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Autoantibodies to neurotypic and gliotypic proteins as biomarkers of neurotoxicity: Assessment of trimethyltin (TMT)

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Abstract

Developing accessible biomarkers of neurotoxic effects which are readily applicable to human populations poses a challenge for neurotoxicology. In the past, the neurotoxic organometal trimethyltin (TMT) has been used as a denervation tool to validate the enhanced expression of GFAP as a biomarker of astrogliosis and neurotoxicity resulting from chemical exposures. In the present study, TMT was used to assess the detection of serum autoantibodies as biomarkers of neurotoxicity. Previous studies in both human and animals have demonstrated the presence of serum autoantibodies to neurotypic [e.g., neurofilament triplet (NF)] and gliotypic proteins [myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP)] as a peripheral marker of neurodegeneration that may be applicable to humans and experimental studies. Male Long–Evans rats (45 days of age) were administered either TMT (8 mg/kg; s) or an equal volume of sterile 0.9% saline. At 1, 2, and 3 weeks post-administration, serum was collected, and rats were sacrificed for the collection of brains. Serum autoantibodies (both IgM and IgG isotypes) to NF68, NF160, NF200, MBP, and GFAP were assayed using an ELISA. Saline only rats did not have detectable levels of autoantibodies. Only sera from TMT-exposed rats had detectable titers of autoantibodies to NFs with IgG predominating starting week 2. Anti-NF68 titers were highest compared to NF160, or NF200. Autoantibodies to MBP and GFAP also were detected; however, there was no significant increase in their titers until week 3. Hippocampal GFAP, detected at these time points, was significantly ($p < 0.05$) higher than in control brains, indicating the induction of astrogliosis as confirmed by immunostaining of brain sections. The detection of anti-NFs, as indicative of neuronal insult, was consistent with loss of hippocampal neurons in CA3 and CA1. Our results suggest that the detection of autoantibodies to neurotypic and gliotypic proteins may be used as peripheral biomarkers to reveal evidence of nervous system neurotoxicity.

Keywords: Nervous system proteins; Neurofilament; Myelin basic protein; GFAP; Autoantibodies; Biomarkers; Neurotoxicity; Trimethyltin

1. Introduction

A great deal of attention is currently being devoted to the development of biomarkers of specific disease states for the purpose of rapidly and accurately screening the efficacy of potential therapeutics (Petricoin et al., 2006). A biomarker approach has long been used as a means to assess many aspects of toxicity, however, one subdiscipline of toxicology that has lagged behind in this effort is neurotoxicology. Few broadly applicable biomarkers sufficient to reveal damage to the diverse targets of neurotoxic agents have been identified, much less implemented on a large scale. Biomarkers of gliosis are perhaps the one notable exception (O'Callaghan and Sriram, 2005).

Astrogliosis, or ''reactive'' gliosis, characterized by astrocytic hypertrophy and accumulation of the intermediate filiament protein, GFAP, occurs in response to all types of neural insults. As a hallmark of astrogliosis, enhanced expression of GFAP, assessed by immunohistochemistry, has been used as a biomarker of reactive gliosis and underlying neural injury (Garman et al., 2001; O'Callaghan and Sriram, 2005). More recently, a GFAP immunoassay has been used to document differences, quantitatively, in the expression of this protein as a marker of dose-, time, and region-dependent damage from exposure to a wide spectrum of neurotoxic agents (O'Callaghan and Sriram, 2005). Although direct measurement of proteins in the brain, such as GFAP, may provide valuable insights into the cellular targets of neuropathology in animal

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studies (O'Callaghan, 1988) they are not applicable to living human populations. Nevertheless, the extant database on biomarkers of neural injury obtained from animal and post-mortem studies should prove invaluable in evaluating potential non-invasive markers of neurodegeneration, including those associated with environmental/occupational neurotoxic exposures.

We have proposed that detection of autoantibodies against neurotypic and gliotypic antigens be a surrogate to document insult to the nervous system (El-Fawal, 1996; El-Fawal et al., 1999). This approach is not without precedent. Serum autoantibodies to nerve growth factor, GFAP and the astrocytic protein S100b have been reported in several mental and neurological disorders, including Parkinson's disease (PD; Poletaev et al., 2000; Orr et al., 2005; Papachroni et al., 2007). In amyotrophic lateral sclerosis (ALS) patients, autoantibodies against Ca^{2+} voltage-operated channels and antineuronal proteins, including neurofilament-68 (NF-68), have been found in sera and cerebrospinal fluid (CSF) (Rowland and Shneider, 2001; Nieborj-Dobosz et al., 2006; Pagani et al., 2006). In Alzheimer's disease (AD) patients, there have been reports of autoantibodies to GFAP, Aβ peptide and other neural proteins in CSF and serum (Frank et al., 2003; Bouras et al., 2005). In these studies, comparison was made with control groups that had no neurological disorders, detectable titers or where there were significant quantitative increases beyond what is referred to as ''natural autoantibodies'' against some autoantigens (Poletaev and Osipenko, 2003). Furthermore, the prevailing isotype associated with neurodegenerative conditions was IgG, the isotype associated with development of immunological memory and pathology (Abbas et al., 2000; Kindt et al., 2007). These studies demonstrate a precedent for autoantibody detection as indicative of nervous system insult. Although these neurodegenerative conditions are not recognized as autoimmune, the presence of autoimmunity does indicate injury to neural tissue. Therefore, in the context of neurotoxicology, evidence of an autoimmune response to neural antigens reveals a potential approach to assess and monitor neurotoxicity (El-Fawal et al., 1999), an approach that may even provide ''memory'' of a neurotoxic events that occurred in the distant past.

The present study was undertaken to evaluate the detection of autoantibodies against neurotypic and gliotypic proteins in blood serum as a peripheral marker of neurotoxicity following exposure to TMT. Although autoantibodies may be detected in CSF as well as serum, serum was used in the present study to assess a more accessible relatively non-invasive approach when applied to humans. The choice of dose and TMT was based on its successful use as a denervation tool for evaluating GFAP immunochemistry and immunoassay as markers of neurotoxicity in brains obtained from experimental animals (Brock and O'Callaghan, 1987; O'Callaghan et al., 1999).

2. Materials and methods

Long–Evans male rats, 6 weeks of age, were obtained from Charles River (Portage, MI). Rats were housed singly in plastic tub cages with aspen shred bedding in a temperature (21 ± 1 °C) and humidity-controlled (50 ± 10%) colony room maintained on a 12 h light/12 h dark schedule. All procedures were performed under protocols approved by the Institutional Animal Care and Use Committee of West Virginia University and the animal colony was certified by the American Association for Accreditation of Laboratory Animal Care. Rats received intraperitoneal injections of vehicle (0.9% saline) or TMT hydroxide (8 mg/kg body weight as the free base). This is the dose previously used to validate brain GFAP as a biomarker of neurotoxicity (Balaban et al., 1988; O'Callaghan, 1991). TMT was obtained from K&K Laboratories, Division of ICN Biomedical, Cleveland, OH. TMT-treated rats and saline controls (for each time point) were sacrificed by decapitation at 1 ($n = 5$), 2 ($n = 5$), and 3 ($n = 16$) weeks after treatment. At these time points serum was collected post-decapitation using a ventricular puncture. A 2 ml blood sample was collected and placed in a coagulation tube for serum separation. Following centrifugation, serum was aspirated into 0.5 ml polyethylene eppendorf tubes and frozen at −85 °C for later assay of autoantibodies. Simultaneous to blood collection, brains were removed from the skull, the hippocampus was immediately dissected and the tissue then was homogenized by sonification in hot (85–95 °C) 1% SDS for subsequent GFAP immunoassay. A separate set of rats ($n = 5$) was administered TMT as described above and they were anesthetized with pentobarbital (200 mg/kg, i.p.; Veterinary laboratories, Lenexa, KS, USA) and perfused transcardially at 21 days post-dosing.

Hippocampal GFAP levels were determined using the sandwich ELISA developed by O'Callaghan (1991, 2002). Although other brain regions are damaged by TMT, previous studies indicate that the hippocampus is particularly vulnerable to TMT (Balaban et al., 1988; O'Callaghan, 1991; Chang, 1996). Briefly, total protein of each brain region was determined colormetrically with an assay kit (BCA, Pierce, Rockford, Illinois). Bovine serum albumin was used as a standard. Samples were normalized for total protein prior to performance of the ELISA. A GFAP standard was generated using a stock of control hippocampus homogenate as previously described (O'Callaghan, 1991, 2002). Flat-bottomed Immulon microtiter plates (Fisher Scientific) were coated with 1.0 μg/100 μg/well polyclonal anti-GFAP (Dako, Carpenteria, California) for 1 h at 37 °C. Microtiter plates were washed with phosphate buffered saline (PBS, pH 7.4), blocked for 1 h with Blotto (5% non-fat dry milk in PBS) then 100 μl of standard or normalized sample were added for 1 h. After washing with PBS-0.5% Triton X-100, plates were incubated for 1 h with monoclonal anti-GFAP (Oncogene Research Products, Boston, MA), washed with PBS-0.5% Triton X-100, then alkaline phospatase-conjugated anti-rat antibody (Jackson Immunoresearch, West Grover, PA) was added for 30 min. Plates were washed with PBS-0.5% Triton X-100. The alkaline phosphatase substrate, *p*-nitrophenylphosphate, was added. The reaction was stopped after 30 min by adding 100 μl of 0.4 N NaOH. Absorbance was read at 405 nm in Spectramax Plus microtiter plate reader (Molecular Devises, Sunnyvale, CA).

Serum levels of autoantibodies to neurotypic and gliotypic proteins were determined as described by El-Fawal et al.