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

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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*

Materail 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)*

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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 immerged 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.*

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อุมาพร นวลไธสง, คงปภพ ปัญญา, สุพจน์ รัชชานนท์, วิภาวี กิตติโกวิท. การลดความเสี่ยงการฝังตัวซ้ำของเซลล์มะเร็ง ภายหลังการผ่าตัดเอากระเพาะปัสสาวะออก ในมะเร็งกระเพาะปัสสาวะชนิด transitional cell carcinoma ด้วยการทำให้เซลล์แตกตัวจากน้ำเปล่า. จุฬาลงกรณ์เวชสาร 2552 ก.ย. – ต.ค.; 53(5): 397 – 403

วัตถุประสงค์ : *เพื่อศึกษาประสิทธิผลของน้ำเปล่าในการทำให้เซลล์มะเร็งแตกตัว ณ เวลาต่างๆ กัน*

รูปแบบการวิจัย : *การศึกษาเชิงทดลอง ชนิดมีกลุ่มควบคุม และกลุ่มทดลองโดยใส่ตัวแปรที่แตกต่างกันในประชากรเดียวกัน แล้ววัดผล ณ เวลาต่างๆ กันหลายครั้ง*

สถานที่ทำการวิจัย : *ฝ่ายศัลยกรรมทางเดินปัสสาวะ และฝ่ายพยาธิวิทยา โรงพยาบาลจุฬาลงกรณ์*

วิธีการศึกษา : *ทำการศึกษา 76 สไลด์เซลล์วิทยาของผู้ป่วยมะเร็งกระเพาะปัสสาวะชนิด transitional cell carcinoma จำนวน 9 คน โดย 7 คนมาผ่าตัดเอากระเพาะปัสสาวะออก และสองคนมาผ่าตัดส่องกล้องผ่านท่อทางเดินปัสสาวะเพื่อตัดเอาเนื้อมะเร็งออก ระหว่างเดือน สิงหาคม 2551 ถึง มกราคม 2552 โดยชิ้นเนื้อมะเร็งที่ได้จากตัวผู้ป่วยจะถูกแบ่งเป็น 2 กลุ่ม นำไปแช่ในน้ำเกลือ (0.9%) และน้ำเปล่า เป็นเวลาต่างๆ คือ 5 นาที, 10 นาทีและ 15 นาที หลังจากนั้นจะนำมาศึกษาทางเซลล์วิทยาเพื่อนับสัดส่วนของเซลล์ที่บวมจนแตก และเซลล์ที่ยังคงสภาพดีอยู่ และนำทั้ง สองกลุ่มมาเปรียบเทียบกัน นอกจากนั้นได้ศึกษาถึงแนวโน้มการแตกตัว ของเซลล์มะเร็งในน้ำเปล่าที่เวลาต่างๆ ตั้งแต่ 0 ถึง 15 นาที*

ผลการศึกษา : *สัดส่วนการแตกตัวของเซลล์มะเร็งที่ถูกแช่ในน้ำเปล่ามากกว่าที่แช่ในน้ำเกลือ อย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) และพบว่าการแช่เซลล์ไว้ในน้ำเปล่าประมาณ 15 นาทีสามารถลดจำนวนเซลล์มะเร็งที่ยังคงสภาพให้เหลือเพียง 20 % โดยประมาณ*

สรุป : *การล้างช่องท้องด้วยน้ำเปล่าหลังผ่าตัดเอากระเพาะปัสสาวะที่มีมะเร็งออกจะสามารถทำให้เซลล์มะเร็งที่หลุดปลิวเข้าสู่ช่องท้องบวมและแตกได้ดีกว่าการล้างช่องท้องด้วยน้ำเกลือ และอาจลดการฝังตัวใหม่ของเซลล์มะเร็งที่หลุดปลิวได้ด้วยเช่นกัน โดยหากแช่ล้างน้ำเปล่าประมาณ 15 นาทีจะช่วยทำให้เซลล์มะเร็งที่ยังคงสภาพเหลือประมาณ 20 %*

คำสำคัญ : *มะเร็งกระเพาะปัสสาวะ, Transitional cell carcinoma, การฝังตัวซ้ำของเซลล์มะเร็ง, การบวมและแตกตัวของเซลล์, 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 cysstectomy 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. [1, 2] 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[3 - 6] while there is insufficient evidence to prove that distilled water impairing bladder cancer reimplantation by interaction with extracellular matrix.[2] 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 ($p < 0.001$), 10 minutes ($p < 0.001$) and 15 minutes ($p < 0.001$).

Figure 4 shows the rate of tumor cell osmolysis which depends on time that is exposed to sterlie 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%.

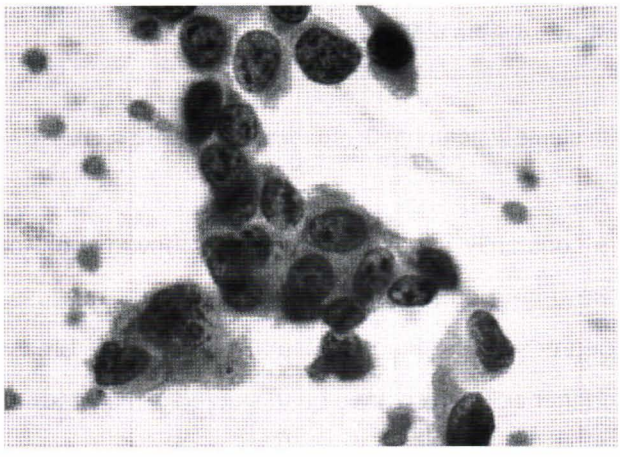

Figure 1. A cluster of urothelial carcinoma cells. These cells have enlarged nuclei, nuclear irregularity and coarse chromatin.

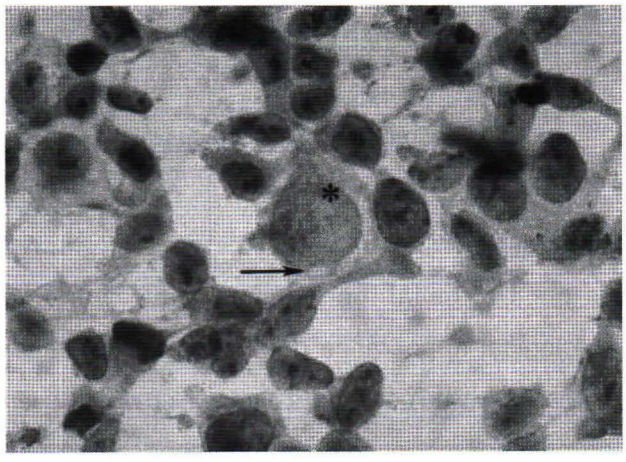

Figure 2. Urothelial cell with hypoosmotic effect (*). This cell contains swelling nucleus which has lost its chromatin detail, compared to the nearby cells. Notice the nuclear leakage (black arrow).

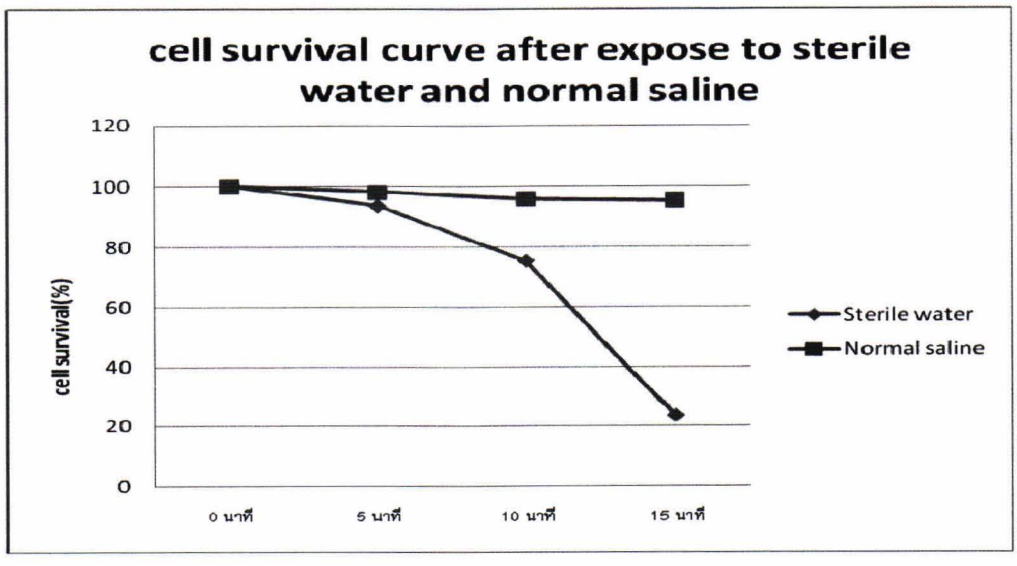

Figure 3. Comparison of tumor cell survival after exposure to NSS and sterile water (mean ± SD). Significant p <0.001

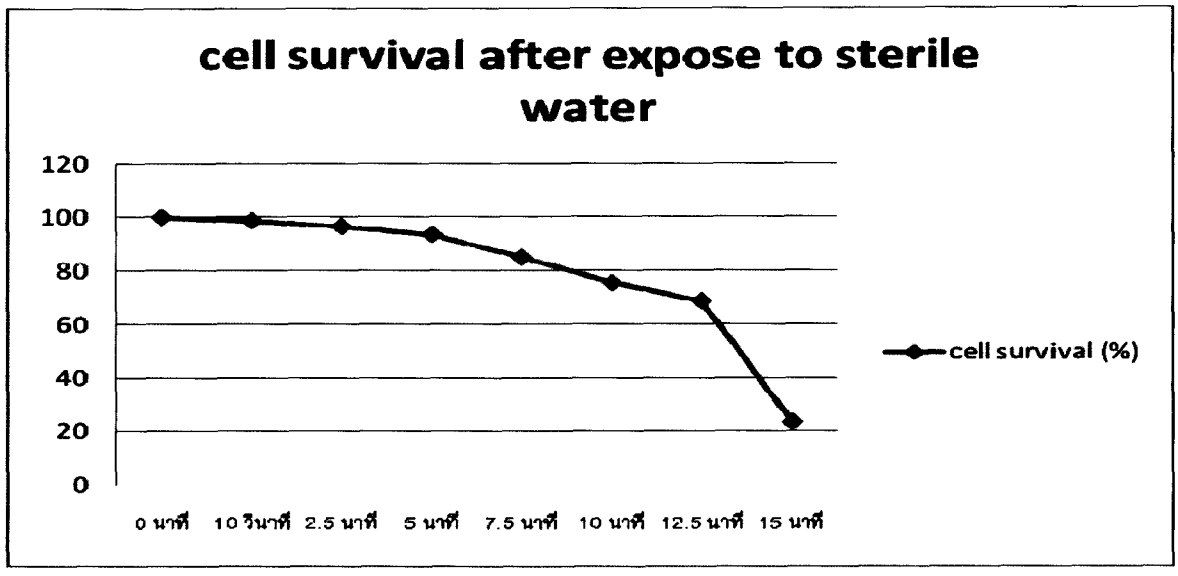

Figure 4. Trend of tumor cell survival after exposure with sterile water form 0 - 15 minutes (mean ± SD).

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

1. Fechner G, Pocha K, Schmidt D, MÜller SC. Reducing recurrence and costs in superficial bladder cancer: preclinical evaluation of osmotic cytolysis by distilled water vs. mitomycin. Int J Clin Pract 2006 Oct; 60 (10): 1178 - 80
2. Abaza R, Keck RW, Selman SH. Intraperitoneal chemotherapy for the prevention of transitional cell carcinoma implantation. J Urol 2006 Jun; 175(6): 2317 - 22
3. Ost MC, Patel KP, Rastinehad AR, Chu PY, Anderson AE, Smith AD, Lee BR. Pneumoperitoneum with carbon dioxide inhibits macrophage tumor necrosis factor - alpha secretion: source of transitional-cell carcinoma port - site metastasis, with prophylactic irrigation strategies to decrease laparoscopic oncologic risks. J Endourol 2008 Jan; 22(1): 105 - 12
4. Ku JH, Yeo WG, Park MY, Lee ES, Kim HH. Metastasis of transitional cell carcinoma to the lower abdominal wall 20 years after cystectomy. Yonsei Med J 2005 Feb 28; 46(1): 181 - 3
5. Kasai T, Moriyama K, Tsuji M, Uema K, Sakurai N. A case of vaginal implantation of transitional cell carcinoma of the bladder. Nippon Hinyokika Gakkai Zasshi 2001 May; 92 (4): 538 - 41
6. Estrada CR, Salanga M, Bielenberg DR, Harrell WB, Zurakowski D, Zhu X, Palmer MR, Freeman MR, Adam RM. Behavioral profiling of human transitional cell carcinoma ex vivo. Cancer Res 2006 Mar 15; 66(6): 3078 - 86
7. McKee G. The kidney and retroperitoneal tissues. In: Gray W, McKee GT, eds. Diagnostic Cytopathology. 2nd ed. Edinburgh: Churchill Livingstone, 2003: 337- 60
8. DeMay RM. Urine. In: DeMay RM, ed. Practical Principles of Cytopathology. Chicago: ASCP Press, 1999: 95 -114
9. Howat AJ, Coghill SB. Normal breast cytology and breast screening. In: Gray W, McKee GT, eds. Diagnostic Cytopathology. 2nd ed. Edinburgh: Churchill Livingstone, 2003: 237-56
10. Molyneux AJ, Coghill SB. Cell lysis due to ultrasound gel in fine needle aspirates; an important new artefact in cytology. Cytopathology 1994 Feb; 5(1): 41-5