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Longitudinal diffusion tensor imaging of the rat brain after hexachlorophene exposure

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ABSTRACT

Longitudinal MRI employing diffusion tensor imaging and T_2 mapping approaches has been applied to investigate the mechanisms of white matter damage caused by acute hexachlorophene neurotoxicity in rats *in vivo*. Male Sprague-Dawley rats were administered hexachlorophene orally once a day for five consecutive days at a dose of 30 mg/kg and were monitored in 7T MRI scanner at days 0 (baseline), 3, 6, 13, and 20 following the first hexachlorophene dose. Quantitative T_2 maps as well as a number of diffusion tensor parameters (fractional anisotropy, radial and axial diffusivity, apparent diffusion coefficient, and trace) were calculated from corresponding MR images. T_2, as well as all diffusion tensor derived parameters (except fractional anisotropy) showed significant changes during the course of neurotoxicity development. These changes peaked at 6 days after the first dose of hexachlorophene (one day after the last dose) and recovered to practically baseline levels at the end of observation (20 days from the first dose). While such changes in diffusivity and T_2 relaxation clearly demonstrate myelin perturbations consistent with edema, the lack of changes of fractional anisotropy suggests that the structure of the myelin sheath was not disrupted significantly by hexachlorophene in this study. This is also confirmed by the rapid recovery of all observed MRI parameters after cessation of hexachlorophene exposure.

1. Introduction

Hexachlorophene (HC), an organochlorine compound and a potent neuro-toxicant typically used to treat burns and prevent *Staphylococcus aureus* infection in infants (Alder et al., 1980; Heath et al., 2000) is known to cause alterations in white matter in the brain. It is still used as a prescription anti-infective topical preparation in the US. Although HC is typically administered externally, there have been reported instances of accidental ingestion of HC, due to its similar appearance to milk of magnesia, which have resulted in spongy degeneration of white matter (Kimbrough, 1973; Mullick, 1973). Several reports, including some dating back to the 70's have described some of the toxic effects of HC (Lockhart, 1972; Kimbrough, 1973; Mullick, 1973; Shuman et al., 1974; Catalano, 1975; Shuman et al., 1975b, 1975a; Paul and Gordon, 1978; Tripier et al., 1981; Vorherr et al., 1988; Flanagan et al., 1995; Boyd et al., 2010; Kanno et al., 2012; Hanig et al., 2014; Itahashi et al., 2014), which include nausea, vomiting, diarrhea, dehydration, hypotension, irritability, weakness of lower extremities, and finally convulsions in human patients, and drowsiness with hyperactivity, hind limb paralysis accompanied with very high cerebrospinal fluid pressure in mice, rats, cats, and monkeys. Almost all clinical and non-clinical observations of HC toxicity were accompanied with brain edema and vacuolation of white matter, described as status spongiosis (Kimbrough, 1973; Mullick, 1973; Hanig et al., 1976). White matter changes caused by HC consistent with edema were also observed *in vivo* using MRI (Igisu and Kinoshita, 2007; Hanig et al., 2014). If human patients survive exposure to HC, the effects are reversible without noticeable long-term implications (Lockhart, 1972). In rats recovery from neurological effects occurs rather rapidly (days to weeks). Neuropathology and behavioral evidence takes longer to return to the normal state (Lockhart, 1972; Hanig et al., 1976; Hanig et al., 1977; Hanig et al., 1984).

Overall there were 15 deaths reported in US (Lockhart, 1972; Kimbrough, 1973; Mullick, 1973; Culliford et al., 1974) and 39 in

Abbreviations: AD, axial diffusivity; cc, Corpus Callosum; Comm, Commissures; DTI, diffusion tensor imaging; fim, Fimbria; HC, hexachlorophene; IC, Internal Capsule; FA, fraction anisotropy; RD, radial diffusivity.

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France (Martin-Bouyer et al., 1982), which were partially attributed to misuse of HC.

The exact molecular mechanisms underlying HC toxicity are unknown. Scarce evidence suggests of its possible interference with liver and brain mitochondrial oxidative phosphorylation (Cammer and Moore, 1972), inhibition of myelin carbonic anhydrase (Cammer et al., 1976), and activation of lipid peroxidation in brain membranes (Rakhit and Hanig, 1984). On the cellular level the formation of spongiform lesions with vast vacuolation and splitting across the myelin sheath in white matter has been reported (Webster et al., 1974; Tripier et al., 1981). Behaviorally HC toxicity is manifested as motor dysfunction (hindlimb paralysis) and also problems with memory and cognition (Kimbrough, 1973).

Despite the fact that HC is clearly known to cause significant white matter vacuolization, some recent studies have investigated potential beneficial effects of this compound, especially in the field of oncology where it is uses to inhibit β-Catenin degradation (Park et al., 2006; Miller et al., 2012; Zheng et al., 2012). However, there are insufficient data on the life cycle of the neurotoxic effects associated with HC to provide for its prudent use, clinically. Non-invasive MRI offers the unique opportunity for longitudinal investigations of the brain toxicity caused by HC, including changes in white matter.

Diffusion Tensor Imaging (DTI) has been widely used to characterize white matter *in vivo* and to provide specific measures that can be related to, or correlated with, axonal damage (Assaf and Pasternak, 2008; Barrick et al., 2010; Amlien and Fjell, 2014). DTI utilizes the directional properties of water diffusion that is determined by the microstructure of the tissue under investigation. Brain white matter–as opposed to gray matter, is also characterized by higher anisotropy, which also depends on the compartmentalization (e.g. myelination) and direction of nerve fibers (Basser et al., 2000; Gulani et al., 2001; Beaulieu, 2002; Mori and Zhang, 2006; Alexander et al., 2007). DTI measurements provide specific scalar metrics including Fractional Anisotropy (FA), Trace, Axial Diffusivity (AD) and Radial Diffusivity (RD). FA provides information about the level of microstructural organization and Trace is highly sensitive to edema and demyelination. AD and RD correspond to the restricted diffusion of water parallel and perpendicular, respectively, to the orientation of the imaging plane, which, if oriented along or across the myelin sheath can provide specific information about changes in white matter (Gulani et al., 2001; Song et al., 2002; Schwartz et al., 2005). Information from the DTI scalars can be compiled to generate probabilistic paths of the fiber tracks based on the directionality of the main diffusion vector (Mori et al., 2002; Mori and van Zijl, 2002; Nucifora et al., 2007). A simpler approach – quantitative T_2 mapping has been used in the past to study the toxic effects of HC (Kinoshita et al., 2000; Hanig et al., 2014). In the current study both DTI and T_2 mapping have been employed to characterize the development of neurotoxic changes and subsequent recovery of white matter after HC exposure.

2. Materials and methods

2.1. Animal preparation

The animal use protocol was approved by the National Center for Toxicological Research Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (N = 15, 329 ± 20 g) were either given HC (N = 10, 30 mg/kg) or pure vehicle (corn oil, N = 5, 1 ml/kg) orally (via gavage), once a day, for 5 consecutive days. Animals were kept under a typical 12/12 h day/night light cycle, watered and fed ad lib a standard rat chow diet and were single-housed in polypropylene cages outfitted with ventilated-top isolators and natural wood chip bedding.

2.2. MRI

MR imaging was performed using a 7 T Biospec Avance III MRI system (Bruker MRI, Billerica, MA) with a 12 cm ID gradient insert (600 mT/m). A four channel split-array rat brain RF coil (Rapid MR International, Columbus, OH) was used for receiving and a 72 mm birdcage RF coil (Bruker MRI, Billerica, MA) was used for transmitting the MRI signals. Animals were anesthetized with isoflurane (3% induction, 1–2% maintenance at 1 L/min in oxygen). Body temperature was kept at 37.3 ± 0.6 °C using a water-heated imaging cradle. For T_2 relaxation mapping of the whole brain, a multi-slice, multi-echo spin echo sequence was used (image matrix 192 × 192 × 24, field of view = 3.84 × 3.84 × 2.4 cm, echo spacing = 15 ms, 16 echoes, repetition time = 6 s, number of averages = 1, total acquisition time ~ 20 min). High-resolution anatomical reference images were acquired using a fast spin echo sequence with the following parameters: rare factor of 8, TR/TE of 9000/17.5 ms, image matrix of 192 × 192 × 56, field of view = 3.84 × 3.84 × 2.8 cm. DTI images were acquired with geometry identical to the anatomical images using a multi-shot 3D spin echo EPI sequence (number of shots 4) with TR/TE of 1000/41.73 ms, b factor of 1000 s/mm^2, bandwidth of 250 kHz, 30 diffusion encoding directions, using the same geometry and matrix as the anatomical reference images.

2.3. T_2 analysis

T_2 maps were calculated using voxel-by-voxel single-exponential fitting of image intensities and analyzed using an arbitrary threshold (Hanig et al., 2014; Liachenko et al., 2015). Voxels with T_2 values equal to or less than 72 ms inclusive were designated as normal tissue and voxels with T_2 values above 72 ms were designated as lesion tissue (Liachenko et al., 2015). Prior to thresholding, skull stripping of all T_2 maps was performed to remove non-brain tissue from the images using semi-automated routines created in-house.

2.4. DTI analysis

Diffusion weighted images were initially pre-processed for intra-subject motion correction using SPM8 software (Wellcome Department of Imaging Neurosceince, UCL, London). Semi-automated skull-stripping and 12-parameter affine transformation registration to a chosen subject (inter-subject) was performed using Medical Image Processing, Analysis and Visualization (MIPAV) software (mipav.cit.hin.gov). The co-registered images were then imported into DTI Studio software (Jiang et al., 2006) to calculate the DTI parameters such as FA, Trace, AD and RD. RGB*FA (FA modulated by the principal eigenvectors) was also generated by multiplying the FA image by the principal Eigen vector images.

2.5. T-Maps

The registered FA, Trace, AD and RD maps were averaged based on time-points and a two-sample, two-tailed *t*-test ($P < 0.05$) was performed using SPM8 as described elsewhere (Ramu et al., 2008). T-tests were performed for unbiased determination of significant regions in the DTI scalars between the time-points. Maps of these significant differences were generated (t-maps).

2.6. ROI analysis

The Region of Interest (ROI) analyses of the DTI scalars were performed using ImageJ software (http://imagej.nih.gov/ij/). Based on published literature, four major white matter structures including the internal capsule (IC), fimbria (Fim), genu of the