Structural MRI as a Tool for the Study of Neurotoxicity and Neurodegenerative Disorders

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Abstract

High-resolution magnetic resonance (MR) imaging affords an unprecedented opportunity to study the severity and distribution of neurodegenerative changes in the human brain. By selecting specific MR sequence parameters (i.e., TE and TR), different MR signals can be received from different tissue types, such as gray and white matter. Through optimization of the contrast between different tissue types, the surfaces and internal structures of brain structures of special interest can be visualized and quantitated. Metrics such as two-dimensional areas, three-dimensional volumes, and three-dimensional shape characteristics have proven to be highly useful for quantitating the effects of toxins on the human brain. Among toxins, the effects of alcohol on the human brain have been most intensively studied using structural MR imaging. Volume losses in the cerebral cortex and other brain regions of interest have been carefully quantitated. However, because exposure to alcohol is almost always repeated over many years, the effects of normal aging must be carefully considered when making comparisons between diseased and healthy populations. In contrast to the literature on alcohol, structural MR imaging has been relatively underutilized in the study of drugs and other chemicals such as MPTP and other drugs of abuse that are toxic to special populations of neurons. However, as the resolution of structural MR continues to improve, the structural characteristics of such neuron populations will be visualized and quantitated, and successful use of structural MR imaging for the study of such toxins will become possible.

Introduction

High-resolution magnetic resonance (MR) imaging of the brain is widely used to identify and quantify changes in brain structure associated with neurovascular, neurodegenerative, and neurodevelopmental diseases. Careful selection of MR sequence parameters and semiautomated methods for image analysis are available for the precise quantitation of areas, volumes, and surfaces in the central nervous system (CNS) that can then be used to identify the subtle effects of neurological disease. Within such regions of interest (ROI), changes in tissue characteristics may also be detectable during the progression of disease. However, this technology has not yet been widely applied to the study of brain damage secondary to neurotoxins.

Neurotoxins may be divided into two broad categories: (1) those that affect all neurons and their processes via their physicochemical or metabolic actions and (2) those that affect particular neuronal populations via their pharmacodynamic properties. Through the selection of specific imaging methodologies, damage to the CNS by either type of neurotoxin should be detectable. Damage to CNS structures after exposure to a generalized neurotoxin might be expected to cause similarly generalized loss of structural volume detected by precise volumetric quantitation following segmentation of the appropriate tissues. Damage to CNS after exposure to a more specific neurotoxin might be expected to show volumes losses only in very small ROI or a change in structural shape caused by the loss of specific populations of neurons.

In this paper, the general principles of MR imaging will be briefly reviewed as they pertain to the quantitation of appropriate structural variables. Then, the literature on the effects of two neurotoxins, one generalized and one specific, on brain structure as demonstrated by MR imaging will be reviewed. Alcohol will be taken as an example of a generalized neurotoxin, and the neurotoxic analogue of morphine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) will be taken as an example of a specific neurotoxin. Finally, the potential of MR imaging for the study of other neurotoxins will be considered with an emphasis on the further development of new methodologies.

Principles of Structural MR Imaging

There are several excellent texts available for the reader who wishes to acquire a more detailed understanding of the principles and methods of MR imaging. The two used in the preparation of this section (1,2) are good examples of such texts. Regarding this paper, this section is included only so that readers who are unfamiliar with MR imaging can understand how the existing studies on the effects of neurotoxins on brain structure were performed and reasonably anticipate other studies that
might be performed in the future. MR imaging can be divided into three main components: (1) the effects of strong magnetic fields on the human body, (2) the perturbation of such fields by a brief pulse of radiofrequency energy, and (3) the way relaxation from this perturbation creates a magnetic echo characteristic of the specific tissues being imaged (Figure 1).

Every atomic nucleus with an uneven number of electrical charges (i.e., protons) can be reoriented into alignment with a strong magnetic field, much as the needle of a compass is re-oriented into alignment with the earth's magnetic field. The magnetic fields produced by the large superconducting electromagnets used in MR imaging reorient all possible atomic nuclei within the body, but because of the overwhelmingly large concentration of water molecules and their hydrogen nuclei, it is these nuclei that are usually assessed by MR imaging. In the presence of the scanner's magnetic field, these nuclei come into alignment to produce a magnetic dipole moment (MDM) and the resultant vector, M. However, the MDM must also wobble or precess around its base, much as a top wobbles around its tip, and with a frequency that is unique to the nuclei being assessed, in this case, the hydrogen nuclei of water molecules. This particular frequency is an important detail because it is the uniqueness of this precession frequency that allows one to tune the scanner for particular atomic species.

Next, a brief pulse of radiofrequency (RF) energy is applied within this magnetic field at a frequency that matches the precession frequency of the atomic nuclei of interest (e.g., in the case of hydrogen nuclei, the desired frequency is about 64 Hz) and at an angle essentially perpendicular to the pre-existing M vector. The RF pulse thus perturbs or reorients the pre-existing M vector into a new orientation that is out of alignment with the prevailing magnetic field of the scanner. However, this perturbation, also called a "flip," is brief and immediately followed by a period of relaxation.

It is during the period of relaxation following the RF pulse that the information needed to produce an MR image is collected. The M vector returns to its original orientation during relaxation in a manner that can be broken into two components, T1 and T2. Roughly speaking, T1 represents the rate of the original M vector becoming reoriented so that it is again aligned with the scanner's magnetic field, and T2 represents the rate of decay of the M vector's alignment with the perturbing pulse. Because different physical properties of the tissue being scanned can affect one component but not the other, T1 and T2 proceed at different rates during relaxation (i.e., they are not the simple inverse of each other).

The T1 and T2 components of relaxation following the RF pulse are specific for each tissue with the human body, and it is these differences that are exploited to differentiate one tissue from another within an MR image. However, these differences must be revealed through the careful selection of parameters within the MR sequence. MR sequences are made up of repetitive series of RF pulses and assessments (i.e., echoes), and the strength of the MR signal is increased by making many assessments within a single scan. The time from the RF pulse to the echo time is called TE. The time between repetitive RF pulses is called TR, and different tissues (e.g., gray matter, white matter, and cerebrospinal fluid [CSF]) will have different T1s and T2s for a given set of sequence parameters (i.e., TE and TR). Thus, by selecting TE and TR, one can maximize the differences between the resultant signal intensities, called contrast. Finally, a T1-weighted scan is one in which tissue contrasts are maximized by selecting TE and TR to exploit the resultant tissue differences in T1, and a T2-weighted scan is one in which tissue contrasts are maximized by selecting TE and TR to exploit the resultant tissue differences in T2.

When scanning the brain, one is almost always preoccupied with optimizing the contrast between gray and white matter because we use the boundaries between these tissues to recognize the brain characteristic structures. T1-weighted and T2-weighted scans will both show contrast between gray and
white matter, although in different ways (i.e., in a T1-weighted scan, gray matter has an intermediate signal intensity and white matter has a greater signal intensity and so gray matter appears "gray" and white matter "white"), whereas in a T2-weighted scan, the signal of gray matter is more intense than that of white matter and so gray matter appears more "white" than white matter). However, subtle differences between subregions within a gray matter structure, such as the hippocampus, or between the gray matter/white matter boundary in the cerebral cortex may be better visualized by choosing one sequence over the other. In addition, the presence of neurological disease, such as multiple sclerosis, can produce patches of increased or decreased signal intensity within a tissue or brain region that is normally homogenous.

Changes in Brain Structure Caused by the Neurotoxic Effects of Alcohol

Ethyl alcohol, or ethanol, is without doubt the most widely consumed neurotoxin. Its effects are both generalized and various, and exemplify the issues involved in studying the effects of a generalized neurotoxin on brain structure using MR imaging (3). Ethanol has direct toxic effects on neurons (4,5), possibly because of its non-oxidative metabolism to fatty acid ethyl esters within these cells (6). However, the consumption of ethanol is also indirectly associated with a variety of other neurotoxic syndromes. Vitamin deficiencies (e.g., thiamine) are common in alcoholics and have been shown to have deleterious effects on specific structures of the mammalian brain, such as the mammillary bodies and thalamus (7). Many chronic alcoholics eventually develop hepatocellular degeneration, which has also been associated with the accumulation of metabolic toxins and encephalopathy, and traumatic brain injuries tend to be more common among alcoholics (6). Finally, the teratogenic effects of alcohol, including those associated with the fetal alcohol syndrome, have been well established (8).

Studies of changes in brain structure caused by ethanol have been of two types. Those studies focused on the direct toxic effects of ethanol have been largely quantitative, utilizing calculated measurements from 3D T1-weighted images. Those studies focussed on the indirect toxic effects of ethanol have been largely qualitative, utilizing a clear image of the specific brain ROI and neuronal populations involved. For example, Pfefferbaum et al. (9) studied cortical gray matter volumes extracted from high-resolution, T1-weighted MR scans in younger and older, more chronic, alcoholics. These investigators found that cortical gray matter volumes, but not white matter volumes, were significantly decreased in younger alcoholics compared to age-matched controls. However, both gray and white matter volumes were decreased in the older alcoholics compared to their age-matched controls, suggesting that as exposure to this neurotoxin increases the long processes of neurons that make up white matter tracts are lost. Moreover, in this same study, the investigators were able to subdivide the cerebral cortex into subregions and show that with more chronic exposure to alcohol (i.e., in the older alcoholics) greater gray and white matter loss was disproportionately found in the frontal lobes.

Brain areas other than the cerebral cortex, especially those related to memory, have also been shown to suffer volume losses following chronic exposure to ethanol. For example, Sullivan et al. (10) demonstrated that the volumes of the left and right hippocampus, especially the anterior portion of this structure, were decreased in older, chronic alcoholics. Finally, these studies illustrate the importance of controlling for the normative effects of aging when trying to elucidate the effects of a toxin which is administered over a long period of time. Pfefferbaum et al. (11) went on to show that while gray matter volumes decreased and brain CSF volumes increased in healthy controls assessed twice over five years, the rate of change in these same measures was exaggerated in alcoholics, especially those who could not maintain abstinence from drinking.

As pointed out above, studies of CNS structural changes in alcoholics who also show the clinical features of Wernicke's syndrome secondary to thiamine deficiency have focussed on finding small but distinct distortions of normal anatomical patterns in high-resolution, T1-weighted and T2-weighted scans (6). In fact, elucidation of selective volume losses and distortions of the mammillary bodies, as well as the cerebellar vermis and increases in the volume of the third ventricle, have been proposed as a means to distinguish the direct effects of ethanol on the cerebral cortex from the more targeted effects of alcoholism associated with thiamine deficiency (6,12). In one study, the collection of T2-weighted images was especially helpful in showing lesions of the thalamus associated with alcoholism and thiamine deficiency (13).

Recently, other techniques of MR scanning have begun to be used to detect the effects of alcohol on the human brain. The reader should recall that conventional MR scanning is based on detecting the magnetic alignment and relaxation of hydrogen nuclei in water molecules within the tissues of the human body. Further, the specificity of the MR sequence for these specific nuclei is achieved by tuning the frequency of the RF pulse to match the precessional frequency of these particular hydrogen nuclei. Thus, hydrogen nuclei in other molecules contained within the body can also be imaged by changing the frequency of the RF pulse to match the precessional frequency of these chemical species.

The technique of using a variety or spectrum of RF pulse frequencies to detect the concentrations of various molecular species within a tissue is called magnetic resonance spectroscopy (MRS). At present, the chemical species detectable by MRS are limited to those that have substantial concentrations within the brain. These species include the precursors to fatty acid components of neuronal membranes (i.e., choline) and common elements of cellular metabolism (e.g., creatine). In addition, some species that are relatively specific to neurons, such as N-acetylaspartate (NAA), are detectable. Thus, many investigators have used the relative concentrations of NAA to choline or creatine as an index of neuronal integrity (2).

Using the technique of MRS, Seitz et al. (14) have recently shown the relative loss of NAA to choline and creatine in the cerebellar vermis of alcoholics. The fact that decreases in NAA concentrations were found in the cerebellum, where conven-
tional MR scanning had previously detected volume losses, suggests that such volume losses are the result of neuronal destruction. Thus, combining MRS with conventional MR imaging allows one to develop a deeper understanding of the cellular basis of changes in brain structure.

In summary, these studies show that the effects of a generalized neurotoxin, such as ethanol, on brain structure can be quantitated. Moreover, these studies show that multiple approaches to scanning and the use of the collected scans (qualitative and quantitative) can be helpful in elucidating the complex and varied toxicities of such a toxin. Generalized losses of cerebral cortical volume were best appreciated using fully quantitative volumetrics, whereas more specific damage to small structures, such as the mammillary bodies, or subregions of larger structures, such as the cerebellum and thalamus, were best appreciated by utilizing a variety of MR sequences and qualitative techniques of measurement. Finally, the cellular basis of gross structural changes was preliminarily investigated with MRS.

Changes in Brain Structure Caused by the Neurotoxic Effects of the Specific Neurotoxin MPTP

MPTP was accidently synthesized in the early 1980s by street drug manufacturers seeking to make a synthetic form of heroin (15). The victims of exposure to this drug developed a sudden and severe form of parkinsonism due to the almost total loss of dopamine-containing neurons in the substantia nigra. The mechanism of MPTP neurotoxicity and its peculiar specificity for dopaminergic neurons was eventually discovered to involve formation of a metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP+), in the dopaminergic nerve terminals by monoamine oxidase (type B) (16). Because monoamine oxidase (type B) is relatively specific for dopamine neurons within the basal ganglia, the specificity of MPTP's toxicity for these dopaminergic neurons was explained (17). However, for reasons that are still largely unexplained, the toxicity of MPTP for dopaminergic neurons is limited to those of the nigrostriatal dopaminergic system and to neurons of the primate brain but not other mammals (17,18).

Because of the close similarity of the clinical syndrome produced by MPTP and idiopathic Parkinson's disease in humans, many investigators began to administer MPTP to non-human primates in attempts to develop an experimental model of Parkinson's disease where hypotheses of disease pathogenesis and therapeutic sensitivity could be tested (19).

Unilateral intracarotid administration of MPTP to monkeys was soon shown to produce a total, but unilateral, loss of dopaminergic neurons and their processes which extend to the basal ganglia (20). The time course of cell death and structural damage ranged from days to weeks. Although MR imaging could not be expected to detect the loss of the dopaminergic neurons in the midbrain, it was hoped that changes in those brain structures that received dopaminergic projections could be detected. Specifically, it was hypothesized that edema, due to an inflammatory response to the degeneration of dopaminergic projections, would alter the water content of subregions of the basal ganglia, and thus the tissue characteristics of these structures when exposed to the magnetic field of the MR scanner.

Miletich et al. (21) showed that the effects of MPTP on the basal ganglia of the primate brain could be demonstrated by MR imaging as a change in signal intensity (i.e., the basal ganglia appeared brighter) on a T2-weighted image. This change in signal intensity appeared six days after MPTP administration and disappeared after two weeks. Similarly, increases in signal intensity were observed in the vicinity of the substantia nigra. Furthermore, these changes were observed to be limited to the nigrostriatal components of the basal ganglia, such as caudate, putamen, and substantia nigra, and to spare the nucleus accumbens and ventral tegmental area, which are the major components of the mesolimbic system. However, using both T1-weighted and T2-weighted MR sequences, Zhang et al. (1999) have more recently shown that structural deformation of the globus pallidum are detectable one year after MPTP administration to monkeys. After sacrifice, areas of structural deformation detected by MR scanning were found to represent areas of gross tissue degeneration and expansion of the CSF spaces.

Taken together, these studies exemplify the potential of MR scanning to detect brain damage due to specific neurotoxins. Although acute changes were observed in those brain areas known to contain the specific populations of neurons destroyed by the toxin, changes were also observed in areas known to contain the projections of these neurons. These changes included the acute appearance of edema, detected as a change in signal intensity on T2-weighted MR images, and chronic changes in the volume of specific brain structures, detected on both T1-weighted and T2-weighted images.

The Potential of MR Scanning to Detect the Effects of Other Neurotoxins

Structural MR scanning has the potential to detect the effects of other neurotoxic substances. These substances include drugs of abuse other than MPTP, food contaminants especially those compounds that have excitotoxic properties, and heavy metals. With respect to other drugs of abuse, 3,4-methylenedioxymethamphetamine (MDMA), an analogue of methamphetamine, has been shown to have neurotoxic effect on serotonergic neurons in the brain (23). The effects of this drug on human subjects with a history of abusing it have been investigated using positron emission tomography and a ligand that specifically labels the serotonin transporter (24) and using single photon emission tomography and a ligand to assess blood flow in the cerebral cortex (25). Given that serotonergic neurons of the nucleus raphe project their axons throughout much of the cerebral cortex, functional changes across this large brain region would not be unexpected. Moreover, structural changes might be observed acutely after administration of the drug, as has been the case after exposure to MPTP and chronically, especially in those brain areas that rich in serotonin neurons or their projections.
Excitotoxins are another category of neurotoxin that are likely to produce changes in brain structure detectable with structural MR imaging. Analogues of the excitatory amino acid neurotransmitters glutamate and aspartate, such as domoic acid and kainic acid, occasionally appear as contaminants in food and are ingested by human beings. Because of the almost universal distribution of the excitatory amino acid neurotransmitters and their receptors throughout the brain, administration of these compounds is well known to be neurotoxic, especially in the cerebral cortex, the hippocampus, and the basal ganglia (26). Also, given the density of such neurons and their receptors in these brain regions, relatively large changes in brain structure volumes and shapes are likely. Use of structural MR imaging to study the distribution and progression of neurotoxicity in humans exposed to such toxins and in animals experimentally administered such toxins to model human disease could be highly useful in establishing relationships between changes in brain structure and patterns of functional impairment.

Finally, structural MR imaging could contribute to our understanding of brain damage following exposure to heavy metals such as lead. Lead has long been known to be neurotoxic to children who are accidentally exposed, with the most substantial evidence of damage found in the prefrontal cortex, hippocampus, and cerebellum (27). Furthermore, some of this toxicity may be mediated by the effects of lead on the expression of excitatory amino acid receptors, especially in the hippocampus where such receptors are densely expressed and known to play a role in the development of one’s capacity for learning and memory (28). Again, structural MR imaging could be invaluable in mapping the distribution and severity of structural brain changes in young children exposed to this toxin. In particular, repeated assessments over an extended period of time could be used to establish the progression of the lesion over time as well as the effects of attempts at treatment and rehabilitation.

References