**////Title: Determining the Safety of the Tobacco Cembranoid 4R as a Neuroprotective Agent**

**////Stand-first**:

Increased use of organophosphate chemicals in industry, agriculture and warfare has led to a rising threat of exposure to these neurotoxins in civilian and military populations. Though their danger has been recognised and efforts made to decrease concentrations used, even at low doses these chemicals can still pose significant risks to exposed individuals. Finding effective treatments to counteract the impact of exposure is becoming increasingly important and is the focus of research by Professor Nadezhda Sabeva and Professor Peter Ferchmin at the Universidad Central del Caribe, Puerto Rico.

**////Body text:**

Organophosphates are the main components of certain herbicides, pesticides, and insecticides and are also commonly found in products used around the home. Certain organophosphates, like sarin and soman, are used as nerve gases in warfare and terrorist attacks. Although their danger has been recognised and effort has been made to decrease their concentrations in civilian applications, approximately 3 million people globally are exposed to organophosphates each year with 10% of those exposures leading to fatalities.

This group of chemicals works by inhibiting the action of acetylcholinesterase, an enzyme essential for the function of the nervous system. When functional, this enzyme breaks down the neurotransmitter acetylcholine, thus regulating the messages among neurons as well as between neurons and muscles. Inhibiting the action of acetylcholinesterase produces an increase of acetylcholine in the brain, causing seizures (similar to epilepsy) and overstimulation of muscles leading to breathing and other disturbances that often are fatal.

Classic antidotes to organophosphate exposure already exist, such as atropine, and have previously been shown to decrease mortality rates. However, patients often continue to develop neuroinflammation, ultimately triggering a form of cell death known as apoptosis leading to brain damage and potentially culminating in the development of neurodegenerative conditions similar to Alzheimer’s disease and Parkinson’s disease. With the rising prevalence of neurodegenerative disorders worldwide, an antidote that can reduce neuroinflammation and neurodegeneration after exposure to organophosphates is urgently needed.

The research in this area has narrowed the focus of investigation down to a group of natural compounds known as cembranoids. These have been found in marine corals, insects, and plants and are thought to act as a chemical defence against predators and competing species. The most popular cembranoids used in research come from the tobacco plant*,* from which over eighty different cembranoids have been isolated and are now being studied. Two candidates, abbreviated as 4S and 4R, have been highlighted so far for their potential as neuroprotective, antibacterial, and anti-tumour agents. 4R, in particular, shows significant neuroprotective effects, meaning it can protect the brain cells from damage.

Professor Nadezhda Sabeva and Professor Peter Ferchmin at the Universidad Central del Caribe have been researching the 4R compound and its potential use as a neuroprotective agent after exposure to organophosphates. Previous work by the group had shown that a single dose of 4R either 1 hour before or 24 hours after exposure to a nerve agent resulted in a noticeable decrease in inflammation and cell death in the brain. However, studies examining the safety of using 4R as a neurotherapeutic – an essential step to move toward clinical trials – have not yet been conducted. With this in mind, Professor Sabeva and colleagues have carried out further research to investigate potential safety concerns associated with using 4R as a neuroprotective agent.

….

To carry out an initial examination into potential safety issues, the group treated both male and female rats with a single injection of either 4R or a control mixture. The injection was given into the upper back of the rats in order to mimic the route of administration that has been considered most effective in clinical settings. Doses of 6, 24, or 98 mg/[per]kg were given to different test groups to test a range of doses despite the dose of 6 mg/kg previously being shown to be effective as a neuroprotective agent. Using higher doses than needed allowed the researchers to gain a full picture of potential safety issues.

The rats were then observed for any symptoms of toxicity immediately after injection and during the week that followed. The symptoms researchers monitored included mortality, signs of illness or pain, injury, allergic responses, alterations in appearance or behaviour and loss of weight. After the 7-day period, the researchers found that the rats maintained body weight and didn’t show any signs of toxicity post-injection. Mild reactions at the administration site, including alopecia, discoloured fur and skin peeling were observed in both 4R-treated rats and the control rats. The fact this was seen in both treatment groups suggests these responses were not specific to 4R.

Blood samples were also collected from the rats on day 3 and day 8 post-treatment which allowed the researchers to examine any changes in the levels of key blood cells or blood biochemistry. Overall, there were minimal changes seen in key cell groups across the 7-day period with the only noticeable changes occurring on day 3 post-treatment. These included an increase in mean platelet volume, which refers to the average size of the small blood cells that are essential for blood clotting in the 6 mg/kg male and 24 mg/kg female groups.

There was also a decrease in mean absolute eosinophils in the high-dose female groups, indicating decreases in a particular type of white blood cell. However, by day 8 these changes had returned to normal levels and were not regarded as significant. Similarly, changes in blood biochemistry were only observed at the earlier timepoints, mainly in the groups that received higher doses of 4R, but these had returned to normal or non-significant levels by day 7.

After day 7, tissue samples were collected to be further examined for any indications of toxicity. Researchers examined sections of major organs such as the liver, spleen, kidney and lungs and found no indications of toxicity or damage to the tissue caused by 4R.

….

The researchers concluded that the cembranoid 4R doesn’t have toxic effects, at least under the conditions studied here. All rats, including those treated with the highest dose, survived the post-injection observation period with no external or internal signs of toxicity.

Although this was a preliminary study, findings clearly support the safety of 4R application in a rodent model and provide strong encouragement for moving the compound closer towards the clinical trials needed to confirm its use in humans.

This SciPod is a summary of the paper ‘In Vivo Evaluation of the Acute Systemic Toxicity of (1S,2E,4R,6R,7E,11E)-Cembratriene-4,6-diol (4R) in Sprague Dawley Rats’, published in Nutraceuticals. DOI: https://doi.org/10.3390/nutraceuticals2020005

For further information, you can connect with Nadezhda Sabeva at nadezhda.sabeva@gmail.com