# Human antibody that targets carfentanil, fentanyl and related opioids reverses overdose effects in preclinical study

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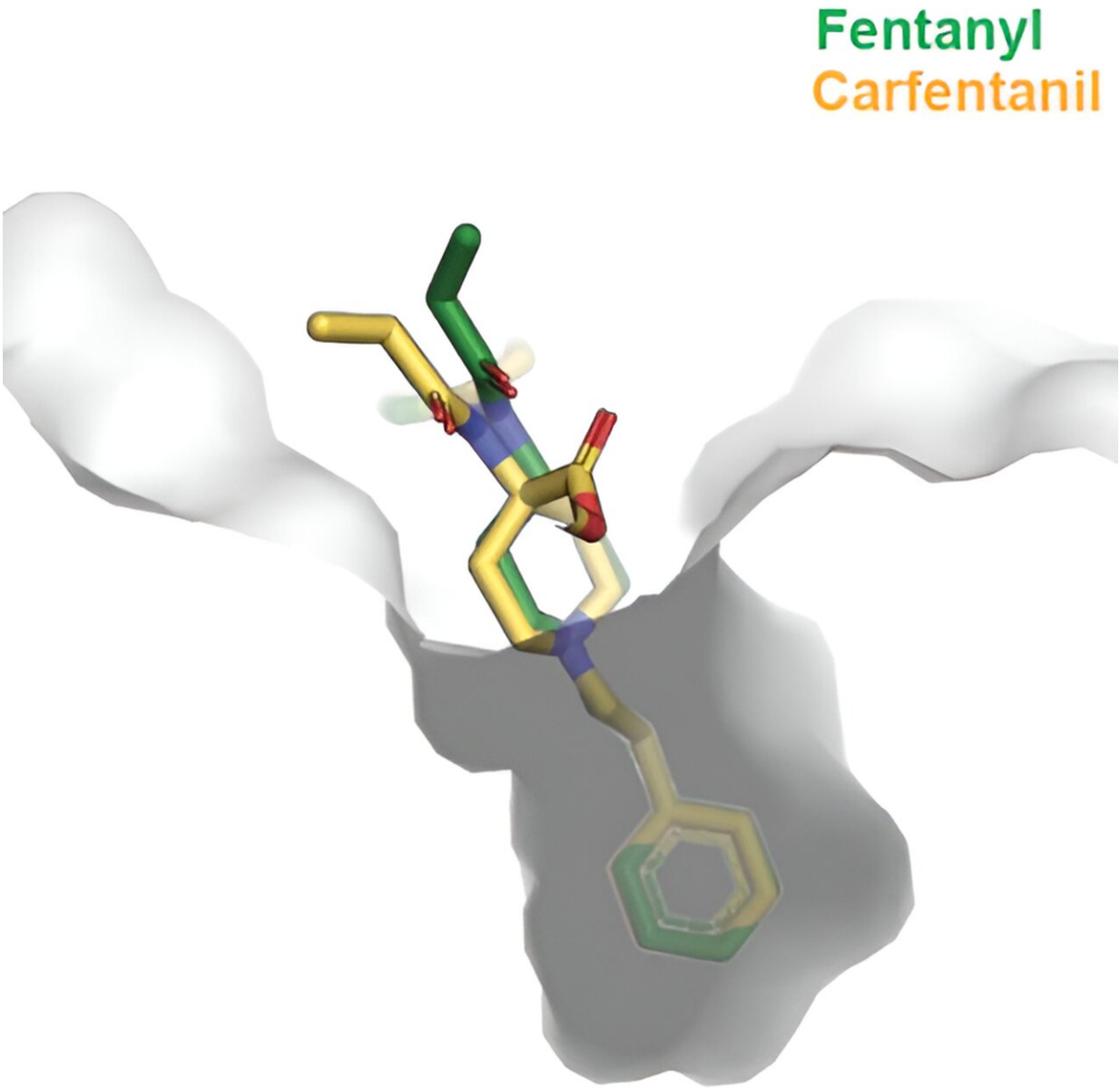


A crystal structure of the optimized antibody, C10-S66K. The binding pocket of C10-S66K with fentanyl and carfentanil is displayed as a gray surface with fentanyl and carfentanil shown as green and yellow sticks, respectively. Credit: Scripps Research

An antibody in single-chain fragment variable (scFv) format that binds to the powerful opioid carfentanil was shown to reverse signs of carfentanil overdose in preclinical tests conducted by scientists at Scripps Research.

Carfentanil is a variant of the synthetic opioid fentanyl, and about 100 times as potent as its chemical cousin. Along with fentanyl and other fentanyl variants, it is commonly mixed with illegal drugs such as heroin and cocaine to enhance their euphoric effects, resulting in many fatal overdoses.

In the study, published in *ACS Chemical Neuroscience* on August 3, 2023, the researchers developed a human antibody that binds very tightly to carfentanil, fentanyl and other fentanyl variants. In rodents, they showed that administering a solution of the antibody shortly after an overdose reverses the potentially deadly respiratory depression caused by carfentanil, the most dangerous of the variants. The results suggest that the antibody could be a more powerful, longer-lasting treatment for synthetic opioid overdose, compared to existing options.

"We expect this antibody to be a valuable new weapon for fighting the opioid crisis," says study senior author Kim D. Janda, Ph.D., the Ely R. Callaway, Jr. Professor of Chemistry at Scripps Research.

The study's first author was Lisa Eubanks, Ph.D., a senior staff scientist in the Janda laboratory.

Opioid drugs, whether synthetic or derived from the opium poppy, bind and activate neuronal receptors called mu-opioid receptors. These receptors are present on different types of neurons across the human nervous system, which is why opioid drugs have multiple effects like pain-relief and euphoria, but also respiratory depression—slower and shallower breathing. Respiratory depression is the immediate cause of

death in the tens of thousands of fatal opioid-related overdoses that occur each year in the U.S.

Carfentanil, after fentanyl, is the next-most common synthetic opioid found in illicit drugs in the U.S. Once available legally as a tranquilizer for large animals, it was pulled from the market by the FDA in 2018 because of its potential for misuse—and its potential lethality at doses measured in micrograms. Carfentanil is so potent that the U.S. government regards it as a possible chemical warfare agent; the Janda lab's early work on the new antibody was aimed at finding antidotes to such weapons.

Fentanyl and carfentanil overdoses currently are treated with the mu-opioid receptor-blocking drugs naloxone and naltrexone, but these treatments are sometimes ineffective against synthetic opioids even at large doses. Moreover, the benefits of these treatments typically last for less than an hour after dosing—potentially allowing respiratory depression from fentanyl or carfentanil (which persist much longer in the body) to resume.

Janda and his team set out to develop an anti-fentanyl antibody that would have three basic features: Firstly, it should bind with very high affinity to fentanyl and its derivatives, pulling them out of the bloodstream and thereby causing them to diffuse out of the brain as well; secondly, it should persist in the body so as to provide reasonably long-term protection; and thirdly, it should be able to get quickly into the bloodstream and be delivered by a simple intramuscular injection, which requires no special training.

To obtain antibodies, Janda and his team vaccinated rodents with a molecule they designed that would elicit antibodies against carfentanil, fentanyl and variants. The rodents were engineered to produce human antibodies (rather than rodent antibodies, which would trigger an

unwanted immune response if administered to humans). Among the resulting antibodies, the researchers were able to identify several that bind to carfentanil with super-high affinity—and bind very strongly to fentanyl and several other fentanyl derivatives. They then selected the most potent of these antibodies, modified it to be more lightweight (so that it would get quickly into the bloodstream), and further altered it so it would persist in the blood for days.

Tests in rodents showed that the optimized scFv, dubbed C10-S66K, did indeed have a powerful effect at reducing carfentanil's actions on the brain—reversing carfentanil-driven respiratory depression when injected 15 minutes after a heavy carfentanil exposure. The effect after about 40 minutes was stronger than naloxone's and was still increasing after two hours, whereas naloxone's peaked at 30 minutes and swiftly declined.

As part of the study, the collaborating laboratory of Ian Wilson, Ph.D., Hansen Professor of Structural Biology at Scripps Research, used X-ray crystallography to determine the near-atomic resolution structures of carfentanil- and fentanyl-bound C10-S66K. These structural data suggest that the antibody should indeed bind well to multiple fentanyl derivatives but should not interfere with the activity of other beneficial opioid molecules such as naloxone and naltrexone.

The U.S. Food and Drug Administration (FDA) has approved a full length IgG version of this antibody termed CSX-1004 for clinical trials, slated to begin this month for the prevention of fentanyl overdose.

**More information:** Lisa M. Eubanks et al, An Engineered Human-Antibody Fragment with Fentanyl Pan-Specificity That Reverses Carfentanil-Induced Respiratory Depression, *ACS Chemical Neuroscience* (2023). DOI: 10.1021/acschemneuro.3c00455

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