REVIEW

Isoniazid resistance among Bacillus Calmette Guerin strains: implications on bladder cancer immunotherapy related infections

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Bacillus Calmette Guerin (BCG) immunotherapy is widely used for treatment of superficial bladder transitional cell carcinoma. Infectious complications while rare can be serious and severe disseminated infections as well as sepsis has been reported. There are no standard guidelines to direct therapy of these complications. Isoniazid is a commonly and widely used component of the various treatment regimens. Various strains of BCG are used for treatment of bladder cancer as well as vaccinations. These strains have evolved because of repeated subcultures in various laboratories in the world and have been shown to exhibit phenotypic differences in their immunogenicity as well as recently in susceptibility to various antimycobacterial agents.

In this article, we review the resistance of BCG strains to various antimycobacterial agents. Some of these strains including the BCG Connaught strain, which is widely used in the United States, Canada and some other parts of the world for bladder cancer therapy exhibit intrinsic resistance to isoniazid. Although the clinical relevance of these differences is unclear, recent studies have questioned the role of isoniazid in treatment of infections after vaccination with these strains. Also, use of isoniazid in combination therapy for these infections may lead to the development of resistance to other antimycobacterial agents.

We conclude that isoniazid may not be a suitable agent for empiric treatment of infections related to intravesical immunotherapy for bladder cancer with these strains and further studies are needed to clarify its role.

Key Words: BCG, isoniazid, resistance, Connaught

Introduction

Transitional cell cancer of the bladder is among the top five most common cancers in the male sex with incidence rates of 18-30 new cases per 100000 men.[1] In 75% of men it is diagnosed in the early superficial stages, with microscopic and gross hematuria being the most common presentation. However, even in these patients there is a 65% recurrence and 30% progression rate necessitating long term surveillance. Various chemotherapy agents including thiotepa, doxorubicin, and mitomycin had previously been tried but the results were at best disappointing.

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Bacille Calmette Guerin (BCG) is one of the oldest and most commonly used vaccines worldwide. BCG had previously been postulated as immunotherapy for various cancers including leukemia, colon cancers, melanoma and lung cancers. In 1976 Morales et al described the successful treatment of a group of bladder cancer patients with intravesical instillation.[2] However, this modality of treatment gained wide acceptance only after the Southwest Oncology group was able to demonstrate benefits in terms of both decreased recurrence rates and increased time to recurrence in patients given this form of immunotherapy after local surgery.[3] However, BCG is not effective in treatment of cancers that invade muscle or that lie deep within the prostrate or upper urinary tract where BCG cannot penetrate. The most widely used regimen consists of a weekly intravesical BCG injection for 6 weeks, which is repeated three more times every 6 months.[2] However additional reinduction courses as well as booster doses are sometimes used.

Mechanism of BCG`s antitumor activity

BCG is believed to exert its antitumor activity via local modulation of immune responses which results in inflammation and subsequent elimination of malignant cells.[4] The interaction of BCG with urothelial cells is thought to result in several immunologically important changes including induction of chemokines such as interleukin 8 (which serve to attract leucocytes to a local site), proinflammatory cytokines (granulocyte-macrophage colony stimulating-factor, tumor necrosis factor α, interleukin 6), and the upregulation of adhesion-molecule expression (intracellular adhesion molecule-1), which promotes effector cell-tumor cell interactions. In addition, infection of bladder tumor cells with BCG retards their growth in vitro.[5] As treatment continues, a marked infiltration of the bladder wall occurs, which is characterized by the presence of T lymphocytes, macrophages, and neutrophils in the urine, as well as further induction on tumor of intercellular adhesion molecule 1, MHC class I and II molecules, and reversion of cytology from positive to negative.[6]

Strains of BCG

BCG was initially produced by attenuation of Mycobacterium bovis over a 13 year period in France. Subsequently it was distributed to various laboratories all over the world where repeated subcultures led to the emergence of phenotypically different strains. These have been stored and are used as seed lots for future vaccination and bladder immunotherapy.[7] These strains have been shown to have differences in their immunogenicity, adverse effects and recently in susceptibility to various antimicrobial agents.[8-10]

Metanalysis have documented no significant difference in efficacy between the Pasteur, Frappier, Connaught, Tice, and RIVM strains in treatment of superficial bladder cancer.[11] In North America BCG Connaught strain (Theracys, Sanofi Pasteur Inc, Swiftwater, PA, USA) and BCG Tice (Schering Plough Inc, Kenilworth, NJ, USA) are the most commonly used strains.

Infectious complications of BCG immunotherapy

While generally well-tolerated, infectious complications of BCG immunotherapy are well-documented. Most of the early manifestations of BCG disease are characterized by a hypersensitivity type of reaction, namely, pneumonitis, hepatitis, or rarely sepsis. Fever, chills, sweats, and malaise within 3 months of instillation are the most common presenting symptoms.[12] In a Cochrane database review comparing transurethral resection of the tumor with BCG immunotherapy the most common reported complications were fever (25%), cystitis (67%), and hematuria (23%).[13] Fatal sepsis is described in the literature at an approximate rate of 1/15000 patients.[14]

Diagnosis and treatment of BCG immunotherapy related infections

Most laboratories do not work up non tuberculous mycobacterium further for speciation and sensitivity to antimycobacterial agents. Though these isolates are often sent over to reference laboratories, susceptibility of mycobacterium bovis strains is rarely done. This may lead to underdiagnosis of resistance as well as potentially ineffective therapy.

Treatment of BCG related infectious complications has not been subjected to any randomized control studies. Most severe or disseminated infections are treated with isoniazid containing regimens consisting of 2 or 3 antimycobacterial agents. Prophylactic isoniazid has not been shown to be effective and is not recommended.[15] Isoniazid has also not been shown to have any significant effect on the frequency of suppurative lymphadenitis after BCG vaccination.[16]

Susceptibility of BCG strains to antimycobacterial agents and its effect on treatment

There have been some recent studies examining the sensitivity of the BCG strains to various antimycobacterial agents. Dureka et al documented the MIC of mycobacterium bovis BCG Connaught strain for isoniazid at 0.125 μg/m.[17]

In a study comparing the Connaught and Tokyo strains, the Connaught strain was found to have low level resistance to isoniazid.[18] The authors in this study expressed the concern that isoniazid may not be "sufficiently useful" for the clinical control of infections due to the BCG Connaught strain.

Ritz et al reported profiles of the comparative susceptibility of seven BCG strains to antituberculous drugs and macrolides.[9] Using the automated BACTEC MGIT 960 system, they examined susceptibility in order to select appropriate antituberculous drugs for the treatment of BCG infections frequently encountered in HIV patients after BCG vaccination in the prevention of tuberculosis. They found that both Connaught and Tokyo strains were susceptible to isoniazid at 0.4 mg/L but resistant at 0.1 mg/L, rifampicin (1 mg/L), ethambutol (5 mg/L), ciprofloxacin and ofloxacin

(1 mg/L), streptomycin and amikacin (1 mg/L), kanamycin (5 mg/L), and capreomycin (2.5 mg/L).

The authors suggested an alternative treatment strategy for complications related to vaccinations with either the BCG Connaught or Denmark strain. They also expressed concern about the rising incidence of BCG related complications associated with the human immunodeficiency virus/AIDS epidemic and the lack of evidence based guidelines for the treatment of local and disseminated complications. It was also suggested that the documentation of various strains is important in interpreting the changes in epidemiology of adverse effects as well as selection of antimycobacterial agents to treat BCG related infections arising from vaccinations.

We also recently reported a case of a central nervous system BCGoma as a rare complication of BCG immunotherapy for bladder cancer.[19] In this case as well as a subsequent case, PCR-based genomic deletion analysis for regions of difference (RD) 1, RD9 and RD 10 identified the isolate as *Mycobacterium bovis BCG* strain.[20] Sensitivity testing using the Bactec MGIT (mycobacterial growth indicator tube) 960 system (Becton Dickinson, MD, USA) was done and demonstrated resistance to isoniazid (at concentration 0.1 μg/mL) and pyrazinamide (at concentration 100.0 μg/mL), Table 1. Our patients were treated with regimens not containing isoniazid and have successfully recovered.

TABLE 1. **Sensitivities of the two BCG isolates to various antimycobacterial agents using Bactec MGIT (mycobacterial growth indicator tube) 960 system (Becton Dickinson, MD, USA)**

Drug	Concentration (μg/mL)	Patient 1 isolate	Patient 2 isolate
Isoniazid	0.1	R	R
Isoniazid	0.2	S	S
Isoniazid	0.4	S	S
Rifampin	1.0	S	S
Rifabutin	1.0	S	S
Ethambutol	5.0	S	S
Ethionamide	5.0	R	-
Streptomycin	1.0	S	S
Capreomycin	10	S	S
Cycloserine	30	S	S
Kanamycin	5.0	S	S
Ciprofloxacin	2.0	-	S
Ofloxacin	1.0	S	-
Amikacin	2.0	S	-
Pyrazinamide	100	R	R

The low level resistance of the BCG Danish strain (MIC 0.4 μg/mL) to isoniazid has previously been acknowledged by the manufacturer. However, the clinical significance of this is unclear and the WHO Global Advisory Committee of Vaccine Safety of the World Health Organization despite reporting five patients with isoniazid-resistant BCG lymphadenitis after immunization with BCG-Denmark had concluded that the identification of low-level isoniazid resistance does not justify any change in BCG immunization policy.[21] No comment, however, was made on treatment strategies.

Mechanism of isoniazid resistance

The mechanism of the low level resistance is not clear. In Mycobacterium tuberculosis 0.025 μg/mL to 0.05 μg/mL is inhibitory and higher concentrations have been described as being bactericidal. Low level isoniazid resistance, defined as the MIC of 0.1 μg/mL is most commonly associated with point mutations or short deletions within the catalase-peroxidase gene (katG), which still produces some enzymatic activity, whereas high level resistance defined as MIC greater than 0.1 μg/mL is associated with major deletions within the gene with loss of all enzymatic activity.[22,23] Mutations in the regulatory region of the gene controlling mycolic acid synthesis (inhA) also confers isoniazid resistance. Previous studies have established association between resistance to isoniazid and ethionamide in other mycobacteria. This resistance was first described for isolates of *M. tuberculosis* from patients who had been on isoniazid monotherapy, suggesting that isoniazid and ethionamide share a common target. Subsequently, the gene *inhA*, whose mutation or over expression leads to resistance against isoniazid and ethionamide, was identified in *Mycobacterium smegmatis*.[24] A recent study found that all *M. tuberculosis* isolates with low level isoniazid resistance that were also ethionamide resistant had mutations in the regulatory region of the *inhA* gene.[25] In contrast, only 5.9% of isolates with high level isoniazid resistance that were also ethionamide resistant had such mutations.

Monotherapy with antimycobacterial agents and potential for development of resistance

The serum concentration of isoniazid after a 10 mg/kg dose in children has been shown to exceed 0.4 μg/mL

and thus the MIC of the low level resistant strain may be exceeded.[26] However the clinical relevance of this has not been established. The potential for development of resistance in mycobacteria when treated with 1-2 agents alone is high. However the rate of mutations in mycobacterial conferring resistance to multiple drugs is very low and this has led to the successful use of combination therapies for tuberculosis.[27,28] Also, combination therapy can better reach bacteria with different levels of metabolic activity at multiple sites in the body. Monotherapy or inadequate therapy can lead to the development of resistance to other agents as well.

This phenomenon has also been demonstrated in *Mycobacterium bovis* BCG strain. Hesseling et al[29] described the case of a HIV infected child who developed axillary lymphadenitis after vaccination with the BCG Danish strain. They demonstrated intrinsic low level resistance to isoniazid in the initial strain and the acquisition of a mutation in codon 531 (Ser531Tyr) in the later isolate leading to at least 8 fold higher MIC to rifampin as compared to the original isolate. They concluded that intrinsic resistance of the Danish BCG strain to isoniazid was a key factor in the subsequent acquisition of rifampin resistance. Prolonged exposure to limited drug regimens, including rifampin, may have predisposed to further resistance, despite good adherence to therapy on directly observed therapy while on two active drugs (rifampin and ethambutol). Thus, the treatment of BCG Connaught strain infections with isoniazid containing regimens may predispose to development of resistance to other agents and maybe a reason not to use isoniazid even in combination therapy.

Conclusion

The BCG Connaught strain which is widely used for intravesical therapy of superficial transitional cell cancer in the United States and certain other parts of the world has been shown to have low level resistance to isoniazid. The clinical significance of this low level resistance is unclear, the mechanism is not well understood and it is unknown if this low level resistance could transform to high level resistance. Use of isoniazid containing regimens could lead to development of resistance to other anti-mycobacterial agents. These concerns have led some recent concerns about use of isoniazid in treating infections related to vaccination with BCG Connaught and Denmark strains and call for alternative regimens. Our review raises similar concerns about using isoniazid in treating infections related to BCG Connaught intravesical therapy and we would like to call for further studies to address these concerns. Fluoroquinolone (particularly moxifloxacin) containing regimens are attractive options given their excellent bioavailability and efficacy against mycobacteria. However; further studies are needed to make recommendations about the most suitable regimens. □

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