Bacillus Calmette–Guérin and Bladder Cancer

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Bladder cancer is the second most common cancer of the urinary tract, and overall it is among the top 10 cancers in men. Transitional cell carcinoma (TCC) is the most common type, with the majority being superficial disease, i.e. the tumour has not gone beyond the lamina propria. The main problem with superficial TCC is the high recurrence rate. Various forms of treatment methods have been attempted to reduce the recurrence rate, with intravesical bacillus Calmette–Guérin (BCG) being the most successful to date. In fact, intravesical BCG is one of the most successful forms of immunotherapy in the treatment of any form of cancer. This article is a general review of BCG in bladder cancer with an emphasis on the indication and mechanism of action in reducing recurrence and progression. [Asian J Surg 2007;30(4):302–9]

Key Words: bacillus Calmette–Guérin, BCG, bladder cancer, transitional cell carcinoma

Introduction

Bladder cancer is the seventh most common cancer among men worldwide. The incidence is much lower in females, with a male to female ratio of 4:1. It is more common in the developed world compared to the developing countries. In South East Asia, the incidence in 2002 was 2.7 per 100,000 in males and 0.8 per 100,000 in females, making it the ninth most common cancer in men. In Europe, bladder cancer accounts for 5–10% of all malignancies among males. In the United States, the estimated number of new cases in 2003 was 57,400, and the estimated number of deaths was 12,500. In the United Kingdom, the number of new cases reported in 2000 was 11,081, and the number of deaths in 2002 was 4,908.

Most bladder cancers are transitional cell carcinoma (TCC), accounting for about 93% of cases. The rest include squamous cell carcinoma and adenocarcinoma. About 70% of bladder tumours are classified as “superficial”, that is, the tumour has not gone beyond the lamina propria on presentation, while the rest are invasive. Among the superficial tumours, 70% are Ta while 20% are T1, with carcinoma in situ (CIS) comprising the remaining 10%. The TNM staging is as shown in Table 1.

Diagnosis and treatment

Haematuria is the most common presenting symptom in patients diagnosed with bladder cancer. These patients are investigated by intravenous urography or ultrasound, urine cytology and flexible cystoscopy. If cystoscopy confirms the presence of a bladder tumour, these patients will subsequently undergo transurethral resection of bladder tumour (TURBT) for histological confirmation and staging. Further treatment depends on the histological stage and grade. Those with invasive tumour, i.e. T2 or T3, will undergo radical treatment, with radical cystectomy and urinary diversion being the most common procedure. Patients who are not suitable for surgery can undergo radiotherapy. However, a combination of chemotherapy and radiotherapy has recently been attempted to preserve the bladder.

The majority of patients will have a Ta or T1 tumour. As shown in Table 2, they can be classified as low risk,
intermediate risk or high risk to guide subsequent treatment. The main problem with these patients is recurrence in the majority of patients, and progression.7

Recurrence and progression

The recurrence rate for low risk patients is around 15% and 31% at 1 and 5 years, respectively, while for high risk patients it is 61% and 78% at 1 and 5 years, respectively. This is based on the extensive review carried out by Sylvester et al of 2,596 patients from seven European Organisation for Research and Treatment of Cancer (EORTC) trials.8 The same review also showed that in low risk patients, the progression rate was 0.2% and 0.8% at 1 and 5 years, respectively, while in high risk patients it was 17% and 45% at 1 and 5 years, respectively.8

Table 1. TNM classification of bladder tumours

<table>
<thead>
<tr>
<th>Tumour type (T)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tx</td>
<td>Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumour</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ, flat tumour</td>
</tr>
<tr>
<td>Ta</td>
<td>Noninvasive primary tumour</td>
</tr>
<tr>
<td>T1</td>
<td>Tumour invades subepithelial connective tissue</td>
</tr>
<tr>
<td>T2</td>
<td>Tumour invades muscle</td>
</tr>
<tr>
<td>T2a</td>
<td>Tumour invades superficial muscle</td>
</tr>
<tr>
<td>T2b</td>
<td>Tumour invades deep muscle</td>
</tr>
<tr>
<td>T3</td>
<td>Tumour invades perivesical fat</td>
</tr>
<tr>
<td>T3a</td>
<td>Microscopically</td>
</tr>
<tr>
<td>T3b</td>
<td>Macroscopically</td>
</tr>
<tr>
<td>T4</td>
<td>Tumour invades any of the following: prostate, uterus, vagina, pelvic wall or abdominal wall</td>
</tr>
<tr>
<td>T4a</td>
<td>Tumour invades prostate, uterus or vagina</td>
</tr>
<tr>
<td>T4b</td>
<td>Tumour invades pelvic wall or abdominal wall</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Regional lymph nodes (N)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nx</td>
<td>Regional nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Metastasis in a single lymph node measuring ≤ 2 cm in greatest dimension</td>
</tr>
<tr>
<td>N2</td>
<td>Metastasis in a single lymph node measuring &gt; 2 cm but not &gt; 5 cm in greatest dimension or multiple lymph nodes, none measuring &gt; 5 cm in greatest dimension</td>
</tr>
<tr>
<td>N3</td>
<td>Metastasis in a lymph node measuring &gt; 5 cm in greatest dimension</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distant metastasis (M)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mx</td>
<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>M1</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M2</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

Table 2. Patient risk classification

<table>
<thead>
<tr>
<th>Risk status</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Single, Ta, G1, &lt; 3 cm in diameter</td>
</tr>
<tr>
<td>High</td>
<td>T1, G3, multifocal or highly recurrent CIS</td>
</tr>
<tr>
<td>Intermediate</td>
<td>All other tumours, Ta, Ta-1, G1-2, multifocal, &gt; 3 cm in diameter</td>
</tr>
</tbody>
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G = grade of tumour and refers to the differentiation of the tumour; G1 = tumours that have least degree of anaplasia compatible with the diagnosis of malignancy, i.e. well differentiated; G3 = tumours with the most severe degree of anaplasia, i.e. poorly differentiated; G2 = tumours in between; CIS = carcinoma in situ.
The most important factors for progression are T category, grade and presence of CIS. To better improve the prognosis of T1 disease, some have suggested the subclassification of T1 into T1a and T1b. T1a is where the lesion has gone into the lamina propria but has not involved the muscularis mucosa, while T1b is where there is involvement of the muscularis mucosa. Hasui et al have shown that the progression rate of T1b is significantly higher than T1a irrespective of the grade, size and number of tumours.9

As far as the grading is concerned, various grading systems have been used, but the one most widely used currently is the original Mostofi grading, which was subsequently adopted by the World Health Organisation (WHO) in 1973. In this system, there are three grades based on the differentiation of the tumour, as described in Table 2. The main criticism regarding this system is that the criteria used to assign a grade are vague and subjective, resulting in poor interobserver reproducibility. Further, the classification of grade 2 was broad and not very specific.10 In view of this, in 1998, the WHO and the International Society of Urologic Pathologists put forward a consensus classification of urothelial neoplasms that has now been adopted as the 2004 WHO grading system. The classification is shown in Table 3. In this system, each grade has a more detailed histological description that should help prevent the interobserver variation noted in the WHO 1973 grading system. As far as prognosis is concerned, there seems to be better correlation with the new system, although the system has been slow to gain acceptability by urologists and pathologists, as most recent publications continue to use the 1973 grading system.11,12 In view of this, there has been a recommendation made to use both classifications together in the interim period to increase familiarization with the new grading system. Table 4 shows the correlation between the two systems.10,12

Another important risk factor is tumour recurrence at the first surveillance cystoscopy 3 months after the primary resection. Many have suggested a repeat TURBT between 2 and 6 weeks after the first resection to ensure that the recurrence is not due to residual tumour. It was shown in a study by Herr that, in a repeat resection done 2–6 weeks after the initial resection, 76% of the patients had residual disease and 29% had a higher stage including invasive disease.13 The presence of residual tumour and higher stage tumour during re-resection increases in T1, higher grade, multiple and solid tumours.14 Thus, second-look transurethral resection (TUR) is currently recommended for patients with T1, high grade, multiple tumours and those for whom complete primary resection was not possible for various reasons.

In an attempt to reduce recurrence and progression, many different types of treatment have been attempted, but the most successful is intravesical treatment. This form of treatment involves instillation of different agents directly into the bladder via a urethral catheter. Many agents have been used, among them thiopeta, adriamycin, mitomycin C, epirubicin and bacillus Calmette–Guérin (BCG).15 Intravesical BCG treatment of bladder cancer has been the most successful immunotherapy of any cancer.

**Development of BCG**

Nocard isolated *Mycobacterium bovis* from a cow with tuberculous mastitis in 1904. In 1908, Calmette and Guérin, 304

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**Table 3. 2004 World Health Organisation classification of urothelial neoplasm**

<table>
<thead>
<tr>
<th>Hyperplasia</th>
<th>Flat hyperplasia</th>
</tr>
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<tbody>
<tr>
<td>Flat lesions with atypia</td>
<td>Reactive (inflammatory) atypia</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>Carcinoma <em>in situ</em></td>
</tr>
<tr>
<td>Papillary neoplasms</td>
<td>Papilloma</td>
</tr>
<tr>
<td></td>
<td>Papillary neoplasm of low malignant potential (PUNLMP)</td>
</tr>
<tr>
<td></td>
<td>Papillary carcinoma, low grade</td>
</tr>
<tr>
<td></td>
<td>Papillary carcinoma, high grade</td>
</tr>
</tbody>
</table>

*May include cases formerly classified as severe dysplasia.*

**Table 4. Relation of World Health Organisation (WHO) 1973 to WHO 2004**

<table>
<thead>
<tr>
<th>WHO 1973</th>
<th>WHO 2004</th>
</tr>
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<tbody>
<tr>
<td>Papilloma</td>
<td>Papilloma</td>
</tr>
<tr>
<td>Grade 1</td>
<td>PUNLMP</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Low grade</td>
</tr>
<tr>
<td>Grade 3</td>
<td>High grade</td>
</tr>
</tbody>
</table>

PUNLMP = papillary neoplasm of low malignant potential.
while working on an antituberculosis vaccine, noted that the culture gradually lost its virulence with each passage. After 231 passages over a period of 13 years, it became avirulent. This special M. bovis strain was named Bacillus of Calmette and Guérin, or BCG. Storage was a problem at that time so it was necessary to subculture, which led to genetic variability and the emergence of various sub-strains. The most widely used substrains are Connaught, Pasteur, Tice and Tokyo. In 1929, Pearl observed that patients who suffered from tuberculosis had lower frequency of cancer, and BCG as a cancer therapy was first suggested, but it was only in the 1960s that it was taken up. Mathe et al in 1969 showed promising results in treating lymphoblastic leukaemia with BCG. However, no further progress was made due to the inability to reproduce the results of the early studies as well as the development of successful modern chemotherapy and radiotherapy.

In 1974, Zbar and Rapp formulated conditions necessary for antitumour effect with BCG:

- ability to develop an immune response to mycobacteria antigens;
- adequate number of living bacilli;
- close contact between BCG and tumour cells;
- the tumour burden must be small.

In 1976, Morales et al noted that all the conditions were present in superficial bladder cancer and was able to show a decrease in recurrence rate in the nine patients he initially treated. After Lamm et al confirmed these findings in a larger study, BCG has been widely used in the treatment of superficial bladder cancer.

**Intravesical BCG treatment**

Intravesical BCG has been used in TCC of the bladder for three main indications. It is most commonly used as a prophylaxis in superficial bladder cancer after initial TURBT to reduce recurrence and progression. It is also used in the treatment of CIS. The other indication is in the eradication of residual tumour not completely cleared during TURBT for various reasons.

The most common treatment regimen today is the one originally introduced by Morales et al but without the simultaneous intradermal BCG. The early practice of using intradermal BCG to enhance the immune response to intravesical BCG has been subsequently shown to be of no benefit. The freeze-dried BCG powder is mixed with 50 mL of saline and instilled into the bladder via a urethral catheter. The patient retains the fluid in the bladder for around 1–2 hours. The treatment consists of once weekly instillation of BCG for 6 weeks. The best method of measuring the strength of the various strains of BCG used is by the number of colony forming units (CFU). This is because the efficacy of the BCG depends on the number of viable bacteria in the instillation used. Kelley et al measured the CFU in each batch of BCG vials and noted that the batch with $6 \times 10^6$ CFU had a higher recurrence rate compared to another batch with 300 billion CFU.

**Prophylactic treatment**

One of the main aims of treating patients with intravesical BCG is to reduce the recurrence and progression rate. Many studies have shown a response rate varying from 40% to 60%. The response also depends on the stage and grade of tumour. The risk groups for superficial TCC of the bladder is as shown in Table 2 based on European Association of Urology guidelines. In one of the early randomized trials, Lamm noted a 52% recurrence rate in the control group compared with a 20% recurrence rate in the BCG group. The recurrence-free interval in the treatment group and control group was 48 months and 24 months, respectively. One of the best results has been from Offenburg, in Germany, where 109 of the 126 patients who were only started on intravesical BCG after repeated resections were found to be tumour-free. After a median follow-up of 4 years, 71% of the patients remained tumour-free after one cycle. This increased to 86% after a second cycle for the initial failures. The reason for the high success rate is most probably due to the low tumour burden before BCG treatment was commenced. A meta-analysis carried out by Sylvester et al for the EORTC showed a relative reduction in risk of progression of 32% and 35% for patients with papillary tumour and CIS, respectively. The decrease in progression rate has been noted to have an impact on the mortality rate. Herr et al noted an improved 5-year survival rate of 87% in the BCG group compared to 63% in the TUR only group. They also showed that cancer deaths were reduced from 37% to 12%. In a Cochrane review, it was noted that in medium-risk and high-risk groups, BCG reduced the risk of recurrence by 54% compared to TUR alone.

BCG has been very successful in the treatment of T1G3 tumours, which has a recurrence and progression
rate of >80% and 50% respectively. Many urologists have suggested radical cystectomy as the treatment of choice. However, BCG has made a great impact in the management of these patients. Currently, most urologists would attempt BCG as their first line of treatment and would only consider radical cystectomy after failure of one or two cycles of treatment. Peyromaure et al showed that patients with a T1G3 tumour treated with BCG had a recurrence rate and progression rate of 42.1% and 22.8%, respectively, after a median follow-up of 53 months. In another study, Brake et al reported 27% recurrence and 16% progression after one or two cycles. Overall, 82% of the patients remained tumour-free after a mean follow-up of 3 years.

CIS

CIS in the bladder is a high-grade noninvasive disease with a progression rate of more than 50%. The complete response rate to intravesical BCG varies between 60% and 79% based on 41 studies with a total of 1,496 patients. Lamm et al showed a complete response rate of 70% with a median duration of response of 39 months when BCG was used in the treatment of CIS; 45% of patients were disease-free after 5 years and 64% of patients who had a complete response remained disease-free for 5 years or more. In another South West Oncology Group (SWOG) study, an additional 3-week course of BCG at 3 months resulted in a further 25% increase in the complete response rate at 6 months, and with further maintenance every 6 months for 3 years, there was an estimated 5-year disease-free rate of more than 75%. With these impressive results, BCG has replaced radical cystectomy as the treatment of choice in the management of patients with CIS.

Residual disease

Eradication of residual disease is another indication for using intravesical BCG. This group of patients form a very small proportion of patients who have BCG treatment and are usually patients who are not fit to undergo endoscopic resection or patients with very extensive Ta or T1 disease. The response rate is usually around 60% to 70%.

BCG vs. intravesical chemotherapy

BCG has been shown to be superior to many of the intravesical chemotherapy agents used. However, very few studies have shown it to be better than mitomycin C (MMC) in terms of recurrence. One of the main reasons for this is that in many of the studies, patients of varying risk groups were analysed together. In the Cochrane review, when BCG was compared to mitomycin C, it was noted that tumour recurrence was significantly reduced with BCG only in the subgroup of patients at high risk of tumour recurrence. However, there was no difference in terms of disease progression or survival. However, others have shown a survival benefit with BCG treatment. Herr et al in their review reported that in high risk and recurrent superficial bladder cancer, patients treated with intravesical BCG had superior disease-free survival. Lamm et al also showed that maintenance BCG improved disease-free survival.

Dosage and schedule

The optimal dose and schedule of BCG has not yet been clearly defined. As noted above, a second cycle of BCG has been found to be beneficial with a further improvement in tumour-free rate of 20–30%. However, the risk of progression and metastases outweighs the benefit of more than two courses of BCG. The risk of muscle invasive tumour was 30% and the risk of metastases was 50% in a study by Catalona et al in patients receiving more than two courses of BCG.

Low-dose BCG has been shown to be as effective as full-dose BCG in a number of studies. The doses used varied from half to quarter strength. It has been shown that low-dose BCG can reduce the side effects without compromising efficacy. Further large-scale studies are required before the optimum dose can be defined.

Side effects are the main reason for discontinuing the treatment in many studies and are especially important in maintenance treatment as discussed below.

Maintenance

The role of maintenance therapy after the induction course of BCG is controversial. Maintenance therapy involves continuous treatment with BCG after successful induction therapy. It consists of shorter cycles given periodically. The EORTC meta-analysis showed that the trials resulting in a reduction in risk of progression were those with maintenance therapy. However, there is no standard maintenance therapy schedule, but the one with the best reported results is that by SWOG from USA. In their schedule, the
maintenance therapy consisted of 3-weekly instillations at 3, 6, 9, 12, 18, 24, 30 and 36 weeks. The main concern regarding maintenance therapy has been the side effects associated with BCG. In the SWOG study, only 16% of patients completed the full course of maintenance mainly due to side effects. However, the EORTC study found that patient drop-out due to side effects mainly occurred in the first 6 months and the local side effects remained fairly constant during the entire treatment period while the systemic side effects were observed more frequently during the first 6 months.

Overall, it seems that maintenance therapy is beneficial if the patient is able to tolerate the side effects. The main side effects are cystitis, dysuria and frequency which can occur in as high as 90% of the patients treated with BCG. Other side effects include haematuria in a third and fever in about 3%. The majority of patients tolerate the treatment well and the side effects are usually self-limiting. The most serious complication is BCG sepsis, which was noted in less than 0.5% and occurs if given immediately after tumour resection or at the time of severe cystitis. This is most probably due to intravascular absorption through an inflamed bladder wall.

**Mechanism of action**

The exact antitumour mechanism of BCG is not clear but it is known that a complex cascade of immunomodulating processes is involved. The importance of an intact immune system in the antitumour activity of BCG was first demonstrated by Ratliff and colleagues. They noted that BCG was not effective in eradicating the disease in athymic mice. It was also shown that animals depleted of T cells were not capable of mediating antitumour activity after BCG treatment. This suggests that T lymphocytes and cell-mediated immunity are important in the immune response to BCG.

After intravesical instillation, live mycobacteria attach to the urothelial lining. This binding is facilitated by fibronectin, which is a component of the extracellular matrix, and BCG undergoes endocytosis. The contact between BCG and the epithelium is important as BCG-induced antitumour activity is localized to the site of contact. This process leaves bacterial cell surface glycoprotein attached to the epithelial cell membrane and this interaction of BCG leads to the activation of urothelial and antigen presenting cells (APC). The APC include macrophages, B lymphocytes, dendritic cells and Langerhans cells. The APC process and present antigens which are then linked to major histocompatibility (MHC) class II molecules for recognition by T helper cells.

Intravesical BCG therapy has been noted to induce local production of various cytokines including interleukin-1 (IL-1), IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-18, interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α). Cytokines are soluble mediators secreted by macrophages, monocytes or lymphocytes. They are mediators of stimulatory or inhibitory signals between cells. Their functions include attracting APC to the site of infection and T cell differentiation and proliferation. Many of these cytokines are known to be involved in the initiation or maintenance of the inflammatory process.

It is thought that the initial production of cytokines, after BCG instillation, is by macrophages and urothelial cells and further production is by activated T cells. The cytokines produced after intravesical BCG have been identified in the urine of patients. The majority of the cytokines produced are mainly the T helper type 1 (TH1) cytokines like IFN-γ, IL-2 and IL-12. It has also been shown that the production of cytokines associated with TH2, like IL-4, are decreased and patients who have a high level of TH2-associated cytokines are more likely to have BCG failure. A few studies have shown that cytokine levels can be used to predict the long-term response to BCG treatment with higher levels indicating a lower recurrence rate and a longer recurrence-free interval.

The main mechanism by which BCG stimulates the immune system to overcome the tumour is by playing a role, either directly or indirectly, in the production of effector cells. These include the stimulation of cytotoxic T lymphocytes (CTL), natural killer (NK) cells, lymphokine-activated killer (LAK) cells and BCG-activated killer cells (BAK). BAK cells are similar to NK and LAK cells but have some differences, as the effects are believed to last longer than those of the other killer cells. The production of effector cells is stimulated by cytokines. The cytokines, especially IFN-γ, also induce the expression and upregulation of MHC class II and intracellular adhesion molecule 1 by the tumour cells. This helps in the recognition and destruction of the tumour by the effector cells.

The peak immune response is usually noted around 6–24 hours after instillation and there is a cumulative increase in response up to the fifth or sixth cycle. The response slowly continues to wane over a period but the exact
duration of response is not clear as cytokine-producing infiltrates have been identified up to 21 months after treatment. This is the rationale for maintenance therapy.

Conclusion

Intravesical BCG is an effective and well established immunotherapy for superficial TCC of the bladder. However, the optimum dose and schedule of treatment is yet to be defined. The other important aspect that needs to be established is the prognostic or predictive factors of treatment response. This is important for selecting patients who will benefit from the treatment to avoid unnecessary radical treatment and at the same time identify non-responders early on so that they can be offered alternative treatment without compromising their prognosis.

References