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IMPROVED DETECTION OF UROTHELIAL CARCINOMA IN SITU WITH HEXAMINOLEVULINATE FLUORESCENCE CYSTOSCOPY

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ABSTRACT

Purpose: In this European multicenter study we compared hexaminolevulinate (HAL) fluorescence cystoscopy and standard white light cystoscopy for the detection of carcinoma in situ (CIS) in patients suspected of having high risk bladder cancer.

Materials and Methods: This study was a prospective controlled, within-patient comparison of standard and HAL fluorescence cystoscopy. Eligible patients received an intravesical instillation of 50 ml HAL 8 mM solution. Cystoscopy was performed using a D light system, which provided white and blue light at 375 to 440 nm. The bladder wall was inspected and mapped, first under white light, followed by blue light. All tumors and suspicious areas identified under white light and by red fluorescence were resected or biopsied. Histological findings were assessed by an independent central pathologist blinded to the identity of the biopsies.

Results: Of 211 evaluable patients 83 (39%) had CIS, of whom 18 (22%) were detected by HAL cystoscopy only, 62 (75%) were detected by standard and HAL cystoscopy, 2 (2%) were detected by standard cystoscopy only and 1 (1%) was detected by nonguided biopsy. Therefore, HAL cystoscopy identified 28% more patients with CIS than standard cystoscopy. The side effects of HAL instillation were negligible and no unexpected events were reported.

Conclusions: HAL fluorescence cystoscopy improves the detection of bladder CIS significantly, which has consequences for clinical management and may improve the patient prognosis. The procedure is easily implemented as an adjunct to standard cystoscopy and it adds no significant risk of complications.

KEY WORDS: bladder, fluorescence, carcinoma in situ, cystoscopy

By definition, carcinoma in situ (CIS) of the bladder is a flat, high grade intraurothelial neoplasm.[1] It may occur as focal lesions, in diffuse form or concurrent with other high risk types of transitional cell carcinoma.[2] CIS indicates a high probability of panurothelial involvement and it is associated with a high risk of tumor progression due to its aggressive, unpredictable nature.[3–6] Its detection has a significant impact on the treatment decision even when associated with infiltrating bladder cancer, and failure to diagnose it seriously threatens the patient. Unfortunately these flat lesions are difficult to identify by standard cystoscopy. Urinary cytology has high sensitivity and specificity for high grade lesions but it provides no information on the location or extent of disease. Moreover, cytopathological findings are highly dependent on the training and expertise of the investigator and, hence, they are frequently underused.[7] As a result, there is an obvious clinical need to improve the detection of CIS.

Porphyrin based fluorescence cystoscopy has been investigated as a diagnostic procedure to improve the detection of bladder tumors. The technique is based on the preferential accumulation of photoactive porphyrins in neoplastic cells, which fluoresce red under blue light illumination and enable visualization of the tumor.[8] Systemic administration of photosensitisers such as dihematoporphyrin ester has been tested but side effects, ie prolonged skin sensitization, limit their use. Intravesical aminolevulinic acid, which has been used for the photodiagnosis of bladder tumors and for fluorescence guided transurethral resection, has shown some promise.[9,10] Hexvix (PhotoCure ASA, Oslo, Norway) hexaminolevulinate (HAL), a more potent ester of aminolevulinic acid, provides better selectivity, brighter fluorescence and permits a shorter instillation time.[11,12] Preliminary studies suggests that HAL cystoscopy has a high detection rate for all bladder tumors, including flat lesions, with an excellent safety profile.[13,14] In this European multicenter study we compared HAL with standard cystoscopy in regard to the detection of CIS in patients suspected of having high risk bladder cancer.

MATERIALS AND METHODS

At 19 European urology centers 286 patients were recruited between October 2001 and April 2002. The primary objective was to determine the proportion of patients in whom additional CIS lesions were detected by HAL compared with standard cystoscopy. By aiming at 42 patients with CIS and assuming a final CIS patient detection rate of 20% the study targeted 210 patients. To become familiar with the procedure at centers where there was no previous experience with fluorescence cystoscopy the first 5 patients were training patients, increasing the total number of patients required to 280. To obtain a study population with CIS in

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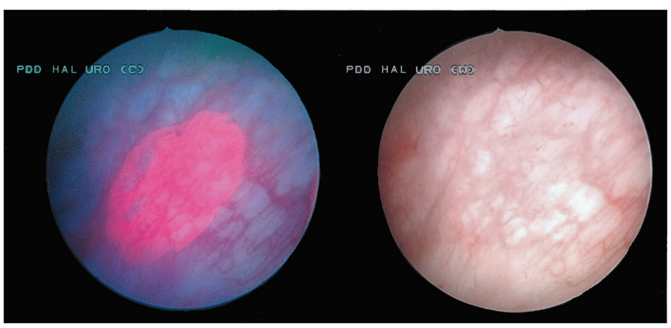
20% enrolled patients had to fulfill at least 1 inclusion criterion, including multiple bladder tumors or suspicious lesions, a tumor greater than 3 cm on primary cystoscopy, positive urinary cytology, a history of invasive tumor (T1 or T2), or pTa tumor grade 2 or 3 and a recurrent bladder tumor at followup examination. Patients with gross hematuria, porphyria, or an allergy to HAL or similar compounds and those who received topical bacillus Calmette-Guerin (BCG) or chemotherapy within the last 3 months, or participated in any other study within 30 days were excluded. Patients were at least 18 years old and women who were pregnant, breast feeding or not on adequate contraceptive measures were excluded.

The study was performed in accordance with ICH guidelines for good clinical practice and the Declaration of Helsinki (revised, Edinburgh, 2000). All patients provided written informed consent prior to study entry.

Study design. The comparison of HAL with standard cystoscopy was performed using a within-patient design by inspecting the bladder first under white light, followed by blue light (fluorescence). No randomization of the procedure sequence was done because HAL cystoscopy was used as an adjunct to standard cystoscopy. A catheter was inserted into the bladder and 50 ml HAL 8 mM phosphate buffer solution was instilled and retained in the bladder for 1 hour. Because cystoscopy was combined with immediate resection/biopsy of suspicious lesions, the patient then received anesthesia. The bladder was evacuated. Standard and HAL cystoscopy was performed using a D light system (Karl Storz, Tuttlingen, Germany), which allowed inspection under white and blue light by simply pushing a button on the endoscope (see figure). D light provides white light from a 300 W xenon lamp with a band pass filter to produce the blue/violet light. The light source is compatible with normal endoscopes but they must be supported by special optics for use in the fluorescing mode.

The number and location of all exophytic lesions and suspicious areas identified under white light were precisely mapped on a bladder chart. D light was then changed to blue light. The number and location of all fluorescing areas were identified and documented on the same bladder chart. Video recording was performed as source verification.

All exophytic and suspicious areas identified under white light were immediately biopsied or resected by transurethral resection. Additional biopsies were then obtained from all fluorescing suspicious lesions not previously identified by white light. One biopsy was taken from normal appearing urothelium as a reference for the pathologist. All biopsies and resected materials were analyzed by a central pathologist blinded to the identity of the lesion. Exophytic lesions were staged and graded according to the 2002 International Union Against Cancer/UICC TNM classification[15] using the 1998 UICC/International Society of Urological Pathology consensus guidelines.[1] Flat lesions were also classified according to that 1998 consensus.[1]



Appearance of urothelial carcinoma in situ under HAL fluorescence and standard white light cystoscopy.

Safety assessments, including physical examination, vital signs and blood sampling for hematology and biochemistry were performed at baseline and 24 hours after HAL instillation. All spontaneously reported and observed adverse events were documented. Patients were followed 7 days after HAL instillation for safety assessments.

HAL preparation. HAL 8 mM solution was prepared by the pharmacy at each hospital by reconstitution of 100 mg HAL hydrochloride powder for intravesical use in 50 ml sterile phosphate buffered saline, pH 5.7–6.2 (Hexvix®). Both HAL hydrochloride powder and the solvent were manufactured by Isopharma ASA, Oslo, Norway. The solution was prepared fresh before use, or stored for a maximum of 24 hours in the refrigerator before use, if needed.

Statistical analysis. For detecting a difference of 20% between standard and HAL cystoscopy using a power of 90% and significance level of 5% an estimated number of 42 CIS cases was considered necessary. All patients who received an intravesical instillation of HAL were included in the safety analysis. Training and nonevaluable patients (no histology or cystoscopy failure) were excluded from efficacy analysis. The primary efficacy parameter was the proportion of patients who had more histologically confirmed CIS lesions detected by HAL cystoscopy than by standard cystoscopy. This value was compared with the proportion of patients who had more CIS lesions found by standard cystoscopy using the exact sign test.[16] The lesion detection rate is reported as the number of lesions detected by HAL cystoscopy out of the total number of lesions detected by HAL and/or standard cystoscopy. The false-positive detection rate was calculated as the number of lesions falsely detected (normal mucosa) by HAL or standard cystoscopy out of the total number found by HAL and standard cystoscopy, respectively. Adverse events, and standard blood hematology and chemistry results were reported for all patients who received HAL.

RESULTS

Of the 286 patients who entered the study 279 received HAL instillation and were included in the safety analysis. Nine patients were not evaluable due to protocol violations and 59 training patients at 13 centers where there was no previous experience with fluorescence cystoscopy were excluded from efficacy analysis.

A total of 169 men (80%) and 42 women with a mean age ± SD of 70 ± 11 years (range 34 to 92) were evaluable for efficacy analysis. Mean patient height was 171 cm and mean body weight was 78 kg. Except for 1 African patient all were white. Of the 121 patients (57%) with recurrent bladder cancer 54 (45%) had had 3 or more recurrences. A total of 33 patients (16%) had received previous topical chemotherapy and 25 (12%) had undergone previous BCG instillations. Median HAL instillation time was 80 minutes (range 5 to 250).

Efficacy. Of the 211 patients with suspected bladder cancer histology showed that 17 had no tumor (normal in 11, hyperplasia in 1 and dysplasia in 5), leaving 194 with CIS, pTa or pT1-4 tumors (table 1). Of the 83 patients (39%) with CIS lesions CIS was detected in 80 (96%) by HAL cystoscopy and in 64 (77%) by standard cystoscopy (table 2). Two CIS cases were found only with white light. Therefore, in essence HAL cystoscopy detected CIS in an additional 28% of patients (18 of 64) compared with standard cystoscopy. Of the 18 patients with CIS only and without concomitant exophytic tumors CIS was diagnosed by light and HAL cystoscopy in 12, and by HAL cystoscopy only in 6 (33%). The proportion of patients with additional CIS lesions detected by HAL cystoscopy was significantly higher as compared with standard cystoscopy findings (55% vs 4%, $p < 0.0001$, table 2). Overall HAL cystoscopy detected 97% of all lesions compared with 78% identified by standard cystoscopy. HAL significantly improved

TABLE 1. *Patient classification*

Biopsy Histology*	No. Pts (%)
Normal	11 (5)
Hyperplasia	1 (0.5)
Dysplasia	5 (2)
CIS	18 (9)
pTa G1/G2 + CIS	8 (4)
pTa G3 + CIS	11 (5)
pT1 G1/G2 + CIS	2 (1)
pT1 G3 + CIS	31 (15)
pT2 + CIS	12 (6)
pT4 + CIS	1 (0.5)
pTa G1/G2	84 (40)
pTa G3	4 (2)
pT1 G1/G2	8 (4)
pT1 G3	6 (3)
pT2	9 (4)

* Highest stage and grade in 211 patients.

TABLE 2. *Patients with CIS and method of detection, and those with additional CIS lesions diagnosed by HAL and standard cystoscopy*

Diagnostic Procedure	No. CIS (%)
Initial CIS:	
Pts	83
Detected by HAL + standard cystoscopy	62 (74.7)
Detected by HAL cystoscopy only	18 (21.7)
Detected by standard cystoscopy only	2 (2.4)
Detected by select nonguided biopsy only	1 (1.2)
Additional CIS:	
More CIS lesions detected by HAL cystoscopy	46 (55.4)
Same number of CIS detected by HAL + standard cystoscopy	33 (39.8)
More CIS lesions detected by standard cystoscopy	3 (3.6)
Detected by select nonguided biopsy only	1 (1.2)

the detection of CIS (97% vs 58%) and pTa (97% vs 88%) lesions compared with standard cystoscopy. The detection of dysplasia was similarly improved for CIS compared with standard cystoscopy (94% vs 53%) (table 3). In 1 of the 83 CIS cases (1.2%) that condition was only detected histologically by nonguided biopsy.

The HAL lesion false-positive detection rate of 13% was similar to the 10% rate for standard cystoscopy. Inflammatory changes were the most common reason for this finding but previous topical BCG or chemotherapy had no effect in this context. Exfoliative urinary cytology of voided urine or barbotage specimens were obtained in 72 of the patients with CIS and 61 (85%) showed positive cytology according to the standard definition.[7] All 11 cytology negative CIS cases were detected by HAL but only 7 were detected by standard cystoscopy.

Safety. All 279 patients who received HAL instillation were included in the safety analysis. In 130 patients (47%) a total of 221 adverse events were reported. The most commonly reported adverse event was postoperative pain, which was reported in 36 patients (13%), followed by hematuria in 14 (5%), abdominal pain in 10 (3.6%), insomnia in 10 (3.6%), urinary tract infection in 9 (3.2%), urinary retention in 8 (2.9%), dysuria in 7 (2.5%) and pyrexia in 7 (2.5%). However, only 3 events in 2 patients were considered to be related to HAL. One patient was in urinary retention with burning sensations after illumination, while the other patient complained of mild bladder spasms. In the 17 patients who reported a total of 19 serious adverse events none was definitely related to HAL instillation (hematuria 5 or 1.8% sepsis in 3 or 1.1%, urinary retention in 3 or 1.1%, and bradycardia, cerebrovascular accident, pneumonia, abdominal pain, lung disorder, death from metastases, bladder perforation and aggravated angina pectoris in 1 or 0.4%, respectively). There were no clinically relevant changes in laboratory safety parameters or vital signs (blood pressure or heart rate).

TABLE 3. *Lesion detection rates of HAL and standard cystoscopy*

Lesion Type	Total No.	No. HAL Cystoscopy (%)	No. Standard Cystoscopy (%)
Dysplasia	68	64 (94)*	36 (53)
CIS	177	172 (97)*	103 (58)
pTa	376	365 (97)*	329 (88)
pT1	82	79 (96)	72 (88)
pT2	29	29 (100)	28 (97)
pT4	1	1 (100)	1 (100)
Totals	733	710 (97)	569 (78)

DISCUSSION

Up to 70% of newly diagnosed bladder tumors present as highly differentiated, superficial tumors that can be readily treated with transurethral resection.[17] Although up to 50% to 80% of patients may have recurrent tumors, at worst 10% ultimately progress to invasive disease.[17,18] In contrast, although it is also a superficial noninvasive lesion, CIS is a potentially aggressive tumor and patients with bladder CIS are at significant risk for cancer progression and death from bladder carcinoma.[5,6] Therefore, the detection of CIS has significant prognostic and therapeutic implications for the patient. Whereas superficial papillary lesions can usually be managed by transurethral resection, evidence for the appearance of CIS alone or with papillary lesions clearly heralds the limits of attempts at transurethral surgical ablation. Topical instillation therapy administered in a timely manner, usually today in the form of topical BCG immunotherapy, provides complete remission in a substantial segment of CIS cases.[18] Even with superficially invasive high grade bladder cancer additional CIS may impact treatment decisions. Whereas with isolated, superficially muscle invasive transitional cell carcinoma a bladder sparing approach may still occasionally be considered, it becomes more problematic with concomitant CIS.

Because it is difficult to detect CIS by standard cystoscopy, voided urinary or barbotage cytology as well as random biopsies are currently used to detect and verify CIS lesions. The value of the former is heavily dependent on the availability and expertise of the cytologist, and the benefit of the latter has been questioned since detection rates are low, they traumatize the urothelium and they may increase the risk of tumor seeding.[19] A better diagnostic procedure is warranted and fluorescence cystoscopy has been suggested to improve sensitivity compared with standard cystoscopy.[20] In this study bladder cancer patients at high risk for CIS lesions were included. The diagnosis of 39% CIS cases confirmed adequate inclusion criteria, selection of reference centers and the benefit of HAL cystoscopy.

The results of this phase III study clearly demonstrate the superiority of HAL over standard cystoscopy for detecting CIS lesions. HAL cystoscopy detected 28% more patients with CIS, that is an additional 18 vs the 64 diagnosed by standard cystoscopy, which obviously altered treatment. Overall HAL cystoscopy detected 67% more CIS lesions than standard cystoscopy. Indisputably detecting additional CIS lesions by fluorescence cystoscopy in patients with papillary lesions only at standard cystoscopy may also result in different management. Also, patients with papillary pTa lesions only benefit from HAL cystoscopy since the detection rate was 97% vs 88% for fluorescence vs standard cystoscopy. Tumors missed at standard cystoscopy were usually smaller and flatter lesions, which may light up brightly during HAL cystoscopy. Although they show a low tendency to progress, they can be missed at transurethral resection and they are one of the main reasons for high early recurrence rates. HAL cystoscopy facilitates early recognition and, hence, more complete transurethral resection with lower early recurrence rates.

Fluorescence from nontumor tissue (false fluorescence) may occur due to inflamed bladder areas but the false detection rate in this study was low, and similar for HAL and standard cystoscopy. In 1 patient CIS was detected by nonguided biopsy of the prostatic urethra. On HAL cystoscopy it is difficult to evaluate the prostatic urethra because tangential light may mimic false fluorescence.

The safety profile of HAL cystoscopy was excellent. We believe that only 3 minor reactions were related to HAL. No relevant changes in biochemistry or hematology parameters were observed. HAL cystoscopy proved simple and it was easy to implement in clinical practice.

CONCLUSIONS

This large multicenter study documents the superiority of fluorescence cystoscopy for detecting CIS lesions compared with standard cystoscopy. The high sensitivity of the procedure for CIS is important for early detection and improved patient treatment to avoid tumor progression to invasive disease. HAL cystoscopy was well tolerated. With standard cystoscopy it may provide the surgeon with a highly sensitive diagnostic method for all lesions.

HAL was provided by PhotoCure.

APPENDIX

Hexvix PCB301/01 Study Group included the additional investigators Steinar Karlsen, Oslo; Dirk Zaak, Munich; Olivier Bouchot, Nantes; Philippe Mangin, Nancy; Thomas Filbeck, Regensburg; Jens Høostmark, Bergen; Didier Jacqmin, Strasbourg; Pierre Conort, Paris; Patrice Jichlinski, Lausanne; Per-Uno Malmstrøm, Uppsala; Christian Pfister, Rouen; Karl-Heinz Kurth, Amsterdam; Ole Damm, Linköping; Gordon Williams, London; and Jean-Luc Descotes, Grenoble.

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