Metronidazole validates drugs targeting hypoxic bacteria for improved treatment of tuberculosis

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Latent tuberculosis infection (LTBI) affects one-third of the world population and poses a major challenge for TB control (1). People with LTBI have a 10% lifetime risk of developing active TB disease, but the risk is greatly increased in immunocompromised conditions such as HIV infection, which has a 10% annual risk. Prophylactic treatment of LTBI is increasingly recognized as an important strategy for prevention of active disease in high-risk populations, such as HIV-positive individuals, and more effective disease control in endemic areas. LTBI, where the number of *Mycobacterium tuberculosis* bacteria in the lesions is considered small, can be treated with the single drug isoniazid (INH) for 6 or 9 mo or rifampin (RIF) for 4 mo, or it can be treated with INH and rifapentine weekly for 3 mo to prevent reactivation of TB in humans (2). In PNAS, the work by Lin et al. (3) shows that metronidazole (MTZ), a drug that is only active against nonreplicating bacteria (persisters) under hypoxic/anaerobic conditions, can prevent reactivation of LTBI to active disease in an anti-TNF antibody-induced reactivation model in macaques, and it could potentially improve treatment of active TB disease.

Activity of MTZ Dependent on Oxygen Content and Lesion Microenvironment

MTZ shows considerable bactericidal activity for *M. tuberculosis* under hypoxic conditions in a nonreplicating persistence model in vitro (4), but it has no apparent activity for growing bacilli in ambient oxygen content in vitro (minimum inhibitory concentration > 512 μg/mL) or ex vivo in macrophages (5). During LTBI and active disease in macaque and rabbit models, where hypoxic lesions with histopathological features resembling the human disease are present, MTZ has significant activity against *M. tuberculosis* (3, 6). However, MTZ had no apparent activity for *M. tuberculosis* in the murine (5, 7) or guinea pig model (8, 9), presumably because there is not sufficiently low oxygen tension in the tuberculous lesions. Nevertheless, MTZ did have a modest effect on reducing bacterial load in a 100-d-old murine chronic TB model (5). It is worth noting that MTZ was shown to improve the treatment of advanced pulmonary TB patients in a regimen of INH/RIF/streptomycin/MTZ in terms of clinical improvement and increased bacterial clearance in sputum compared with the control regimen without MTZ (10). It is quite likely that advanced chronic pulmonary lesions may contain pockets of hypoxic bacteria that could be killed by MTZ. However, unfortunately, no follow-up study on this topic in humans is available. The findings by Lin et al. (3) that MTZ alone given for 2 mo is almost as effective as INH/RIF for 2 mo in preventing LTBI from reactivation suggest that, during LTBI, the nonreplicating bacteria in hypoxic environments that are killed or inhibited by MTZ serve as the root for subsequent reactivation of LTBI to active disease. This finding is of particular interest, because drugs that target nonreplicating bacteria in hypoxia alone have not been shown to be effective in preventing reactivation of LTBI. In this sense, it would be of interest to test if pyrazinamide (PZA), a frontline TB drug that has no activity for growing bacilli but kills nonreplicating bacilli (persisters) in acidic and hypoxic environments and shortens therapy (11, 12), could also prevent reactivation of LTBI like MTZ in macaques.

MTZ alone given for 2 mo is almost as effective as INH/RIF for 2 mo in preventing LTBI from reactivation.

Although MTZ was shown to prevent reactivation of LTBI, MTZ seems to fare less well than INH/RIF for the 2-mo treatment and especially less well than INH for 6 mo in terms of gross pathology, bacterial burden, and dissemination used to measure reactivation of LTBI (3). Because the study did not evaluate INH or RIF alone given for 2 mo like MTZ, it is not possible to determine if MTZ is as effective as INH or RIF in preventing reactivation. In addition, it is likely that the timing of MTZ administration, the level of bacterial burden, and the lesion oxygen tension when MTZ is given will all affect the therapeutic effect of MTZ and the frequency of anti-TNF–induced reactivation. The effect of MTZ may be better seen with a low bacterial load in hypoxia, such as in LTBI, but it may not be seen easily during active disease when there are large numbers of bacilli residing in lesions with varying oxygen contents. Considering the heterogeneity of LTBI (13) and the lesions of varying stages of development, it is possible that MTZ may have varying effects on the treatment outcome of LTBI. Thus, based on these considerations, it is quite likely that MTZ alone may not be relied on for effective treatment of LTBI. It would be of interest to determine if MTZ combined with other drugs, such as INH or RIF, will be more effective than MTZ alone and the current treatment of LTBI (see below). It is intriguing that, although assessment of bronchoalveolar lavage samples and gastric aspirates as a surrogate for sputum bacterial count revealed that all monkeys treated with INH/RIF/MTZ converted from positive to negative cultures compared with only 30% conversion in the INH/RIF group after 2-mo treatment, no difference was observed in the bacterial counts in infected organs between the two treatment groups (3). The lack of therapeutic benefit of MTZ when added to INH/RIF in treating active disease (3) may have resulted from the high potency of INH/RIF in eliminating the bacterial population against which MTZ is active, or the oxygen tension in the lesion may not be sufficiently low for MTZ to work. This finding may suggest a limited use of MTZ for treating active disease, which can have variable results in individual patients containing lesions of varying oxygen contents. Future studies may need to determine if MTZ or perhaps more appropriately, INH + RIF + MTZ can treat human LTBI as effectively as in macaques and explore the use of MTZ in the treatment of chronic and advanced TB and multidrug resistant TB (MDR-TB) or extensively drug-resistant TB (XDR-TB) combined with other agents.

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The anti-TNF antibody-induced reactivation of LTBI is a promising model to study the fascinating but difficult problem of LTBI. The length of anti-TNF antibody administration of 5 wk used to induce reactivation of LTBI may be appropriate for reactivation of untreated LTBI but may not be sufficiently long to allow the reactivation of the drug-treated persisters to occur. The drug-treated bacilli may be damaged, and they may take substantially longer to reactivate than untreated bacilli in the control, which may reactivate more easily under the influence of anti-TNF antibody. Future studies may need to examine the effects of extended period of anti-TNF treatment and perhaps, its combination with steroids on prevention of reactivation by MTZ in comparing regimens for treatment of LTBI.

Heterogeneous Bacteria During LTBI Affected by Different TB Drugs

Perhaps the most intriguing feature about LTBI is that its treatment takes almost as long as the treatment of active disease (i.e., treating LTBI is like treating a miniature disease). This finding is thought to be an expression of bacterial persistence during infection process, which seems to be a common feature exhibited in other bacterial infections such as syphilis, where initial penicillin treatment had little effect during latent infection but more dramatic effect for active disease (14). Although the number of bacteria underlying LTBI is thought to be small, they seem to be quite heterogeneous, consisting of growing, slow-growing, and nongrowing bacteria, similar to the kind of bacterial heterogeneity during active disease. This finding is shown by the fact that INH (a drug that is only active against growing bacteria), RIF (a drug that kills slow-growing bacteria), and MTZ (a drug that kills nongrowing bacteria under hypoxia) can all be used to treat LTBI. A yin–yang model was proposed to express the heterogeneity of the bacterial populations during LTBI and active TB, explain the two-phase TB chemotherapy, and explain why INH could be used to treat LTBI (15, 16). Thus, future treatment of LTBI, despite a relatively small number of bacilli, may need to take this heterogeneity into consideration by using drug combinations that kill heterogeneous bacterial populations instead of just using INH (active only against growing bacilli) alone or RIF alone as currently practiced. Indeed, INH plus rifapentine given weekly for 3 mo has recently been shown to be as effective as 9 mo of INH treatment (2). It would be of interest to determine if MTZ improves on INH and rifapentine in preventing reactivation of LTBI.

The fact that MTZ has varying activity against *M. tuberculosis* in different animal models with different pathologies and oxygen contents, which can affect its therapeutic efficacy, has implications for evaluating TB drugs using animal models. The lack of antituberculous activity of MTZ in the mouse model (5, 7) raises some question about the validity of this model for evaluating TB drugs or drug regimens in some cases, despite the fact that murine models have historically been quite useful in evaluating treatment regimens that prove mostly valid for humans. For example, moxifloxacin had superior efficacy to replace INH combined with RIF and PZA in the mouse model (17) but this efficacy was not subsequently proven in human clinical study (18). Thus, in evaluation of new TB drugs or regimens, caution is advised with data obtained in the murine model, and drugs or regimens that are effective in mice should be further validated in rabbit or monkey models that more resemble human disease.

Need for Understanding Persisters and LTBI and Developing Drugs for Persisters in Hypoxia

Drugs that have activity against nonreplicating tubercle bacilli (persisters) under hypoxic conditions, such as PZA (11), RIF (19), TMC207 (20), and PA-824 (21), are known to be important for shortening the duration of TB treatment (22). The above drugs that shorten treatment inhibit bacterial pathways of varying importance for persister survival and thus, have varying ability to kill persisters and shorten therapy (16). MTZ, a prodrug that is activated by a nitroreductase enzyme to reactive species known to damage DNA, kills a population of bacteria under hypoxic conditions. Although MTZ may not be the ideal drug to kill persisters and shorten therapy for TB or LTBI, it serves as a tool compound and validates the principle that drugs targeting nonreplicating persisters in hypoxic environment are important for improved treatment of TB. PA-824, an MTZ derivative, is a more powerful agent in current clinical development that has activity against both growing and nongrowing bacilli under hypoxic conditions (21). In this sense, PA-824 or TMC207 might be a more promising drug candidate for the treatment of LTBI than MTZ. However, more effective treatment of LTBI as well as active TB requires a combination of drugs that target both growing and nongrowing bacterial populations. Much remains to be learned about the biology of LTBI, such as how it is established, why only a small proportion of LTBI goes on to develop active disease whereas the majority does not, how to identify people at high risk to develop from LTBI to active disease, how LTBI is cured by a single drug like INH, and how to more effectively cure LTBI. Future efforts are needed to shed new light on these questions and develop new TB drugs that target nonreplicating persisters, such as those persisters residing in hypoxic environment, for more effective treatment of TB and LTBI.

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