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Improved Detection of Nonmuscle Invasive Urothelial Carcinoma of the Bladder Using Pirarubicin Endoscopy: A Prospective, Single-Center Preliminary Study

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Abstract

Background and Purpose: Fluorescence cystoscopy (FC) with intravesical instillation of a photosensitizing agent has emerged as an adjunctive and safe diagnostic tool with high sensitivity and reasonable specificity; however, it has not been widely accepted, because it is time-consuming and expensive. The aim of the present study was to determine whether the use of the fluorescent dye pirarubicin [(2″R)-4′-O-tetrahydropyranyl doxorubicin] (THP) in endoscopy can improve detection of nonmuscle invasive urothelial carcinoma of the bladder.

Patients and Methods: Forty-eight patients with known or suspected bladder urothelial carcinoma were enrolled in this prospective study between January 2008 and April 2009. The Storz D-light system was used to detect fluorescence 15 minutes after intravesical instillation with 30 mg THP. Endoscopic findings, histopathologic evaluation of biopsy lesions, and adverse effects of THP were recorded.

Results: After THP uptake, the lesions appear bright orange under white light, and produce bright red fluorescence under blue light. Among 238 biopsies evaluated (84 malignant, 20 dysplasia, and 134 benign), sensitivity of overall tumors, carcinoma *in situ* (CIS), and dysplasia detection using FC was 96% (81/84), 100% (6/6), and 90% (18/20), respectively. The specificity of FC was 74.7% (115/154), and its false-positive rate was 32.5% (39/120). No significant systemic side effects or allergic reactions were observed other than a few cases of mild cystitis.

Conclusion: THP endoscopy may improve the detection of nonmuscle invasive urothelial carcinoma of the bladder, especially CIS and flat lesions. Results indicate that THP is a promising fluorescent dye for diagnosis and follow-up of nonmuscle invasive bladder carcinoma. Moreover, it is inexpensive, easily available, simple to administer, and is associated with few side effects.

Introduction

Noninvasive urothelial carcinoma of the bladder manifests as papillary or flat neoplasia; the latter may comprise dysplasia or carcinoma *in situ* (CIS). Up to 24% of random biopsies from patients with T_a and T_1 superficial bladder carcinoma show dysplasia or CIS.[1] CIS is multifocal in up to half the cases and has a high likelihood of progressing if untreated, with spread occurring in up to 83% of cases.[2,3]

The gold standard for detection and follow-up of bladder carcinoma is white light cystoscopy (WLC).[4] Small and flat urothelial malignancies, however, are difficult to visualize and may be missed during conventional WLC; therefore, sensitivity varies from 50% to 75%, depending on the experience of the investigator. In addition, detection by WLC is affected if the bladder is overdistended and trabeculated, or ongoing cystitis is present.[5] Thus, regular WLC and random biopsy are unable to detect all urothelial neoplasms in patients who are at high risk of recurrent bladder cancer.[6]

To overcome this problem, photodynamic diagnosis (PDD) allows visualization of malignant cells or tissues by accumulation of photosensitive fluorescent dyes. Since the early 1990s, various studies have reported a higher sensitivity of bladder cancer detection using PDD with 5-aminolevulinic acid (5-ALA)-induced porphyrin fluorescence.[5,7–10] New fluorescent dyes, such as hexaminolevulinate (HEX, also referred to as HAL) and hypericin, are considered more effective in the detection and diagnosis of bladder cancers.[11,12]

The current guidelines of the European Association of Urology recommend fluorescence cystoscopy (FC) as a valuable aid for follow-up and endoscopic treatment of nonmuscle invasive urothelial carcinoma of the bladder. HAL is the only

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PDD agent approved by respective agencies to date[13]; however, HAL is commercially available only in Europe, needs longer intravesical dwelling time, and is readily photobleached.

Pirarubicin hydrochloride [(2″R)-4′-O-tetrahydropyranyl doxorubicin] (THP) is an anthracycline anticancer agent that was approved in Japan and China as clinical prophylactic chemotherapy for nonmuscle invasive urothelial carcinoma of the bladder after transurethral resection (TUR). THP is pigmented and selectively absorbed by bladder tumor cells. In a preliminary study, Uchikoba and associates[14] reported the utility of intravesical instillation of THP in the macroscopic detection of flat malignant lesions, such as CIS. THP is similar to the color of the bladder mucosa, however, which can make discrimination of targeted cells difficult, especially when bleeding or cystitis is present. When THP is absorbed by tumor cells, 69% to 74% localizes to the nuclear surface and is metabolized in the nucleus.[15] Fluorescence from THP in the nucleus can be detected by fluorescence microscopy, which enhances the contrast of malignant lesions to normal bladder mucosa urothelium. Through the use of blue light cystoscopy, enhanced visual contrast would make it much easier for the inspector to discriminate targeted lesions.

The aim of the present study was to evaluate whether the use of THP as a fluorescent dye in endoscopy improves detection of nonmuscle-invasive urothelial carcinoma of the bladder.

Patients and Methods

Patients

Between January 2008 and April 2009, we enrolled (Department of Urology, Sun Yat-sen Memorial Hospital) 48 consecutive patients (mean age, 58.8 years; age range 36–86 years; men, n = 40; women, n = 8) suspected of having primary or recurrent bladder cancer as determined by cystoscopy, cytology, and/or ultrasonography. Inclusion criteria were: Primary or recurrent bladder cancer without recent (<6 months) history of intravesical chemotherapy or immunotherapy, age >18 years, declaration of consent, and no anesthesia risk. Exclusion criteria were: Urethral strictures, detrusor instability, small-capacity bladder, pregnancy, renal failure or liver insufficiency, THP allergy, macroscopic hematuria, or mental health problem.

Chemicals and instruments

THP was obtained commercially from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China). A 300W xenon short arc lamp (D-Light AF system; Karl Storz, Tuttlingen, Germany) was used for white light illumination, and the blue light for excitation was filtered by a band-pass filter (375–440 nm). Illumination and observation of tissues were performed with a modified endoscope integrated with a long-pass filter (cutoff wavelength, 550 nm) to reduce excitation light and improve the contrast of red fluorescing tumors and surrounding tissue. A foot switch regulated the filter for blue or white light. The optical device allowed a 30-degree or 70-degree angle view.

Clinical procedures

Endoscopy was performed under local spinal anesthesia. The bladder was first assessed carefully by WLC to inspect the dome, posterior bladder wall, right lateral wall, left lateral wall, and trigone. Macroscopic WLC findings based on tissue morphology were documented on a bladder map. Bladder mucosa morphology was classified as normal, papillary, or exhibiting nonspecific inflammation (the latter being hypervascularized, erythematous, erosive, or edematous areas of the mucosa). Then the bladder was completely emptied by catheterization. THP (30 mg, freshly dissolved in 40 mL distilled water) was instilled into the bladder and retained for 15 minutes. After emptying the bladder of THP, it was irrigated with 500 mL physiologic saline.

FC was used to examine the bladder with blue light. FC findings were documented on the same map for each bladder site examined. Cold cup biopsies and/or deep biopsies were taken by standard resection from fluorescent and nonfluorescent areas of the bladder under either blue or white light from all five sites of the bladder wall, regardless of whether

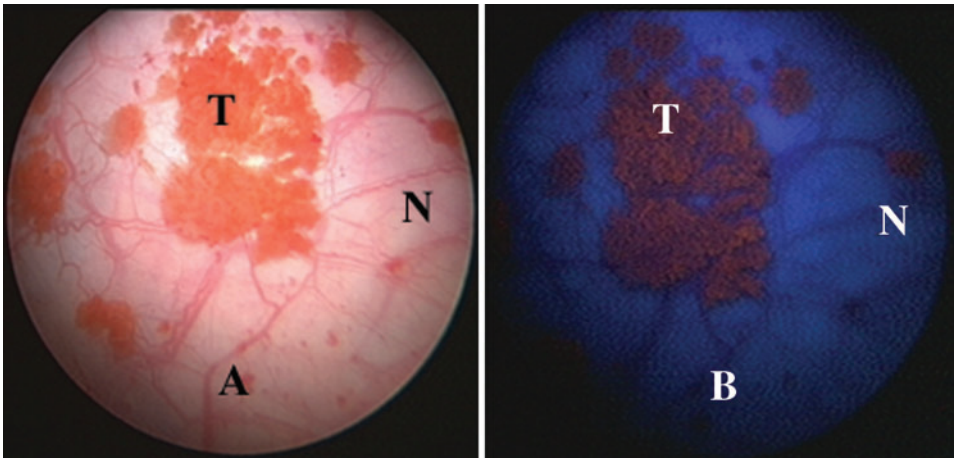

FIG. 1. Endoscopic images of the bladder after instillation of pirarubicin hydrochloride. **(A)** Bladder mucosa under white light, and **(B)** under blue light. Note tumor (T), which is bright orange in **A** and produces bright red fluorescence in **B**, compared with normal (N) bladder.

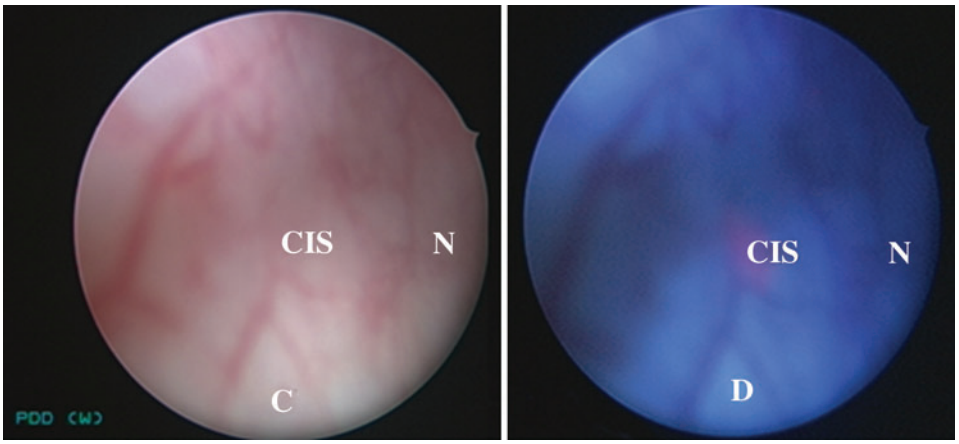

FIG. 2. Endoscopic images of the bladder after instillation of pirarubicin hydrochloride. (**C**) Bladder mucosa under white light, and (**D**) under blue light. Note tumor (CIS), which is invisible in **C** and produces red fluorescence in **D**, compared with normal (N) bladder. CIS = carcinoma *in situ*.

the bladder mucosa appeared normal. Tumors were staged and graded according to the 2004 World Health Organization diagnostic criteria by a pathologist blinded to endoscopic findings. CIS lesions were classified as high grade. All adverse events related to the drug were described.

Sensitivity and specificity were calculated according to guidelines of the European Agency for the Evaluation of Medicinal Products (EMEA; CPMP/EWP/1119/98). The study was approved by the local ethics committee and was performed in accordance with the Declaration of Helsinki. All patients provided written informed consent before undergoing the procedure.

Results

After THP uptake, the lesions with much THP uptake in the bladder appear bright orange under white light and produce bright red fluorescence under blue light (Fig. 1), interestingly; minor lesions with a little THP uptake also produce red fluorescence under blue light while obscure under white light (Fig. 2). A total of 238 biopsies from 48 patients were evaluated (Table 1). Tumors were categorized as T_a, $T_1 + T_2$, or CIS according to the degree of malignancy as determined by histologic findings. There were a total of 84 malignant specimens, 20 cases of dysplasia, and 134 benign specimens (including hyperplasia of the transitional epithelium, glandular cystitis, and nonspecific inflammation).

Table 1. Pirarubicin Hydrochloride Uptake in Biopsied Tissue from 48 Patients

	Number of biopsies	*THP+*	*THP−*
Stage			
Dysplasia	20	18	2
T_a	27	24	3
T_1-T_2	51	51	0
CIS	6	6	0
Grade			
Low	40	37	3
High	44	44	0
Hyperplasia	3	3	0
Glandular cystitis	11	11	0
Nonspecific inflammation	7	7	0
Normal urothelium	113	0	113

THP = (2″R)-4′-O-tetrahydropyranyl doxorubicin, pirarubicin; CIS = carcinoma *in situ*.

Sixteen malignant lesions in 12 cases were detected only by FC, including three flat malignancies overlooked after THP instillation for WLC (four malignancies in 1 patient, two in 1 patient, and one each in 10 patients); three malignant lesions in 3 patients were missed by FC (Table 2). The sensitivity of overall tumor detection was 96% (81/84) for FC and 82% (69/84) for WLC (Table 3). Six malignancies were classified as CIS. Notably, the sensitivity of FC in detecting CIS was 100%, whereas WLC showed a sensitivity of only 33%; the sensitivity for detection of dysplasia was 90% (18/20) compared with 35% (7/20) for WLC.

The specificity of FC was calculated to be 74.7% (115/154) compared with 80.1% (113/141) for WLC. The false-positive rate of FC was 32.5% (39/120) compared with 28.9% (28/97) for WLC. Among 39 false-positive biopsies obtained by FC, 3 were hyperplasia of urothelium; 11 were glandular cystitis; 7 were benign tissue with nonspecific inflammation; 18 were dysplasia. Sensitivity of FC with respect to tumor grading was 92% for low-grade and 100% for high-grade tumors compared with 80% for low- or high-grade tumors for WLC.

Fluorescence remained stable and constant during inspection. No significant systemic side effects or allergic reactions caused by the instillation of THP solution were observed other than a few cases of mild cystitis.

Discussion

Early diagnosis of bladder cancer is crucial to improving treatment outcomes and increasing survival rates. Urinary cytology and conventional cystoscopy with bladder mapping have been the gold standard diagnostic tools for many years. Urine cytology, however, has a sensitivity of less than 60%[16] and cannot provide information regarding the location and extent of the tumor. WLC is reliable for exophytic tumors;

Table 2. Overview of Identified and Overlooked Malignancies Using Fluorescence Cystoscopy and White Light Cystoscopy

Results	*Number of patients*	*Patients (%)*	*Number of biopsies*	*Biopsies (%)*
Identical malignancies detected by FC and WLC	33	70	65	77
Overlooked malignancies by WLC	12	25	16	20
Overlooked malignancies by FC	3	6	3	4

FC = fluorescence cystoscopy; WLC = white light cystoscopy.

however, flat carcinomas (particularly CIS), dysplasia, multifocal growth, and microscopic lesions are much more difficult to detect using this method.

About 15 years ago, the photodynamic agent 5-ALA was first introduced into urology practice, and porphyrin-induced FC has been considered a safe diagnostic method for bladder cancer detection with high sensitivity and considerable specificity.[5,7–10] 5-ALA is unstable at room temperature, needs longer intravesical dwelling time because of its low lipophilicity, and is readily photobleached, so its hexyl ester derivative HAL (more lipophilic, stable at room temperature, less vulnerable to photobleaching, and needs shorter indwelling time) was introduced and is more powerful. Robust evidence from various multicenter, prospective, controlled, or randomized trials on hundreds of patients revealed that photodynamic diagnosis using these agents improves the detection rate of nonmuscle-invasive papillary bladder tumors (T_a-T_1 and CIS), and decreases the amount of residual cancer after TUR of the bladder tumor,[17] leading to a reduction in rates of tumor recurrence at 9 months.[18] Up to now, HAL is the sole product that is commercially available in Europe and for which FDA approval is pending in the United States.

In spite of this evidence, PDD cannot be routinely performed because of high start-up expenses (dedicated equipment), cost and availability of fluorescent drugs, and need to plan the procedure in advance.[19] The ideal diagnostic tool for bladder cancer detection and monitoring should be affordable and simple to perform and should provide the highest levels of sensitivity and specificity.

Table 3. Sensitivity of Fluorescence Cystoscopy and White Light Cystoscopy for Detection of Malignancies of Different Stages and Tumor Grading

	Number of biopsies	*Sensitivity*	
		FC (%)	*WLC (%)*
Staging			
Dysplasia	20	90	35
T_a	27	90	85
T_1-T_2	51	100	89
CIS	6	100	33
Malignancies + dysplasia	104	94	71
Malignancies	84	96	82
Grading			
Low	40	92	80
High	44	100	80

FC = fluorescence cystoscopy; WLC = white light cystoscopy; CIS = carcinoma *in situ*.

THP is an anthracycline derivative that exerts a stronger antitumor effect than adriamycin. Intravesical instillation of THP after TUR may significantly decrease the recurrence rates of nonmuscle-invasive urothelial carcinoma of the bladder.[20] After absorption by the bladder tumor, THP pigment and anthracene nuclear fluorescence are easily detected. In the present study, papillary and flat lesions were stained orange by THP and easily found macroscopically. Furthermore, inspection under blue light detected additional malignant lesions (especially CIS) by enhancing contrast of abnormal lesions with normal tissues. Dysplasias, which were usually determined to be precancerous lesions, were easily detected as well. THP was taken up by benign lesions including glandular cystitis, hyperplasia, and nonspecific inflammatory urothelia, although fluorescence intensity was low. The sensitivity and specificity of THP uptake for malignant mucosal lesions in our study was 96% and 74.7%, respectively, which is identical to that of previously documented photodynamic diagnosis.[21] Uchikoba and colleagues[14] reported sensitivity and specificity of up to 100% and 97.5%, respectively, using THP for malignant mucosal lesions; however, this difference may be attributed to different samples, diagnostic instruments, and THP dosage and retention time.

THP is quickly and selectively absorbed by tumor cells,[22] and fluorescence intensity is proportional to the level of uptake. In previous studies, THP was absorbed within 3 to 4 minutes, reaching peak absorption in 30 minutes, but retention of THP in the bladder for 5 to 15 minutes using intravesical chemotherapy appears to be a better technique.[23,24] Combining the benefits of preoperative intravesical chemotherapy and enhanced tissue contrast, we injected THP into the urinary bladder for 15 minutes and used the conventional dose of 30 mg, which appears to be the optimal dose with respect to drug efficacy, prevention of side effects, and fluorescence interference.

Protoporphyrin IX fluorescence-PDD exploits the photoactive nature of certain compounds to enhance the visual demarcation between normal and neoplastic tissue. Currently used photosensitizers, such as 5-ALA and its derivative HAL, accumulate preferentially in tumor tissue, with a ratio of around 20:1 compared with normal tissue. The mechanisms are still not fully understood, but studies suggest that accumulation is not from selective uptake by cancerous cells. Rather, 5-ALA is taken up similarly by all cell types, but the processes of conversion and elimination are different in malignant cells, leading to a concentration gradient between neoplastic and normal tissue.[25] For that reason, a relatively long time between drug instillation and FC is needed to obtain

adequate concentrations of the photosensitizer. Moreover, fairly rapid photobleaching, the difficult preparation of drug instillation, and high cost limit clinical applicability.

In contrast, THP is quickly and selectively absorbed by tumor cells and does not undergo an intracellular conversion process. Thus, the time between drug instillation and endoscopy is very short and need not interfere with the surgical procedure. THP is commercially available with easy preparation of instillation and low cost. Instillation of THP during the TUR procedure not only improves detection of malignant lesions under white or blue light, but also serves as a preoperative chemotherapy that prevents replantation of tumor cells, which may reduce tumor recurrence and improve treatment outcomes. Although a case of anaphylactic shock induced by intravenous injection of pirarubicin has been reported,[26] early intravesical chemotherapy of pirarubicin with 30 mg after TUR may cause mild or moderate chemical cystitis. including pain with micturation, urinary frequency/urgency, and macroscopic hematuria,[27] but that resolved by conservative management. As we know, a severe adverse side effect such as anaphylactic shock caused by intravesical instillation with THP has not been reported to date.

In the present study, no serious side effects were observed with 30 mg THP intravesical injection, and it demonstrated high sensitivity and specificity for detecting bladder cancer. Thus, we recommend THP instillation to correctly diagnose flat or papillary bladder carcinoma.

Conclusions

THP endoscopy may improve the detection of nonmuscle-invasive urothelial carcinoma of the bladder, especially CIS and flat lesions. Results indicate that THP is a promising fluorescent dye for diagnosis and follow-up of nonmuscle-invasive bladder carcinoma. Moreover, it is inexpensive, easily available, simple to administer, and is associated with few side effects.

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Disclosure Statement

No competing financial interests exist.

References

1. Bostwick DG, Ramnani D, Cheng L. Diagnosis and grading of bladder cancer and associated lesions. Urol Clin North Am 1999;26:493–507.
2. Cheng L, Cheville JC, Neumann RM, et al. Survival of patients with carcinoma in situ of the urinary bladder. Cancer 1999;85:2469–2474.
3. Hudson MA, Herr HW. Carcinoma in situ of the bladder. J Urol 1995;153:564–572.
4. Pytel A, Schmeller N. New aspect of photodynamic diagnosis of bladder tumors: Fluorescence cytology. Urology 2002;59:216–219.
5. Kriegmair M, Baumgartner R, Knüchel R, et al. Detection of early bladder cancer by 5-aminolevulinic acid induced porphyrin fluorescence. J Urol 1996;155:105–110.
6. May F, Treiber U, Hartung R, Schwalbold H. Significance of random bladder biopsies in superficial bladder cancer. Eur Urol 2003;44:47–50.
7. Jichlinski P, Wagnières G, Forrer M, et al. Clinical assessment of fluorescence cytoscopy during transurethral bladder resection in superficial bladder cancer. Urol Res 1997; 25(suppl 1):S3–S6.
8. D'Hallewin MA, Vanherzeele H, Baert L. Fluorescence detection of flat transitional cell carcinoma after intravesical instillation of aminolevulinic acid. Am J Clin Oncol 1998;21: 223–225.
9. Riedl CR, Plas E, Pflüger H. Fluorescence detection of bladder tumors with 5-amino-levulinic acid. J Endourol 1999;13:755–759.
10. Zaak D, Kriegmair M, Stepp H, et al. Endoscopic detection of transitional cell carcinoma with 5-aminolevulinic acid: Results of 1012 fluorescence endoscopies. Urology 2001;57: 690–694.
11. D'Hallewin MA, Kamuhabwa AR, Roskams T, et al. Hypericin-based fluorescence diagnosis of bladder carcinoma. BJU Int 2002;89:760–763.
12. Grossman HB, Gomella L, Fradet Y, et al. A phase III, multicenter comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of superficial papillary lesions in patients with bladder cancer. J Urol 2007;178:62–67.
13. Burger M, Stief CG, Zaak D, et al. Hexaminolevulinate is equal to 5-aminolevulinic acid concerning residual tumor and recurrence rate following photodynamic diagnostic assisted transurethral resection of bladder tumors. Urology 2009;74:1282–1286.
14. Uchikoba T, Horiuchi K, Oka F, et al. Diagnosing the location of carcinoma in situ (CIS) of the urinary bladder using pirarubicin hydrochloride. Urol Int 2005;74:235–239.
15. Kunimoto S, Miura K, Takahashi Y, et al. Rapid uptake by cultured tumor cells and intracellular behavior of 4′-O-tetrahydropyranyladriamycin. J Antibiot (Tokyo) 1983;36: 312–317.
16. Balaji KC, McGuire M, Grotas J, et al. Upper tract recurrences following radical cystectomy: An analysis of prognostic factors, recurrence pattern and stage at presentation. J Urol 1999;162:1603–1606.
17. Witjes JA, Douglass J. The role of hexaminolevulinate fluorescence cystoscopy in bladder cancer. Nat Clin Practice Urol 2007;4:542–549.
18. Mynderse L, Stenzl A, Denzinger S, et al. Hexaminolevulinate fluorescence cystoscopy improves detection and resection of papillary bladder cancer lesions and reduces early recurrences. J Urol 2009;181(suppl. 4):689.
19. Colombo R, Naspro R, Bellinzoni P, et al. Photodynamic diagnosis for follow-up of carcinoma in situ of the bladder. Ther Clin Risk Manag 2007;3:1003–1007.
20. Okamura K, Ono Y, Kinukawa T, et al. Randomized study of single early instillation of (2″R)-4′-*O*-tetrahydropyranyldoxorubicin for a single superficial bladder carcinoma. Cancer 2002;94:2363–2368.
21. Hungerhuber E, Stepp H, Kriegmair M et al. Seven years' experience with 5-aminolevulinic acid in detection of transitional cell carcinoma of the bladder. Urology 2007;69:260–264.
22. Masuda H, Hirai M, Ohtawara Y, et al. Infiltration and tissue concentration of intravesically instilled (2″R)-4′-O-tetrahydropyranyladriamycin or adriamycin in rat bladder tumor induced by BBN. Nippon Hinyokika Gakkai Zasshi 1990;81:577–582.

23. Saika T, Tsushima T, Nasu Y, et al. Relationship of intracellular concentration and duration of contamination of pirarubicin and adriamycin in human bladder cancer cell lines and human bladder normal mucosa cell line. Gan To Kagaku Ryoho 1996;23:905–909.
24. Isobe H, Ishida T, Yamazaki K, et al. Flow cytometric detection of the rate of intracellular anthracycline-accumulation. Gan To Kagaku Ryoho 1994;21:2073–2075.
25. Krieg RC, Messmann H, Rauch J, et al. Metabolic characterization of tumor cell-specific protoporphyrin IX accumulation after exposure to 5-aminolevulinic acid in human colonic cells. Photochem Photobiol 2002;76: 518–525
26. Mitsuhashi M, Iwata H, Kiyota A, et al. A case of anaphylactic shock induced by pirarubicin hydrochloride. Hinyokika Kiyo 2004;50:257–259.
27. Okamura K, Ono Y, Kinukawa T, et al. Randomized study of single early instillation of (2"R)-4′-O-tetrahydropyranyldoxorubicin for a single superficial bladder carcinoma. Cancer 2002;94:2363–2368.

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Abbreviations Used

CIS = carcinoma *in situ*
FC = fluorescence cystoscopy
5-ALA = 5-aminolevulinic acid
HAL = hexaminolevulinate
PDD = photodynamic diagnosis
THP = (2″R)-4′-O-tetrahydropyranyl doxorubicin, pirarubicin
TUR = transurethral resection
WLC = white light cystoscopy