

BRAIN GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) AS A MARKER OF NEUROTOXICITY DURING INHALATION EXPOSURE TO TOLUENE

Health and Environmental Sciences Department
Publication Number 4647
June 1997



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Brain Glial Fibrillary Acidic Protein (GFAP) as a Marker of Neurotoxicity During Inhalation Exposure to Toluene

Health and Environmental Sciences Department

API PUBLICATION NUMBER 4647

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ACKNOWLEDGMENTS

THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

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Technical assistance at New York University was provided by Zhaolong Gong, Dawn Gray, Alvin Little, Kenneth Magar, and Dr. Cheng Wang.

Dr. Hassan El-Fawal contributed to the planning and interpretation of the GFAP assay.

Dr. Bernard Jortner provided neuropathology studies of our specimens in his laboratory at Virginia Polytechnical University.

Dr. J. P. O'Callaghan of the United States Environmental Protection Agency provided helpful suggestions on the GFAP method.

Dr. Udai Singh assisted with the assays of corticosterone.

Dr. Carroll Snyder contributed to the inhalation exposure methods.

Dr. Ronald W. Wood and Dr. John G. Graefe provided the system for measurement of locomotor behavior during inhalation exposure.

Supported in part by an Environmental Health Science Center Grant at NYU Medical Center (EH-00260).

ABSTRACT

Glial fibrillary acidic protein (GFAP) was measured during and after sub-chronic exposure to toluene. Rats received inhalation exposure to air or 100 - 3,000 ppm toluene, 6 hr/day, 5 days/wk for up to 42 days. Toluene, in concentrations that are low for the rat (100 to 1,000 ppm), altered GFAP and motor behavior without affecting body weight or producing overt signs of neurotoxicity. However, the declines in GFAP concentration during toluene exposure differ from the more commonly reported toxicant-induced pattern of increased GFAP. At a higher concentration (3,000 ppm), toluene produced increased GFAP concentrations, observable neurological signs and weight loss. These results are discussed in relation to methodological issues and the relevant scientific literature. GFAP can provide an index of toxicity, even with exposures below the level which produce overt signs of toxicity. For toxicity screening with animals, a battery including GFAP as well as behavioral and neurochemical measures would be useful. Implications for future research are discussed.

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EXECUTIVE SUMMARY

Measures of brain cell-specific proteins show promise as markers of neurotoxicity in animals, particularly after exposure to heavy metals. One such marker is glial fibrillary acidic protein (GFAP). Increased GFAP indicates reactive gliosis following neuronal injury from toxic exposures. Modern biochemical techniques for measurement of GFAP may prove to be faster, less expensive and more quantitative than classical neuropathological examination, and thus may be useful for evaluating potential neurotoxins. The purpose of this study was to determine whether an immuno-assay for GFAP in the rat's brain can provide practical evidence of toluene-induced neurotoxicity. The U.S. Environmental Protection Agency (USEPA, 1994, 1995) has suggested that a Radio-Immune-Assay (RIA) of brain GFAP be used in the screening for neurotoxicity of chemicals. Previous findings reported to API that an Enzyme-Linked-Immuno-Sorbant Assay (ELISA) of GFAP yielded results similar to results from the older RIA method and that the ELISA was sensitive to repeated oral exposure to lead (Pb) at exposure levels which produced behavioral and histological evidence of neurotoxicity (Evans, 1994a). The ELISA method has two advantages over the RIA method: freedom from radioactive materials, and simplicity. Although GFAP was a useful marker of Pb-induced neurotoxicity, GFAP was a less useful marker of Pb exposure than traditional indices such as blood lead concentration (Evans, 1994a).

Toluene was chosen as a model neurotoxicant for these studies because its neurotoxicity in the rat has been characterized. The present studies documented changes in GFAP concentration during subacute inhalation exposure to toluene. Adult male F344 rats, at approximately 47 days of age, received inhalation exposure to room air or 100, 300, 1,000 or 3,000 ppm toluene, 6 hr/day, 5 days/wk for up to 42 days. These exposures approximate an occupational exposure schedule. During and after exposure, the concentration of GFAP was determined in four brain regions. These changes in GFAP were compared with standard neurotoxicity criteria: behavioral or neuropathological changes. Body weight was monitored as a sign of general toxicity.

The toluene concentration-effect data for GFAP concentration suggest that 50% of brain samples are affected by an exposure of at least 3 days to 1,000 ppm toluene. At concentrations that are quite low with respect to the literature on the laboratory rat (100 to 1,000 ppm), toluene altered GFAP concentration without affecting body weight, brain pathology or producing overt signs of neurotoxicity. Changes in GFAP were seen as early as the third day of exposure; however, the declines in GFAP concentration differ from the more commonly reported toxicant-induced pattern of increased GFAP. GFAP was affected by toluene concentrations as low as 100 ppm, within the range of occupational exposures for humans. In contrast, a much higher concentration (3,000 ppm) of toluene impeded growth and caused observable neurological signs in the rats, confirming previous reports of toluene's toxicity at high concentrations. Increased GFAP after 7 days exposure to 3,000 ppm is suggestive of reactive gliosis, but cellular damage was not investigated at 3,000 ppm. At 1,000 ppm, cellular damage could not be seen at the light microscopic level.

The time-effect data suggest that, as toluene exposure continued, significant changes in GFAP appeared, then reversed as exposure duration continued. There was no evidence of permanent nervous system damage or functional impairment. For example, significant increases in GFAP at 42 days of exposure to 1,000 ppm toluene had returned to control levels by 14 days after exposure. No behavioral changes could be detected in the home cage in the 24 hours after the most recent exposure.

The information provided by GFAP is partly correlated with, but not redundant to, that available from standard assays of behavior and general signs of toxicity such as body weight. GFAP was clearly more sensitive to toluene than histopathology indicates at the light microscopic level. GFAP was nearly equal to the sensitivity of behavioral measures, keeping in mind that the most sensitive behavioral index was recorded during toluene inhalation, whereas GFAP was measured 24 hours or more after the last exposure, at a time when behavior in the home cage and neuropathological indices were unaffected. GFAP was of similar sensitivity to physiological

indices of inhaled toluene, as reported in the literature. The most sensitive indices at present are those reflecting changes in brain neurotransmitter function.

Because the direction of changes in GFAP concentration was inconsistent as repeated toluene exposure continued, GFAP alone may not provide a practical marker of the effects of short term occupational exposure to toluene. Measurement of GFAP concentration with an ELISA should be an element in toxicity screening batteries, along with behavior and indices of neurotransmitter function. An inter-laboratory workshop would be useful to advance the standardization of the GFAP ELISA. Further research is needed to identify how toxicant-induced changes in GFAP concentration are influenced by sub-types of astrocytes, changes in expression of the GFAP gene, adrenal cortical steroid production, or neurotransmitter function.

Section 1

INTRODUCTION

The nervous system is a target organ for inhaled toluene (ATSDR, 1994; Morata *et al.*, 1995) and for many other organic solvents (Arlie-Soborg, 1992). Exposure to solvents has been alleged in neurobehavioral disorders. The cellular and molecular mechanisms by which inhaled toluene causes changes in function of the central nervous system are not well understood (ATSDR, 1994). This is not surprising, since little more is known about the cellular and molecular mechanisms of action of inhaled volatile anesthetics, despite the routine use of those chemicals in human surgery (Pocock and Richards, 1993; Snyder and Andrews, 1996).

The detection of the effects of inhaled toluene, and an understanding of the mechanisms of neurotoxicity, may be indicated by molecular markers. Solvents and their metabolites are cleared rapidly from the body (Brugnone *et al.*, 1995) and markers of solvent neurotoxicity have not been validated for peripheral media, e.g., blood, urine (ATSDR, 1994; Tardif *et al.*, 1991).

Most promising as markers of neurotoxicity in animals are measures of brain cell-specific proteins (Evans, 1995; Aschner and Kimelberg, 1996). One such marker is GFAP, the major intermediate filament of astrocytes. When reactive gliosis accompanies brain injury, GFAP is increased. Gliosis is observed in the brains of humans who died of solvent inhalant abuse (Kornfeld *et al.*, 1994). GFAP has been suggested as a marker for solvent-induced neurotoxicity in animal studies (Arlie-Soborg *et al.*, 1992). Two reports indicate that inhalation of organic solvents may affect brain GFAP content in rats (Rosengren *et al.*, 1986; Rosengren and Haglid, 1989). Inhalation of toluene was observed to increase or decrease the concentration of several protein markers of astrocytes in the rat's brain, although GFAP was not studied (Huang *et al.*, 1990 and 1992).

Modern biochemical techniques for measurement of GFAP may prove to be faster, less expensive and more quantitative than classical neuropathological examination, and thus may be