Decreased Dopamine Transporter Binding in Machado-Joseph Disease

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The aim of this study was to use ^{99m}Tc-TRODAT-1 brain SPECT for investigation of the binding of dopamine transporter (DAT) in the nigrostriatal dopaminergic pathway of symptomatic Machado-Joseph disease (MJD) and to compare the results with the abnormal cytidylate, adenylate, and guanylate (CAG) expansion in the MJD1 gene and other clinical factors. **Methods:** Ten symptomatic MJD patients (8 women, 2 men; age range, 20–71 y; mean age ± SD, 36.4 ± 10.6 y; mean duration of illness, 9.8 ± 5.4 y) and 21 healthy volunteers (age range, 24–71 y; mean age, 47.6 ± 20.1 y) were examined. Brain SPECT images were acquired 4 h after injection. The ratio of specific to nonspecific nigrostriatal ^{99m}Tc-TRODAT-1 binding was measured and compared with the clinical symptoms, duration of illness, and size of abnormal expanded CAG repeats. **Results:** All nigrostriatal ^{99m}Tc-TRODAT-1 ratios were significantly lower in MJD patients than in healthy volunteers ($P < 0.05$). Discriminant function analysis of all MJD patients showed that the decreased binding of ^{99m}Tc-TRODAT-1 in the putamen was not significantly different from that in the caudate nucleus. Eight of 10 MJD patients had significantly decreased ^{99m}Tc-TRODAT-1 uptake. Of these 8, 2 had extrapyramidal signs and 6 had no obvious extrapyramidal signs. The other 2 patients, who had normal ^{99m}Tc-TRODAT-1 uptake, had no obvious extrapyramidal signs. **Conclusion:** Our findings indicate that ^{99m}Tc-TRODAT-1 brain SPECT is an appropriate method for evaluating damage to the nigrostriatal DAT in symptomatic MJD patients with and without extrapyramidal signs. The decreased binding of ^{99m}Tc-TRODAT-1 in the nigrostriatal dopaminergic pathway in symptomatic MJD patients correlates with the phenotype of extrapyramidal signs but not with the abnormal CAG repeat length, age at disease onset, or disease duration.

Key Words: TRODAT-1; SPECT; Machado-Joseph disease

J Nucl Med 2000; 41:994–998

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disease characterized by cerebellar ataxia associated in varying degrees with pyramidal signs, extrapyramidal signs, or peripheral amyotrophy (*1,2*). The molecular abnormality has recently been described as abnormal expansion of the cytidylate, adenylate, and guanylate (CAG) repeats in the MJD1 gene on chromosome 14q32.1 (*3*). The number of CAG trinucleotide repeats may be correlated with some aspects of clinical pathology (*2*). This repeat is located in an exon, as with Huntington's disease and similar neurodegenerative disorders (*4*). CAG codes for glutamine and may cause mitochondrial excitotoxicity (*5,6*).

Although the molecular mechanism of MJD has been described, few studies have investigated the use of MRI, ^{123}I-iomazenil brain SPECT, or PET to study the nigrostriatal dopaminergic pathway in MJD patients (*7–11*), and the findings of most of those studies were nonspecific. The removal of free dopamine from the synaptic cleft is one of the primary mechanisms for regulating dopaminergic tone. The reuptake of free dopamine from the synaptic cleft is mediated by dopamine transporter (DAT) embedded in the presynaptic neuron membrane (*12*). Drugs such as cocaine can bind the DAT and block the reuptake of dopamine from the synaptic cleft to the presynaptic neuron (*13*). TRODAT-1 is a cocaine analog that can easily be labeled with ^{99m}Tc and bind to the DAT site on the presynaptic neuron membrane (*14–25*). In this retrospective study, we wished to test the power of ^{99m}Tc-TRODAT-1 in evaluating damage of the nigrostriatal dopaminergic DAT site in symptomatic MJD patients with various degrees of extrapyramidal signs. In particular, we wished to determine whether transporter levels allow differentiation between healthy volunteers and patients, reflect the severity of certain symptoms, and are related to CAG repeats.

MATERIALS AND METHODS

Patients

Ten patients (from 9 families) with symptomatic MJD (8 women, 2 men; age range, 20–71 y; mean age± SD, 36.4 ± 10.6 y; mean duration of illness, 9.8 ± 5.4 y), who were referred to us by our neurologic department, were included in this study. A definite diagnosis of MJD was made in these 10 patients using both molecular evidence (proven abnormal expansion of the CAG repeats [ranging from 74 to 85 repeats] in the MJD1 gene on chromosome 14q32.1) and the family pedigree (*26*). The 10 patients were clinically evaluated to estimate the degree of cerebellar ataxia, pyramidal signs, extrapyramidal signs, or peripheral amyotrophy in each. ^{99m}Tc-TRODAT-1 brain SPECT and MRI

Received May 17, 1999; revision accepted Sep. 14, 1999.

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were performed with the informed consent of each patient, for both quantitative analyses (imaging registration) and qualitative comparison. Twenty-four healthy volunteers (age range, 24–71 y; mean age, 47.6 ± 20.1 y) were included as a control group.

^{99m}Tc-TRODAT-1 Brain SPECT

The ^{99m}Tc-TRODAT-1 was prepared from a research kit from the Institute of Nuclear Energy Research. The intravenous injection dose was 925 MBq (25 mCi). Imaging was performed 4 h later. SPECT images were obtained using a Multispect 3 γ camera (Siemens Medical Systems, Inc., Hoffman Estates, IL) with a fanbeam collimator, 128 projections over 360°, 60 s per stop, and a 128 × 128 matrix. Individual images were reconstructed with backprojection using a Butterworth filter, with a cutoff of 0.3/cm and an order of 10. The data were corrected for the effects of photon attenuation using Chang's first-order method, with the attenuation ellipses defined on the summed images of the entire dataset and applied, without modification, to all the images individually. The summed image was reoriented to give transverse slices parallel to the orbitomeatal line, and then the same transformation parameters were applied to every other image in turn. Pixel size was 2.9 × 2.9 mm, and slice thickness was 2.9 mm. All images were reviewed by 3 nuclear physicians who were unaware of clinical data, and all decisions were confirmed by at least 2 of the 3 reviewers.

MRI

Patients with MJD, patients with Parkinson's disease, and healthy volunteers were examined using 0.5-T MRI. T1-weighted axial images (repetition time, 500 ms; echo time, 25 ms) and T2-weighted axial images (repetition time, 3500 ms; echo time, 120 ms) were obtained in the transaxial plane (6-mm slice thickness and 0.6-mm gap). Measurements were performed separately by 3 neuroradiologists who did not know the clinical or genetic status of the subjects.

Data Processing

For analysis of the nigrostriatal dopaminergic pathway of ^{99m}Tc-TRODAT-1 binding, the ratio of specific to nonspecific binding was calculated by summing 2 transverse slices representing the most intense nigrostriatal DAT binding. Analyses were performed by investigators unaware of the clinical data. A standard region-of-interest template (using a stereotactic shape obtained from an MRI atlas and including regions of the putamen, caudate nucleus, and occipital cortex) and additional regions of interest for the entire striatum were placed bilaterally on the acquired images. Estimates of specific striatal binding were made by subtracting occipital counts from nigrostriatal counts. The ratio of specific to nonspecific striatal ^{99m}Tc-TRODAT-1 binding was then calculated by dividing the specific nigrostriatal uptake by occipital binding.

Statistics

All data were analyzed using the computer software package JMP 3.0 (SAS Institute Inc., Cary, NC) on a Macintosh personal computer (Apple Computer, Cupertino, CA). Differences between the groups were examined by ANOVA. Multiple regression analysis was used to examine the relationship between the ^{99m}Tc-TRODAT-1 binding measurements, clinical severity, abnormal expansion of the CAG repeats, age at disease onset, and disease duration. $P < 0.05$ was accepted as significant, because this level of significance was commonly chosen for relevant studies reported in the literature and multiple comparisons were not evaluated.

RESULTS

Table 1 shows the clinical data of the patients. Their age at onset of MJD ranged from 11 to 40 y (mean age, 27.6 ± 7.7 y). The duration of disease ranged from 2 to 31 y (mean duration, 9.8 ± 5.4 y). Chromosomal molecular analysis showed abnormal expansion of CAG repeats (from 74 to 85 repeats; mean, 79.8 ± 2.8) in chromosome 14q32.1. All patients had cerebellar signs. Seven had pyramidal signs, and 2 had extrapyramidal signs. None had amyopathy. Table 2 shows the striatal specific-to-nonspecific binding ratios calculated from ^{99m}Tc-TRODAT-1 brain SPECT results of the patients. Stratum analysis by age and in comparison with healthy volunteers indicated that 2 had almost normal ^{99m}Tc-TRODAT-1 uptake in both the putamen and the caudate nucleus. The other 8 had significantly decreased ^{99m}Tc-TRODAT-1 uptake in both the putamen and the caudate nucleus (Fig. 1). The 2 MJD patients who had extrapyramidal signs had more obviously decreased ^{99m}Tc-TRODAT-1 uptake. Grading of the decreased ^{99m}Tc-TRODAT-1 uptake in the nigrostriatal pathway did not show a correlation with age at disease onset, disease duration, or abnormal expansion of CAG repeats. Nine MJD patients had normal MRI results. Only 1 had cerebellar atrophy, shown with MRI, and a mildly decreased ^{99m}Tc-TRODAT-1 uptake. In healthy volunteers, ^{99m}Tc-TRODAT-1 binding was found to decrease 6% per decade with aging. Table 3 shows the mean results of ^{99m}Tc-TRODAT-1 brain SPECT measurements in the patients and healthy volunteers. All nigrostriatal ^{99m}Tc-TRODAT-1 ratios were significantly lower in the patients than in the healthy volunteers ($P < 0.05$). For the ipsilateral putamen, the mean value in patients was 31.1% of that in healthy volunteers; for the contralateral putamen, 30.7%; for the ipsilateral caudate nucleus, 28.9%; and for the contralateral caudate nucleus, 29.9%. Discriminant function analysis in all patients showed that decreased binding of

TABLE 1
Clinical Data for 10 Symptomatic MJD Patients

Parameter	Patient no. 1	2	3	4	5	6	7	8	9	10
Sex	F	F	F	M	F	M	F	F	F	F
Age (y) of onset of MJD	40	31	18	27	22	36	11	22	29	40
Duration (y) of MJD	15	2	8	3	10	10	9	4	6	31
CAG repeat number	84	81	81	78	85	79	76	74	78	82
Symptoms and signs										
Cerebellar ataxia	+	+	+	+	+	+	+	+	+	+
Pyramidal signs	+	+	+	−	+	−	+	+	+	−
Extrapyramidal signs	−	−	−	−	+	−	−	−	+	−
Amyopathy	−	−	−	−	−	−	−	−	−	−

TABLE 2
Binding Ratio of ^{99m}Tc-TRODAT-1 in Striatum of 10 Symptomatic MJD Patients

Site	Patient no.									
	1	2	3	4	5	6	7	8	9	10
Putamen										
Contralateral	1.97	1.66	1.91	1.66	1.64	1.80	2.07	1.79	1.40	1.80
Ipsilateral	1.80	1.62	1.85	1.66	1.49	1.68	1.86	1.57	1.43	1.67
Caudate										
Contralateral	2.09	2.29	2.29	2.25	1.92	2.36	2.45	2.38	1.92	1.89
Ipsilateral	2.06	2.20	2.18	2.10	1.84	2.30	2.27	2.18	1.81	1.75

Ipsilateral = side of initial presentation of motor signs; contralateral = side opposite to that of initial presentation of motor signs.
Binding ratio is binding in region of interest divided by binding in occipital cortex.

^{99m}Tc-TRODAT-1 in the putamen was not significantly different from that in the caudate nucleus.

DISCUSSION

MJD is a progressive disorder whose most prominent clinical symptom is cerebellar ataxia. Recent MRI studies of symptomatic MJD patients gave nonspecific findings, including progressive atrophy of the cerebellum, brain stem, frontal and temporal lobes, and globus pallidus (*2,7*). PET studies in MJD patients using raclopride showed that the striatal D_2 receptors were normal (*27*). However, the severity of nigral damage varied, as shown by ^{18}F-dopa. Taniwaki et al. (*28*) found significantly decreased uptake in the putamen and relative sparing in the caudate nucleus, whereas Shinotoh et al. (*27*) found variable uptake. Because fluorodopa uptake can be influenced by multiple factors and occurs by a mechanism different from TRODAT-1 uptake, we used TRODAT-1 to evaluate the damage of DAT in the nigrostriatal dopaminergic pathway of MJD patients. Our study showed a significantly lower striatal ^{99m}Tc-TRODAT-1 binding ratio in symptomatic MJD patients than in healthy volunteers, with no significant difference between caudate nucleus and putaminal activity. These findings were different from those in Parkinson's disease patients we have studied, in whom damage to dopaminergic neurons appeared preferential in the putamen rather than in the caudate nucleus (*29–32*).

Abnormal FDG metabolism was found in MJD carriers, indicating preclinical changes (*10*). Eight of our 10 MJD patients had an obviously low uptake of DAT in the nigrostriatal pathway, with no significant difference between uptake in the putamen and uptake in the caudate nucleus. In 6 of these 8 patients, preclinical changes were shown by ^{99m}Tc-TRODAT-1 brain SPECT. The other 2 patients, who had extrapyramidal signs, had an extremely low uptake of ^{99m}Tc-TRODAT-1. These findings probably reflect diffuse degeneration of neurons in the nigrostriatal dopaminergic pathway in MJD patients. The genetic anomaly may produce functional disturbance, which may be evident before any anatomic change. We believe that the DAT-specific radioligand may permit a powerful in vivo nigrostriatal DAT imaging method, thus allowing recognition of early MJD in patients or, preclinically, in those who have the potential to develop extrapyramidal signs.

We selected the occipital lobe as the reference region for subtraction for 3 reasons: the cerebral and cerebellar cortices and inferior olives are unaffected in MJD (*33*); normal FDG metabolism was noted in both clinical and preclinical MJD

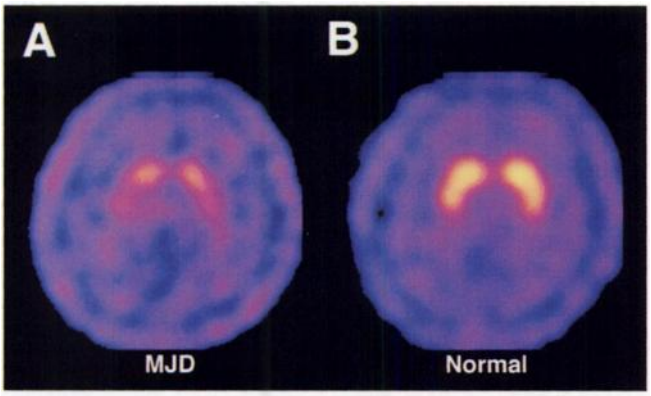

FIGURE 1. (A) A 26-y-old MJD patient. Diffusely decreased ^{99m}Tc-TRODAT-1 uptake is seen in both putamen and caudate nuclei. (B) A 26-y-old healthy volunteer. Normal ^{99m}Tc-TRODAT-1 uptake is seen in both putamen and caudate nuclei.

TABLE 3
Mean Binding Ratio of ^{99m}Tc-TRODAT-1 in Striatum of 10 Symptomatic MJD Patients and 21 Healthy Volunteers

Site	Symptomatic MJD patients	Healthy volunteers
Putamen		
Contralateral	1.77	2.54
Ipsilateral	1.66	2.41
Caudate		
Contralateral	2.18	3.11
Ipsilateral	2.07	2.91

Ipsilateral = side of initial presentation of motor signs; contralateral = side opposite to that of initial presentation of motor signs.
Binding ratio is binding in region of interest divided by binding in occipital cortex.

(*10*); and ^{99m}Tc-TRODAT-1 uptake could be calculated in the striatum as well as in the occipital lobe from the same planar image, allowing better comparison of data between these 2 regions.

By showing DAT abnormalities in patients both with and without extrapyramidal signs, one may be able to predict that an MJD patient has a preclinical disturbance of extrapyramidal signs before anatomic changes are evident. Because of different mechanisms of neuron degeneration in the nigrostriatal dopaminergic pathway, ^{99m}Tc-TRODAT-1 is likely to be useful in differentiating Parkinson's disease (preferentially decreased uptake in the putamen compared with the caudate nucleus) from MJD (significantly decreased uptake in the striatum, but no significant difference between putamen and caudate nucleus) in those who have similar clinical manifestations (*29–32*). We will soon perform further studies, such as of the relationship between changes in TRODAT-1 uptake, the onset of extrapyramidal signs, the role of TRODAT-1 in MJD gene carriers, and MJD families with TRODAT-1. Other research, which could further test the power of ^{99}Tc-TRODAT-1 in predicting expression of the disease, its early diagnosis, and its monitoring after treatment, could be performed profitably in asymptomatic MJD patients.

In this preliminary study, we found a correlation between a decrease in TRODAT-1 uptake and the presence of extrapyramidal signs in MJD patients. However, the decrease did not correlate with patient age at disease onset and abnormal CAG repeat expansions, perhaps because age at disease onset and CAG repeat length correlate with the severity of brain stem and cerebellar atrophy and because DAT was the uptake site of TRODAT-1 (*18,34*). However, these findings are from single, unreplicated studies of few patients.

CONCLUSION

In conclusion, because of the phenotypic and genotypic heterogeneity of dominantly inherited cerebellar ataxias, recognition of MJD based solely on clinical grounds may sometimes be misleading. Moreover, the extrapyramidal signs may be masked in MJD patients with dominantly spinocerebellar ataxia (*35–39*). We believe that ^{99}Tc-TRODAT-1 brain SPECT is an appropriate way of evaluating damage to the nigrostriatal DAT in symptomatic MJD patients with and without extrapyramidal signs but does not correlate with abnormal CAG repeat length, age at disease onset, or disease duration.

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