthermia. Cancer Res. 1979;39:2264–2268.

[17] Iitaka D, Shiozaki A, Ichikawa D, et al. Blockade of chloride ion transport enhances the cytocidal effect of hypotonic solution in gastric cancer cells. J Surg Res. 2012;176:524–534.

[18] Sakai Y, Fujii Y, Hyochi N, et al. A large amount of distilled water ineffective for prevention of bladder cancer cell implantation at the time of transurethral resection. Hinyokika Kiyo. 2006;52:173–175.

[19] Mstzkin H, Rangel MC, Soloway MS. In vitro study of the effect of hyperthermia on normal bladder cell line and on five different transitional cell carcinoma cell lines. J Urol. 1992;147:1671.

[20] van der Heijden AG, Verhaegh G, Jansen CF, et al. Effect of hyperthermia on the cytotoxicity of 4 chemotherapeutic agents currently used for the treatment of transitional cell carcinoma of the bladder: an in vitro study. J Urol. 2005;173:1375–1380.

[21] Lammers RJ, Witjes JA, Inman BA, et al. The role of a combined regimen with intravesical chemotherapy and hyperthermia in the management of non-muscle-invasive bladder cancer: a systematic review. Eur Urol. 2011;60:81–93.

[22] Hahn GM, Li GC. Interactions of hyperthermia and drugs: treatments and probes. Natl Cancer Inst Monogr. 1982;61:317–323.

[23] Kosuga T, Shiozaki A, Ichikawa D, et al. Pleural lavage with distilled water during surgery for esophageal squamous cell carcinoma. Oncol Rep. 2011;26:577–586.

[24] Bijalwan P, Pooleri GK, Thomas A. Comparison of sterile water irrigation versus intravesical mitomycin C in preventing recurrence of nonmuscle invasive bladder cancer after transurethral resection. Indian J Urol. 2017;33:144–148.

[25] de Jong JJ, Hendricksen K, Rosier M, et al. Hyperthermic intravesical chemotherapy for BCG unresponsive non-muscle invasive bladder cancer patients. Bladder Cancer. 2018;4:395–401.