Wednesday 25 June 10.30–11.30
Poster Session 7: Bladder Cancer 1
Chairmen: N. Clarke and P. Harnden

P061

Entonox (50% nitrous oxide/oxygen) as an analgesic during flexible cystoscopy in men aged <55 years: a randomized double-blind controlled trial

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INTRODUCTION

Despite current best practice of the urethral instillation of 20 mL of 2% lignocaine 15 min before cystoscopy [*Br J Urol* 1994; **80**: 69–71] flexible cystoscopy in men aged <55 years is painful. An alternative agent would be useful which would improve tolerance, act rapidly, be easily reversible and have minimal adverse effects. Entonox is such an agent and significantly reduces pain during TRUS-guided prostate biopsy [*J Urol* 2002; **168**: 116–20]. We report our experience of using Entonox in flexible cystoscopy.

PATIENTS AND METHODS

Twenty-six men were randomized to a control (20 mL of 2% lignocaine gel for 15 min, and air) and 26 to a treatment (same lignocaine but plus Entonox) group. Both groups inhaled gas via similar breath-activated devices. Vital signs were recorded before, immediately after and 15 min after cystoscopy. Visual analogue pain scores (VAS) for gel insertion and cystoscopy were recorded.

RESULTS

There were no significant differences between the groups in age, VAS scoring of gel insertion ($P = 0.344$) or resting pulse rate ($P = 0.869$). The mean cystoscopy VAS scores and peroperative pulse rates were significantly lower in the Entonox group ($P < 0.01$) than in the control group, both suggesting better analgesia. There were no significant complications with the use of Entonox. Four of the Entonox group felt they needed more analgesia, vs 14 of the air group. One of the group inhaling Entonox and 10 of the air group would not have a repeat procedure.

CONCLUSIONS

This safe, inexpensive and well-established agent dramatically reduced pain and should improve patient compliance. We feel that this should be the analgesic of choice for outpatient flexible cystoscopies in men aged <55 years.

P062

The use of Raman spectroscopy to differentiate between benign and malignant bladder pathologies *in vitro*

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INTRODUCTION

Raman spectroscopy uses molecular-specific, inelastic scattering of light photons to interrogate biological tissues. When tissue is illuminated with laser light photons donate energy to intra-molecular bonds. This less energetic photon exits the tissue at a longer wavelength than the original laser light. When all the altered wavelengths, produced by photons hitting different bonds, are added together they form a Raman spectrum. The spectrum gives an objective measure of pathology, in contrast to subjective histological examination.

MATERIALS AND METHODS

Bladder samples collected during cystoscopic procedures were snap-frozen and a section taken for histological examination. Samples were classified as normal, cystitis, carcinoma in situ (CIS), TCC and squamous cell carcinoma (SCC). Scanning was carried out on an optimised Raman system, using an acquisition time of 10 s. In all, 1685 spectra were recorded from 76 patients (590 benign and 1095 malignant spectra). These spectra were analysed using principal-component fed linear-discriminant analysis, to construct a diagnostic algorithm. The algorithm was

tested for its accuracy in predicting the histological diagnosis.

RESULTS

The accuracy achieved by the algorithm for normal, cystitis, CIS, TCC and SCC, were respectively (sensitivity), 91%, 79%, 86% and 84%, and 98% and (specificity) 96%, 92%, 97%, 96% and 100%.

CONCLUSION

Raman spectroscopy can differentiate between different bladder pathologies *in vitro* and therefore shows potential as an objective method for assessing bladder pathology. *In vivo* probes are currently being developed.

Funding: Medlink 152

P063

A comparative phase III study of hexyl-aminolaevulinate fluorescence and standard cystoscopy in detecting carcinoma *in situ* of the bladder

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INTRODUCTION

Fluorescence cystoscopy has been reported to improve the detection of carcinoma *in situ* (CIS) lesions but this has not been verified in large multicentre studies. In this study, recruiting patients from 19 centres in eight European countries, we compared the detection of CIS using hexyl-aminolaevulinate (HAL) fluorescence and standard white-light cystoscopy.

PATIENTS AND METHODS

Patients with high-risk bladder cancer received an instillation with 50 mL of HAL (8 mmol/L) for 1 h before cystoscopy. The bladder was inspected in white and then blue light (dual inspection by D-light, 375–440nm, Karl Storz, Germany). All suspicious areas were biopsied and tumours resected. The samples were assessed by a central pathologist.

RESULTS

Of 211 evaluable patients (80% men) 83 (39%) had CIS; in 17 (21%) patients HAL detected CIS lesions not seen under white light. In 54% of patients with CIS, HAL detected additional CIS lesions compared to standard cystoscopy, and overall HAL detected significantly more CIS lesions (97%) than standard cystoscopy (58%). Of 92 patients with Ta tumours, HAL detected additional lesions in 20. Only negligible side-effects were reported, including changes in blood biochemistry and haematology.

The detection rates of 730 lesions in 211 patients were:

Lesion	Blue light	White light	Total
CIS	169 (97)	101 (58)	174
Dysplasia	64 (94)	36 (53)	68
Ta	365 (97)	329 (88)	376
T1	79 (96)	72 (88)	82
T2	29 (100)	28 (97)	29
T4	1	1	2

CONCLUSION

HAL fluorescence cystoscopy significantly improved the early detection of CIS of the bladder.

Funding: Study Sponsor: PhotoCure ASA, Oslo, Norway

P064

BCG therapy for high-risk superficial bladder cancer

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OBJECTIVES

The aim of this prospective study was to evaluate the effects and results of a BCG therapy scheme (6+3) in the treatment of high-risk superficial bladder cancer, and to find predictive factors for the response to treatment.

PATIENTS AND METHODS

In all, 155 high-risk patients were enrolled in a randomized study of transurethral resection

(TUR) alone (53) or combined with intravesical BCG (102) for superficial bladder cancer. BCG was administered for 6 consecutive weeks followed by 3-weekly instillations. Flow cytometry analysis of DNA ploidy and S-phase analysis was performed on bladder washing and tissue specimens received from 105 patients with TCC. From 85 patients samples were immunohistochemically stained with p53 monoclonal antibodies.

RESULTS

After a median (range) follow-up of 23 (6–42) months, 83 of the 102 BCG-treated patients (81%) were disease-free, vs 24 of those treated by 53 TUR alone (45%). There was also a significant difference in tumour progression and time to progression between the groups. Disease progressed in eight patients (8%) with BCG and in 12 (23%) with no BCG therapy.

CONCLUSION

This intravesical BCG immunotherapy maintenance schedule (6+3) seems to be effective in reducing recurrence and progression in high-risk superficial bladder cancer. The independent risk factors for progression and failure of BCG therapy were grade, DNA aneuploidy and high S-phase. Also, when the over-expression of p53 was apparent after BCG instillation it predicted the failure of therapy.

P065

Optimizing conditions for intravesical mitomycin therapy

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INTRODUCTION

The efficacy of mitomycin C may be influenced by the residual urine volume at the time of treatment, urine production rate and urinary pH. We previously showed that 6-h fasting and/or desmopressin reduce urine production, but urinary pH during the treatment period has been poorly documented despite mitomycin C treatment being optimal at pH 7.

PATIENTS AND METHODS

Fifty patients receiving intravesical chemotherapy for superficial bladder cancer were assessed (129 episodes). Before treatment, fluids were omitted for 6 h; after voiding a catheter was inserted and the residual volume and urinary pH recorded. The bladder was then assessed by ultrasonography to measure the residual after catheterization and subsequently the patients were ambulant for 5 min with a catheter and leg-bag to ensure complete bladder emptying; again the volume was recorded.

RESULTS

The mean (range) volume drained on catheterization after voiding was 36.5 (0–150) mL. Ultrasonography after this showed a mean (range) of 1.7 (0–95) mL remaining in the bladder. Ambulation for 5 min produced < 15 mL in all but two patients, who both had an initial residual after catheterization of > 100 mL. The mean (range) pH before and after mitomycin C instillation was 5.6 (4.9–7.1) and 5.9 (4.9–7.9), respectively.

CONCLUSION

Catheterization when supine empties the bladder in most patients but may fail to do so in those with residuals of > 100 mL. We recommend a short period of ambulation in those individuals before instillation. In most cases urinary pH is suboptimal and as mitomycin C degradation is 10 times higher at pH 5 than 7, we recommend routine alkalinization of urine.

P066

MUC1, MUC2 and MUC5AC expression in superficial bladder cancer

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INTRODUCTION

Mucins are found in mucus secretions, including in the urinary bladder, and their expression is frequently altered in cancer. We studied the expression of MUC1, 2 and 5AC in carcinoma *in situ* (CIS), papillary urothelial carcinomas (pTa) and normal bladder specimens, to examine their use as tumour markers in superficial bladder cancer.

MATERIAL AND METHODS

Samples from 65 patients with bladder cancer were investigated, including CIS from 25, pTa from 40 (G1 in six, G2 in 30 and G3 in four)

and normal bladder specimens (seven). Immunohistochemistry was performed using antibodies NCL-MUC1, MUC2 and MUC5AC. Specimens were scored for the presence or absence, proportion and depth of staining. The results were analysed using the chi-squared test.

RESULTS

MUC1, 2 and 5AC were expressed in 24 (96%), eight (32%) and two (8%) of the CIS specimens, respectively ($P < 0.001$). pTa specimens expressed MUC1 and MUC2 in 37 (93%) and 13 (33%) respectively, with no expression of MUC5AC ($P < 0.001$). There was no difference in the incidence of MUC1, 2 or 5AC expression in CIS and pTa specimens but CIS specimens expressed MUC1 in a higher proportion of cells ($P = 0.004$) and stained more deeply ($P = 0.041$) than in pTa specimens. Normal urothelium expresses MUC1 but not MUC2 or 5AC.

CONCLUSION

We report for the first time MUC1, 2 and 5AC expression in CIS of the human bladder by immunohistochemistry. These mucins are expressed differently in superficial bladder cancer, with greater expression of MUC1 and 2 than in normal urothelium. The identification of specific mucin expression patterns in superficial cancers might help to identify those patients at increased risk of recurrence and progression.

Funding: Grampian University Hospitals Endowment Grant

P067

The role of maspin and p53 in TCC of the bladder

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OBJECTIVE

To investigate expression of maspin and p53 tumour-suppressor gene products in different grades of bladder TCC.

PATIENTS AND METHODS

Previously resected TCC tissue specimens were re-graded and immunohistochemically stained for p53 and maspin expression using a standard avidin-biotin complex technique. Expression was assessed semiquantitatively and graded (0, absent; 1, weak; 2, strong). Clinical data including patient age at first presentation and recurrence rate after initial resection was recorded.

RESULTS

Bladder tumour tissue from 75 (57 men and 18 women) random patients was analysed; the mean (range) age at first presentation was 66.7 (14–89) years. Seventeen tumours were grade I, 20 grade II, 18 grade III and 20 were classed as invasive. There was a statistically significant increase in p53 expression with increasing tumour grade ($P < 0.001$). Conversely, there was a decline in maspin expression as tumour grade progressed ($P < 0.01$). The most significant change in maspin and p53 expression was between grades II and III. There was no statistically significant difference between patient age at first presentation, whether positive or negative for either maspin or p53 expression. Recurrence rates were not significantly different in tumours with or without maspin or p53 expression. Maspin positivity was detected in both cell nuclei and cytoplasm. Overexpression of p53 was exclusively nuclear.

CONCLUSION

The tumour suppressor genes maspin and p53 appear to have a role in the development of TCC, with p53 expression increasing and maspin expression decreasing with increasing tumour grade. Larger prospective studies are needed to clarify their function as potential predictors of tumour behaviour and prognosis in bladder TCC.

P068

The effect of gefitinib (Iressa™, ZD1839) and trastuzumab (herceptin) on the human bladder cancer cell line 5637

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INTRODUCTION

ErbB tyrosine kinase receptors are key regulators of cell behaviour and are implicated in the development of human cancers. Gefitinib (Iressa™, ZD1839), an epidermal growth factor receptor, EGFR, tyrosine kinase inhibitor), has cytostatic and pro-apoptotic effects in vitro. Trastuzumab (Herceptin, a monoclonal antibody directed against c-erbB-2) is currently used as a treatment option in breast cancer. We assessed the effect of gefitinib and trastuzumab in the EGFR/erbB-2-positive human bladder cancer cell line 5637.

MATERIALS AND METHODS

Cells (seeded at 2×10^3 and 4×10^3 in 200 μL growth medium) were exposed to gefitinib (0.19–24 μmol/L; 96 h) or trastuzumab (300–500 μg/mL; 140 h), or combinations of gefitinib (5 μmol/L) and trastuzumab (500 μg/mL; 230 h). Growth was assessed using the sulphorhodamine-B assay.

RESULTS

Gefitinib inhibited the proliferation of 5637 cells in a dose-dependent manner, as assessed using the growth assay ($IC_{50} = 9.57$ μmol/L) and under light microscopy. Scanning electron microscopy confirmed reduced invadopodia activity on the surface of treated cells. Trastuzumab did not reduce cell proliferation nor result in distinct morphological changes; indeed, there was a mild stimulatory effect at >10 μg/mL. Combined treatment was anti-proliferative, although this effect was not additive or synergistic.

CONCLUSIONS

Gefitinib, but not trastuzumab, caused reproducible, anti-proliferative effects and marked changes in surface topography in 5637 cells. These effects indicate that gefitinib could be used to influence the invasive behaviour of bladder tumours.

Funding: Newcastle University Hospitals Research Award

P069

Flow cytometry in the follow-up patients with TCC of the bladder

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INTRODUCTION

Flow cytometry allows the rapid, objective and accurate measurement of cellular DNA content. The aims this study were to assess any correlation of cystometric variables with tumour stage, grade, papillary status and the recurrence and progression rate in patients with TCC of the bladder.

MATERIAL AND METHODS

Flow cytometric analysis of DNA ploidy was performed on bladder washing and tissue specimens received from 105 patients with bladder TCC. The DNA content of single cell nuclei was analysed and DNA histograms classified as diploid or aneuploid, with one or more aneuploid cell populations.

RESULTS

The DNA histograms were classified as diploid in 52 and aneuploid in 53, with one or more aneuploid cell populations. The degree of ploidy in relation to histological grade and stage showed an increasing frequency of the aneuploid pattern in grades 2 and 3, and in invasive stages. DNA measurement was related to the clinical course of the disease; patients with diploid tumours had a lower recurrence rate (40% vs 90% for aneuploid tumours). Patients with diploid tumours developed almost no tumour progression (6%) while 64% of the aneuploid tumours progressed.

CONCLUSION

The results of prospective studies of flow cytometry DNA analysis of bladder washing and biopsy simultaneously indicate that ploidy status is a useful complement to the clinical and histological classifications of urothelial bladder carcinoma. The widest clinical application for flow cytometry has been the monitoring and prognosis of stage Ta–T1 carcinoma.

P070

The F344/AY27 orthotopic bladder cancer model: a reproducible preclinical system for assessing molecular strategies and response

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INTRODUCTION

The aims of this study were first to characterise the AY-27 rodent bladder cancer cell line for a panel of key oncoproteins (Bcl-2, Bcl-X, Bax, ras, p53 and retinoblastoma) known to be implicated in TCC of the bladder, and second, to establish the Fischer F344/AY-27 orthotopic TCC bladder model in Belfast. A reproducible model system that mimics human disease is essential for the preclinical evaluation of novel molecular strategies in bladder cancer.

MATERIALS AND METHODS

Key protein expression was investigated in the AY-27 cell line using immunocytochemistry, confocal microscopy and flow cytometry. AY-27 cells were added to the bladders of 40 adult female F344 rats after pre-sensitization of the bladder mucosa. Bladders were removed after 16 days for histopathological assessment.

RESULTS

The AY-27 cell line expressed Bcl-2, Bcl-X, Bax and ras, using the methods described above. To date we have not identified either p53 or retinoblastoma in the cell line. Thirty-two of 40 animals (80%) developed TCC of the urinary bladder with many having evidence of multifocal disease. These animals had variable stage and grade including, dysplasia (one), CIS (one), superficial G2 (eight), muscle-invasive G2 (one), superficial G3 (one), muscle-invasive G3 (18) and superficial indeterminate grade (two).

CONCLUSIONS

This new characterization of the AY-27 cell line, together with the broad spectrum of disease encountered, fully supports this highly reliable and reproducible system for preclinical evaluation of new treatment methods, within the immunocompetent host.

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