NTP Update on
Vinpocetine: Genetic Toxicity Assays
May 2017

Synopsis

Vinpocetine is a dietary supplement that is asserted to enhance memory. The National Toxicology Program (NTP)[1] tested vinpocetine for its ability to cause genetic damage by using an assay that measures the induction of micronuclei in red blood cells (erythrocytes) of female mice. Micronuclei contain small fragments of genetic material, and their presence in red blood cells is an indication of the capacity of a chemical to damage chromosomes. NTP concluded that none of the vinpocetine doses tested caused genetic damage because the frequency of red blood cells with detectable micronuclei after oral treatment of female mice for 3 days did not increase.

Levels of DNA damage in white blood cells and in cells from the liver and stomach were measured in the female mice using the comet assay. The comet assay is used to identify a genetic hazard that might indicate a risk for mutations or chromosomal damage, although the type of DNA damage detected in the comet assay usually is repaired rapidly. NTP found no increases in DNA damage in blood cells or stomach cells of these mice, but a small dose-related increase in DNA damage was observed in liver cells that might be related to vinpocetine exposure.

NTP also tested vinpocetine for its ability to cause mutations, or permanent changes in DNA sequence, using the bacterial mutagenicity, or Ames, test. The Ames test assesses the ability of a chemical to induce mutations in any of several different strains of bacteria. A positive test in any strain indicates the chemical is mutagenic and, therefore, has the potential to cause cancer. The Ames test results showed that Vinpocetine did not cause mutations.[2]

Background

Micronucleus and Comet Assays

The in vivo rodent erythrocyte micronucleus assay has been used widely for over 30 years as a standard test to evaluate the potential of a chemical or other environmental exposure (e.g., radiation) to cause genetic damage. This assay assesses a chemical's ability to cause chromosomal damage, measured as micronuclei, in rapidly dividing precursors of red blood cells in the bone marrow. Although the genetic damage initially occurs in red blood cells in the bone marrow, the cells move from the bone marrow into the blood as they mature. Thus, either bone marrow or blood samples can be used to measure the frequency of micronucleated red blood

[1] NTP is a federal, interagency program that has as its goal to safeguard the public by identifying substances in the environment that might affect human health. NTP is headquartered at the National Institute of Environmental Health Sciences, which is part of the National Institutes of Health. For more information about NTP and its programs, visit http://ntp.niehs.nih.gov/.

[2] The June 2015 NTP update for the bacterial mutagenicity study can be found at http://ntp.niehs.nih.gov/ntp/research/areas/wvspill/bacterial_mutagenesis_update_508.pdf.

cells. Chemicals that induce micronuclei formation in red blood cells of rodents have a high likelihood of causing cancer in rodents.[3]

The in vivo single-cell gel electrophoresis or comet assay[4,5] has been used for more than 20 years to measure the potential for a chemical or other environmental exposure to induce DNA damage in any of a variety of different cell types within an organism. Although the damage detected by the comet assay typically is repaired rapidly by the cell, an increased level of damage suggests that a particular exposure poses a risk for damage that might be converted into a mutation or chromosomal damage.

Bacterial Mutagenicity Assays

Bacterial mutagenicity (Ames) tests have been used widely for many years to determine if a chemical has the potential to cause mutations, which are permanent changes in the DNA sequence of the bacteria. These tests are performed regularly in the chemical and pharmaceutical industries and are accepted by regulatory agencies as a standard method of assessing the mutagenic potential of chemicals.

The Ames test employs several different strains of bacteria. NTP routinely uses three strains of bacteria in the test: two strains of *Salmonella typhimurium* and one strain of *Escherichia coli*. Each strain might react differently to chemical exposure, so using multiple strains increases the opportunity for detecting a mutagenic chemical. Chemicals are tested using five or more, widely spaced concentrations that are determined by preliminary trials in each bacterial strain.

Findings from the In Vivo Micronucleus and Comet Assays

NTP conducted a 3-day study in female mice to evaluate the ability of vinpocetine to cause chromosomal damage and DNA damage. Vinpocetine was mixed in 0.5% methylcellulose (in water) and administered orally once daily for 3 days to separate groups of female mice at the following doses: 0, 350, 500, 750, or 900 mg/kg/day. Blood samples were obtained from the mice 3 hours after the third treatment (~48 h after the first treatment), and the frequency of micronucleated red blood cells was measured using standard procedures.[6] The results were negative.

Comet assays also were performed in the same female mice to assess DNA damage in three different cell types (blood leukocytes, liver, and stomach epithelium). The results from the comet assays were negative in blood and stomach cells, indicating that vinpocetine does not induce DNA damage in these cell types. In liver cells, a small, dose-related increase in DNA damage was observed that was judged equivocal (neither positive nor negative).

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[3] Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT. 2000. Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environ Mol Mutagen. 36(3):163–94.
[4] OECD (Organisation for Economic Co-operation and Development). 2014. Test No. 489: In Vivo Mammalian Alkaline Comet Assay, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.
[5] Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, Tice RR. 2003. Recommendations for conducting the in vivo alkaline comet assay. 4th International Comet Assay Workshop. Mutagenesis 18:45–51.
[6] http://ntp.niehs.nih.gov/testing/types/genetic/invivo/mn/index.html.

Findings from the In Vitro Bacterial Reverse Mutation Assays

NTP tested vinpocetine with the Ames test to determine its ability to mutate bacterial DNA. Vinpocetine was tested at a minimum of five concentrations using standard procedures.[7] Vinpocetine did not cause mutations in any of the bacterial strains used in the test.

Summary

The genetic toxicity of vinpocetine was tested in animals and in bacteria. The results from these studies indicate that vinpocetine is negative for genetic toxicity, with the exception of a dose-related increase in DNA damage in liver cells assessed in the comet assay that was judged equivocal.

Next Steps

A comprehensive report of these studies and those evaluating the toxicity of vinpocetine in pregnant rats and rabbits is currently in progress. The draft report is anticipated to be released for external peer review and public comment in late summer 2017.

[7] Information about the test is available on the NTP website at http://ntp.niehs.nih.gov/testing/types/genetic/invitro/sa/index.html.