How long does it take to reduce the risk of spillage
TCC implantation by distilled water peritoneal
irrigation to complete cytolysis?

Umaphorn Nuanthaisong* Kongpaphop Panya**
Supoj Ratchanon* Wipawee Kittikowit**


Objective : Tumor cells spillage from bladder during radical cystectomy increases the risks of seeding in the peritoneum. The current therapy is irrigation with sterile water as rare of strong supportive investigation and unknown extent of clinical benefit. We investigated rate of tumor cell survival after cells immerge in normal saline compare to distilled water. Additionally, we studied in time for near complete osmolytic cytolysis effect of tumor cells.

Study design : Repeated Measures design
Setting : Department of Urology and Department of Pathology, King Chulalongkorn Memorial Hospital
Materials and Methods : Seventy - six specimens from 9 patients who underwent to perform radical cystectomy due to invasive TCC of bladder and bladder biopsy of recurrent TCC were recruited to the study. The subjects were recruited from August 2008 to January 2009. Multiple small pieces of tumor from 9 patients (6 - 12 specimens per patient)
were separated to immerge and oscillate in NSS and distilled water for 5 min, 10 min and 15 min, respectively. Then each treated sample was sent for cytological reports (8 - 20 fields/slide) from one pathologist to report tumor survival rate. Additionally, we investigated the rate of tumor cytolysis in every 2.5 minute after the specimens were immersed in distilled water for 15 minute from 2 patients.

**Results**

Distilled water irrigation showed significant decrease the rate of tumor cells survival (p < 0.001). After 15 minutes, most cell viability decreased to approximately 20%.

**Conclusion**

Intraperitoneal distilled water irrigation after radical cystectomy decrease TCC viable cell spillage cells and it is assumed to decrease the rate of intraperitoneal spillage tumor implantation. Peritoneal irrigation with sterile water for at least 15 minutes after radical cystectomy for 15 minutes decreases spillage survival tumor to approximately 20%.

**Keywords**

Transitional cell carcinoma, Tumor cell seeding, Osmolysis, Sterile water, Cytolysis.
อุมาพร นวลโพรง, คงภพ ปัญญา, สุพจน์ รัชชานนท์, วิภา ภิศบดีกิจวิ. การแสดงความเสี่ยงการสัมผัสช่องทางและเริ่มรักษาแบบมีประสิทธิภาพ transitional cell carcinoma ผ่านวิธีการทำให้เซลล์แตกตัวจากน้ำเปล่า. จุฬาลงกรณ์เวชสาร 2552 ก.ย. – ต.ค.; 53(5): 397 – 403

วัตถุประสงค์ : เพื่อศึกษาประสิทธิผลของน้ำเปล่าในการทำให้เซลล์มะเร็งแตกตัว ณ เวลาก่อนต่างๆ กัน

รูปแบบการวิจัย : การศึกษาชี้ขาดโดย ชนิดมีกลุ่มควบคุม และกลุ่มทดลองโดยใช้สิ่งแวดล้อมที่แตกต่างกันในระดับการต่างกัน แล้ววัดผล ณ เวลาก่อนต่างๆ กันหลายครั้ง

สถานที่ทำการวิจัย : ห้องศูนย์การทางเดินปัสสาวะและวิทยาทักษะ โรงพยาบาลจุฬาลงกรณ์

วิธีการศึกษา : ทำการศึกษา 76 ตัวอย่างเซลล์กระชับของผู้ป่วยมะเร็งระยะเฉพาะปัสสาวะชนิด transitional cell carcinoma จำนวน 9 คน โดย 7 คนมีอาการมาจากระยะเฉพาะ ปัสสาวะระยะแยก และสองคนหนึ่งมีอาการจากผู้ป่วยที่ถูกแบ่งเป็น 2 กลุ่ม น้ำเปล่าในน้ำเกลือ (0.9%) และน้ำเปล่าเป็นน้ำทะเล คือ 5 นาที, 10 นาทีและ 15 นาที หลังจากนั้นจะน้ำมันศึกษาทางเดินปัสสาวะเพื่อนับสิ่งผสมของเซลล์ที่บริเวณจุดตะกอน และเซลล์ที่อยู่ในสภาพดีอยู่ และน้ำทะเล สองกลุ่มจะมีการเปลี่ยนแปลงกัน นอกจากนี้ได้ศึกษาเกี่ยวกับมัยการแตกตัวของเซลล์มะเร็งในน้ำเปล่าที่เวลาต่างๆ คือ 0 ถึง 15 นาที

ผลการศึกษา : สังเกตการณ์ระดับต่ำของเซลล์มะเร็งที่ถูกแช่ในน้ำเปล่ามากกว่าที่แช่ในน้ำเกลือ อย่างมีนัยสำคัญทางสถิติ (p < 0.001) และพบว่าการแช่เซลล์ไว้น้ำเปล่าประมาณ 15 นาทีสามารถทำให้เซลล์มะเร็งที่อยู่ในสภาพดีอยู่เป็นน้ำเปล่าประมาณ 20% โดยประมาณ

สรุป : การสัมผัสของท้องด้านบนทำให้เซลล์มะเร็งแยกตัวออกจากเฉพาะของเซลล์มะเร็งที่ของด้านในของเซลล์มะเร็งที่อยู่ในสภาพดีอยู่แล้ว โดยที่สัมผัสท้องด้านบนของเซลล์มะเร็งที่มีนัยสำคัญทางสถิติ (p < 0.001) และสามารถทำให้เซลล์มะเร็งที่อยู่ในสภาพดีอยู่เป็นน้ำเปล่าประมาณ 20% โดยประมาณ

คำสำคัญ : มะเร็งระยะเฉพาะปัสสาวะ, Transitional cell carcinoma, การสัมผัสช่องทางเซลล์มะเร็ง, การวิเคราะห์, การปรับและแตกตัวของเซลล์, น้ำเปล่า, น้ำเกลือ.
Radical cystectomy is the gold standard treatment of invasive transitional cell carcinoma (TCC). During the procedure, however, tumor spillage may occur due to necrotic tumor or technique error. In either situation tumor cells spillage from bladder during cystectomy increase the risk of seeding into the peritoneal cavity. A common practice in the setting of this potentially lethal complication is irrigation of the abdomen with copious amount of sterile water. Cytotoxic effects of distilled water are mainly cause by osmotic cytolysis. Almost twenty years ago, bladder cancer growth inhibition by distilled water was described for the first time. The incidence of TCC reimplantation is not shown exactly because it is difficult to identify between tumor recurrent and spillage tumor reimplantation. However, evidences of transitional cell carcinoma reimplantation has been mentioned in many previous studies while there is insufficient evidence to prove that distilled water impairing bladder cancer reimplantation by interaction with extracellular matrix. Therefore, we investigated in the efficacy of this unproved strategy.

Materials and Methods

Seventy-six specimens from 9 patients who underwent to perform radical cystectomy due to invasive TCC of the bladder and bladder biopsy of recurrent TCC (biopsy under normal saline before transurethral resection tumor was done) were recruited to the study from August 2008 to January 2009.

Multiple small pieces of the tumor were collected immediately after being removed from the patient. Grossly viable pieces of the tumor tissue, approximately 0.5 cm in the greatest dimension were retrieved. The specimens were separated to immerse and oscillate in sterile water and 0.9% sodium chloride solution for 5, 10, and 15 minutes, respectively. The treated specimens were smeared onto the slides in the same manner that the time limit was reached. (Additional slides at 10 seconds, 2.5, 7.5, 12.5 minutes in sterile water were done on specimen No. 8 and 9). All slides were quickly fixed in 95% alcohol and stained with Papanicolaou staining within 12 hours after fixation.

Protocol for cell counting

Every slide was scanned for validity evaluation. Hypoosmotic - affected cancer cells were defined as cancer cells with nuclear and/or cytoplasmic swelling, loss of chromatin detail and nuclear and/or cellular leakage. Cells were count in 8 to 20 HPFs per slide by a pathologist (KP), under the supervision of another superiorly - experienced pathologist (WK). Only clusters of cancer cells with monolayer distribution were counted. Air-dried cells and crushed cells were avoided. The ratio and percentage of hypoosmotic - affected cells and total viable tumor cells were calculated.

The data were computed by Stata Ver.10; pair t-test analysis was done. And $p < 0.05$ was set for statistic significance.

Result

The average age of the subjects was 57 years old. There were 8 males and 1 female. Seven of the nine patients underwent radical cystectomy due to high grade invasive TCC. Two patients received transurethral resection of recurrent bladder tumors and the pathologic reports showed that one was low grade TCC and the other was high grade TCC.
The cytological study and interpretations are shown as figure 1 and figure 2. Hypoosmolytic effect on tumor cells after being exposed to sterile water is hereby shown.

Figure 3 shows comparison of tumor cell survival after being treated with normal saline (NSS) and sterile water. The study shows significant decrease of tumor cell survival by osmotic cytolysis effect of sterile water irrigation at 5 minutes ($\rho < 0.001$), 10 minutes ($\rho < 0.001$) and 15 minutes ($\rho < 0.001$).

Figure 4 shows the rate of tumor cell osmolyisis which depends on time that is exposed to sterile water. At 2.5 minutes after being exposed to sterile water, there are only 2.92% (97.08% viable cells) were osmolyzed. However, after 15 minutes of sterile water exposure, viable tumor cells were decreased to 23.53%.

**Figure 1.** A cluster of urothelial carcinoma cells. These cells have enlarged nuclei, nuclear irregularity and coarse chromatin.

**Figure 2.** Urothelial cell with hypoosmotic effect (*). This cell contains swelling nucleus which has lost its chromatin detail, compared to the nearby cells. Notice the nuclear leakage (black arrow).

**Figure 3.** Comparison of tumor cell survival after exposure to NSS and sterile water (mean ± SD). Significant $\rho < 0.001$.
**Discussion**

Urothelial carcinomas have variable cytological appearance depending on the tumor grade. However, common features which can be seen on cytology specimen are increased cellularity, nuclear enlargement and coarse chromatin.\(^{(7,8)}\) Osmotic lysis of such cells has not ever been established in any textbooks or studies. Therefore, the characteristics of the mammary cancer cells that undergo osmotic lysis by mean of ultrasound transmission jelly, including nuclear and cytoplasmic swelling and leakage, loss of chromatin detail and cell explosion\(^{(9,10)}\) are adapted to describe the hypoosmotic effect on urothelial cancer cells. Exploded cells as well are ignored in this study because they are potentially indistinguishable from necrotic debris in high grade urothelial carcinomas and artificially crushed cells. This could explain why the percentages of osmotic - affected cells by total cells as the time passes are much less than expect. In our opinion, the urothelial nature of the tumor which is constantly exposed to hypotonic environment of the urine could attribute to any, much if not less, degrees of resistance to osmotic lysis, unlike breast cancer cells which have been studied in Molyneux *et al*. However, this study shows promising use of more reliable and reproducible, yet more complicated and expensive methods in determining the guideline for appropriate intraoperative irrigation time. The same direction of previous, well-designed study of Fechner *et al.* using measured numbers of cultivated cells, immersing in different solutions, staining with specific agent and measuring viable cells with light absorbance could be the appropriate solution for this clinical problem.

The peritoneum shares poor absorptive capacity for large molecules, allowing high local concentrations of chemotherapy agents such as mitomycin\(^{(5,6)}\) with low systemic toxicity. On this regard, there are previous studies that investigated intraperitoneal chemotherapy agent irrigation to decrease the risk of tumor seeding. Nevertheless,
there has not been any in vivo study to confirm the safety of this treatment. On the other hand, sterile water intraperitoneal lavage was mentioned and practiced for many years. So far, no one wonders whether this procedure is safe to the patients. Water intoxication as was reported in transurethral resection of a prostate syndrome (TUR-P syndrome). However, it has not shown the effect in sterile water intraperitoneal lavage. This can be explained by a low pressure to raw surface so small amount of water that was absorbed.

**Conclusion**

According to the result, we found significant decrease of the rate of tumor cell survival by distilled water and if we want to decrease spillage survival tumor to approximately 20 %, peritoneal irrigation with sterile water after radical cystectomy at least 15 minutes is suggested.

**References**


