Researchers decipher modus operandi of potential Alzheimer's drug

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The chemical compound known as "methylene blue" is a potential candidate for treating Alzheimer's, as it prevents the harmful clumping of so-called tau proteins typically associated with this disease. However, until now it was unknown why methylene blue had this effect. Researchers from Goettingen and Bonn in Germany have now shed light on this microscopic process.

The study published in *Angewandte Chemie* might help to work out

strategies for developing potential drugs. As the team of scientist including Markus Zweckstetter and Eckhard Mandelkow report, methylene blue inactivates molecular residues that promote the bonding of tau proteins.

Methylene blue is a multi-talented substance with a long history. The synthetic compound was first produced in 1876, and since then has served not only as a blue dye, but also as a medical drug – for example to treat malaria and prevent urinary tract infections. It is now also being debated as a potential treatment for Alzheimer's disease.

Methylene blue works in many ways. With regard to Alzheimer's, it is interesting to note that it prevents the clumping of "tau proteins". Such aggregates are typical in numerous forms of dementia: The protein clumps accumulate in the brain cells, disrupt their function, and can even kill them.

"Tau proteins are actually extremely important, because they stabilize the transport routes inside each nerve cell," explains Prof. Eckhard Mandelkow, who works at the German Center for Neurodegenerative Diseases (DZNE) and the caesar research center in Bonn. "However, in cases of Alzheimer's, they stop doing their job. The transport routes inside the cells break down, and supplies essential for the survival of the cells can no longer reach their destination. In addition, the tau proteins stick together. These aggregates are also harmful and are a typical characteristic of the disease."

Such characteristics can be reproduced in animal studies. Previously, another team of scientists led by Dr. Eva-Maria Mandelkow was able to prove that methylene blue is able to alleviate the symptoms of an illness in mice and threadworms. However, no significant data from human patients has been collected so far. Furthermore, to date it was unknown, why methylene blue had the observed effect. "Methylene blue inhibits

the aggregation process," Eckhard Mandelkow emphasizes. "But the way in which this happens was unknown until now."

The study now published in *Angewandte Chemie* reveals the nature of this process: Markus Zweckstetter's research group at the DZNE site in Göttingen and the Max Planck Institute for Biophysical Chemistry in Göttingen in collaboration with the Mandelkow team have been able to prove that methylene blue deactivates molecular residues which promote the bonding of tau proteins. Moreover, the researchers found indications that the substance acts as a spacer to keep the proteins apart. These findings could lead to the development of modified forms of methylene blue and new types of treatment.

Methylene blue tackles sulphur groups

NMR spectroscopy, a powerful technique for investigating biomolecules, was centrally important to the current study. "We found that methylene blue reacts with certain elements in the tau proteins called cysteines," Prof. Zweckstetter summarizes.

This reaction is highly effective. Methylene blue specifically modifies the tau proteins at critical spots: Of the up to 441 elements which a tau protein can consist of particularly the two cysteines are modified. The elements directly modified are the so-called SH groups, molecular appendages comprising sulphur and hydrogen which are typical of cysteines. Oxygen atoms now couple with them.

"This chemical transformation prevents tau proteins from bonding together," says Zweckstetter. "Otherwise SH groups from different proteins would react and form a so-called disulfide bridge. Now, this is no longer possible, because the reaction with methylene blue eliminates the SH groups."

In a healthy organism, the formation of these disulfide bridges is suppressed naturally. "The cell tries to prevent harmful reactions with the help of antioxidants," says Eckhard Mandelkow. "However, with age and in cases of neurodegenerative diseases such as Alzheimer's, this protective system weakens allowing tau proteins to aggregate."

Beta sheets also important

Zweckstetter stresses that along with the disulfide bridges, another mechanism is important for the clumping of tau proteins. "Tau proteins aggregate particularly quickly when disulfide bonds form. These work like a trigger. However, tau proteins can also aggregate without these bridges, albeit more slowly."

This is due to the structure of the molecule, the backbone of which can fold like an accordion in some places. Such regions can pile up to "beta sheets" when two proteins come together closely enough and in the appropriate orientation. "Our experiments also show a distinct effect of methylene blue on the regions that want to form these beta sheets." Thus, methylene blue, particularly its derivatives "Azure A" and "Azure B", which are expected to be predominantly present in the body, also appear to inhibit the aggregation of beta sheets. "Steric hindrance occurs," Zweckstetter guesses. "When an inhibitor attaches to a beta sheet region of the tau protein, no other tau molecule can lock on."

There are other substances besides methylene blue that can suppress the aggregation of tau proteins. Some of them focus explicitly on preventing the build-up of beta sheets. The researchers believe that an effective treatment could ultimately require a combination of various substances: "Certainly, one conclusion of our study is that there are different ways to disrupt the pathogenic aggregation of tau proteins."

More information: Mechanistic Basis of Phenothiazine-driven

Inhibition of Tau Aggregation, Elias Akoury, Marcus Pickhardt, Michal Gajda, Jacek Biernat, Eckhard Mandelkow, Markus Zweckstetter, *Angewandte Chemie*, DOI: 10.1002/anie.201208290