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Sensitivity to Isoniazid of *Mycobacterium bovis* BCG Strains and BCG Disseminated Disease Isolates[▽]

Intravesical *Mycobacterium bovis* BCG instillation therapy is commonly used to prevent recurrences of carcinoma *in situ* (CIS) and/or Ta/T1 papillary tumors of the urinary bladder (8). A rare and serious complication associated with BCG treatment of bladder cancer is the development of disseminated BCG disease (1, 6). Current recommendations for bladder cancer patients (being treated with BCG) who develop persistent fever or experience an acute febrile illness consistent with a mycobacterial infection include administration of combination therapy with two or more antimycobacterial agents (3).

Recently, U.S. federal public health authorities were notified of two cases of disseminated BCG disease resulting after bladder cancer therapy with BCG Connaught. Drug sensitivity testing (DST) of these strains suggested that both of these strains were resistant to low concentrations of isoniazid (0.2 μg/ml isoniazid [INH]), the primary drug used to treat *Mycobacterium tuberculosis* complex infections. These DST results raised concerns about acquired resistance *in vivo* or the generation of drug-resistant bacteria during manufacturing. Interpretation of the DST data was confounded because the levels of sensitivity to INH for many BCG strains were poorly defined.

To address these concerns, the sensitivities to INH of 13 different BCG strains were determined using the standard proportion method (7). DST analysis showed that four of the BCG strains (Tokyo, Moreau, Russia, and Sweden) and two *M. tuberculosis* controls were extremely sensitive to INH at the 0.05-μg/ml level (Table 1). In contrast, the other 9 BCG strains tested (including BCG Connaught) consistently had 2- to 4-fold-higher MICs for INH. For these BCG strains, the MICs ranged from 0.1 to 0.2 μg/ml INH. Importantly, the MICs of INH for the patient isolates and the parental BCG Connaught strain were essentially identical.

TABLE 1. Drug sensitivity testing of *M. bovis* BCG strains

Species and strain	MIC for INH (μg/ml)	Yr obtained from Pasteur Institute
M. tuberculosis		
H37Rv	0.05	
Erdman	0.05	
M. bovis BCG		
Russia	0.05	1924
Moreau (Brazil)	0.05	1925
Tokyo	0.05	1926
Sweden	0.05	1926
Birkhaug	0.1	1927
Danish	0.2	1931
Prague	0.1	1931 to Denmark and then 1947 to Prague
Glaxo	0.1	1931 to Denmark and then 1953 to England
Tice	0.1	1934
Frappier	0.1	1937
Connaught	0.2	1937 to Frappier and then 1948 to Connaught
Phipps	0.1	1938
Pasteur	0.2	Lyophilized 1961

Interestingly, our DST analysis of the different BCG strains indicated that all BCG strains obtained by international public health officials from the Pasteur Institute after 1926 had moderately elevated levels of resistance to INH (Table 1). Following the original attenuation of BCG at the Pasteur Institute from 1908 to 1921, BCG strains were maintained by serial propagation in different laboratories (1921 to 1961) until frozen seed lot systems were developed. Behr et al. have reported that all BCG strains obtained from the Pasteur Institute after 1926 had a point mutation in the *mma3* gene which resulted in impaired methoxy mycolic acid production (2). Since the target for INH antituberculosis activity is the mycolic acid biosynthetic pathway, it has been speculated that this mutation would cause decreased sensitivity to INH for BCG strains obtained after 1926. These data support this hypothesis since every strain obtained from the Pasteur Institute after 1926 has a modestly elevated MIC for INH.

The increasing frequency of drug-resistant *M. tuberculosis* disease worldwide has enhanced public health concerns about the adequacy of antituberculosis therapeutic regimens and the overall effectiveness of tuberculosis control programs (4, 5). Within this environment, the identification of two BCG isolates with apparent increased resistance to INH from patients with disseminated mycobacterial disease was worrisome. However, these DST data suggest that the decreased sensitivity to INH seen in the patient isolates (relative to that seen in sensitive *M. tuberculosis* strains) was likely not caused by acquired resistance *in vivo* or a genotypic modification during the manufacturing process. The modestly decreased sensitivity to INH seen in these BCG strains likely resulted from a mutation in a gene involved in mycolic acid biosynthesis which occurred more than 8 decades ago.

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Kristopher Kolibab
Steven C. Derrick
Sheldon L. Morris*
Laboratory of Mycobacterial Diseases and Cellular Immunology
FDA/CBER
Bethesda, Maryland 20705

*Phone: (301) 496-5978
Fax: (301) 435-5675
E-mail: sheldon.morris@fda.hhs.gov

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