

Research Highlight

Novel Proteasome Inhibitors as Potential Drugs to Combat Tuberculosis

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***Mycobacterium tuberculosis* is one of the most notorious killers worldwide. These pathogens have evolved to infect human beings in a so-called dormant form that is extremely difficult to treat. New work, however, suggests that mycobacterial proteasomes, multi-component structures that protect the microbe from damaging effects of nitric oxide generated by the host, can be selectively and specifically blocked by small molecules.**

Mycobacterium tuberculosis is responsible for ~9 million new tuberculosis disease cases and over 2 million deaths annually. In the majority of infected individuals, the bacterium lies dormant and causes no immediate health problems. However, different environmental triggers (malnutrition, deterioration of general health status and HIV infection) can reactivate the tubercle bacilli resulting in active tuberculosis.

Upon infection via the airways, *M. tuberculosis* is internalized into alveolar macrophages that reside within the lung. Within these macrophages, phagocytosed *M. tuberculosis* resides within the so-called mycobacterial phagosomes, thereby avoiding killing within lysosomal organelles through various strategies (Pieters, 2008). Macrophages harboring mycobacteria can remain within the lungs of the infected host or, alternatively, disseminate to other organs in the body. In most healthy individuals, the immune defense system is well capable to control the infection by *M. tuberculosis* such that disease cannot develop; in ~10% of the infected individuals, however, tuberculosis develops with potential deadly outcome. One reason why healthy individuals are well able to control tuberculosis is because mycobacteria are being sequestered within granulomas, which are structures harboring macrophages infected with living tuberculosis within the center, surrounded by macrophages that are being

kept in an activated state by surrounding T cells. Although the precise conditions within granulomas are still largely undefined, it is becoming clear that the granuloma represents a balance in which *M. tuberculosis* is actively kept in check by macrophages and T cells (Cosma et al., 2003).

Several reasons exist why *M. tuberculosis* is such a difficult bacterium to eradicate. First, as mentioned, in many infected individuals, the bacilli are not actively replicating but are metabolically inactive causing a latent infection. Therefore, classical antibacterial agents that typically target growth and proliferation pathways (DNA replication, gene transcription, protein translation and cell wall formation) fail miserably. Secondly, *M. tuberculosis* is characterized by an especially thick and rigid cell wall, which makes it very difficult for compounds to access the mycobacterial cell. Thirdly, *M. tuberculosis*, with a doubling time of ~18–22 h, is a very slowly growing organism, resulting in frequent development of drug resistance, including multi and extensive drug resistance. On top of these strategies, *M. tuberculosis* has evolved highly successful strategies to avoid destruction within macrophage lysosomes, by actively blocking phagosome–lysosome fusion (Nguyen and Pieters, 2008).

Given these obstacles, it is not surprising that no drugs against tuberculosis have made it to the clinic in recent years.

There is, however, some momentum gathering, in that several drug targets are being identified and analyzed for potential inhibition by small molecules (Butler, 2000; Warner and Mizrahi, 2007). One of these recently revealed drug targets is the mycobacterial proteasome.

Proteasomes are large multisubunit complexes that are primarily responsible for the proteolysis of cytoplasmic proteins. Proteasomes are present in virtually all organisms, highly conserved and essential in all eukaryotes. They consist of a core complex made up of four stacked rings, each made up of seven proteins (α and β subunits), giving the appearance of a barrel-like structure. Within the barrel, the central rings consist of seven proteases, with the active sites of these proteases oriented toward the center of the ring (Zwickl et al., 2001). This way, proteins can only be degraded at the inside of the barrel. In eukaryotes, targeting of proteasome substrates is coordinated by a regulatory particle or cap, which consists mainly of ATPases of the AAA family of proteins. Cytosolic proteins destined for proteasomal degradation are modified on lysine residues with ubiquitin, a small (76 amino acid residues long) polypeptide. Proteins need to be modified with at least four ubiquitin molecules to be recognized by the proteasome. Such recognition occurs at the cap structure, by still poorly defined mechanisms, after which the protein is unfolded and translocated to

the inner core of the proteasome barrel where it is proteolysed by the active proteases present within the barrel. Small peptide fragments then leave the barrel again, and are further metabolized by as yet unknown processes.

Although proteasomes are present in certain parasites, *M. tuberculosis* is thus far the only known bacterial pathogen possessing proteasomes. The mycobacterial proteasomes seem to have a similar organization as its eukaryotic counterpart. A function for the mycobacterial proteasome was revealed several years ago following a screen for mutant bacteria hypersensitive to acidified nitrite, such as present within mycobacterial phagosomes, in which *M. tuberculosis* is believed to survive and lie dormant. Of the several mutants that were identified, five contained insertions in genes coding for proteasome-associated molecules (Darwin et al., 2003), strongly implicating the mycobacterial proteasome in resistance to reactive nitrogen intermediates. Indeed, silencing of proteasome subunits results in an inability of *M. tuberculosis* to persist in mice (Gandotra et al., 2007). Therefore, it seems that mycobacterial proteasomes are involved in the resistance against nitric oxide-induced stress. Importantly, this work suggested that proteasomes are especially important during the dormant phase of *M. tuberculosis*, making the proteasome an attractive target for compounds that may interfere with dormant *M. tuberculosis*.

The promise for proteasome inhibitors as potential drugs to treat tuberculosis has been dampened by the high degree of conservation of the mycobacterial proteasome with those from their mammalian host cells. Indeed, although proteasome inhibitors are currently being tested as agents against a variety of diseases, including cancer and neurodegenerative disorders (Huang and Chen, 2009), the idea of using proteasome inhibitors as anti-infectives has thus far been curbed because it has been impossible to develop proteasome inhibitors that avoid the inherent toxicity because of the important function for proteasomes in normal cellular homeostasis.

In the current work, however, Lin et al. (2009) zoom in on a certain class of small molecules, the oxathiazol-2-one

compounds. Using an *in vitro* assay based on proteasome activity, Lin et al. screened 20 000 compounds and identified two inhibitors of the oxathiazol-2-one class. These compounds permeated the mycobacterial cell wall, and were highly effective as inhibitors of *M. tuberculosis* growth in liquid cultures. Importantly, these inhibitors synergized with low amounts of nitric oxide in efficient killing of *M. tuberculosis*.

As stated, one major disadvantage of all known proteasome inhibitors is their inherent toxicity due to blocking proteasome activity of the host. However, the oxathiazol-2-one compounds, although specifically and irreversibly inhibiting the mycobacterial proteasome, were >1000-fold less effective against human proteasomes, and were inactive toward a series of different proteases, including trypsin, cathepsin B and metalloproteases.

The biochemical analysis showed that the oxathiazol-2-one compounds modified the mycobacterial proteasome by covalent attack on the active site threonine (Thr 1) of the core complex β -subunit. As a result, Thr 1 undergoes cyclocarbonylation, thereby drastically modifying the environment of the active site. With the knowledge of how the oxathiazol-2-one compounds modify the proteasome, the authors then used structural analysis to determine whether this modification can explain the exquisite specificity of the oxathiazol-2-one compounds toward mycobacterial proteasomes, although not affecting mammalian proteasomes. To that end, Lin et al. solved the structure of proteasome complexes, prior to and after exposure to oxathiazol-2-one compounds. This analysis first of all confirmed the modification of Thr 1 by the inhibitors, but also revealed another important consequence of modifying Thr1 by oxathiazol-2-one compounds. The substrate-binding pocket of the proteasome undergoes a major conformational change which involved several residues outside the active loop. According to the structural analysis, this newly formed moiety on Thr 1 causes an alternative protein confirmation in which the substrate-binding pocket is disturbed. Therefore, the substrates of mycobacterial proteasome fail to gain access to the proteasome resulting in the accumulation of toxic proteins and peptides within mycobacterial cells.

Importantly, the substrate-binding pocket of human proteasomal β -subunits is speculated not to be changed by the modification of the oxathiazol-2-one compounds. The authors suggest that this is because the residues involved in maintaining the altered conformation are extensively non-conserved, and therefore not subject to inactivation through cyclocarbonylation (Lin et al., 2009). This of course allows the highly selective inhibition of *M. tuberculosis* proteasomes, without blocking host proteasomal activity.

The irreversible inhibition of proteasomes by the oxathiazol-2-one compounds may prove to be of significant benefit under conditions where protein synthesis is drastically reduced, such as during dormancy, or blocked, such as is the case during antibiotic treatment. Indeed, as Lin et al. note, perhaps the most promising therapeutic options will consist of a combination of proteasome inhibitors with existing drugs that block protein synthesis at stages of transcription or translation.

One of the most difficult issues in developing drugs to combat tuberculosis is the fact that *M. tuberculosis* can adopt a dormant form that is virtually impossible to eradicate with existing treatments. Although there is presently no information of the effectiveness of oxathiazol-2-one compounds in an *in vivo* setting, the work by Lin et al. promises to provide desperately needed compounds that can be developed into drugs to treat tuberculosis.

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