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Quantitative mapping of trimethyltin injury in the rat brain using magnetic resonance histology

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ABSTRACT

The growing exposure to chemicals in our environment and the increasing concern over their impact on health have elevated the need for new methods for surveying the detrimental effects of these compounds. Today's gold standard for assessing the effects of toxicants on the brain is based on hematoxylin and eosin (H&E)-stained histology, sometimes accompanied by special stains or immunohistochemistry for neural processes and myelin. This approach is time-consuming and is usually limited to a fraction of the total brain volume. We demonstrate that magnetic resonance histology (MRH) can be used for quantitatively assessing the effects of central nervous system toxicants in rat models. We show that subtle and sparse changes to brain structure can be detected using magnetic resonance histology, and correspond to some of the locations in which lesions are found by traditional pathological examination. We report for the first time diffusion tensor image-based detection of changes in white matter regions, including fimbria and corpus callosum, in the brains of rats exposed to 8 mg/kg and 12 mg/kg trimethyltin. Besides detecting brain-wide changes, magnetic resonance histology provides a quantitative assessment of dose-dependent effects. These effects can be found in different magnetic resonance contrast mechanisms, providing multivariate biomarkers for the same spatial location. In this study, deformation-based morphometry detected areas where previous studies have detected cell loss, while voxel-wise analyses of diffusion tensor parameters revealed microstructural changes due to such things as cellular swelling, apoptosis, and inflammation. Magnetic resonance histology brings a valuable addition to pathology with the ability to generate brain-wide quantitative parametric maps for markers of toxic insults in the rodent brain.

1. Introduction

The increasing presence of toxic compounds in the biosphere is elevating our need to understand their impact on the nervous system. Environmental factors have been implicated in neurologic diseases ranging from autism (Bushnell, 2013; LaSalle, 2013; Matsuzaki et al., 2012), Parkinson's (Willis et al., 2010), and Alzheimer's (Campdelacreu, 2012). The use of agricultural chemicals is growing nearly exponentially with wide-ranging potential impact on the nervous system (Costa et al., 2008; Thany et al., 2013). An ever-widening array of pharmaceuticals comes with an increasing potential for unwarranted neurologic side effects (Ekici et al., 2011; Nevin, 2011). As the prevalence of these insults increases, the challenge to early identification of their impact on the nervous system grows ever more daunting.

The traditional approach for histopathologic evaluation of neurologic toxicology has recently been expanded from three slices to include seven carefully selected slices, providing a more robust method for assessing the impact of chemicals of concern (Bolon et al., 2013; Rao et al., 2011, 2013). Even with this increase, the total volume of tissue studied is <1% of the total brain volume. The simultaneous need to screen many more compounds and a desire to perform a more comprehensive coverage of the brain compels us to consider new approaches.

Over the last 30 years, magnetic resonance imaging (MRI) has revolutionized the clinical domain. The idea that one can view the

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brain noninvasively in three dimensions with exquisite soft tissue delineation seemed almost like science fiction, but MRI is now in widespread daily use, with more than 8000 MRI systems in the United States (OECD, 2011). The closing sentence of Paul Lauterbur's seminal paper describing the invention of MRI clearly predicted its widespread adoption in the basic sciences: "Zeugmatographic (imaging) techniques should find many useful applications in studies of the internal structures, states and composition of microscopic objects" (Lauterbur, 1973).

MRI can provide a particularly powerful approach to high-throughput scanning for neurotoxicologic evaluation, with wide use for clinical evaluation of neurotoxic insults at spatial resolution of 1–3 mm. There are numerous live animal studies at spatial resolution ~0.1–1 mm. The suggestion that MRI could be applied to histopathology at resolution of <0.1 mm was first made in 1993 (Johnson et al., 1993). Magnetic resonance histology (MRH), i.e. the study of fixed tissues, is perfectly suited for neurotoxicology. MRH is non-destructive, is sensitive to many different types of injury, provides full three-dimensional (3D) coverage of the specimen, and is inherently digital, which present particularly appealing opportunities for high-throughput, automated analysis. Lerch et al. have published a particularly insightful analysis of the tradeoffs between live animal and ex vivo studies. When longitudinal information is not required, the lower cost and higher spatial resolution of MRH is preferred over in vivo studies (Lerch et al., 2012).

MRH was first employed to survey chemically-induced neuropathology in 2000 (Lester et al., 2000). It has helped elucidate the pathogenesis of carbonyl sulfide (Morgan et al., 2004; Sills et al., 2004), and has become routine for structural neuroanatomical phenotyping (Badea et al., 2007, 2009; Ellegood et al., 2012; Johnson et al., 2007). But, the pathologic changes studied to date have for the most part been relatively large, focal lesions. For MRH to be useful for high-throughput screening, it must be able to detect more subtle pathology. The focus of this work is to assess the sensitivity of MRH for detecting such subtle, diffuse pathology.

As part of an ongoing effort to assess the potential for MRH, the International Life Sciences Institute (ILSI)/Health and Environmental Sciences Institute (HESI) Project Committee on Imaging for Translational Safety Assessment has been established to explore the utility of MRH in safety assessment (http://www.hesiglobal.org/i4a/pages/Index.cfm?pageID=3494). This group generated a list of potential compounds that would create diffuse lesions. Trimethyltin hydroxide was chosen because it is well studied and produces a range of injury.

Trimethyltin (abbreviated TMT) is a selective toxicant, affecting several organs, including the central nervous system (CNS), where it has particular affinity for neurons containing the protein stannin (Toggas et al., 1992). TMT is an organometal compound used for insect, bacteria, and fungus control, as well as for preserving wood, textiles, paints, and plastics. As polyvinyl chloride (PVC) products have become popular, reports of toxicity caused by organotin compounds used to stabilize such plastics (dimethyltin [DMT] and trimethyltin [TMT]) have emerged. Exposures to high doses of TMT, usually work-related, have been associated with behavioral and anatomical changes in the brain (Kreyberg et al., 1992). The larger population may also be affected in cases of fires and contaminations. A recent study suggests that even in low doses, cumulative effects of alkyltins, such as DMT/TMT, can affect the population, e.g. through DMT/TMT leaching into drinking water from PVC pipes. We thus chose to study the CNS effects of TMT, known to be a toxicant for humans, as well as other mammals such as hamsters, gerbils, and marmosets (Brown et al., 1984; Gozzo et al., 1993), and the usual small animal mouse and rat models (Tang et al., 2013; Toggas et al., 1992).

While its mechanism is not fully understood, the spatio-temporal effects of TMT in the rat brain have been previously described (Dyer et al., 1982). Early effects of TMT on the rat brain (1–3 days post-exposure) include neurodegeneration starting in the lateral septal nucleus, followed by the septo-hippocampal nucleus, septo-hypothalamic nucleus, anterior olfactory nucleus, bed nucleus of stria terminalis, endopiriform nucleus, parafascicular nucleus, superior colliculus, interstitial nucleus of the posterior commissure, inferior colliculus, pontine nuclei, raphe nuclei, spinal trigeminal nucleus, nucleus tractus solitatiris, vagal motor nucleus, the piriform cortex, enthorhinal cortex, and layers V and VI of the neocortex (Balaban et al., 1988). Most studies focus on the striking effects, detected as early as 2–3 days post-exposure (Balaban et al., 1988), on the hippocampal areas, including the granule (Bruccoleri et al., 1998; Fiedorowicz et al., 2008) and pyramidal cells in the CA areas and dentate gyrus, as well as subiculum (Brown et al., 1979; Dyer et al., 1982; Kim et al., 2013; Mignini et al., 2012; Shirakawa et al., 2011). Changes in structures, including the piriform cortex and amygdala, have also been reported (Brown et al., 1979). These anatomical changes in neuronal cell populations and glial cells (Brock and O'Callaghan, 1987; McPherson et al., 2014) are accompanied by behavioral changes collectively known as "TMT syndrome," including seizures, vocalizations, self-mutilation, whole-body tremors, and hyper-reactivity to touch (Brown et al., 1979; Dyer et al., 1982), as well as spatial memory impairment (Mignini et al., 2012).

Because the injury is diffuse and widespread, TMT is a perfect candidate for a test of MRH and its ability to detect such injury. If, in fact, MRH can provide reasonable sensitivity and full coverage, it may allow expanded insight into TMT and its diverse effects. Conventional histological studies usually require some a priori knowledge of the areas to be sampled, or else it would be prohibitively time-consuming. Reproducing the same study in multiple animals, treated in different ways or doses, adds more to the burden. While lower in spatial resolution, MRH offers full three-dimensional (3D) coverage of the brain, and the ability to quantify the differential effects due to TMT toxicity. The focus of our study was to test the hypothesis that MRH could detect and quantitate the diffuse injury that is seen in TMT.

2. Materials and methods

All protocols were approved by the Duke University Institutional Animal Care and Use Committee. Dosing, tissue preparation, scanning, and image analysis were performed at the Duke Center for In Vivo Microscopy, and conventional histology was performed at the National Institute of Environmental Health Sciences (NIEHS) in the Research Triangle Park, North Carolina.

TMT hydroxide (Strem Chemical, Inc., Newburgport, MA) was dissolved in 0.9% saline. Twenty-four male Sprague Dawley rats (~325 g) (Charles River, Wilmington, MA) were divided into three groups – saline control ($n = 8$), low TMT dose (8 mg/kg) ($n = 8$), and high TMT dose (12 mg/kg) ($n = 8$). Animals received one IP injection of either saline or TMT. Animals in the saline and 8 mg/kg-dose groups were sacrificed 6 days after dosing. Animals in the 12 mg/kg-dose group were sacrificed 3 days after dosing, because their clinical symptoms were sufficient to cause humane concern. All the animals were perfusion-fixed using a protocol to actively stain the tissue to enhance the signal for MRH. Animals were anesthetized to a surgical plane and perfused through the left ventricle with outflow from a cut in the right atrium. The initial flush was 0.9% saline/0.1% heparin. This was followed by fixation with 1:10 ProHance (Gadoteridol, Bracco Diagnostics, Monroe Township, NJ) in 10% phosphate buffered formalin. The heads were removed and placed in formalin for one day and then transferred to a 1:100 ProHance/PBS solution to rehydrate the tissue. This active