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Accumulation of methylsulfonylmethane in the human brain: identification by multinuclear magnetic resonance spectroscopy

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Abstract

Methylsulfonylmethane (MSM) is a widely available 'alternative' medicine. In vivo magnetic resonance spectroscopy (MRS) was used to detect and quantify MSM in the brains of four patients with memory loss and in three normal volunteers all of who had ingested MSM at the recommended doses of 1–3 g daily. MSM was detected in all subjects at concentrations of 0.42–3.40 mmole/kg brain and was equally distributed between gray and white matter. MSM was undetectable in drug-naïve normal subjects ($N = 25$), patients screened for 'toxic exposure' ($N = 50$) or patients examined with 1H MRS for the diagnosis of probable Alzheimer Disease ($N = 520$) between 1991 and 2001. No adverse clinical or neurochemical effects were observed. Appearance of MSM in significant concentrations in the human brain indicates ready transfer across the intact blood-brain barrier, of a compound with no known medical benefits.

Keywords: Magnetic resonance spectroscopy; Methylsulfonylmethane; Complementary medicine; Health food supplements; Human brain metabolism; Toxicology; Blood-brain barrier

1. Introduction

The exposure to toxins that cross the intact blood-brain barrier (BBB) is a possible cause of neurological disorders including Parkinson's Disease (Betarbet et al., 2000), Gulf War Syndrome (Haley et al., 2000), Chronic Fatigue Syndrome (CFS; Racciatti et al., 2001) and Alzheimer's Disease (Cummings and Benson, 1992). Use of xenobiotic compounds without adequate screening for possible neurotoxic effects has increased dramatically in the last decade (Eisenberg et al., 1993). Screening for the presence of xenobiotics in the human brain would be of great value in toxicology. In the course of exploring the value of multinuclear (1H, 31P, 13C) magnetic resonance spectroscopy (MRS) for such a screening program, several patients were incidentally discovered to have a novel peak in the proton-brain spectrum. The purpose of our study was to iden-

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tify the resonance, its cerebral distribution and its origin in medications or dietary supplements and to exclude possible neurotoxicity. We defined the compound as methylsulfonylmethane (MSM); an unapproved 'over-the-counter' product recently promoted as a remedy for joint pain and memory loss.

2. Patients/methods

The study population was drawn from subjects referred by their physician to the clinical MRS Unit of Huntington Medical Research Institutes between January 1991 and March 2001, for diagnosis of common neurological disorders including mild cognitive impairment, Alzheimer's Disease, stroke, brain tumor, Parkinson's Disease, infection, CFS, hepatic and toxic encephalopathies.

2.1. Group 1

Comprised four patients drawn from a consecutive series of 44 patients of Group 3 examined between January 2001 and May 2001, who demonstrated an unusual metabolite in 1H MR spectra of the brain. A list of current medications was compiled in interviews with patients, their caregivers and the referring physician. All of the patients had ingested significant amounts of the suspected agent, MSM prior to the 1H MRS examination.

(1) *Patient A:* A 64 years old female with memory loss, mental confusion of undetermined origin, and ataxia treated by cervical laminectomy. Current drug consumption included 17 medications with 87 identified active and inactive xenobiotic ingredients. This patient was examined with 1H MRS on four occasions, in three different locations (Fig. 1) over 60 days, and once with proton de-coupled 31P MRS.

(2) *Patient B:* A 40 years old female with systemic lupus erythematosis, fibromyalgia, high titer of anti-nuclear antibodies, hypertension controlled by medication, and recent onset of memory loss. This patient was examined once with 1H MRS, in gray matter (posterior cingulated gyrus; PCG) and white matter (right occipital posterior parietal WM).

(3) *Patient C:* A 79 years old male presenting with memory loss and sleep apnea. He was prescribed seven different medications and self-medicated with several others. This patient was examined once with 1H MRS in the PCG.

(4) *Patient D:* A 79 years old male with a left parietal stroke confirmed by magnetic resonance imaging (MRI), recent onset of memory loss and aggressive behavior. In addition to several prescribed medicines, this patient admitted to consuming 'many' unnamed herbal remedies. PCG and WM were each examined once with 1H MRS.

Normal healthy volunteers: One female and two males (mean age 48 years) each voluntarily ingested MSM (2.0–3.0 g/day) for related to joint discomfort. The healthy female (age 51 years) provided informed consent for the study and took three 1000 mg doses of over-the-counter MSM (Jarrow Formulas, Los Angeles, CA) each day to a total of 200 g of MSM over 2.5 months. 1H, 13C MR spectra were acquired prior to ingestion of MSM and then weekly in the gray matter of the PCG, until the amount of MSM measured by 1H MRS in the brain reached steady-state. Two weeks later, natural abundance 13C MRS (Bluml, 1999) and three-dimensional (3D) proton chemical shift imaging (1H CSI; Bailles et al., 1987) were performed.

2.2. Group 2

Retrospective chart-review of 50 consecutive patients (mean age 43 years; $M = 19$, $F = 43$) in whom 1H MRS of the brain was performed for diagnosis of CFS ($N = 35$) or toxic exposure ($N = 15$) between 1998 and 2000.

2.3. Group 3

Retrospective chart-review of 520 consecutive patients with memory loss examined with 1H MRS for the diagnosis or exclusion of Alzheimer Disease between 1991 and 2001.

MRI and multinuclear MRS: MRI and quantitative 1H MRS were performed on a GE Signa, 1.5 T routine clinical MR scanner using stimulated echo mode localization (STEAM) with echo times (TE) of 30 and 135 ms, repetition time (TR)

of 1500 ms in a voxel volume of between 8 and 12.5 cc (Kreis et al., 1993). The locations of the voxels selected are indicated in Fig. 1. 3D proton CSI (Bailles et al., 1987) was performed using STEAM, TE = 30 ms, 10×10 matrix in a volume of $14 \times 14 \times 2$ cm^3 processed with SA/GE software (GE, Milwaukee, WI). Proton de-coupled 31P MRS was performed with a dual tuned volume head coil (Hoang et al., 1998). In vivo natural abundance proton de-coupled, 13C MRS was performed using a dual-tuned surface coil applied to the occipital lobe (Bluml, 1999).

Model solutions: A phantom containing 50 mM of 99% pure MSM (Jarrow Formulas) and 50 mM of creatinine (Sigma, Los Angeles, CA) was used to test 1H (short and long echo) and 13C MRS acquisitions. In vivo cerebral MSM concentrations were measured in proton MRS examinations by comparison with the measured peak areas of the internal reference NAA and the external reference, 2-(trimethylsilyl) ethanol (Kreis et al., 1993) and calculated using the known chemical structures and T2 relaxation times of these chemicals.

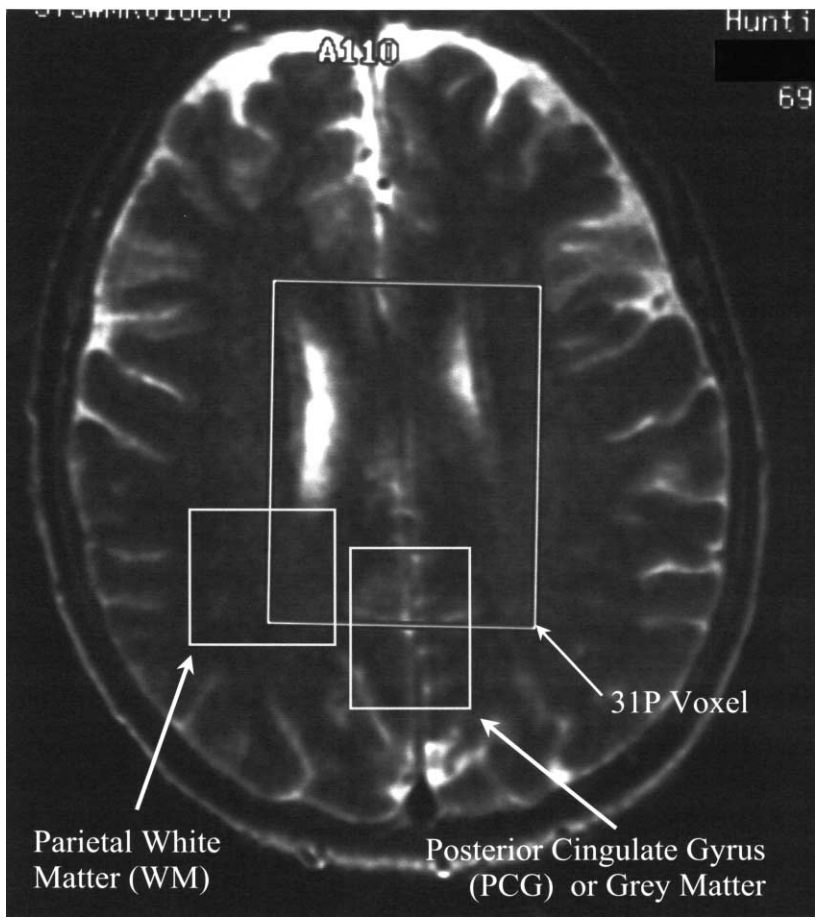

Fig. 1. Axial MRI of human brain demonstrates locations of voxels selected for proton (1H) and 31-Phosphorous (31P) spectroscopy. Patients and normals were examined with 1H MRS in the PCG ($2.1 \times 2.7 \times 2.0$ cm^3) and in the left or right parietal white matter ($2.5 \times 2.5 \times 2.0$ cm^3). The midbrain (MB) location is not shown in this axial slice. 31P spectroscopy, which is less sensitive than 1H MRS was acquired from a much larger voxel ($5.0 \times 6.5 \times 3.0$ cm^3) that was placed in the prefrontal and superior parietal lobes as indicated.

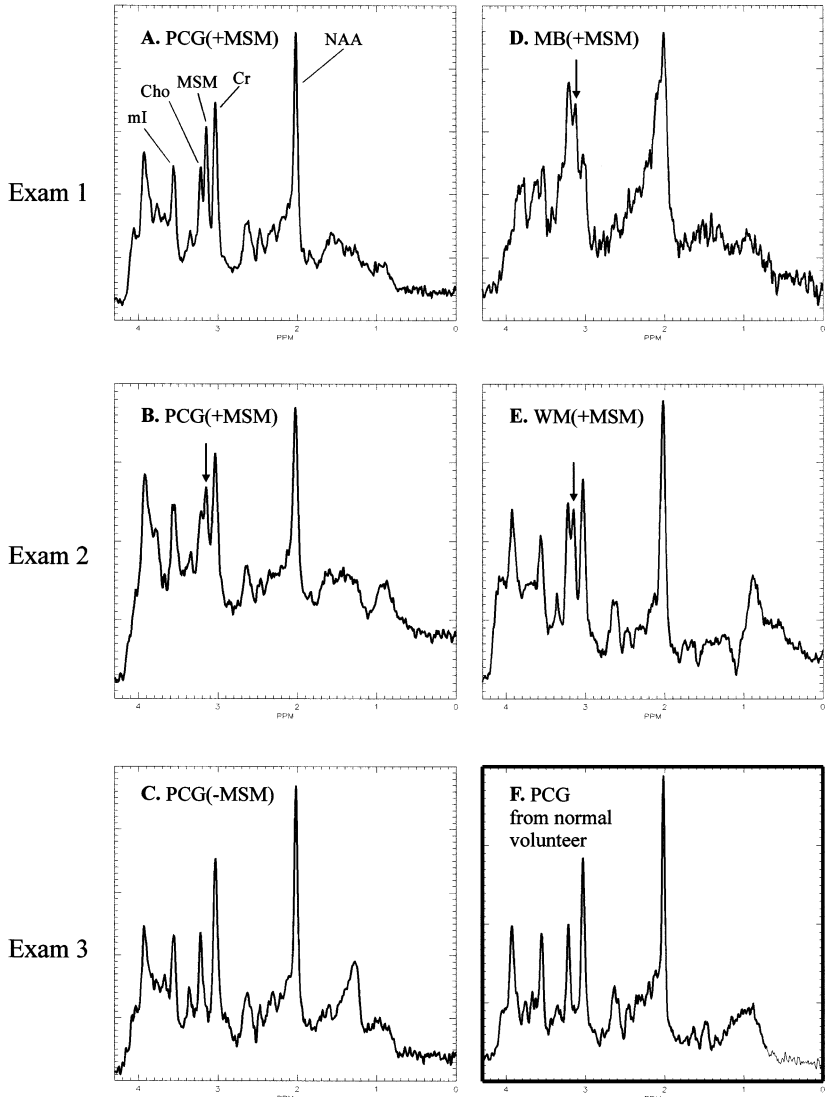

Fig. 2. Cerebral 1H MRS in a patient consuming MSM. Spectra were acquired on three occasions at intervals of 6 weeks. An additional peak at 3.15 ppm (identified as MSM) was present in the PCG, MB, and WM. Between Exam 2 and 3, the patient discontinued MSM, at which time the 1H MRS (C) was indistinguishable from normal (F). Intrinsic metabolites in the brain are not changed by the presence of MSM in the brain.

3. Results

3.1. Identification of MSM in the human brain

1H MR spectra confirmed the major metabolites detected in the normal brain, *N*-acetyl aspartate (NAA; 2.02 ppm), creatine (Cr; 3.02 ppm), choline (Cho; 3.22 ppm) and myoinositol (mI; 3.56 ppm). In addition, each of the four patients exhibited a previously unknown resonance, a well-resolved singlet peak between the Cr and Cho resonances at 3.15 ppm, in all of their brain 1H spectra (Fig. 2). The identity of the peak at 3.15 ppm was first surmised by cross-correlation of the

medications prescribed or self-administered to each of the four patients. The common denominator was MSM (a.k.a. MSM dimethyl sulfone, methyl sulfone, $DMSO_2$:$C_2H_6O_2S$ (Budavari, 1989)). This novel peak also disappeared from the brain spectrum in one of the four patients, who was re-examined after she voluntarily discontinued several medications, including MSM (Fig. 2). The assignment was next confirmed by acquiring a 1H MR spectrum, with the same acquisition-parameters, from the model solution of 50 mM MSM in water. Finally, an in vivo study of three normal volunteers, who were asked to ingest MSM under controlled conditions, showed an identical resonance at 3.15 ppm. Studies of the model solution verified the assignment of MSM: a single resonance at 3.15 ppm in 1H MRS and at 42.64 ppm in 13C MRS (NIMHC, 2001). 13C MRS performed in one of the normal subjects, who had ingested MSM was normal apart from the presence of a peak at 42.64 ppm. Normal cerebral concentrations of the high-energy phosphates ATP and phosphocreatine and the myelin metabolites phosphocholine, phosphethanolamine, glycerophorylcholine, and glycerophosphorylethanolamine, but no novel resonances were identified in a 31P MRS examination performed in one of the patient receiving MSM. As expected, model spectra of MSM (which has no phosphate moiety) were also negative.

3.2. Distribution of MSM within the brain

3D-CSI obtained in one of the normal volunteers showed uniform distribution of MSM in the parietal lobe (Fig. 3). Based on the locations of single-voxels examined with MRS in patients and normal subjects, MSM was evenly distributed throughout the brain including the brainstem (Fig. 2) with equal concentrations of MSM in gray and white matter ($P > 0.05$).

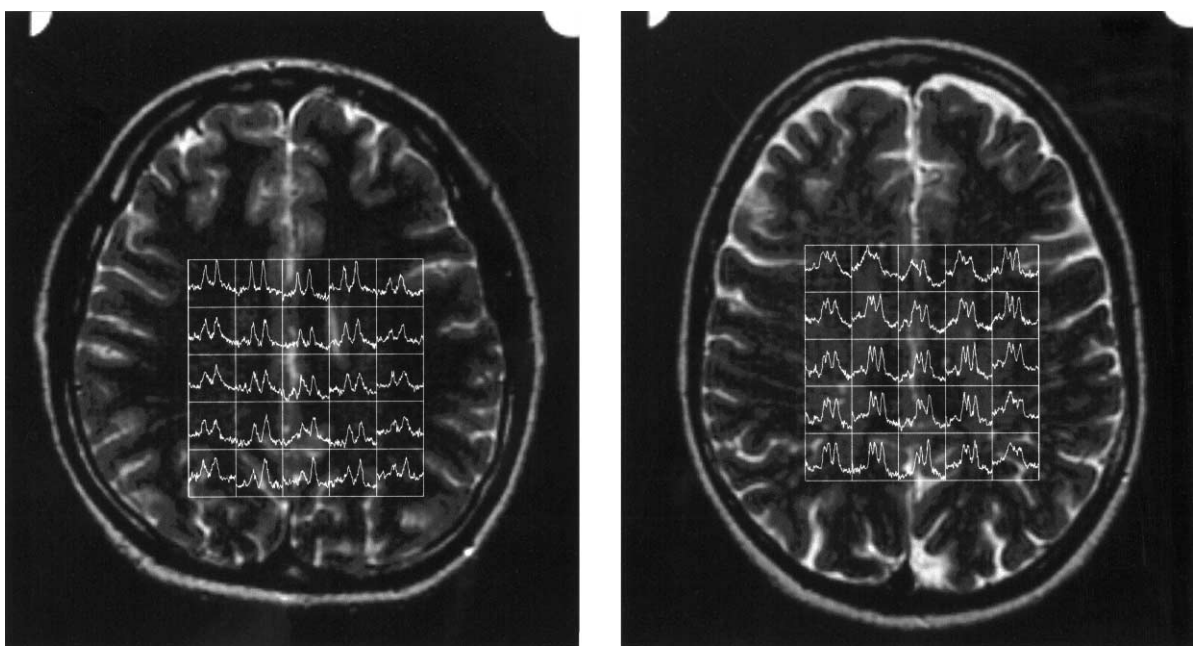

Fig. 3. Regional distribution of MSM. CSI of the bilateral parietal region in a normal volunteer (left) and in a volunteer taking MSM (right) are displayed in a range of 2.75–3.4 ppm. The MSM peak seen at this resolution indicates that the distribution of MSM in the parietal lobe is uniform.

Table 1
Cerebral concentrations of MSM in individual examinations of patients and normal subjects

	Voxel location	Cerebral (MSM) Conc. (mM)	Dosage/duration	
			Duration (time)	Daily dose g (g/kg body wt.)
Patient A	PCG	1.95	2 years	1.5 (0.04)
	White matter	1.65		
	MB	0.72		
Patient B	PCG	1.35	2 years	6.0 (0.1)
	White matter	1.34		
Patient C	PCG	0.42	24 hours	3.0 (0.03)
	Frontal CG	0.25		
Patient D	PCG	3.40	>2 years	~3.0 (0.04)
	White matter	3.38		
Control 1	PCG	2.33	7 weeks	3.0 (0.05)
Control 2	PCG	0.70	5 weeks	2.0 (0.03)
Control 3	PCG	0.70	1 year	2.2 (0.03)

MSM was determined using localized in vivo 1H MRS from the peak area of the metabolite at 3.15 ppm compared to an internal and external reference (Kreis et al., 1993).

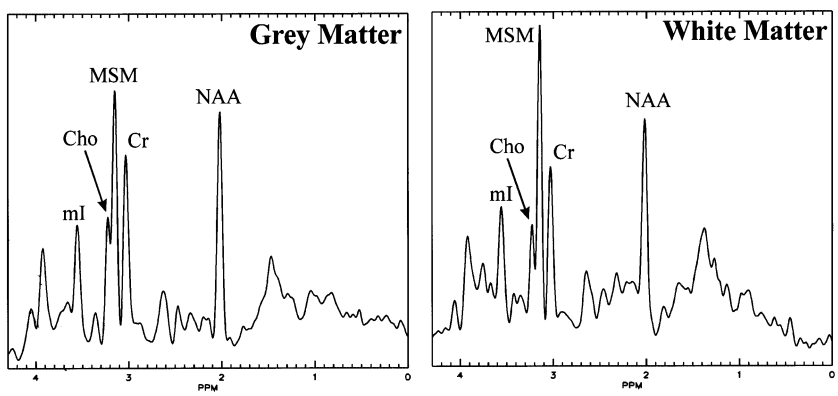

Fig. 4. Higher cerebral concentration of MSM in a patient after stroke. In one patient, high levels of MSM were detected throughout the brain with 1H MRS. Peak intensity of MSM was approximately two times that of NAA. It is possible that prior pathology may result in increased accumulation of MSM in the brain.

3.3. Quantitation of intra-cerebral MSM

The four patients exhibited MSM in all 1H spectra irrespective of brain location. In patients A and B, the steady-state intra-cerebral concentration of MSM was 1.67 ± 0.36 mM (Table 1), with somewhat lower concentration in Patient C, who had began taking MSM only 1 day before the MRS examination. In Patient D, with evidence of prior stroke on MRI a significantly higher MSM concentration (3.39 ± 0.01 mM, $P <$ 0.05) was observed in the brain (Fig. 4). The time course of accumulation of MSM in the brain was demonstrated in one normal subject, receiving the average daily dose of 44 mg/kg recommended by the manufacturer. Cerebral MSM concentrations increased rapidly and reached steady-state of 2.33 ± 0.24 mM, within 2 weeks (Fig. 5). The concentrations of the intrinsic cerebral metabolites (NAA, Cr, Cho, mI, and Glx) remained normal throughout the trial (Fig. 6). The remaining normal volunteers demonstrated the charac-

teristic resonance at 3.15 ppm at somewhat lower intensity (Mean cerebral MSM = 1.24 ± 1.33 mM; $N = 3$).

3.4. Neurochemical composition in patients ingesting MSM

MSM had no systematic effect on brain metabolites as measured with 1H or dc31P MRS. Small, expected differences from normal NAA and mI were detected in patients with memory loss due to Alzheimer Disease or other pathologies (Shonk et al., 1995) compared to normal subjects. However, there were no systematic abnormalities of 1H MR spectra in this small series of subjects ingesting MSM compared to the appropriate patient or normal controls, who had not consumed MSM (results not shown).

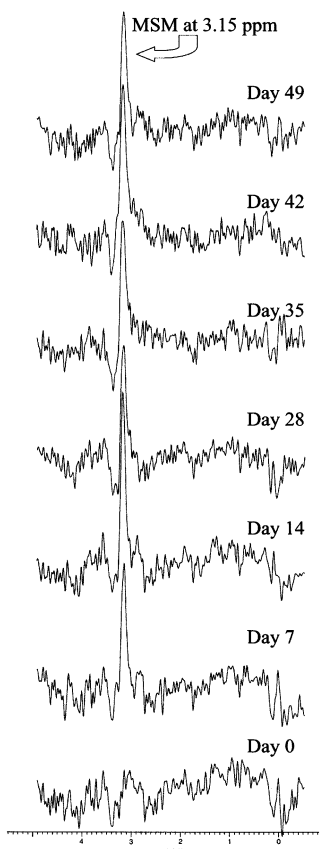

Fig. 5. Time course of accumulation of MSM in the normal human brain. Stack plot of difference spectra of the PCG during trial period is shown. Difference spectra, where the 'normal' baseline spectra is subtracted from the spectra obtained during MSM ingestion at the intervals indicated show the peak at 3.15 ppm to be prominent 7 days after MSM consumption begins and to persist for 49 days.

4. Discussion

MSM occurs naturally in the human blood at about 0.2 ppm (3 μM; Lawrence, 1998), two-orders of magnitude below the limit of detection with the present in vivo MRS method. Thus, MSM was undetectable in brain spectra of a large numbers of patients in whom there was no history of MSM-consumption. The four patients with detectable amounts of MSM in the brain represent an incidence of 9.1% in the past 5 months. Prior to the present study, MSM had not been observed in any of the more than 10 000 brain spectra acquired at this institution over the past 10 years. The rapid increase in detection of MSM is probably attributable to vigorous Internet marketing of MSM in the past year (Phillips, 2000).

Only after complete identification of MSM during the course of this study was it possible to conduct a literature search (MEDLINE). This revealed a single case study identifying MSM in the brain of a normal subject at a concentration of 2.36 mM, taking 181 mg/kg per day of MSM (Rose et al., 2000). MSM is an increasingly popular dietary supplement sold over-the-counter for the treatment of arthritis, emphysema, carpal tunnel, asthma, back pain, acne, fibromyalgia and memory loss (Jacob et al., 1999). MSM is a metabolic by-product of dimethyl sulfoxide (DMSO; Hucker et al., 1966), an FDA-approved treatment for chronic inflammatory genitourinary disorders (Jacob and Herschler, 1983). MSM may have value in treatment of interstitial cystitis (Childs, 1994) and in degenerative arthritis (Murav'ev et al., 1991; Lawrence, 1998), but there is no clinical proof that this substance ameliorates other illnesses (Phillips, 2000). MSM itself has not been FDA-approved for any treatment. Some effect on murine autoimmune lymphoproliferative disease (Morton and Siegel, 1986) and cancer

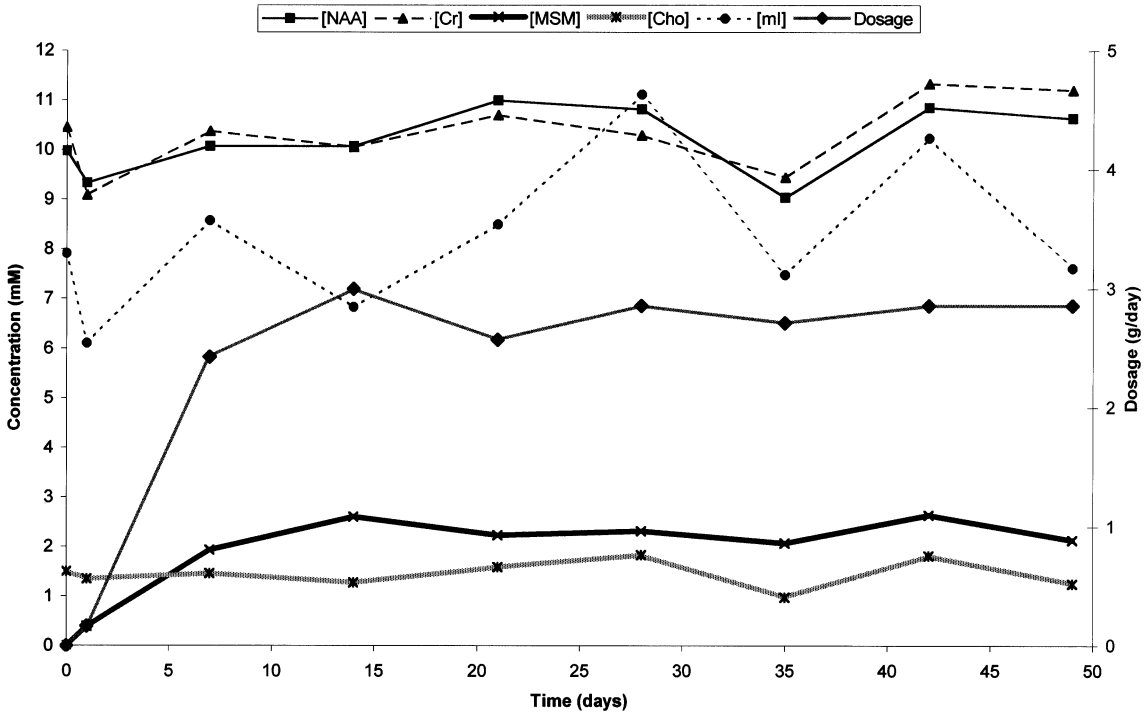

Fig. 6. Concentrations of cerebral metabolites in normal volunteer ingesting MSM for a period of 49 days. NAA, Cho, Cr, mI, and MSM concentrations determined weekly are displayed on the left value scale. MSM (g/day) taken the week prior to each acquisition is shown on the right. Despite a significant increase of MSM, there was no significant effect on the concentrations of the intrinsic cerebral metabolites.

(McCabe et al., 1986; O'Dwyer et al., 1988) has been demonstrated in animals. Despite the lack of large-scale human clinical trials of efficacy, MSM is readily available at health food stores and the Internet. It is often combined with glucosamine as a natural alternative pain medication and is consumed by increasing numbers as a homeopathic remedy (Canedy, 1998).

MSM is relatively harmless (LD50 = 8000 mg/kg) in mice (Morton and Siegel, 1986). In 1H MRS studies, neurotoxicity may be reflected in the presence of lactate, increased Cho, or, in neurodegenerative disorders and acute brain injury, a decrease in concentration of the neuronal and axonal marker NAA (Danielsen and Ross, 1999). Lack of correlation between cerebral MSM concentration and the concentrations of intrinsic cerebral metabolites (NAA, Cr, Cho, mI, and Glx) indicates that MSM does not appear to have any adverse neurochemical effect in man. Considering the very large amounts of MSM consumed (200 g = 3 moles MSM in one volunteer) it is perhaps surprising that the maximum cerebral concentration of MSM reached only 2.6 mM. The lack of serious neurotoxicity may be attributable to an active mechanism for the removal of MSM from the brain. Of clinical significance therefore is the considerably higher concentration of MSM found in one patient with a history of stroke. It is possible that the breakdown of the normal BBB may allow MSM to enter brain even more readily or alternatively, may interfere with the normal export mechanism. It is unknown at this point whether higher intra-cerebral MSM concentrations could have a long-term neurotoxic effect.

Acknowledgements

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References

Bailles, D.R., Bryant, D.J., Bydder, G.M., Case, H.A., Collins, A.G., Cox, I.J., Evans, P.R., Harman, R.R., Hall, A.S., Khenia, S., McArthur, P., Oliver, A., Rose, M.R., Ross, B.D., Young, I.R., 1987. Localized phosphorus-31 NMR spectroscopy of normal and pathological human organs in vivo using phase-encoding techniques. J. Mag. Reson. 74 (10), 158–170.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 3 (12), 1301–1306.

Bluml, S., 1999. In vivo quantitation of cerebral metabolite concentrations using natural abundance ^{13}C MRS at 1.5 T. J. Mag. Reson. 136 (2), 219–225.

Budavari, S. (Ed.), 1989. The Merck Index, 11th ed. Merck, New Jersey, p. 3250.

Canedy, D., 1998. Real medicine or medicine show? Growth of herbal remedy sales raises issues of value. NY Times, D1.

Childs, J., 1994. Dimethyl sulfone in treatment of interstitial cystitis. Urol. Clin. North Am. 21, 85–88.

Cummings, J.L., Benson, D.F., 1992. Dementia: A Clinical Approach. Butterworth-Heinemann, Boston, pp. 71–72.

Danielsen, E., Ross, B.D., 1999. Magnetic Resonance Spectroscopy Diagnosis of Neurological Diseases. Marcel Dekker, New York, pp. 293–300.

Eisenberg, D.M., Kessler, R.C., Foster, C., Norlock, F.E., Calkins, D.R., Delbanco, T.L., 1993. Unconventional medicine in the United States – prevalence, costs, and patterns of use. N. Engl. J. Med. 328 (4), 246–252.

Haley, R.W., Marshall, W.W., McDonald, G.G., Daugherty, M.A., Petty, F., Fleckenstein, J.L., 2000. Brain abnormalities in Gulf War Syndrome: evaluation with 1H MR spectroscopy. Radiology 215 (3), 807–817.

Hoang, T.Q., Bluml, S., Dubowitz, D.J., Moats, R., Kopyov, O., Jacques, D., Ross, B.D., 1998. Quantitative proton-decoupled 31P MRS and 1H MRS in the evaluation of Huntington's and Parkinson's diseases. Neurology 50 (4), 1033–1040.

Hucker, H.B., Ahmed, P.M., Miller, E.A., Brobyn, R., 1966. Metabolism of dimethyl sulphoxide to dimethyl sulphone in the rat and man. Nature 209, 619–620.

Jacob, S.W., Herschler, R., 1983. Biological actions and medical applications of dimethyl sulfoxide. Ann. NY Acad. Sci. 411, xii–xvii.

Jacob, S., Lawrence, R.M., Zucker, M., 1999. The Miracle of MSM: The Natural Solution for Pain. Penguin-Putnam, New York.

Kreis, R., Ernst, T., Ross, B.D., 1993. Absolute quantitation of water and metabolites in the human brain II. Metab. Conc. B102, 9–19.

Lawrence, R.M., 1998. Methylsufonylmethane: a double-blind study of its use in degenerative arthritis. Int. J. Anti-Aging Med. 1 (1), 50.

McCabe, D., O'Dwyer, P., Sickle-Santanello, B., Woltering, E., Abou-Issa, H., James, A., 1986. Polar solvents in the chemoprevention of dimethylbenzanthracene-induced rat mammary cancer. Arch. Surg. 121, 1455–1459.

Morton, J.I., Siegel, B.V., 1986. Effects of oral dimethyl sulfoxide and dimethyl sulfone on murine autoimmune lymphoproliferative disease. Proc. Soc. Exp. Biol. Med. 183 (20), 227–230.

Murav'ev, V., Venikova, M.S., Pleskovskaia, G.N., Riazantseva, T.A., Sigidin, Ia.A., 1991. Effect of dimethyl sulfoxide and dimethyl sulfone on a destructive process in the joints of mice with spontaneous arthritis. Patol. Fiziol. Eksp. Ter. 2, 37–39.

NIMHC, 2001. Integrated spectral data base system for organic compounds. http://www.aist.go.jp/RIODB/SDBS/sdbs/owa/sdbs_sea.cre_frame_disp?sdbsno = 3049.

O'Dwyer, P.J., McCabe, D.P., Sickle-Santanello, B.J., Woltering, E.A., Clausen, K., Martin, E.W., 1988. Use of polar solvents in chemoprevention of 1,2-dimethylhydrazine-induced colon cancer. Cancer 62, 944–948.

Phillips, J., 2000. MSM: does it work? Harvard Health Letter.

Racciatti, D., Vecchiet, J., Ceccomancini, A., Ricci, F., Pizzigallo, E., 2001. Chronic fatigue syndrome following a toxic exposure. Sci. Total Environ. 270 (1–3), 27–31.

Rose, S.E., Chalk, J.B., Galloway, G.J., Doddrell, D.M., 2000. Detection of dimethyl sulfone in the human brain by in vivo proton magnetic resonance spectroscopy. Mag. Reson. Imag. 18, 95–98.

Shonk, T.K., Moats, R.A., Gifford, P., Michaelis, T., Mandigo, J.C., Izumi, J., Ross, B.D., 1995. Probable Alzheimer disease: diagnosis with proton MR spectroscopy. Radiology 195 (1), 14–15.