BI
BIOMEDICINE INTERNATIONAL
2013; 4: 58-71.

ORIGINAL ARTICLE

Antitumor Effect of Bacillus Calmette-Guérin in Bladder Cancer

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ABSTRACT

Bacillus Calmette-Guérin (BCG) is currently the standard therapy for treating high grade non-invasive bladder cancer. BCG is superior to intravesical chemotherapy. The antitumor effect of BCG seems to be related to cellular immunological mechanisms. However, its precise mode of action is unknown. High-risk non-invasive bladder tumors in patients will progress to muscle-invasive bladder cancer despite BCG treatment. Traditional prognostic factors are mainly based on histopathological characteristics. However, there are no clear definitive markers for the response to therapy, partly because of a lack of knowledge concerning the mechanism of action utilized by BCG to mediate the observed clinical response. In response to the inflammatory process, the urothelial and tumor cells upregulate the expression of important surface proteins, such as major histocompatibility antigens, adhesion molecules, and death receptors. These proteins might serve as markers and potential therapeutic targets to enhance BCG efficacy. The genetic and molecular changes that occur in transitional cell carcinoma (TCC) of the bladder are numerous. Recent advances in carcinogenesis research will permit the discovery of new markers in patients treated with BCG that are directly involved in the pathogenesis of bladder cancer. Further investigations of the antitumor effect of intravesical BCG will lead not only to a potential enhancement of the BCG response, but probably also to a better understanding of bladder carcinogenesis. *Biomed. Int.* 2013; 4: 58-71.

Key words: Bladder, immunity, malignancy, tuberculosis

INTRODUCTION

In 1921, a bacteriologist named Albert Calmette and a veterinarian named Camille Guérin attenuated the bovine tuberculosis bacillus, *Mycobacterium bovis* (*M. bovis*) at the Pasteur Institute in Lille, France.[1] A virulent strain of *M. bovis* was obtained from the udder of an infected cow, and the bacilli were cultured in a medium consisting of cow bile, potatoes, and glycerin. Loss of virulence was achieved gradually and a genetically stable, non-virulent form of *M. bovis* was obtained; this unique strain was named bacillus Calmette-Guérin (BCG).[2] This bacillus was orally administered to an infant whose mother had died of tuberculosis; no side effects were detected, and the baby did not contract tuberculosis. Eventually, the original Pasteur strain vaccine obtained by Calmette and Guérin could be lyophilized and stored. Subsequently, the Pasteur Institute began mass production of the vaccine, and it was distributed worldwide.

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Submitted September 10, 2012; accepted March 7, 2014.
For online access, see www.bmijournal.org

In 1929, a low frequency of cancer was observed among patients suffering from tuberculosis at the Johns Hopkins Hospital, and Raymond Pearl, a professor of biology, suggested that this was due to a mutual antagonism between tuberculosis and cancer.[3] In 1950, the first direct evidence of the antitumor effect of BCG was demonstrated at the Sloan-Kettering Institute in New York, where mice infected with BCG showed increased resistance against transplantable tumors. Furthermore, BCG was demonstrated to activate macrophages that could inhibit or destroy cancer cells, and to induce hemorrhagic necrosis in murine tumors. In 1969, the use of BCG as an adjuvant therapy for malignant melanoma was established[4], and Jean DeKernion utilized cystoscopic injections of BCG to treat melanoma of the bladder.[5]

BCG and Bladder Cancer

Immunotherapy could be useful in the treatment of non-invasive bladder cancer owing to the antigenicity of such tumors.[6] In 1975, Bloomberg et al. reported that immunostimulation with agents such as BCG could be used as an adjunctive treatment in patients with bladder cancer and suggested a role for immunopotentiators in the future.[7]

In 1976, the first report demonstrating that BCG could be used to treat bladder cancer was published. Morales et al. reported that seven out of nine patients with recurrent superficial stage Ta-1 bladder cancer were successfully treated using intravesical BCG once a week for six weeks.[8] Before BCG therapy began, the nine patients collectively had a total of 22 recurrences during 77 months. After vaccination, only one recurrence occurred during the 41-month follow-up period. The authors concluded that intravesical BCG altered the pattern of evolution of bladder cancer in patients with persistent tumor recurrence without severe side effects. The Southwest Oncology Group (SWOG) confirmed the efficacy of the Morales regimen against non-muscle-invasive bladder cancer[9], and a randomized controlled trial carried out at the Memorial Sloan-Kettering Cancer Center demonstrated that BCG markedly reduced the frequency of tumor recurrence compared with treatment with transurethral surgery alone.[10]

Brosman observed that patients with superficial bladder cancer treated with six weekly instillations of Tice strain BCG followed by additional instillations every two weeks for three months and subsequently every month for two years presented with a reduction in tumor recurrence rate. Furthermore, an ablative effect was evident in some patients with incomplete resection of the bladder tumors.[11] Herr et al. have suggested that intravesical therapy with BCG delays tumor progression and reduces specific cancer mortality in patients suffering from superficial bladder cancer.[12] In 1990, the American Federal Drug Administration (FDA) approved the general use of intravesical BCG for the treatment of patients with superficial bladder tumors, and BCG is now the standard therapy for treating high grade non-invasive bladder cancer. Approval was based on Dr. Lamm's study in which treatment with BCG was compared with treatment with intravesical doxorubicin.[13]

Strains of BCG

Multiple strains of BCG, including Pasteur, Armand-Frappier, Connaught, Tice, CoRIVM, Glaxo, and Tokyo, are used to treat superficial bladder cancer worldwide. Each strain retains a similar antigenic potency[2], and there are no clinical studies demonstrating superiority of one BCG strain over the others. A recent meta-analysis concluded that there are no

significant differences in efficacy among the Pasteur, Armand-Frappier, Connaught, Tice and CoRIVM strains.[14]

Shelley et al. published a meta-analysis of a large number of randomized controlled trials evaluating the benefit of adjuvant intravesical BCG following transurethral resection (TUR) compared with TUR alone in intermediate-risk and high-risk patients. In this study, Tokyo, Connaught, and Pasteur strains were analyzed and the authors concluded that BCG reduced the risk of recurrence regardless of the strain used.[15] Akaza reported that the Connaught strain resulted in complete response rates of 63.5% in Ta and T1 bladder cancer patients and 77.8% in bladder cancer patients with carcinoma in situ (CIS), findings comparable to the complete response rate of 66.4% reported for Ta and T1 bladder cancer patients treated with the Tokyo 172 strain.[16] In 2005, Quirino et al. analyzed the use of the lyophilized Moureau-Rio de Janeiro BCG strain in 114 patients with non-invasive bladder cancer and reported tolerance and activity similar to those of other strains.[17]

Thalmann et al. reported that the recurrence rates in patients treated with the Connaught strain were significantly lower than in patients treated with the Tice strain, yet both groups had a comparable incidence of side effects.[18] However, most studies conclude that the different strains of BCG have similar efficacy.

Dosage

The first intravesical BCG dose utilized (Armand-Frappier strain) was empirically determined to be 120 mg, based on the observation that this dose was tolerated by intradermal scarification. To optimize the effectiveness of intravesical BCG, randomized studies have investigated varying the dose and duration of BCG administration in order to find an effective low dose that is not toxic.[19]

The Spanish Oncology Group (CUETO) compared the efficacy of a three-fold reduced dose (RD, 27 mg) of intravesical BCG (Connaught strain) with the standard dose (81 mg). The authors analyzed recurrence, progression, and toxicity in 500 patients with superficial bladder cancer (Ta, T1, CIS, Tis) that were randomly assigned 81 mg or 27 mg intravesical BCG following TUR. Instillations were repeated once a week for six consecutive weeks and thereafter once every two weeks for a total of 12 weeks. 71 patients receiving the standard dose (28%) and 76 patients receiving the reduced dose (31%) developed recurrences; there was no significant difference between the groups ($p = 0.586$). Furthermore, there was no significant difference in terms of recurrence in patients considered at high-risk (T1G3 or Tis, relapsing often, multifocal or large tumor), although only 29.5% presented with recurrence when administered the standard dose compared with 36.7% treated with the reduced dose ($p = 0.14$). In terms of tumor progression, there was no significant difference between the groups, with progression occurring in 11.5% of patients receiving the standard dose and in 13.3% of patients receiving the reduced dose. In addition, there was no significant difference in overall survival after five years. However, in the group of patients with high-risk tumors (multifocal disease), the standard dose was more effective at preventing progression than the reduced dose ($p = 0.048$). It was concluded that the lower dose was significantly less toxic than the standard dose, but there was no significant difference between the lower and standard doses in terms of recurrence and progression. Therefore, the authors proposed the use of the standard dose for the induction cycle in high-risk tumors, and the reduced dose for the induction cycle in intermediate-risk tumors and for all maintenance schedules.[20]

In 2005, the CUETO group studied the efficacy of the reduced dose for T1G3 and Tis neoplasms in 155 patients. 73 patients received the reduced dose (27 mg) and 82 patients received the standard dose (81 mg). Recurrence was evident in a total of 65 patients (41.9%). There was no significant difference between the groups ($p = 0.405$), with 39% of those receiving the standard dose and 45% of those receiving the reduced dose demonstrating recurrence. There was no significant difference between the groups in terms of progression ($p = 0.7997$). Progression was evident in 39 patients (25.3%), including 20 (24.3%) receiving the standard dose and 19 (26%) receiving the reduced dose. The authors concluded that the reduced dose is as effective as the standard dose against progression in patients with high-risk (T1G3 and Tis) superficial bladder carcinoma, and the standard dose was proposed to be the dose of choice for general purposes and in maintenance schedules.[21]

Bohle compared the reduced dose of intravesical BCG with a very low dose (13.5 mg) and concluded that the 13.5 mg dose was significantly less effective.[22] Another trial compared the low dose of BCG (27 mg) with a dose of 13.5 mg and with a dose of 30 mg of mitomycin C (MMC). The disease-free interval was significantly better for primary disease treated with BCG 27 mg, but there was no difference in the time to progression among the three groups.[23] Madhu et al. published the results of a trial that used three different doses of BCG to determine whether lowering the dose could reduce toxicity without compromising efficacy. Patients (Ta, T1) received 40 mg, 80 mg, and 120 mg of modified Danish strain 1331 BCG via weekly instillations for six weeks, followed by a maintenance schedule of monthly instillations for a year. No significant difference in the recurrence rate was evident ($p > 0.05$), and no progression to invasive disease was observed in any patients. However, the reduced dose (40 mg) was less toxic than the intermediate dose (80 mg).[24]

The European Organization for Research and Treatment of Cancer (EORTC) recently completed a randomized study comparing standard-dose BCG with long-term maintenance, BCG at one-third of the standard dose with long-term maintenance, and standard-dose BCG with short-term maintenance in intermediate-risk and high-risk patients who had Ta or T1 papillary carcinoma of the bladder, but the results have not been published.[25]

Subsequent research has demonstrated that BCG is superior to intravesical mitomycin C and that maintenance BCG is better than the standard induction cycle, which involves treatment for six weeks. Additional research and meta-analysis of published trials have shown that BCG reduces not only tumor recurrence, but also reduces progression to muscle-invasive disease when maintenance therapy is used.[13,14,18,25-55]

Tumor Recurrence

It is possible to remove non-invasive bladder tumors surgically, but 50% to 70% of patients have a recurrence within one to two years. To prevent this, patients are treated after TUR with intravesical chemotherapy using drugs such as adriamycin, epirubicin, mitomycin, and BCG. BCG is considered to be the most effective intravesical agent for preventing recurrence in non-muscle-invasive urothelial cell carcinoma of the bladder[19], and multiple meta-analyses have demonstrated a statistically significant reduction in recurrence rates of bladder cancer with the use of BCG.

Shelley et al. published a systematic review of randomized controlled trials directly comparing TUR alone with TUR and intravesical BCG for Ta and T1 bladder cancer, and con-

cluded that TUR with intravesical BCG provided significantly better prophylaxis for tumor recurrence in Ta and T1 bladder cancer. Furthermore, a 56% reduction in recurrence was attributable to BCG.[15] A study by Han et al. in which 25 trials and 2,342 patients underwent BCG therapy also concluded that BCG reduced recurrence; 40.5% of patients had tumor recurrence when treated with BCG compared with 49.7% when BCG was not administered. Furthermore, the combined results demonstrated a statistically significant difference in the odds ratio for tumor recurrence between the group treated with BCG and the group not treated with BCG. The authors concluded that BCG with maintenance treatment is effective for prophylaxis against tumor recurrence.[26] In T1G3 carcinoma, Shahin et al. observed that disease recurred in 70% of patients treated with BCG and in 75% of patients treated with TUR alone, suggesting that BCG treatment for high-risk tumors could delay the time to recurrence, with a median follow-up of 5.3 years.[27]

Comparing the effectiveness of BCG with chemotherapy, Lamm observed that intravesical chemotherapy reduced tumor recurrence by 15%, but that after five years the number of patients with recurrence was comparable to patients treated with surgery alone. In patients treated with BCG, complete tumor regression was achieved in 50% or more of patients with papillary tumors and in more than 70% of patients with CIS. Moreover, the response persisted for five years or more.[28] Other randomized trials have compared intravesical BCG with intravesical mitomycin C (MMC), and Malmström demonstrated that there was no difference in the time to the first recurrence ($p = 0.09$) between patients treated with BCG and patients treated with MMC. However, trials with BCG maintenance demonstrated a 32% lower risk of recurrence than with MMC treatment ($p < 0.0001$). Conversely, there was a 28% increased risk ($p = 0.006$) for patients treated with BCG in trials without maintenance. Malmström concluded that BCG with maintenance was more effective than MMC for treating patients regardless of whether patients had previously received chemotherapy.[29]

There is clearly a role for BCG in the setting of tumor recurrence, but the optimal schedule has not been determined. Bohle et al. compared six studies utilizing BCG maintenance or MMC and concluded that for optimal efficacy BCG must be administered in a maintenance schedule. These authors observed that a minimum of 12 instillations in a year were necessary to achieve superiority over MMC regardless of tumor risk status.[30] Lamm et al. published a randomized study with a no-maintenance arm and a maintenance arm. Maintenance therapy consisted of intravesical BCG administered weekly for three weeks at three, six, 12, 18, 24, 30 and 36 months from initiation of induction therapy. The results suggested that maintenance BCG immunotherapy was beneficial to patients with CIS and a selected group of patients with Ta and T1 bladder cancer.[31] Sylvester et al. concluded that in patients with intermediate-risk and high-risk stage Ta and T1 bladder cancer the same maintenance schedule prolonged the time to first recurrence, the time to distant metastases, overall survival, and disease-specific survival.[32]

Tumor Progression

The invasion of the detrusor muscle (increase from Ta, T1, or Tis to stage T2 or higher) is considered to be progression of a superficial bladder cancer (non-muscle-invasive tumor). Muscle-invasive bladder cancer is a potentially life-threatening neoplasm. Although less than 7% of superficial bladder carcinomas progress to muscle-invasive disease, high-grade T1 tumors have a progression rate of 30% to 50%.[33]

BCG maintenance therapy reduces the risk of progression when compared with other treatment strategies. Sylvester et al. published a meta-analysis of 24 trials with information on progression and compared TUR with intravesical BCG with resection alone and resection with a treatment other than BCG. The authors observed a reduction of 27% in the odds of progression in the group treated with BCG and concluded that BCG reduces the risk of progression after TUR in patients with superficial bladder cancer who receive maintenance treatment.[14] In a meta-analysis of nine trials, Bohle et al. compared the effect of intravesical BCG with MMC on tumor progression. Relative risk of progression was reduced by 23% with BCG therapy, but this was not statistically significant. However, in the subgroup receiving BCG maintenance, there was a statistically significant decrease in progression compared with the group receiving treatment with MMC.[30] In a recent meta-analysis of long-term outcomes of randomized studies comparing intravesical MMC to BCG, maintenance BCG was not more effective than MMC in terms of preventing tumor progression.[29] Therefore, the capacity of BCG to prevent progression remains controversial.

It cannot be concluded that BCG reduces progression in low-risk and intermediate-risk non-muscle-invasive bladder cancer[19], but maintenance BCG is currently the most effective therapy for high-risk non-muscle-invasive bladder cancer as concluded from a meta-analysis of nine randomized trials that included 700 patients with CIS. The results demonstrated that maintenance BCG reduced risk of progression by 26% and is a superior treatment compared with MMC. In conclusion, maintenance BCG is the most effective intravesical therapy for preventing progression of non-invasive bladder cancer to invasive bladder cancer.[34]

Toxicity

Local and systemic side effects due to BCG can occur. Symptoms begin between two and four hours after instillation and usually resolve over the next 24 to 48 hours. Pyuria and microscopic hematuria are the most common side effects, and are accompanied by irritative symptoms. These symptoms can be controlled with acetaminophen, non-steroidal anti-inflammatory drugs, and anti-spasmodics. Systemic manifestations include fever, a flu-like malaise, and occasional arthralgias.

Intravasation of BCG into the bloodstream, or BCGosis, results in systemic effects within two hours of BCG instillation. These effects can be treated initially with fluoroquinolones and antipyretics. However, if fever begins after 24 hours and persists for more than 48 hours, an established BCG infection, or BCGitis, should be suspected. In such cases, the patient frequently requires hospitalization and the administration of triple-drug therapy, such as a combination of isoniazid, rifampicin, and ethambutol.[35]

Recently, adverse events of BCG have been grouped into four classes.[36,37] Class 1 adverse events include low-grade fever and local irritative symptoms such as dysuria, frequency, and hematuria that subside within 48 hours and do not require specific treatment. BCG treatment usually requires no modification. Class 2 and 3 adverse events include moderate or intermediate severe systemic or local symptoms, or symptoms that last more than 48 hours. Class 4 adverse events include contracted bladder, granulomatous prostatitis or epididymitis, pneumonitis, hepatitis, osteomyelitis, systemic infections, and serious complications related to septicemia or immunoallergic reactions.[37]

Hall et al. combined adverse events into several broad categories, classifying symptoms of the same category that presented simultaneously in a patient as "maximal overlap", and symptoms of the same category that presented separately in a patient as "minimal overlap".[38] It has been suggested that class 2 side effects of BCG were significantly reduced by administering ofloxacin after each instillation of BCG. In a study carried out by Colombel et al., the number of class 3 side effects requiring anti-tubercular treatment was reduced in patients who had received ofloxacin.[39] These studies suggest that the administration of 200 mg of prophylactic ofloxacin twice after BCG instillation improves the tolerability of BCG treatment without affecting the treatment's efficacy.

Some authors have suggested a correlation between the local and systemic side effects of BCG and its efficacy. Orihuela et al. suggested that BCG toxicity was primarily due to a cell-mediated immune response[40], and thus the time till first recurrence would be significantly longer for patients who experienced local BCG side effects. However, other studies refute such conclusions. For example, Sylvester et al. published the results of a study carried out to determine if toxicity was responsible for improved efficacy and observed that patients with a better outcome remained in the study for a longer period of time and received more BCG, thus increasing their risk for side effects. The authors concluded that a correlation between BCG toxicity and efficacy exists but that there is no evidence that BCG toxicity is responsible for an improved outcome.[41] In support of these conclusions, van der Meijden et al. observed that the majority of local and systemic side effects are seen during induction and the first six months of maintenance, and concluded that toxicity does not increase during maintenance therapy with BCG and that instillations are usually well tolerated.[42]

In 1992, Lamm et al. reported on the incidence and variety of toxicities in 2,602 patients treated with intravesical BCG. The choice of treatment depended upon the severity of toxicity, and treatment options ranged from delaying or withholding instillations to administering anti-tubercular drugs for up to six months, and 95% of the patients had no serious side effects. Recognition of risk factors, particularly traumatic catheterization or concurrent cystitis that results in systemic absorption of BCG, and prompt treatment of early side effects should significantly decrease the incidence of severe toxicity.[43] Major complications appear after systemic absorption of BCG, and BCG should not be administered for the first two weeks following TUR in patients with hematuria or following traumatic catheterization.[44] The majority of side effects are mild and self-limiting, but potentially life-threatening complications can develop, including pneumonitis, hepatitis, and systemic infection with BCG.

Once they have been recognized, virtually all side effects can be treated successfully. Treatment options include delaying or withholding BCG instillations or using antipyretics and analgesics to reduce bladder-related symptoms. Treatment with tuberculostatic drugs for up to six months may be necessary in cases of severe systemic or local toxicity. BCG is very susceptible to most antibiotics and tuberculostatic agents *in vitro*, and some authors suggest that prophylactic use of tuberculostatic agents such as isoniazid or reduction in the dose of BCG can prevent side effects without impairing the efficacy of BCG.[45] However, Vegt et al. analyzed the influence of isoniazid on the incidence and severity of side effects of intravesical BCG treatment in a prospective randomized multi-center study of patients with pTa and pT1 bladder tumors and concluded that there were no differences in local or systemic adverse reactions after intravesical BCG treatment between patients who received

prophylactic isoniazid and patients who did not receive prophylactic isoniazid. However, analysis of liver function tests after treatment with BCG and isoniazid demonstrated greater toxicity than treatment with BCG alone. Prophylactic administration of isoniazid during BCG instillations does not decrease any known side effects. In fact, transient liver function disturbances are encountered more frequently when isoniazid is administered, and the use of prophylactic isoniazid in patients treated with BCG is not recommended.[46]

Immune Mechanisms in Bacillus Calmette-Guérin Therapy

After the original Pasteur strain vaccine was developed by Calmette and Guérin, further passages were obtained, which resulted in a number of additional strains with different phenotypes that retained the same antigenic potency as the original. The current view is that different strains do not vary in efficacy, although few comparative studies exist.[47] A meta-analysis published in 2002 demonstrated no significant differences in efficacy among the Pasteur, Armand-Frappier, Connaught, Tice, and CoRIVM strains.[14] The exact mechanism of action of BCG's antitumor effect is unknown. The initial step involves the binding of mycobacteria to the urothelial lining, which probably depends on the interaction of an attachment protein on the bacterial surface with fibronectin on the bladder wall. The high density of mycobacteria decreases the proliferation and viability of tumor cells, and the presence of BCG leads to activation of urothelial cells and antigen-presenting cells. Immunotherapy with BCG provokes an extensive local immune response characterized by induced cytokine expression in bladder tissue and migration of monocytes, granulocytes, and mononuclear cells into the bladder wall. A cascade of pro-inflammatory cytokines sustains the immune response.[48]

The bladder is not normally infiltrated by large numbers of immune cells. Before therapy ensues, low numbers of leukocytes can be detected in the suburothelial stroma of non-tumor-bearing areas of the bladder. Following intravesical instillation, BCG provokes an early influx of innate immune cells, including polymorphonuclear neutrophil granulocytes (PMN's) and Th1 helper cells.[49] A large set of cytokines, including tumor necrosis factor-α, granulocyte macrophage colony-stimulating factor, interferon-γ (IFN-γ), and various interleukins (IL), such as IL-1, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, and IL-18, have been detected in the urine of patients treated with intravesical BCG. Many of these cytokines are involved in the initiation or maintenance of inflammatory processes. Therefore, the antitumor effect of BCG during the induction period is likely to be non-specific, as normal urothelial cells *in vivo* and tumor cells *in vitro* are damaged. Some authors have reported that a high number of eosinophils in the urine could indicate failure of therapy.[50]

BCG instillation appears to induce a long-lasting immune response in the bladder. Given the importance of cellular immunity and interferons for the effectiveness of BCG therapy, various immunological events could determine the outcome of therapy. After repeated BCG instillations and early accumulation of granulocytes, an influx of macrophages and lymphocytes can be observed.[48] The bladder wall is infiltrated by mononuclear cells, which consist predominantly of monocytes, macrophages, CD4+ and CD8+ T lymphocytes, and natural killer (NK) cells, and these cells form chronic, granuloma-like cellular infiltrates in the suburothelial stroma.[51]

NK cells are critical for the antitumor effect of BCG *in vitro* and *in vivo*.[52] These cells recognize major histocompatibility complex (MHC) class I molecules through inhibitory NK cell receptors, which transduce inhibitory signals and deactivate NK cell cytotoxicity.

Therefore, NK cells lyse targets that have lost MHC class I molecules or that express insufficient amounts of MHC class I molecules, such as bladder tumor cells. In most neoplasms, MHC class I molecules are down-regulated during transformation of normal cells into tumor cells, thereby rendering tumor cells potential targets for NK cells. This molecular mechanism allows NK cells to discriminate between normal cells and malignant cells.[53] Suttmann et al. state that the expression of MHC class I molecules on bladder tumor cells is of primary importance for prognosis and for responsiveness to BCG immunotherapy. BCG immunotherapy induces the expression of various cytokines including IFN-γ, which regulates the expression of MHC class I molecules on tumor cells. The expression of MHC class I molecules on tumor cells influences susceptibility to lysis by cytotoxic T cells and NK cells, and the expression of these MHC class I molecules is modulated by BCG-induced cytokines. These cytokines also modify the activity of antitumor effector cells such as NK cells.[54] Activated NK cells are crucial for effective BCG immunotherapy and possibly for the success of other immunotherapy regimens. Current studies concerning the successful clinical use of IFN-γ to treat patients with bladder cancer demonstrate the importance of basic research for the development of treatments in the future.

Ayari et al. investigated whether tumor-infiltrating dendritic cells (TIDCs) and tumor-associated macrophages (TAMs) could aid urologists in selecting patients for early aggressive therapy.[55] On the basis of a retrospective analysis, it was concluded that immunohistochemical analysis of tumor specimens demonstrated that maintenance therapy was highly effective in patients with low levels of CD83+ TIDCs. In addition, it was observed that CD86+ TAMs were associated with an increased risk of recurrence and that the level of infiltration of the tumor by these cells is important for the response to BCG.

Molecular Markers for the Response to Bacillus Calmette-Guérin Therapy

High-risk non-invasive bladder tumors will progress to muscle-invasive bladder cancer despite BCG treatment. Patients who fail BCG therapy and require radical surgery have a worse prognosis than patients who are admitted for immediate cystectomy, and this fact underlines the clinical importance of identifying prognostic markers for the response to BCG therapy.[47] The challenge is to identify post-TUR patients who will respond to BCG instillations and to individualize BCG treatment according to the patient's immune status. Gender and age have been reported to be associated with the response to BCG.[56] In addition, numerous tumor factors have been linked to the prognosis of transitional cell carcinoma (TCC) of the bladder after BCG therapy.

Traditional prognostic factors are based on histopathological characteristics.[56] However, current evidence does not support the use of molecular markers to predict the response to BCG in clinical practice. There are no definitive markers for the response to BCG therapy, partly owing to a lack of knowledge concerning the mechanism of action utilized by BCG to mediate the observed clinical response.

Granulomas present in biopsies of the bladder after BCG therapy have been associated previously with a lower risk of tumor recurrence, but recent studies involving long-term follow up and large numbers of patients refute such claims. In these studies, other markers such as leukocyturia and Ag-85 correlate with recurrence and progression.[57,58] Zlotta et al. demonstrated that a good response to BCG therapy was associated with lymphoproliferation in response to Ag-85[58], and Saint et al. demonstrated that leukocyturia could be a promising prognostic factor because it correlates with time to recurrence.[59]

Several studies have concluded that urinary markers such as cytokines could be predictive of the response to BCG, and argue that levels of such markers reflect the local immunological reaction in the bladder. Urinary IL-2 is the most promising of these markers and has been linked to time to recurrence[58,60], but not to time to progression. Production of IL-2 by peripheral blood lymphocytes after administration of BCG was associated with a longer time interval before recurrence in a multivariate analysis published by Kaempfer et al.[61] Calais et al.[62] carried out a study involving 90 patients with superficial bladder carcinoma and evaluated cytokine gene polymorphisms after BCG therapy and TUR. There were 72 responders to the treatment regimen (80%) and 18 non-responders in whom tumor recurrence was evident (20%). The results demonstrated that certain alleles for tumor necrosis factor-α, IL-1, and IL-10 were at significantly higher levels in patients with bladder carcinoma recidive after BCG therapy, suggesting that the genetics of immunoregulatory molecules play a role in predicting the response to BCG therapy for bladder cancer.

The relevance of urinary IFN-γ, a marker for local activation of Th1 cells in the bladder wall following instillations of BCG, to the prognosis of superficial bladder cancer was demonstrated by Zoumpos et al.[63] The soluble fraction of this cytokine was measured in urine in order to monitor the immune response linked to the mechanism of action of BCG. There was a statistically significant difference in urinary IFN-γ levels in favor of responders at the time of the fifth and sixth instillations of BCG. There were no significant differences in the urinary IFN-γ levels irrespective of tumor stage and grade. Regression analysis demonstrated that urinary IFN-γ levels at the sixth instillation of BCG could predict the response to treatment with 70.6% accuracy, confirming its potential as a possible prognostic marker.

A recent study evaluated the clinical utility of fluorescence in situ hybridization (FISH), a multi-target test that can be performed on bladder cells and that detects up to four chromosomal aberrations, for surveying the response of bladder cancer patients to BCG therapy.[64] Patients with a positive test after treatment with BCG were at almost threefold higher risk for tumor recurrence than patients with a negative test. In addition, patients who maintained a positive FISH result before and after treatment with BCG had a risk for tumor recurrence 2.96 times higher than patients who changed from a positive result to a negative result after treatment. However, the test was not useful for predicting progression.

Membrane expression of the protein ezrin is associated with the clinical outcome for patients treated with BCG for high-grade non-muscle-invasive tumors.[65] Ezrin interacts with membrane proteins and the cytoskeletal protein actin. Ezrin is involved in the pathogenesis of bladder cancer and should be investigated further. Expression patterns of ezrin have been associated with tumor progression in T1G3 disease. Therefore, differential expression of ezrin discriminates patients who respond to BCG therapy from patients who may require more aggressive therapy.

CONCLUSION

There is sufficient evidence to support using BCG as maintenance therapy in patients with high-risk non-invasive bladder tumors in order to reduce the recurrence and progression rates of these tumors. However, in a significant proportion of patients, high-grade stage T1 bladder cancer progresses to muscle-invasive disease despite treatment with BCG.

In response to inflammation, urothelial and tumor cells upregulate the expression of important surface proteins, such as major histocompatibility antigens, adhesion molecules, and death receptors. These proteins might serve as potential therapeutic targets to enhance the efficacy of BCG. However, no tumor biomarker evaluated to date is sufficiently sensitive or specific to predict a patient's response to BCG therapy. In the future, serial markers associated with the response to BCG and nomograms based on host, tumor, and immunological characteristics could help clinicians identify patients who may or may not respond to BCG instillations and treat them accordingly.[57] New molecular discoveries such as ezrin require further investigation in prospective trials to evaluate their potential as tools for making therapeutic decisions. Moreover, the improvement of the efficacy of BCG therapy should be based on mechanistic considerations. Enhancing the Th1 cytokine cascade remains an attractive proposition. IFN-γ and non-steroidal anti-inflammatory drugs have been shown to decrease the inhibitory mediators IL-10 and prostaglandin E2, respectively. The addition of stimulatory cofactors such as IFN-γ, IL-2, and granulocyte macrophage colony-stimulating factor has been shown to increase the Th1-related effects of BCG. Improving BCG attachment using anti-fibrinolytic therapy has recently emerged as an exciting new method for enhancing the effect of BCG.[66]

The molecular changes that occur in TCC of the bladder are numerous. It is becoming apparent that an accumulation of genetic and molecular changes ultimately determines a tumor's phenotype and subsequent clinical behavior. Intense research efforts are under way to identify and characterize different biological markers more effectively. Recent advances in carcinogenesis research will lead to the discovery of new markers directly involved in the pathogenesis of bladder cancer, such as ezrin, in patients treated with BCG. In the future, convergent pathways of the immunological response to BCG and tumor development will be described in patients with bladder cancer. Further investigation of the antitumor effect of intravesical BCG will lead not only to improvements in BCG therapy, but also to a better understanding of the pathogenesis of bladder cancer.

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