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METHANESULFONYL FLUORIDE:
A CNS Selective Cholinesterase Inhibitor

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INTRODUCTION

Senile dementia of the Alzheimer type (SDAT) is a complex disease process that produces gross neuropathology and generalized deterioration of brain function (Wisniewski, Merz, Wen, Iqbal, and Grundke-Iqbal, 1985). The severity of the pathology is correlated with widespread reduction in regional cerebral glucose metabolism (Metter, Riege, Benson, Kuhl and Phelps, 1985). In addition to reduction in cholinergic markers, SDAT has also been associated with specific changes in many neurotransmitters including somatostatin (Davies, Katzman,and Terry, 1980; Rossor, Emson, Mountjoy, Roth and Iverson, 1980; Beal, Mazurek, Tran, Chattha, Bird, and Martin, 1985), monoamines (Adolfsson, Gottfries, Roos, and Winblad, 1979; Gibson and Ball, 1983), and various amino acids (Arai, Kobayashi, Ichimiya, Kosaka, and Iizuka, 1984). It is, therefore, an oversimplification to characterize the dementia associated with SDAT as anticholinergic dementia.

Even though SDAT involves much more than deterioration of cholinergic function in the central nervous system (CNS), the cholinergic hypothesis of dementia has stimulated clinical and basic research. The cholinergic hypothesis is simply that dementia is the result of insufficient cholinergic function within the CNS. The rationale for this hypothesis is based on the classic discovery that indicators of cholinergic neurotransmission are markedly reduced in brains from Alzheimer's patients as compared to age-matched controls (Davies and Maloney, 1976; Perry, Gibson, Blessed, Tomlinson, and Perry, 1977). Because the cells in the nucleus basalis of Meynert and related nuclei are thought to provide important cholinergic projections to the hippocampus and cortex, loss of these neurons could reduce cholinergic activity in the cortex of Alzheimer's patients (Whitehouse, Price, Struble, Clarke, Coyle, and DeLong, 1982). The neuropathological evidence demonstrating an extensive loss of cholinergic function in the basal forebrain and cortex in SDAT has been widely confirmed by many investigators and is a relatively common feature of the disease.

Treatment strategies intended to facilitate cholinergic function in the CNS to treat dementia assume that a reduction in cholinergic function is the cause of dementia. There is, in fact, some support for this hypothesis. The degree of dementia and memory impairment that occurs in SDAT is correlated with the decrement in cortical cholinergic transmission (Perry, Tomlinson, Blessed Bergmann, Gibson, and Perry, 1978). In addition, however, loss of the

cholinergic system related to the nucleus basalis of Meynert and other basal forebrain nuclei is associated with dementia in other disorders including Parkinson's and Korsakoff's diseases (Nakano and Hirano, 1984; Arendt, Bigl, Arendt and Tennstedt, 1983), parkinsonism-dementia complex of Guam (Nakano and Hirano, 1983) and dementia pugilistica (Uhl, McKinney, Hedreen, White, Coyle, Whitehouse, and Price, 1982). These studies show that the loss of basal forebrain cholinergic neurons is not a neuropathological feature specific to SDAT. Therefore, cholinergic therapies developed for SDAT may also be useful in other clinical applications.

It is the apparent association between deterioration of the cholinergic system and dementia that is the basis for the cholinergic strategies for treating SDAT. In fact, some marginal facilitation of memory performance has been obtained in clinical tests with SDAT patients with physostigmine (Brinkman and Gershon, 1983; Davis and Mohs, 1982), a cholinesterase inhibitor that crosses the blood-brain barrier and has, therefore, central as well as peripheral effects. In another study, twelve patients with SDAT were treated with various oral doses of physostigmine, up to 2 mg, every 2 hours for 3-5 days. Of the ten patients who completed the study, three showed significant improvement at the highest dose that could be tolerated. Cortisol measures showed that clinical improvement was correlated with enhanced central cholinergic activity (Mohs, Davis, Johns, Mathe, Greenwald, Horvath, and Davis, 1985). In addition, there has been a report that tacrine (tetrahydroaminoacridine, THA), a reversible cholinesterase inhibitor with peripheral as well as central effects, produced cognitive improvement in several patients with SDAT (Summers, Majovski, Marsh, Tachiki, and Kling, 1986). At the present time, cholinesterase inhibitor therapy appears to be a promising approach to treating SDAT.

One of the major problems related to the development of anticholinesterase therapy in SDAT is the great potential for severely toxic effects. The somatic motor and autonomic nervous systems are greatly influenced by cholinesterase inhibitors which produce very disturbing and toxic peripheral side effects. One way to limit peripheral toxicity has been to propose direct intraventricular delivery of cholinergic drugs (Harbaugh, Roberts, Coombs, Saunders, and Reeder, 1985; Mattio, McIlhany, Giacobini, and Hallak, 1986). However, in the dog, more rapid and higher levels of cholinesterase inhibition in the cortex were produced by intravenous rather than intraventricular injection of physostigmine. Not surprisingly, intraventricular injections produced higher levels of physostigmine in regions located closely to the ventricular surface (Mattio et al., 1986; Andjelkovic, Beleslin, and Vasic, 1971). Another, more practical suggestion may be, however, the development of a cholinesterase inhibitor that has an inherent selectivity for CNS enzyme (Davies, 1981; Moss, Rodriguez, Selim, Ellett, Devine, and Steger, 1985b).

SULFONYL FLUORIDES

The purpose of the research reported here was to develop very long-lasting CNS selective cholinesterase inhibitors that might be suitable for treatment of SDAT and other CNS diseases that involve a decline in cholinergic function. Some sulfonyl fluorides have been discovered to be cholinesterase inhibitors that meet these criteria and, furthermore, appear to have remarkably low general toxicity (Moss et al., 1985b; Moss, Rodriguez, Herndon, Vincenti

and Camarena, 1986). Because of these special characteristics, these compounds may have some potential as therapeutic agents in SDAT and related CNS diseases.

The sulfonyl fluorides that react with cholinesterase are irreversible inhibitors that form a covalent sulfonyl-enzyme complex similar to the phosphonyl-enzyme complex formed by the organic phosphates (Fahrney and Gold, 1963; Kitz and Wilson, 1962; Myers and Kemp, 1954). These substances are, however, generally less reactive than organic phosphates (Fahrney and Gold, 1962; Myers and Kemp, 1954) and this may account for their selectivity. The sulfonyl fluorides, being less reactive, also to produce fewer noncholinesterase side effects such as "Ginger Jake" paralysis, a delayed neurotoxic effect produced by certain organophosphorus anticholinesterase agents in humans. This organophosphate-induced condition is characterized by polyneuritis, flaccid paralysis of the arms and legs, degeneration of myelin sheaths and axons of the spinal cord, somatomotor neurons and medulla and it does not appear to be the result of cholinesterase inhibition (Brimblecombe, 1974). The sulfonyl fluorides do not, however, produce peripheral neuropathy (Caroldi, Lotti, Masutti, 1984) and can, in fact, be used to protect against neurotoxic organophosphates (Johnson, 1980).

EXPERIMENTS IN RODENTS

Of the thirty-six compounds tested in vivo in rats, only five show significant inhibition of cholinesterase and all of these inhibit brain enzyme to a greater degree than cholinesterases from heart, ileum and pectoral muscle (Moss et al., 1985b; 1986). The reason for the apparent selectivity toward the CNS is unknown and, furthermore, there is no obvious relationship between the molecular structure and selectivity or activity against cholinesterase (Moss et al., 1986).

The two inhibitors that have been studied in greatest detail are phenylmethylsulfonyl fluoride (PMSF, phenylmethanesulfonyl fluoride, alpha-toluenesulfonyl fluoride) and methanesulfonyl fluoride (MSF). Both of these compounds produced an average of 90% inhibition of brain cholinesterase with less than 30 to 35% inhibition of enzyme in peripheral tissues when the drug was administered daily at a low dose (Figure 1)(Moss et al., 1985b). It is important to note that MSF produced this effect at 0.5 mg/kg as compared to 85 mg/kg for PMSF. MSF may, therefore, be the most clinically useful compound.

The remarkable difference between the central and peripheral effects observed in these experiments is the result of two factors: 1) the inherent selectivity of MSF and PMSF to inhibit CNS enzyme; and 2) the relatively slow rate at which CNS enzyme is synthesized. The direct effects produced by a single dose of PMSF, including the selectivity toward brain and the relatively slow resynthesis of CNS enzyme are shown in Figure 2. As with recovery from organophosphates, the rates of recovery are limited by the rates of synthesis of new enzyme in each tissue. From Figure 2, the synthesis of new enzyme in brain appears to occur with a mean half-time of approximately 11 days compared to 1, 3 and 6 days for ileum, heart and pectoralis, respectively.

In vital organs, there is a substantial excess of cholinesterase above the amount required for normal function, and less than 50%

inhibition is generally regarded as not pharmacologically significant (Brimblecombe, 1974). Using this figure as a rough guide, direct measurements of enzyme activity show that MSF and PMSF both produce pharmacologically significant CNS cholinesterase inhibition with limited peripheral effects. These data are supported by observations in rats which show there are no losses of strength or coordination and remarkably low general toxicity even after high doses over a long period of time (Moss et al., 1985b).

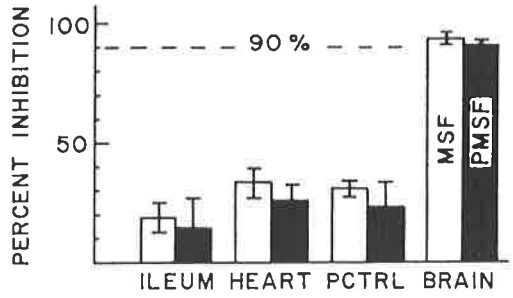

Figure 1. Cholinesterase inhibition in rat ileum, heart, pectoral muscle and brain produced by five daily administrations of PMSF (85 mg/kg) and MSF (0.5 mg/kg) by gavage The error bars represent one SEM. [Redrawn from Moss et al., 1985b]

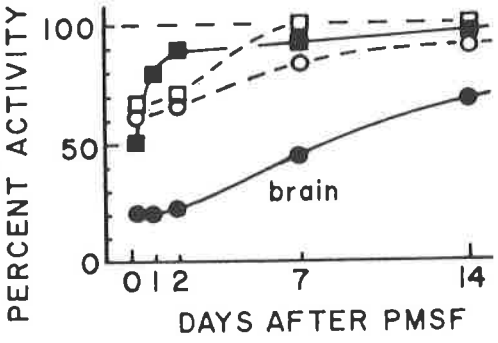

Figure 2. Time course of cholinesterase inhibition produced in rats by one injection of PMSF (100 mg/kg) in various tissues A time point of 4 hours is shown between 0 and 1 day. Ileum is represented by filled squares, heart by open squares, skeletal muscle (pectoralis) by open circles, and brain by filled circles. [Redrawn from Moss et al., 1985b].

In addition, the effects of MSF on mouse brain cholinesterase and acetylcholine content have been assessed. Treatment with 1.5 mg/kg MSF per day for three days caused up to 87% inhibition of forebrain cholinesterase and a significant increase in free acetylcholine levels for more than five days after the end of treatment[1]

[1]Personal communication, H. Kobayashi, Iwate University, Morioka Japan. To be presented to the Japanese Pharmacological Society (March 1988), by T. Nakano, H. Kobayashi, D.E. Moss and A. Yuyama.

EXPERIMENTS IN MONKEYS

In order to prepare for possible human tests with MSF as a therapeutic agent, preliminary experiments were undertaken to assess the efficacy and toxicity of this compound on young male monkeys (M. fasicularis). In this experiment on the effects of MSF on cerebrospinal fluid (CSF) cholinesterase, the monkeys were anesthetized (ketamine) and CSF was taken by lumbar puncture from each subject. Control samples, separated by several days, were taken to establish the level of cholinesterase activity present in each monkey prior to MSF treatment. Two days after the second control CSF sample was taken, all four monkeys were injected with one dose of 1.5 mg/kg MSF. CSF was sampled and analyzed for cholinesterase activity for several days after MSF treatment in order to establish the degree of inhibition produced by a single injection of 1.5 mg/kg MSF and, in addition, the rate at which enzyme is replaced in primates.

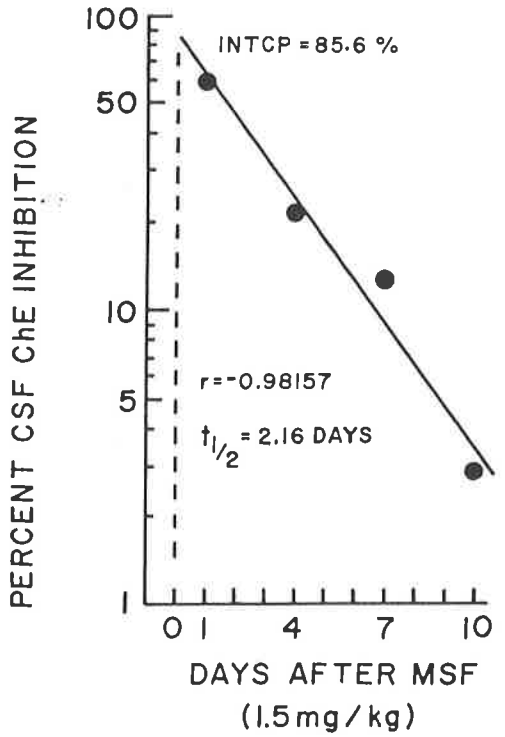

Figure 3. A pseudo-first order plot of recovery of CSF cholinesterase activity in CSF after 1.5 mg/kg MSF. 86% inhibition was produced by the single injection and the half-life for the recovery of enzyme activity (rate of new synthesis) was approximately 2.2 days.

After several months of no drug treatments, a long-term toxicity experiment was conducted. Intramuscular injections of MSF in sterile peanut oil were begun in two of the monkeys. Two others were injected with vehicle only and served as controls. The dose was increased over two months to 1.5 mg/kg which was continued for the following twelve injections (approximately five weeks). During

treatment with 1.5 mg/kg by intramuscular injections two to three times per week, blood samples were drawn several times. Erythrocyte cholinesterase levels in the MSF treated monkeys were so low that they could not be determined reliably on some days. However, there was no loss of appetite, no loss of body weight, and no general behavioral indications of toxicity (e.g., no lethargy or unusual behaviors).

Two to three days after the last injection, all of the monkeys were subjected to cortical biopsy to determine the CNS effect of MSF treatment. The subjects were anesthetized and a sample of about 0.05 grams of cortex was removed. Cortex samples taken from MSF treated monkeys showed approximately 80% inhibition of cholinesterase activity relative to the cortex samples taken from the untreated controls. In view of the data presented in Figure 3 showing 2.2 days as the half-time for the recovery of CSF cholinesterase, 80% inhibition of cortex enzyme up to three days after the last dose of MSF can only be explained by cortex enzyme being replaced more slowly than cholinesterase in the CSF.

At the time the cortical biopsies were taken, a sample of blood was again drawn from the femoral vein for the determination of a full clinical chemical blood profile identical to pretreatment profiles. There were no significant changes in the clinical blood profiles of the treated monkeys as compared to their pretreatment profiles or the untreated monkeys.

After a drug-free period of several weeks, a pilot dose-response experiment was conducted. In this experiment, monkeys were injected with various doses of MSF from 0.5 mg/kg to 3.0 mg/kg. Twenty four hours after the injection, CSF samples were taken and analyzed for cholinesterase activity. Using the pseudo first-order model (Figure 3), it was estimated that 54, 73, 86, and virtually 100 percent inhibition of CSF enzyme was produced by 0.5, 1.0, 1.5, and 3.0 mg/kg MSF. The level of CSF cholinesterase inhibition was linear with log-dose with a correlation of +0.9593 over the dose range.

The lowest dose tested, 0.5 mg/kg MSF, produced an effect similar to that estimated to be necessary for an optimum therapeutic effect in humans insofar as about 50% inhibition of CSF cholinesterase has been correlated with maximum memory improvement (Thal, Fuld, Masur, Sharpless, 1983.). At much higher doses, however, no toxic effects were observed. Even at the 3.0 mg/kg dose that resulted in an estimate of virtually 100 percent inhibition at the time of the injection and an experimental determination of 78 percent inhibition 24 hours later, there were no clear signs of cholinesterase inhibition toxicity. The highest dose, 3.0 mg/kg, did appear to produce lethargy and some illness but these symptoms were gone at 24 hours and did not require veterinary attention.

DISCUSSION

Methanesulfonyl fluoride was effective in inhibiting CSF cholinesterase well over 80% in primate cortex and CSF without toxic side effects as measured either by general behavior or clinical blood analysis. Except at 3.0 mg/kg MSF, the monkeys remained vigorous and active throughout the experiment. It is, therefore, possible to produce a pharmacologically significant level of CNS cholinesterase inhibition from MSF treatment without apparent toxicity

from peripheral cholinesterase inhibition. The monkeys used in the MSF toxicity tests have shown no motor signs of delayed neurotoxicity or other problems in the eleven months during which they received MSF.

The sulfonyl fluorides appear to be efficacious cholinesterase inhibitors with an inherent selectivity for the CNS. However, they have not been as thoroughly studied as other compounds. Although it has been shown that sulfonyl fluorides do not affect norepinephrine, dopamine, or serotonin content in the cortex of rats even after several weeks of administration (Moss et al., 1985b), other parameters have not been examined. The most important of these include assessment of the effects of MSF and PMSF on brain choline acetyltransferase, choline uptake, brain proteases, and direct effects on CNS acetylcholine receptors. Evaluation of these noncholinesterase effects will be informative insofar as PMSF and MSF do not, for example, produce the same extrapyramidal motor effects in rats as those observed with physostigmine (Moss, Rodriguez, and McMaster, 1985a; Rodriguez, Moss, Reyez, and Camarena, 1986). The reason for this behavioral difference between sulfonyl fluorides and physostigmine is not clear.

The sulfonyl fluorides as therapeutic agents in SDAT, of course, share some of the problems inherent in all cholinesterase inhibitor therapies. The first is that anticholinesterase therapy can be expected to be effective only in the presence of sufficient amounts of endogenous acetylcholine. Anticholinesterases can, therefore, be expected to be effective only relatively early in the disease, before deterioration of the CNS cholinergic system has progressed too far. Also, anticholinesterase agents may only have therapeutic effects when used in combination with other therapies. For example, the best results with anticholinesterase agents may be obtained when they are used with M2 muscarinic receptor antagonists to prevent the M2 receptors on presynaptic membranes from defeating the effect of the cholinesterase inhibitor by feedback inhibition of acetylcholine release (Mash, Flynn and Potter, 1985). Similarly, combining a cholinesterase inhibitor with precursors like choline or lecithin with drugs that enhance oxidative metabolism in the CNS may prove to produce significant enhancement of cognitive function (Bartus, Dean, Sherman, Sherman, Friedman, and Beer, 1981). Lastly, of course, the most successful use of anticholinesterase agents would be as an adjunct to other treatments that effectively stop the progression of the underlying pathophysiology of the disease, preferably early enough that the CNS cholinergic system is still largely intact.

Despite the strong rational basis and limited experimental support for the cholinergic hypothesis, research in cholinergic pharmacology has not produced an effective long-lasting treatment for Alzheimer's dementia. Several factors could account for this lack of success. Alzheimer's disease is a complex process that involves many neurotransmitter systems. Because of this, it may be that no therapeutic strategy based on one system can be successful. Similarly, the well documented loss of cholinergic function within the CNS is probably an epiphenomenon that occurs as a consequence of neuronal death. It may be unreasonable to expect any important therapeutic effects to be observed unless the underlying disease process can be arrested in an early stage, while enough of the essential neurotransmitter systems are still responsive to

therapeutic interventions. In spite of the marginal results obtained to date, however, further research is warranted and the results may have significant future clinical applications in SDAT or other diseases characterized by dementia and reductions in CNS cholinergic function.

CONCLUSIONS

Certain sulfonyl fluorides, particularly methanesulfonyl fluoride, are highly effective long-lasting CNS selective cholinesterase inhibitors that are remarkably low in general toxicity. These special qualities may make these unique compounds useful in treating SDAT.

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