JAMA HIV/AIDS HIV/AIDS Resource Center
The Journal of the American Medical Association

Newsline

The Failure of AZT — An Enzyme Bottleneck

By Dennis Blakeslee, PhD
(Posted August 6, 1997)

AZT, the first antiretroviral drug to be approved for treatment of HIV, went into clinical use with high hopes and expectations. In the laboratory, AZT, originally developed as an anti-cancer agent, had been shown to be very efficient at preventing the HIV reverse transcriptase (RT) enzyme from doing its job — making DNA copies of the viral RNA (Note 1). Clearly, if it could act as well in infected cells, virus replication would be effectively shut down, preventing the spread of HIV within the cells of the immune system.

Thus the disappointment was all the greater when AZT monotherapy did not live up to the hope. In the body, AZT did indeed inhibit the function of the RT enzyme — but not enough. HIV replication was only partially blocked, leaving the virus with more than ample opportunity to produce mutant strains resistant to AZT's inhibitory effects.

The response of clinicians was to accept these shortcomings and to abandon AZT as monotherapy, instead using the drug only in combination with other antiretroviral agents (Note 2).

However, scientists who investigate the activity and fate of drugs within the body were by no means ready to give up on AZT. Some wanted to know why the drug was not as effective as the laboratory experiments predicted. In the lab, the HIV reverse transcriptase enzyme readily incorporates AZT into growing DNA chains, they noted, and at the doses administered, should be very effective in aborting the synthesis of DNA copies of the HIV genome.

Studies of why drugs behave in unexpected ways when given to patients often lead to insights into metabolic processes, sometimes uncovering previously unknown pathways (for an example, see our backgrounder on P450 cytochromes). The result can be suggestions for ways to make the drugs more effective or develop new agents that will work even better.

Thus, the reasons behind AZT's relatively poor performance as an antiretroviral monotherapy have been pursued in several research laboratories. Now, a group of investigators in Germany has announced some interesting results (Note 3). The researchers discovered what they describe as a bottleneck in the processing of AZT within cells and, as a result, not enough of the drug is available to entirely shortcircuit the activity of HIV reverse transcriptase.

The Phosphate Connection

AZT, is a prodrug, which means that the substance taken by patients must be converted to an active form by metabolic events within cells. It is this active form of AZT that then works to inhibit HIV replication.

To get to this active state, AZT must undergo three separate chemical reactions, each adding a small cluster of atoms called a phosphate group to the molecule. In the first reaction, one phosphate is attached to AZT directly (ie, AZT-p). Then, a second phosphate is added to the first (AZT-p-p). Finally, a third phosphate is attached to form the triphosphate form of AZT (AZT-p- p-p). It is in this form that AZT (and other nucleosides) is ready to be linked together into a DNA molecule by HIV reverse transcriptase (Note 4).

By carefully examining this sequence of reactions, the authors found that the second step, the attachment of the second phosphate group, is very slow. In fact, it's about 200 times slower than the addition of a second phosphate to the nucleotide that AZT mimics, thymidine. Thus, the single or monophosphate form of AZT accumulates in the cell and very little becomes available for insertion into DNA by reverse transcriptase. This, the slow second reaction, is the bottleneck.

The researchers then examined in detail the enzyme that catalyzes this second phosphate addition reaction, a protein called thymidylate kinase, or TmpK (Note 5). Using the technique of x-ray crystallography, they determined the complete three-dimensional structure of the enzyme and discovered precisely why it was so poor at attaching a second phosphate group to AZT. The modification that lets AZT act as an inhibitor of HIV reverse transcriptase is bulky. Because of its size, it distorts the catalytic site of TmpK and thereby inhibits the enzyme's activity (Note 6).

Moreover, a failure to product enough AZT triphosphate for incorporation into DNA may not be the only consequence of the poor performance of TmpK with respect to AZT. The authors suggest that the build-up of the monophosphate form of AZT, caused by the TmpK bottleneck, is responsible for many and perhaps even most of AZT's noxious side-effects.

Lessons

Unfortunately, this analysis of AZT and its inability to inhibit HIV replication as well as it theoretically should does not provide any obvious solutions. There is no way to change the activity of an enzyme other than by replacing it. The authors do suggest that perhaps in the future, gene replacement therapy could be used to supply human patients with a version of the TmpK enzyme that could more efficiently convert AZT to its active form. However, this is more speculation than anything else. Such therapy would likely be enormously expensive and difficult to achieve, and even then might be less beneficial than other drugs.

However, one notion that does emerge from these finding is that it might be possible to lower the AZT doses that patients take with no loss of the drug's activity, less than optimal though it is. If, in cells, AZT can only be

converted to its active form at the speed of the TmpK bottleneck, it is possible that the same amount of AZT diphosphate would be made even if there less AZT there in the first place.

But there will be one very real gain from this study. By showing how the bulky modification of AZT distorts the TmpK enzyme binding site and slows its catalytic reactivity, scientists can now try to design new nucleoside analogs that will not have this disadvantage (Note 7). Perhaps such design will allow more effective and potent nucleoside analogues to be synthesized in future.

Note 1. AZT does not inhibit the function of RT in the sense that it prevents the enzyme from functioning, which in this case is the stringing together of subunits called nucleosides into a DNA molecule. Rather, the drug mimics and competes with one of the DNA subunits (thymidine). When it inserts into the growing chain in place of thymidine, no further subunits can be added and the chain growth ends before it is completed. Thymidine and AZT are identical except for a single alteration.

Note 2. The exception is in pregnancy. AZT monotherapy is recommended to some expectant women to reduce the risk of transferring the HIV to their newborns.

Note 3. Lavie A, Schlichting I, Vetter IR, Konrad M, Reinstein J, Goody RS. The bottleneck in AZT activation. *Nat Med.* 1997;3:922.

Lavie A, Vetter IR, Konrad M, Goody RS, Reinstein J, Schlichting I. Structure of thymidylate kinase reveals the cause behind the limiting step in AZT activation. *Nat Structural Biol.* 1997;4:601.

Note 4. These phosphate groups play two roles in the synthesis of DNA from individual nucleosides. The phosphate group attached directly to AZT (or another nucleoside) eventually forms the molecular link between adjacent nucleosides in the growing DNA chain. In contrast, the other two phosphates are there to provide the energy needed to forge the phosphate bridge between nucleosides.

In all living organisms, most of the energy released from the metabolism of nutrients is captured in the chemical bonds between phosphate groups, a ready supply of chemical energy that is then transported throughout the body in the form of small molecules substance called ATP (adenosine triphosphate). When energy is needed to forge new bonds during the synthesis of proteins, carbohydrates, and nearly all other substances in the body, ATP supplies it by giving up one or two of its phosphate groups. The high-energy phosphate groups attached to AZT and other nucleotides are donated by ATP.

Note 5. In this study, the TmpK enzyme from yeast was used rather than human TmpK. The human version of the enzyme was not available for study. However, the authors point out that the yeast and human TmpK are overall very similar. So they have little doubt that the human enzyme would behave toward AZT in the exact same way.

Note 6. In thymidine, the nucleoside that AZT mimics, there is ahydroxyl group on the sugar component of the molecule that acts as one of the attachment points for the phosphate bridges between nucleosides in a DNA chain. In AZT, this hydroxyl is replaced by an azo group (consisting of three nitrogen atoms) that cannot bind phosphate, thereby causing termination of a growing DNA chain. Azo groups are considerably larger than hydroxyl groups (-OH).

The normal function of TmpK is to bind molecules of the nucleoside thymidine monophosphate (TMP) and an ATP molecule and promote a reaction between them. Both fit into a groove or catalytic site in the enzyme surface and, once there, a phosphate group is transferred from the ATP to the TMP. Thus, the products of the reaction are now the diphosphate form of thymidine (TDP) and the diphosphate of adenosine, ADT. In addition, as these two molecules bind, a short loop of amino acids within the enzyme folds over the binding site, thereby holding the reactants in place and greatly accelerating the pace of the reaction.

This essential folding over of the loop is where the AZT bottleneck occurs. Because of the bulky azo group on AZT, the loop is prevented from folding to its full extent, and thus the reaction between the AZT monophosphate and ATP proceeds very slowly and inefficiently.

Note 7. Interestingly, the existing nucleoside reverse transcriptase inhibitors drugs ddC and ddI do not contain a bulky azo group. Rather, they block the activity of the phosphate-binding hydroxyl group by simply removing it. However, experiments to show how efficiently these drugs are phosphorylated by other TmpK-like enzymes have not yet been done.

Back to top