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Study finds link between HIV treatment and neuronal degeneration

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Antiretroviral drugs have been life-changing therapies for HIV patients, but they can have significant side effects.

Mounting evidence has implicated these drugs in contributing to HIV-associated neurocognitive disorders, or HAND, which can be manifested as forgetfulness, confusion and behavioral and motor changes. Yet an explanation for how the drugs take a toll on the brain has been lacking.

Researchers from the University of Pennsylvania have now pinpointed some of the key players in causing neuronal damage. Their work suggests that certain protease inhibitors, among the most effective HIV drugs, lead to the production of the peptide beta amyloid, often associated with Alzheimer's disease. The drugs prompt an increase in levels of the enzyme that cleaves the amyloid precursor protein, APP, to produce beta amyloid, which is responsible for the damage to neurons.

Notably, inhibiting that enzyme, called BACE1, protected human and rodent brain cells from harm, suggesting that targeting this pathway with a new drug could minimize damage to neurons in patients on antiretroviral therapies.

"Protease inhibitors are very effective antiviral therapies, but they do have inherent toxicities," said Kelly Jordan-Sciutto, chair and professor in the Penn School of Dental Medicine's Department of Pathology and senior author on the study. "Our findings may cause us to rethink how we're using these drugs and even consider developing an adjunctive

therapy to reduce some of these negative effects."

The study appears in the *American Journal of Pathology*.

Protease inhibitors such as ritonavir and saquinavir are a key part of the drug cocktail that has reduced mortality in HIV-infected people by 50 percent. Though newer compounds form the frontline treatments for patients in the United States, these protease inhibitors remain widely used in Africa and other developing areas hit hard by HIV/AIDS. They work by blocking viral enzymes necessary for creating infectious particles that allow the virus to spread through the body.

Previous investigations by Jordan-Sciutto's team have suggested, however, that protease inhibitors can have toxic effects on the central nervous system. One study, for example, demonstrated that they triggered the activation of stress-response pathways, including oxidative stress and a process called the unfolded-protein response, or UPR. UPR results when the cell senses misfolded or modified proteins, causing a halt in protein translation. It's meant to protect a cell from aberrant proteins, but, when chronically activated, it can lead to cellular damage or death.

Even after these studies, it wasn't clear whether UPR seen in HIV patients was induced primarily as a result of the virus or of the treatment, and what molecules mediated it. In addition, the researchers were intrigued by the findings of colleague and coauthor Robert Vassar of Northwestern University, who showed that stress-induced UPR led to activation of beta-site APP cleaving enzyme 1, or BACE1, an enzyme that snips apart APP to produce beta amyloid.

"The study emerged from these three lines of converging evidence," Jordan-Sciutto said. "We knew UPR was activated in HIV patients both on and off antiretroviral therapy; we knew that, despite antiretroviral

therapy, cognitive impairment persisted in these patients; and we knew that activation of UPR lead to an increase in BACE1."

To determine whether and how neuronal damage arises from drug treatment and to ascertain BACE1's role, the team investigated the effects of protease inhibitors in two animal models, then probed the mechanism of action in cells in culture.

First, to confirm that the drugs themselves, and not the underlying HIV infection, were responsible for neuronal damage, they examined a population of macaques, some of which had SIV, a retrovirus very similar to HIV that affects non-human primates. The researchers found that SIV-positive animals that had been treated had increased expression of APP in their neurons, a sign of damage, as well as increased BACE1 compared to the untreated animals.

They further confirmed that the drugs were the culprit in causing these changes by administering ritonavir and saquinavir to healthy adult mice. Again, they observed singificant increases in BACE1.

Turning to cells in culture, they found that administering ritonavir or saquinavir at doses equivalent to those seen in the blood of treated humans led to dramatic increases in molecular markers associated with UPR as well as increases in BACE1 expression. Furthermore, they demonstrated that the increase in BACE1 led directly to an increase in processing of APP. Applying an inhibitor of BACE1 to rat brain cells in culture prevented the damage that ritonavir treatment otherwise induced.

"Putting this together with our earlier findings on oxidative stress, it appears that the drugs are triggering oxidative stress that is damaging proteins and inducing the unfolded protein response," said Cagla Akay Espinoza, a research scientist in Jordan-Sciutto's lab and a coauthor. "The virus itself provides a stress, but the drugs are causing additional

stress and damage to neurons, in part by BACE1 leading to downstream processing of amyloid precursor protein."

A final set of experiments showed that an enzyme called PERK, a major player in UPR, helped mediate the increase in BACE1 expression in neurons triggered by protease inhibitors.

"We're very interested in the role of PERK in this process," said Jordan-Sciutto. "Targeting PERK and/or BACE1 could help contribute to a therapeutic approach to treat drug-associated cognitive disorders."

The new findings open up a number of avenues for future research. The team would like to explore whether this pathway of neuronal damage applies to other HIV drugs and how the UPR differs depending on whether the virus or the drugs are inducing it. Also, given the connection between beta amyloid, APP and Alzheimer's disease, the team is curious to learn more about how these peptides contribute to the disorders seen both in that disease and in HAND.

More information: Patrick J. Gannon et al, HIV Protease Inhibitors Alter Amyloid Precursor Protein Processing via β-Site Amyloid Precursor Protein Cleaving Enzyme-1 Translational Up-Regulation, *The American Journal of Pathology* (2017). DOI: 10.1016/j.ajpath.2016.09.006

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