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Methanesulfonyl Fluoride (MSF): A Double-Blind, Placebo-Controlled Study of Safety and Efficacy in the Treatment of Senile Dementia of the Alzheimer Type

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Summary: The purpose of the present study was to evaluate methanesulfonyl fluoride (MSF), a very long-acting CNS-selective acetylcholinesterase (AChE) inhibitor, as a palliative treatment for senile dementia of the Alzheimer type (SDAT). In experiment I, MSF (0.03–0.18 mg/kg) was administered orally to 10 normal volunteers to measure toxicity and establish dose/response function in erythrocyte AChE. MSF produced a dose–response function of %inhibition = (40)(Log_{10}[MSF mg/kg] + 51.7) with no toxicity at these doses. Experiment II was a 16-week double-blind, placebo-controlled study of the safety and efficacy of MSF in doses of up to 0.18 mg/kg given three times per week in 5 men and 10 women (60–82 years), with Mini-Mental State Examination (MMSE) scores of 9–24, who had SDAT. MSF produced a mean of 89.5% inhibition of erythrocyte AChE in patients and improved cognitive performance as measured by the MMSE, Alzheimer Disease Assessment Scale-Cognitive Subscale (ADAS-COG), Global Deterioration Scale, and the Clinical Interview Based Impression of Change (CIBIC). Most of the improvement on the ADAS-COG was maintained 8 weeks after ending MSF. No patients left the study because of drug-related adverse events and there were no toxic effects. MSF may be a safe and effective palliative treatment for SDAT and further clinical trials in larger groups of patients are warranted. **Key Words:** Alzheimer disease—Methanesulfonyl fluoride (MSF)—Dementia—Acetylcholinesterase—ChEI.

Reduced cholinergic function in the basal forebrain and cortex in senile dementia of the Alzheimer type (SDAT) is a common neuropathological finding that may be responsible, in part, for cognitive decline in this disease (Davies and Maloney, 1976; Whitehouse et al., 1982). Many cholinesterase inhibitors have been studied including physostigmine (e.g., Davis and Mohs, 1982; Thal et al., 1983; Mohs et al., 1985), tacrine (e.g., Chatellier and Lacomblez, 1990; Gauthier et al., 1990; Åhlin et al., 1991; Davis et al., 1992; Knapp et al., 1994), metrifonate (Becker et al., 1996), donepezil (Rogers and Friedhoff, 1996), and rivastigmine (Corey-Bloom et al., 1998). Overall, the results have been disappointing or only moderate improvement has been shown.

One of the problems, however, in evaluating the potential clinical efficacy of cholinesterase inhibitors has been to develop an inhibitor that is selective for the brain. Brain selectivity would allow a therapeutic effect in the brain and minimize inhibition in the peripheral tissues, which may be responsible for the nausea, vomiting, and diarrhea that often limit therapeutic doses.

The pharmacodynamics of methanesulfonyl fluoride (MSF) inhibition cause the drug to be highly selective for the brain. As a very long-acting inhibitor, recovery

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from MSF inhibition requires new synthesis of acetylcholinesterase (AChE). The rate of new synthesis of AChE in brain is less than one-tenth of that in the smooth muscle of the intestines (Moss et al., 1988). Synthesis in cortex is slower than in most other brain areas (McDonough et al., 1983). The slowness with which the brain resynthesizes AChE causes MSF-induced inhibition to accumulate to high levels in the brain compared with peripheral tissues. For example, after repeated injections of small doses over several days, MSF produced 80–90% inhibition in rodent and monkey brain with less than about 35% inhibition in peripheral tissues and without toxicity (Moss et al., 1988).

MSF is also highly selective for AChE (Pacheco et al., 1995). To the degree that AChE is the major cholinesterase in the brain (Mesulam and Geula, 1991), selectivity toward AChE (compared with butyrylcholinesterase, BChE) improves selectivity for the brain. AChE is also the enzyme that appears to be associated with memory (Deutsch, 1971; Drachman and Glosser, 1981).

In summary, MSF appeared to be a brain-selective AChE inhibitor that might be useful as a palliative treatment of SDAT. Therefore, in the first use of MSF in humans, experiment I was conducted on a group of 10 normal volunteers to establish dose/response data and assess toxicity. Experiment II was a preliminary test of MSF as a palliative treatment in 15 patients with SDAT.

These experiments were approved by the Institutional Review Board at the University of Texas at El Paso, the Comité Etica and the Comité de Investigación, Facultad de Medicina, Universidad Autonoma de Chihuahua. This research was also reviewed and approved by the Director General de Control de Insumos y Medicamentos, Secretaria de Salud, D.F., Mexico.

EXPERIMENT I

Subjects

Experiment I was a phase I trial of MSF in a group of 12 normal subjects. Two subjects were dropped from the protocol because of absences (business trips). Seven women and three men with an average age of 45.8 years (SD 6.7 years, range 38–60 years) weighing an average of 75.45 kg (SD 9.8 kg, range 63.5–98 kg) completed the protocol. All were free of other medications, had normal clinical blood profiles and electrocardiograms (EKG), and gave informed consent.

Drug Preparation

MSF was custom synthesized by MTM Chemical Co. (Blythewood, SC, now Lancaster Synthesis) to meet the U.S. Food and Drug Administration (FDA) requirements for identity, purity, and strength. The MSF was diluted into peanut oil (U.S.P./N.F., Spectrum Chemical Mfg. Corp., Gardena, CA) and given orally in a gelatin capsule.

Cholinesterase Assays

Erythrocyte AChE was rapidly assayed at 1.0 mM substrate (Ellman et al., 1961) at pH 7.4 from a drop of blood drawn by finger prick. The Michaelis constant (K_m) for erythrocyte AChE was 102.5 μM with acetyl-ß-methylthiocholine. Erythrocyte AChE V_{max} was 9.2552 (SEM = 0.6386) $\times 10^{-6}$ moles/g per minute. Single oral doses of MSF up to 0.22 mg/kg in a preliminary subject produced no measurable inhibition of plasma BChE and, therefore, BChE activity was not assayed further.

Procedures

Baseline erythrocyte AChE levels were established by triplicate assays. All subjects were given three doses of MSF per week at 0.03, 0.06, and 0.12 mg/kg the first week and then three doses of 0.18 mg/kg the second week. An interview for side effects and an assay for accumulated inhibition of erythrocyte AChE accompanied each dose.

Results

The dose/response function by least squares linear regression was:

$$\%\text{INH} = (40.0)(\text{Log}_{10}[\text{MSF mg/kg}]) + 51.7, r = +0.90,$$

where %INH is the percentage of inhibition of the remaining active enzyme produced by each dose. MSF is active after oral administration and produces an orderly dose–response relationship without toxicity at these doses. There were no changes in any clinical blood values.

EXPERIMENT II

The purpose of experiment II was to test the safety and efficacy of MSF in patients with SDAT. This study was conducted in an outpatient clinic in Chihuahua, Mexico, and all psychometric evaluations were in Spanish.

Patients

The patients met the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable Alzheimer disease (McKhann et al., 1984) and had cognitive deterioration in two areas, in addition to memory, without a specific

etiological factor that could be demonstrated by clinical history or supporting examinations, including computed tomography (CT) or magnetic resonance imaging (MRI) studies. Patients with a history of psychiatric illness preceding the dementia syndrome, associated neurological illness (e.g., extrapyramidal disorders), history of cerebrovascular illness, hypertension higher than 180/100, previous abuse of alcohol or drugs of addiction, or evidence of liver pathology were excluded. Informed consent was obtained and a full clinical history was taken.

Twenty-one patients were enrolled in the study. Of those, 15 patients finished the protocol. No patients left the protocol because of a drug-related adverse event. Of the 6 patients who left the protocol, 3 were unable to complete the protocol because of transportation problems, 1 patient left Chihuahua to live in the United States, 1 patient had colitis before enrollment and during the protocol (including placebo) and elected to leave the study, and 1 patient was noncompliant.

The 15 patients who completed the protocol were 5 men and 10 women with the following characteristics: mean age 69.73 years (SD = 7.4, range 60–82 years); Mini-Mental State Examination (MMSE; Folstein et al., 1975) mean of 16.67 points (SD = 4.5, range 9–24); Global Deterioration Scale (Reisberg et al., 1982) mean of 4.4 (SD = 0.83, range 3–6); and Alzheimer Disease Assessment Scale-Cognitive (ADAS-COG; Rosen et al., 1984) mean score of 27.4 errors (SD = 13.1, range 9–54). The 6 patients who left the protocol, by comparison, had an ADAS-COG mean score of 16.6 errors (SD = 9.8, range 9.8–35.3), suggesting higher functioning patients had a tendency to drop out. Four of the 6 patients who dropped out were randomized into the placebo/MSF sequence and 3 of those never received MSF.

MSF Dosing and Treatment Schedule

Because of the pharmacodynamics of MSF discussed above, the strategy was to use small doses given over a period of weeks to accumulate an adequate level of inhibition in the brain while accumulating minimum inhibition in peripheral tissues. The dose/response data from Experiment I indicated that 0.14 mg/kg was the dose required to produce 18% inhibition of residual active enzyme each time the dose was given, the effect required to produce a minimum asymptotic level of 50% inhibition of brain AChE when given three times per week (Tallarida and Murray, 1986). At this dose, a minimum of 3 weeks would be required to accumulate a therapeutic level of AChE inhibition (i.e., more than 50%) in the brain. This dose was expected to be safe insofar as monkeys have been treated with 1.5 mg/kg (intramuscularly), three doses per week, for several weeks without toxicity (Moss et al., 1988).

The first seven patients were given 0.03, 0.07 mg/kg and then administered 0.14 mg/kg three times per week for all 8 weeks of treatment in the protocol. The other eight patients received identical treatment for the first 4 weeks but, because there were no adverse events at 0.14 mg/kg, were raised to 0.18 mg/kg for the last 4 weeks of MSF treatment. Except for dosage, drug preparation was as described for experiment I.

Clinical Evaluation

The main outcome measures used to evaluate efficacy of MSF were changes in ADAS-COG (a scale of 0–70 errors), MMSE, global deterioration scale, and the clinical interview-based impression of change (CIBIC; Knopman et al., 1994). The patients were evaluated three times: at entry into the protocol (baseline), after the first 8 weeks of treatment, and at the end of the 16-week protocol. To ensure uniformity, reliability, and objectivity, all clinical evaluations (CIBIC and Global) were conducted by the same physician independently from the ADAS-COG and MMSE, which were given by a trained psychometric technician. The patients, the families, and the investigators doing the clinical evaluations were blind with regard to treatment throughout the protocol. All patients were subjected to regular clinical blood testing to measure toxicity throughout and at the end of the protocol.

Experimental Design

Subjects were randomly assigned to the protocol. Some patients received MSF the first 8 weeks and placebo (gelatin capsules with oil) in the second 8 weeks (group A), whereas others received placebo the first 8 weeks and MSF the second 8 weeks (group B).

Statistical Evaluation

There were two statistical evaluations: (1) analysis of overall performance relative to baseline (entry into protocol); and (2) change during the MSF period (8 weeks) compared with change during the placebo period (8 weeks). Comparisons were made with Student's *t* test for paired observations or independent samples (Bruning and Kintz, 1977), the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956), the Wilcoxon rank sum test, or the sign test (Bradley, 1968).

Results

MSF treatment accumulated a mean level of 85.3% (SEM = 2.5%) inhibition of erythrocyte AChE after 8 weeks of 0.14 mg/kg MSF. In those patients who

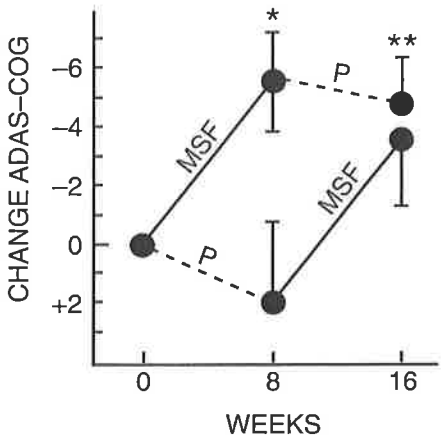

FIG. 1. Differences in Alzheimer Disease Assessment Scale-Cognitive Subscale (ADAS-COG) scores throughout the 16-week protocol from baseline. MSF and P signify the period in which the patients received either MSF (methanesulfonyl fluoride) or placebo, respectively. At 8 weeks, the MSF-treated group was significantly (*$p < 0.05$) more improved than the placebo-treated group. At 16 weeks, both groups combined were significantly improved relative to entry into the protocol (**$p < 0.01$). The groups were not different from each other at 16 weeks.

received 0.18 mg/kg during the last 4 weeks, 89.5% (SEM = 1.1%) inhibition was obtained. The patients showed 17.3% (SEM 1.9%) inhibition of erythrocyte AChE with each dose of 0.14 mg/kg MSF.

There were no patients who suffered from nausea, vomiting, diarrhea, or other cholinergic side effects. One patient who had experienced vomiting on tacrine (60 mg/day) in an earlier attempt at treatment had no problem on MSF. There were no changes in clinical blood profiles.

Figure 1 shows differences in ADAS-COG scores throughout the protocol relative to entry scores. There was substantial improvement of cognitive performance (reduction in errors) during MSF treatment and a slight decline in performance (increase in errors) during the 8 week placebo phase. At 8 weeks, the MSF-treated group A ($n = 9$) was significantly better than the placebo-treated group B ($n = 6$) on the ADAS-COG ($p < 0.05$ two-tailed t test for independent samples and Wilcoxon rank sum test). The difference in size between group A and group B was caused by randomly losing more patients (4) who received placebo first than patients (2) who received MSF first in the protocol. The ADAS-COG performance of both groups combined at 16 weeks was significantly better than at the beginning of the protocol ($p < 0.01$, two-tailed paired t test and Wilcoxon matched-pairs signed ranks test).

MSF and placebo were also compared by a difference score based on the cognitive performance at the beginning of the 8 week period of MSF or placebo with performance at the end of the period. MSF produced significant improvement on the ADAS-COG (mean of 5.6 fewer errors, SEM = 1.3) compared with the placebo (mean of 1.2 more errors, SEM = 1.3; $p < 0.01$ two-tailed paired t test and Wilcoxon matched-pairs signed-ranks test). MSF also improved performance on the MMSE (mean of +2.7 points, SEM = 0.5) compared with placebo (mean change of 0.0 points, SEM = 0.7; $p < 0.01$ two-tailed paired t test and Wilcoxon matched-pairs signed-ranks test). Nine of the 15 patients improved 3 points or more (two improved 6 and one improved 7 points).

Because of the strong carryover effect, the ratings on the CIBIC and Global Deterioration at the end of the protocol were simply compared with baseline ratings at the beginning of the protocol. At the beginning of the protocol, all patients were assigned a CIBIC rating of 4.0; improvement was rated as 3, 2, or 1, whereas deterioration was rated 5, 6, or 7. Patients at the end of the protocol had a mean CIBIC of 2.98 (SD = 1.13), indicating improvement ($p < 0.01$, two-tailed paired t test and the sign test). At the beginning of the protocol, the mean Global Deterioration rating was 4.4 (SEM = 0.21), whereas at the end the mean score was 3.1, an improvement of 1.3 (SEM = 0.33) on this scale ($p < 0.01$, two-tailed paired t test and sign test).

DISCUSSION

In this first use of MSF in humans, the initial objective was to estimate the dose required to test the therapeutic potential. The effect of MSF, a very long-acting inhibitor, is determined by three factors: (1) the percentage of inhibition of AChE produced with each dose; (2) the rate at which the brain resynthesizes AChE to overcome inhibition; and (3) how often the drug is given.

Animal studies have shown that increments of inhibition produced by MSF in the brain are approximately equal to increments of erythrocyte AChE inhibition (Moss, unpublished). Second, the rate at which the human brain recovers AChE can be assumed to require a half-time of about 12 days based on experiments in rodents and monkeys (Moss et al., 1988), whereas human erythrocyte AChE recovery requires a half-time of about 45–60 days (Huser, 1970), the rate at which erythrocytes are replaced. Survival of erythrocytes is unaffected by AChE inhibition (Metz et al., 1961; Goldin et al., 1964; Moriearty et al., 1991). The actual half-time for recovery of erythrocyte AChE was 43 days in a person monitored for 80 days after 60% MSF-induced inhibition (Moss, unpublished). These data support the use of erythrocyte AChE to estimate the effects in the brain as long as the difference in half-time for recovery is considered.

Young normals and older patients showed about the same response to MSF as measured by erythrocyte AChE inhibition. Data from young normals (experiment

I) predicted 18% and older patients actually experienced 17.3% (SEM = 1.96%) inhibition with each dose of 0.14 mg/kg. Calculations based on young normals predicted that 0.18 mg/kg would produce 22% inhibition of the remaining enzyme with each dose. However, to accumulate 89.5% inhibition of erythrocyte AChE over 8 weeks, 0.18 mg/kg had to actually produce about 24% inhibition with each dose (Tallarida and Murray, 1986).

With MSF, our strategy was to use a three times per week dose that would maintain 85–90% inhibition of erythrocyte AChE, corresponding to 66% inhibition in the brain with a half-time of 12 days (Tallarida and Murray, 1986; Moss et al., 1988). With three doses per week, the maximum and minimum inhibition were within 5% of this mean value (Tallarida and Murray, 1986). After 70% inhibition in the brain, increasing doses produce diminishing increments of asymptotic inhibition (Tallarida and Murray, 1986). Without dose-limiting side effects, our strategy was simply to use a theoretically adequate dose. We made no attempt to find a best dose and the benefits of higher or lower doses were not explored. Therefore, maintaining 85–90% inhibition of erythrocyte AChE was expected to provide a good test of the clinical efficacy of MSF.

The absence of side effects, especially nausea, vomiting, and diarrhea, is probably the benefit from minimal inhibition of intestinal smooth muscle AChE with MSF treatment. Intestinal smooth muscle and brain resynthesize AChE with half-times of 1 and 12 days, respectively (Moss et al., 1988). Calculations using these half-times (Tallarida and Murray, 1986) suggest that when MSF accumulates about 70% inhibition in the brain, there would be only between about 10% and 25% inhibition in the intestines. Secondly, avoiding BChE inhibition by using an AChE-selective inhibitor may also reduce the risk of peripheral smooth muscle toxicity (Reutter et al., 1987).

The unexpectedly long duration of the MSF-induced cognitive enhancement (about 5 points improvement maintained during the subsequent 8 weeks of washout on placebo, Fig. 1) is not easily explained. Crossover studies of cholinesterase inhibitors (e.g., tacrine) have shown carryover effects of 3–4 weeks (Chatellier and Lacomblez, 1990; Gauthier et al., 1990; Eagger et al., 1992) and a washout period of 5 weeks has been considered sufficient to make a substantial carryover effect unlikely (Åhlin et al., 1991). The 8-week placebo (washout) period after MSF treatment in group A was expected to be sufficient to avoid a carryover effect in the final cognitive assessment. However, the long carryover MSF effect in group A supports the suggestion that cholinesterase inhibitors may have some long-acting noncholinergic effects (Becker et al., 1996).

Parallel groups design can be used when carryover effects are likely (Schneider, 1990). Because the patients randomized into group A did not differ from group B on baseline ADAS-COG errors, the first 8 weeks could be viewed as a parallel groups design. As shown in Fig. 1, there is a significant improvement in MSF-treated patients compared with placebo controls at 8 weeks ($p < 0.05$, two-tailed t test).

In this small study, it appears that MSF may have less toxicity and equal or better efficacy than other compounds. In conclusion, in this first use of a sulfonyl fluoride in humans, MSF appears to be a safe and effective palliative treatment for SDAT and further clinical trials in larger groups of patients are warranted.

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