Anti-Alzheimer's Drug Mechanism Revealed With X-Rays

Using x-rays produced by the NSLS, a team of scientists has gained new insight into the effects of a newly approved drug, called rivastigmine, in the treatment of Alzheimer's disease – a debilitating brain disease causing memory loss and other cognitive deficits in about 10 percent of the elderly. The new results, which may provide important information for generating improved drugs for this as-yet incurable neurodegenerative disease, were published in the March 19, 2002 issue of the American Chemical Society journal *Biochemistry.*

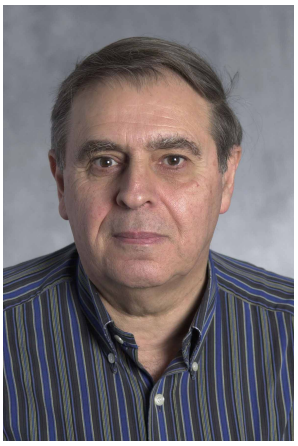
"We were very surprised by our results," says Joel Sussman, a structural biologist at the Weizmann Institute of Science in Rehovot, Israel, and the lead author of the study. "They show that we can safely treat Alzheimer's disease with much lower quantities of rivastigmine, thus minimizing unwanted adverse effects."

Though the drug is currently available under the trade name Exelon, its mechanism of action at the atomic level had not been studied until now. So, the team of scientists, composed of Sussman's team and scientists from Novartis, a pharmaceutical company based in Basel, Switzerland, decided to take a close look at how the drug helps to slow the memory loss of Alzheimer's patients.

One of the main pathological phenomena in Alzheimer's disease is the deterioration of nerve cells releasing acetylcholine, a chemical that helps to carry messages among brain cells. The inadequate supply of acetylcholine in Alzheimer's patients is compounded by the action of an enzyme called acetylcholinesterase (AChE), which breaks down acetylcholine in the body at the rapid rate of 20,000 molecules per second.

The desired effect of potential Alzheimer's treatments, such as rivastigmine, is to inhibit AChE long enough to offset the absence of acetylcholine. But rivastigmine and other anti-Alzheimer's drugs have side effects and may merely slow deterioration rather than halt it.
To look at the action of the drug over time, Israel Silman, a neurochemist at the Weizmann Institute of Science and a co-investigator on the study, together with Pazit Bar-On, a joint graduate student with Sussman and Silman, tested the drug on various types of AChE, extracted from an electric ray, the fruit fly, and human beings. "We wanted to see how long it takes AChEs to go back to normal, or become 'reactivated,' after being inhibited by the drug," Silman says.

Joel Sussman



Israel Silman

The scientists were very surprised to notice an "extremely low reactivation" of the AChEs from all three organisms. "Inhibition of AChE by rivastigmine appears to be almost irreversible, with little reactivation over a period of days," Silman says.

To explain what happens at the molecular level, the scientists took "snapshots" of rivastigmine while it was binding to AChE, using a method called x-ray crystallography. They projected x-rays produced at the NSLS on crystals of rivastigmine combined with AChE. They then determined the structure of the complex rivastigmine-AChE by looking at how the x-rays scattered off the crystal. By reconstructing the positions of these scattered x-rays, the scientists established a molecular map that revealed the locations of all the atoms of AChE and rivastigmine in three dimensions.

"When we looked at this map, things became clearer," Sussman says. "We had suspected that rivastigmine was binding very tightly to AChE, preventing surrounding fluid – mainly water – from breaking this bond quickly, as it usually does."

By looking closely at the AChE "active site" – the part to which rivastigmine binds to AChE – Sussman and his colleagues noticed that rivastigmine was broken in two, each part being ensconced comfortably in the active site (**Figure 1**). The scientists also precisely determined how each part was

bound to the surrounding AChE atoms and moved other AChE atoms, which slowed down reactivation of AChE (**Figure 2**).

"The x-ray molecular maps allow us to see how every atom of rivastigmine interacts with the atoms of AChE's active site," Silman says. "This information will be important in designing new chemicals that will target specific atomic sites in AChE, possibly leading to better drugs that last longer and have less undesirable effects on Alzheimer's patients."

"I am very excited by the perspectives offered by these results," Sussman says. "By fine-tuning the properties of anti-Alzheimer's drugs or their targets at the molecular level, we can truly hope to find a cure for Alzheimer's disease in the future."

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P. Bar-On, et al., "Kinetic and Structural Studies on the Interaction of Cholinesterases with the Anti-Alzheimer Drug Rivastigmine," *Biochemistry*, **41**, 3555-3564 (2002).
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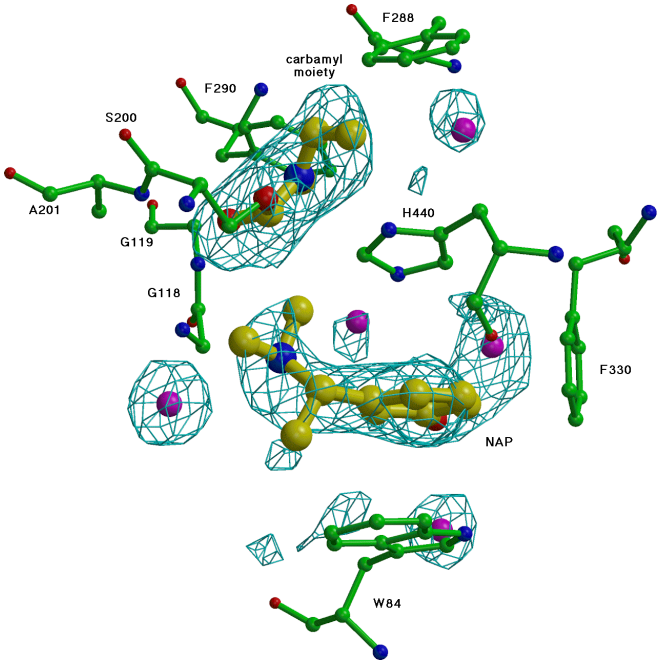

***Figure 1**. Close-up view of how the drug rivastigmine binds to the active site of the acetylcholinesterase (AChE) from the electric ray* Torpedo californica*. After binding to the active site of AChE, the drug is broken into two parts, called carbamyl moiety and NAP. Rivastigmine is rendered as a ball-and-stick model, with carbon atoms colored yellow, oxygen atoms colored red, and nitrogen atoms colored blue. Selected key molecules in the vicinity of rivastigmine are also rendered in ball-and-stick format, with carbon atoms colored green.*

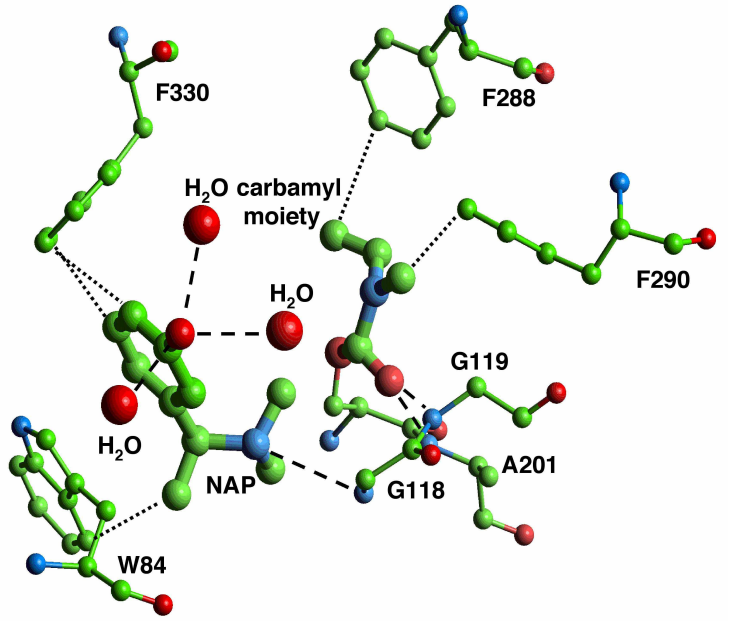

***Figure 2**. Active site of acetylcholinesterase (AChE) from the electric ray* Torpedo californica *after AChE is inhibited by the drug rivastigmine. Both parts of rivastigmine, the carbamyl moiety and NAP, are depicted with larger spheres and thicker lines for emphasis. Amino acids within the active site of AChE that may interact with the drug are shown. The carbamyl portion of rivastigmine is positioned to make two H-bonds (dashed lines) with the amide nitrogens of A201 and G119, as well as non-bonded contacts (dotted lines) with F288 and F290. NAP, the second part of rivastigmine, makes H-bonds with three water molecules (large red spheres) and the amide nitrogen of G118, as well as non-bonding contacts (dotted lines) with W84 and F330. (A, F and G represent the amino acids alanine, phenylalanine and glycine, respectively).*