Niemann-Pick Type C disease is a homozygous recessive disorder resulting in errant intracellular cholesterol metabolism and the accumulation of intracellular unesterified cholesterol and sphingolipids. Although no current effective treatment exists for Niemann-Pick Type C disease, a number of therapies are under development in animal models. As therapies are brought into clinical trials, it will be extremely helpful to have a reliable means to track the progression of the disease and to monitor its response to therapy. In this effort, diffusion tensor imaging has been applied to investigate the white matter in a Niemann-Pick Type C patient, and the results compared to those from age-matched control subjects. Diffusion tensor imaging enables quantitative measurement of water diffusion in white matter, which is sensitive to the architecture and integrity of the tissue. Compared with control subjects, significant reductions in fractional anisotropy values were observed in regions of white matter, most prominently in the corpus callosum. The results from this case study suggest that diffusion tensor imaging may allow progression of the disease to be quantitatively measured and may be able to play a role as a surrogate marker in clinical trials.

© 2005 by Elsevier Inc. All rights reserved.


Introduction

Niemann-Pick Type C (NPC) disease is a homozygous recessive disorder resulting in defective trafficking of intracellular lipids, including cholesterol and sphingolipids [1]. In addition to cholesterol, neutral glycolipids such as glucosylceramide and lactosylceramide, and the monosialogangliosides, GM2 and GM3, accumulate in the brains of NPC patients [2]. These lipid disturbances are predominantly localized to the gray matter, but are accompanied by considerable neuroaxonal dystrophy with formation of axon spheroids containing neurofilaments, organellar debris, and lipid storage, demyelination, neurodegeneration, and marked brain atrophy. In older patients neuropathologic changes also include neurofibrillary tangles resembling those of Alzheimer’s disease ultrastructurally and antigenically. Remarkably, neurofibrillary tangles develop over two to three decades earlier in NPC than in Alzheimer’s disease [3].

Although there is currently no effective treatment of NPC, encouraging responses to pharmacologic agents have been obtained in animal models of the disease. Nifedipine and probucol, effectively reduced liver cholesterol but did not alter the progression of central nervous system disease in npe1−/− mice [4]. A recent study found that oral or intrathecal delivery of cholesterol-mobilizing cycloexetrins decreased liver cholesterol storage in npe1−/− mice, but had only slight or no effect on onset of neurologic symptoms [5]. The combination of tamoxifen and vitamin E also had only slight effect [6]. An inhibitor of glycosphingolipid synthesis, N-butyldeoxynojirimycin (NB-DNJ), has been demonstrated to delay the onset of symptoms and significantly increase life span in NPC1 mice and cats [7]. More recently, early administration of the neurosteroid allopregnanolone was demonstrated to significantly increase the life span of npe1−/− mice and delay the onset of neurologic deficits [8]. As more successful therapies are demonstrated in animal models, there will be a desire to carry out clinical trials in children affected with NPC. This eventuality necessitates methods by which therapeutic responses can be monitored safely, reliably, and longitudinally in humans.

Magnetic resonance imaging is a desirable imaging tool with which to study human disease because it is noninva-
sive and can be carried out repeatedly without harmful effects. Diffusion tensor imaging is a relatively new magnetic resonance imaging methodology that allows quantitative investigation of the anisotropic motion of water in living tissue [9]. By measuring water diffusion in multiple directions, the tensor describing the three-dimensional movement of water can be determined [9]. From this tensor, quantitative anisotropy parameters can be calculated that are sensitive to the geometry and integrity of the tissue. Numerous diffusion tensor imaging studies have been carried out in human brain and have documented changes in anisotropy resulting from white matter disease and injury [10-12]. Because of the neurodegeneration and demyelination associated with NPC [2,13-15], there is the possibility that diffusion tensor imaging can be used to quantitatively determine the presence and progression of NPC and evaluate its response to therapy. This case report describes the first application of diffusion tensor imaging in an NPC patient and compares results with those obtained in age-matched control subjects.

**Methods**

One NPC patient and three control subjects underwent a magnetic resonance imaging examination including $T_1$-weighted gradient-echo imaging, $T_2$-weighted spin-echo imaging, and diffusion tensor imaging of the brain. The 15-year-old female patient was diagnosed with NPC at age 4 after identification of NPC in an older sibling. Because our patient had splenomegaly, a skin biopsy was obtained that revealed the delayed cholesterol esteriﬁcation and excessive cholesterol storage diagnostic of NPC. Lowering of blood cholesterol levels was achieved with a low cholesterol diet, cholestyramine, lovastatin, nicotinic acid, and primrose oil, but because symptoms progressed these medications were eventually discontinued. The patient continued a typical neurodegenerative course and at the time of this study was severely hypertonic, wheelchair bound, but able to communicate with head movement and hand signals. Informed consent was obtained from all subjects. One control subject was age-matched with the patient, and the other two control subjects were 10 and 13 years of age. Healthy subjects within this age range should exhibit similar magnetic resonance imaging and diffusion tensor imaging findings, and the two additional control subjects served to assess the consistency of the diffusion tensor imaging parameters.

All subjects were imaged using a General Electric VH3 3T magnetic resonance imaging scanner equipped with self-shielded 40 mT/m gradients. A quadrature head coil was used for excitation and reception. Subjects were imaged in a supine position and were outfitted with headphones and video goggles (Resonance Technologies Inc.) that allowed them to watch and listen to a DVD movie while being scanned. Sedation was not employed in this study, and being able to watch and listen to a movie was essential to keeping the subjects comfortable and injury. Informed consent was obtained from all subjects. One control subject was age-matched with the patient, and the other two control subjects were 10 and 13 years of age. Healthy subjects within this age range should exhibit similar magnetic resonance imaging and diffusion tensor imaging findings, and the two additional control subjects served to assess the consistency of the diffusion tensor imaging parameters.

Results

Sagittal $T_1$-weighted spoiled gradient-echo images of the NPC patient and an age-matched control subject are presented in Figure 1. Compared with the control subject, the NPC patient manifests enlargement of the ventricles; atrophy of the cerebrum and cerebellum, especially the vermis; and reduction in the size of the corpus callosum. Representative $T_2$-weighted images, apparent diffusion coefficient maps, fractional anisotropy maps, and directional encoded color maps of the NPC patient and an age-matched control subject are presented in Figure 2. There is an obvious increase in ventricular size and reduction of brain parenchyma in the NPC patient as evidenced by the increased volume of hypointense cerebrospinal fluid in the $T_2$-weighted image. Correspondingly, the apparent diffusion coefficient maps contain extensive regions with high apparent diffusion coefficient values reflective of unrestricted diffusion as is found in cerebrospinal fluid. The fractional anisotropy and directional encoded color maps of the NPC patient indicate a general decrease in the amount of brain tissue exhibiting high anisotropy. Most notably, the high anisotropy of the corpus callosum, a feature of all young healthy brains, is not present in the NPC patient. The directional encoded color maps indicate the preferred direction of water motion and are useful in determining the primary direction of white matter tracts in brain. The reduction in anisotropy in the corpus callosum of the NPC patient corresponds to a reduction of color (red) in the directional encoded color maps. The fibers in the posterior limb of the internal capsule (including corticobulbar and corticospinal tracts, denoted in blue), however, exhibit relatively higher anisotropy and directionality than the corpus callosum and are similar to corresponding regions in the control subject. All of the young healthy control subjects studied had similar results (not shown).

Histograms of apparent diffusion coefficient and fractional anisotropy values from the NPC patient and an age-matched control subject are shown in Figure 3. Figure 3A presents the histogram of apparent diffusion coefficient values within the cranial vault. Compared with the control subject, the NPC patient exhibits significantly more voxels with higher apparent diffusion coefficient values and less voxels exhibiting lower apparent diffusion coefficient. Correspondingly, the NPC patient exhibits significantly fewer voxels with high fractional anisotropy values and more voxels with lower anisotropy (Fig 3B). The histograms from two other young control subjects were virtually identical to the age-matched control subject included in the figures (not shown). A region of interest analysis of apparent diffusion coefficient and fractional anisotropy in the genu and splenium of the corpus callosum is included in Figures 3C and 3D, respectively. The regions of interest were manually prescribed on the fractional anisotropy maps to include regions of the genu and splenium of the corpus callosum as seen in multiple axial.
Care was taken not to include any voxels containing cerebrospinal fluid such that the histogram values of apparent diffusion coefficient and fractional anisotropy represent the lowest and highest values, respectively, in the corpus callosum. The regions of interest initially determined on the fractional anisotropy maps were applied to the apparent diffusion coefficient maps to obtain the histogram values of identical regions. As can be readily appreciated from the histograms, the values of apparent diffusion coefficient are significantly increased in the NPC patient compared with the age-matched control subject, and the variation in the values is considerably larger. The mean values of apparent diffusion coefficient in genu and splenium of the corpus callosum in the age-matched healthy control subject were $0.772 \pm 0.168$ and $0.719 \pm 0.178 \, \mu m^2/\mu s$, respectively. The mean values in the NPC patient were $1.41 \pm 0.41$ and $1.44 \pm 0.43 \, \mu m^2/\mu s$, respectively. Mean values in the other control subjects (combined) were $0.726 \pm 0.111$ and $0.683 \pm 0.146 \, \mu m^2/\mu s$ for the genu and the splenium of the corpus callosum, respectively. These values are within statistical error of the age-matched control and in agreement with values reported in literature. There are also significant differences in fractional anisotropy values with the corpus callosum between the age-matched control subject and the NPC patient (Fig 3D). The fractional anisotropy in the age-matched control subject has a mean value of $0.713 \pm 0.099$ and $0.762 \pm 0.111$ for the genu and splenium of the corpus callosum, respectively, whereas the NPC patient has values of $0.31 \pm 0.84$ and $0.361 \pm 0.096$. The fractional anisotropy values from the other control subjects were $0.749 \pm 0.101$ and $0.743 \pm 0.105$, statistically similar to the age-matched control and consistent with values reported in the literature [17].

Histograms of apparent diffusion coefficient and fractional anisotropy measured in the posterior limb of the internal capsule of the NPC patient and age-matched control subject are presented in Figures 3E and 3F, respectively. In this case, the NPC patient exhibits larger values of fractional anisotropy than in the corpus callosum, and the values are much more similar to the age-matched control subject. The apparent diffusion coeffi-
cient and fractional anisotropy values in the internal capsule of the NPC patient were $0.750 \pm 0.170$ $\mu$m$^2$/ms and $0.573 \pm 0.102$, respectively. For the age-matched control subject, the apparent diffusion coefficient and fractional anisotropy values in the internal capsule were $0.588 \pm 0.145$ $\mu$m$^2$/ms and $0.643 \pm 0.097$, respectively. Values of apparent diffusion coefficient and fractional anisotropy and in the other control subjects were $0.602 \pm 0.51$ $\mu$m$^2$/ms and $0.632 \pm 0.150$, respectively.

**Discussion**

The results of a diffusion tensor imaging study in an NPC patient have been reported for the first time. When looking at the entire cranial volume, there was a general increase in average diffusivity (apparent diffusion coefficient) and a decrease in diffusion anisotropy (fractional anisotropy) in the NPC patient compared with control subjects. This finding is not surprising in light of the significant atrophy observed in the T1-weighted and T2-weighted images, because cerebrospinal fluid has high diffusion and low anisotropy. Region of interest analysis of the corpus callosum demonstrated highly significant increases in apparent diffusion coefficient and reductions in fractional anisotropy compared with healthy control subjects. These results correlate well with the known degeneration of white matter associated with NPC [3]. Significant atrophy of the corpus callosum was also observed in the NPC patient, which is consistent with previously reported findings [18]. However, care was taken to only evaluate apparent diffusion coefficient and fractional anisotropy in voxels that were fully volumed within the corpus callosum and contained little or no regions of gray matter or cerebrospinal fluid. In this way, we think that the apparent diffusion coefficient and fractional anisotropy represent changes within the corpus callosum and not simply atrophy. Because these changes should be independent of the starting size of the corpus callosum, they should be more accurate indicators of disease-related changes in the tissue. Interestingly, the fibers of the posterior limb of the internal capsule appear to maintain high values of anisotropy. Although there was atrophy of the internal capsule as observed in the T2-weighted images, the level of anisotropy within the existing tissue was nearly normal. Because the apparent diffusion coefficient and fractional anisotropy within the corpus callosum can be reliably and quantitatively measured over time, they could be a reliable surrogate marker to assess progression of the disease and response to therapy. The availability of diffusion tensor imaging acquisition and analysis software on most modern commercial magnetic resonance imaging systems allows rapid and reliable determination of quantitative diffusion properties and anisotropy parameters in the clinical setting. Quantitative apparent diffusion coefficient and fractional anisotropy maps can be quickly computed at the end of the diffusion tensor imaging examination and be available to radiologists along with conventional magnetic resonance imaging. Other magnetic resonance imaging methodology, such as T2-weighted imaging, fluid-attenuated inversion-recovery imaging, and magnetization...
transfer imaging, may also be sensitive to demyelination, inflammation, or neurodegeneration, and may exhibit qualitative differences in NPC patients compared with healthy control subjects. However, these methodologies are not typically quantitative, in the sense that they do not provide measurable parameters that can be quantitatively compared in patients over time and between patient groups. Diffusion tensor imaging is highly sensitive to microstructural changes in white matter, and has demonstrated abnormalities in white matter that appears both normal and abnormal on T2-weighted images [19]. Magnetization transfer ratio imaging and T2-mapping can be carried out in a quantitative manner, but these techniques have not been carried out extensively in human brain imaging and sensitivity to the changes associated with Niemann-Pick type C disease has yet to be established.

In this study, we have demonstrated that significant differences in brain water diffusion can be measured in a patient profoundly affected with Niemann-Pick Type C disease when compared with age-matched healthy control subjects. It remains to be established whether or not the differences in apparent diffusion coefficient and fractional anisotropy observed in this patient with advanced NPC disease have increased over time or were present early in the disease. If differences in apparent diffusion coefficient and fractional anisotropy increase with the severity of the disease, then these parameters might be useful for noninvasively monitoring disease progression and response to therapy. However, if differences in apparent diffusion coefficient and fractional anisotropy already exist early in the disease, they may be diagnostically useful, but not suitable as a measure of progression or response. Future longitudinal studies in multiple NPC patients are required to assess these issues.

Figure 3. Histograms of the apparent diffusion coefficient (ADC) (A, C, E) and fractional anisotropy (B, D, F) from an NPC patient and age-matched control subject from various regions of the brain. The solid and dashed lines in C and D correspond to histograms from the genu and the splenium of the corpus callosum, respectively.
The authors would like to thank Mr. Scott Squire for his assistance in carrying out the magnetic resonance imaging examination and image analysis. This study was supported by NIH grant R01 EB000343 and the Ara Parseghian Medical Research Foundation.

References


