////Title: Are Amyloid Peptides Potential Therapeutics for Sepsis?

////Stand-first:

Amyloids [am-y-loids] are aggregates of polymerised [**pol**-uh-muh-rahyz-ed] proteins. The polymerised proteins do not fold as they should and adopt shapes that enable multiple copies to stick together. In humans, these clusters of proteins form fibrils and the presence of these amyloid protein clusters are associated with disease pathologies. In a recent study, Dr Sidharth Mahapatra (sid-har-thh ma-ha-pa-trah) and colleagues at Stanford University assessed their hypothesis, that, contrary to much of the work in this area, in some cases, amyloids may be beneficial in treating inflammation caused by serious, life-threatening conditions, such as sepsis.

////Body text:

Amyloids are frequently associated with disease conditions, such as Alzheimer's disease and Multiple Sclerosis, where the plaque bundles of varying types are found within the neuronal cells and structures of the central nervous system. It has been long considered that in these neurological diseases, the amyloids induce an inflammatory response from the immune system, which leads to irreversible damage.

Contrarily, when testing the amyloid most frequently associated with Alzheimer's disease – beta-amyloid – researchers discovered that this protein exhibited anti-inflammatory properties in a mouse model of Multiple Sclerosis. Other researchers tested a different form of amyloid (derived from HspB5) in mouse models of a number of inflammatory conditions, including Multiple Sclerosis, stroke, and cardiac injury, and found that administration of this protein, once again, calmed inflammation by inducing an anti-inflammatory immune reaction.

The work conducted by Dr Sidharth Mahapatra and colleagues at Stanford University focused on a specific hexapeptide, called amylin. Comprised of six amino acids in its unaggregated form, amylin has the capability of self-associating into amyloid fibrils. Evidence from a mouse model of Multiple Sclerosis showed that these fibrils were anti-inflammatory. On this basis, Dr Mahapatra and colleagues tested amylin in a mouse model of sepsis to determine what, if any, effects were present during systemic inflammation across the entire body.

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To achieve this, Dr Mahapatra and colleagues conducted an experiment on mice, whereby the control mice received no amylin and the treatment mice received two injections of a specific dose of amylin, one at 6 and another at 12 hours prior to stimulating the inflammatory response.

To initiate inflammation, treatment and control mice were injected with lipopolysaccharide [li-po-poly-sac-cha-ride] (LPS), which is the component of E. coli bacteria that causes significant systemic inflammation and tissue damage.

To determine the effect of treatment, the mice were checked every six hours over a three-day period to determine illness severity and survival. Inflammation was measured by testing blood samples, taken at regular times in the 24 hours after the LPS injection. The blood samples were tested for a range of cytokines [si-to-kins] which are immune system proteins, including those known to cause inflammation and those which are known to dampen down inflammation.

Given the team's interest in lung damage in sepsis, they also assessed the effects of treatment by testing the barrier functionality of the pulmonary artery endothelial cells. To do this, they assessed the structure and permeability of the tissue and also conducted tests for caspase 3 activity, which measures apoptosis and indicates if programmed cell death cascade has been initiated.

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Dr Mahapatra and colleagues uncovered some highly promising findings. Mice that had been treated with amylin hexapeptide before injection with LPS showed a statistically significant lower rate of mortality than mice that had not. When mortality was assessed at the end of the 72-hour experiment, the control group had a mortality of 61%, while the amylin-treated group had a mortality of less than half, at only 28%. Furthermore, when scoring the level of illness, the amylin-treated mice were healthier than the control mice. More specifically, the severity of the illness increased over the entire 72 hours of the experiment in the control group, whereas, the peak illness occurred at 12 hours following LPS treatments in the amylin-treated group and, thereafter, these mice were found to improve.

Looking at the cytokine assays, Dr Mahapatra and colleagues found that levels of pro-inflammatory cytokines responsible for causing inflammation were reduced in amylin-treated mice compared to the controls. In some cases, the amylin-treated mice produced four-fold lower levels of inflammatory cytokines compared to the controls. Conversely, levels of interleukin 10, an anti-inflammatory cytokine, were significantly higher in the treatment group than the controls, with the treatment group producing almost six times more than the controls. Furthermore, these levels in the amylin treatment group remained higher for substantially longer than in the controls.

In terms of tissue damage determined by the presence of extravasated fluid in the lung tissue and by caspase 3 activity, which measures lung cell death, Dr Mahapatra and colleagues found that pre-treatment with amylin before the LPS injection decreased the damage to the lung tissue. In comparison to the controls, the amylin-treatment group exhibited lower levels of lung fluid and significantly lower levels of caspase 3. Interestingly, the caspase 3 levels were comparable to those of the 'baseline' mice not receiving any treatment whatsoever.

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Overall, Dr Mahapatra's work demonstrates that the amylin hexapeptide tested possesses physiologically significant systemic anti-inflammatory properties, which are evident even when the animal is exposed to a substantial pro-inflammatory attack. This is the first evidence to demonstrate the effect of these amyloid peptides on the systemic immune response since much of the work to date has described the impact of these peptides on autoimmune and neuroinflammatory conditions.

In combination, the data described here indicate a possible therapeutic role for amylin hexapeptides in diseases leading to systemic inflammation. The team tentatively suggests that even sepsis may be treated by these peptides. One key issue with the testing conducted so far, however, is that amylin was provided only prior to exposure to LPS. This is a substantial limitation for the treatment of sepsis, given its unpredictable nature.

However, Dr Mahapatra and colleagues indicate a possible role for this peptide in clinical situations where the occurrence of inflammation is predictable, such as after long surgeries or with the initiation

of dialysis or even ECMO [pronounced Ek-Mo], and point to the need for further work to investigate amylin hexapeptides in these models of inflammation.

This SciPod is a summary of the paper 'An amyloidogenic hexapeptide derived from amylin attenuates inflammation and acute lung injury in murine sepsis' published in the open access journal PLoS One. doi: 10.1371/journal.pone.0199206

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You can learn more about Dr. Mahapatra's research at: https://www.unmc.edu/pediatrics/divisions/critical-care/faculty/mahapatra.html

Key collaborators in this project included Dr. Lawrence Steinman, Professor of Neurology and Neurological Sciences, and Dr. David Cornfield, Professor of Pulmonary and Critical Care Medicine, Stanford University.