Fluorofelbamate

Bryan A. Roecklein,* Harry J. Sacks,* Henry Mortko,* and James Stables†

**MedPointe Pharmaceuticals, Somerset, New Jersey 08873; and †National Institutes of Health, National Institute of Neurological Disorders and Strokes, Bethesda, Maryland 20892*

Summary: The incidence of refractory seizures has remained at 30–40%, even with the approval of nine new anticonvulsants over the past 12 years. In attempts to reduce seizure frequency and severity, physicians routinely resort to combining two or more anticonvulsants, ideally with different mechanisms of action. These combinatorial therapies are difficult to administer for both patient and caregiver and often result in tolerability issues. Hence, a broad spectrum anticonvulsant, with multiple mechanisms of action, that is well tolerated, would provide physicians with an important option in their armamentarium to control seizures. Felbamate initially fit this profile and was demonstrated to effectively control both partial and generalized seizures in clinical studies supporting registration.[1–5] Unfortunately, unanticipated idiosyncratic toxicity was observed after approval and the drug is now relegated to second or third line therapy, depending on patient history and seizure type. Epileptologists still prescribe this drug for refractory seizures, and a recent communication indicates that 35,000 to 46,000 new patients have tried Felbatol (MedPointe Pharmaceuticals, Somerset, NJ) since 1995.[6] The continued utilization of Felbatol, in light of its risk:benefit issues, highlights the need for new efficacious therapeutic options. Fluorofelbamate (MedPointe Pharmaceuticals), a phase I drug candidate, was designed to retain the broad spectrum multimechanistic activity of felbamate, with a modified metabolism that has demonstrated, *in vitro*, to avoid the production of the reactive metabolite believed to cause the idiosyncratic toxicity. This drug candidate is one of several carbamates either in development or currently on the market for treatment of seizures and other CNS disorders. **Key Words:** Carbamates, fluorofelbamate, felbamate, Felbatol, dicarbamates, anticonvulsant, metabolism, atropaldehyde, glutathione.

INTRODUCTION

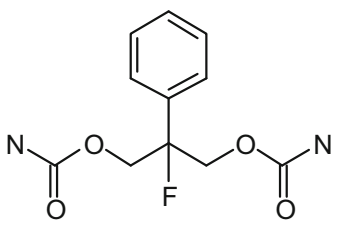
Fluorofelbamate (FFBM; MedPointe Pharmaceuticals, Somerset, NJ) is a new chemical entity that has demonstrated potent anticonvulsant and antiepileptic activity in animal pharmacology models. Based on rational design, it is one of numerous felbamate (FBM) analogues designed to have clinical efficacy similar to FBM without the idiosyncratic toxicity of the latter. Relative to monocarbamates, the dicarbamates consistently demonstrated superior seizure control in the animal models. The FFBM dicarbamate was selected for further development based on its broad spectrum of activity and physical properties. This new chemical entity differs from FBM in that fluorine is substituted for hydrogen in the two position of the propane chain (FIG. 1). This substitution increased the solubility, lowered the ED_{50} in numerous animal seizure models, and significantly modified the metabolism. As designed, it retains broad spectrum anticonvulsant activity and avoids the hydrolytic metabolic pathway that generates a reactive aldehyde (atropaldehyde) from FBM.

Address correspondence and reprint requests to: Bryan Roecklein, MedPointe Pharmaceuticals, 265 Davidson Avenue, Somerset, NJ, 08873. E-mail: BRoecklein@medpointpharma.com.

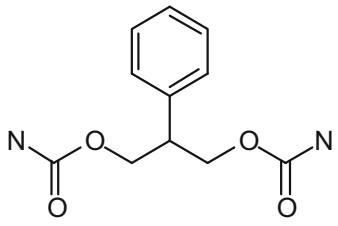
ANTICONVULSANT PROFILE

The anticonvulsant profile of FFBM was established using standardized *in vivo* and *in vitro* tests and the results compared with FBM and other antiepileptic drugs (not shown here). Fluorofelbamate is effective in all tested models of electrically induced seizures (TABLES 1 and 2), in particular the maximal electroshock test (MES) and 6 Hz models. In the 6 Hz model, 32 mA of current are typically used to induce seizures, as indicated in the legend for Table 1. However, investigators have subsequently demonstrated that stimulation at higher currents generates more refractory seizures that are often not inhibited by standard antiepileptic drugs. In contrast, FFBM further demonstrated reduction in seizure frequency when induced at a higher current, 44 mA, suggesting that it may, like its analogue felbamate, be effective against some refractory seizures.

Fluorofelbamate attenuates clonic seizures in mice induced by subcutaneous (s.c.) administration of the



Fluorofelbamate (FFBM)



Felbamate (FBM)

FIG. 1. Fluorofelbamate (FFBM) and felbamate (FBM) chemical structures. Fluorofelbamate contains a fluorine atom on the 2 position of the propane chain resulting in alternative metabolism and retention of broad spectrum anticonvulsant activity.

Cl^- ion channel blocker, picrotoxin. It also blocks sound-induced seizures in the audiogenic seizure-prone Frings mouse (TABLE 1). Although showing moderate protection around 100 mg/kg for reducing clonic seizures induced by subcutaneous pentylenetetrazol, an ED_{50} could not be calculated for FFBM at doses up to 160 mg/kg, which were greater than the median toxic dose (TD_{50}). It was ineffective against seizures induced by the $GABA_A$ antagonist bicuculline.

Fluorofelbamate was tested in the hippocampal kindled rat model of focal seizures. Rats were kindled to stage 5 behavioral seizures using a 10-sec train of 1 ms biphasic 200 μA (50 Hz) delivered (via electrodes implanted in the ventral hippocampus) every 30 min for 6 h (12 stimulations per day for 5 days). Fluorofelbamate reduced generalized seizures and after-discharge duration in a dose-dependent manner. At the low dose (25 mg/kg), FFBM attenuated generalized seizures and at the highest dose tested (100 mg/kg) inhibited focal seizures. The median effective dose was calculated to be 69 mg/kg. These data are summarized in Table 3.

When administered at doses of 100 and 200 mg/kg early in a self-sustaining status epilepticus animal model, FFBM reduced cumulative seizure duration from 393 $\pm$ 10 min to 15 $\pm$ 8 min and 2.4 $\pm$ 0.5 min, respectively. Fluorofelbamate (200 and 300 mg/kg) also significantly attenuated seizures when administered at a late stage of self-sustaining status epilepticus, which is typically refractory to treatment with conventional anticonvulsants.[7] Further, early treatment with FFBM delayed time to development of 5 spontaneous seizures and the frequency decreased over time with no seizures recorded after 2 months in contrast to control animals. This observation demonstrates that FFBM has disease modification properties in this status epilepticus animal model. Overall, the results of these studies support the concept that the broad spectrum anticonvulsant profile of FFBM is similar or slightly better than that of FBM. The ED_{50} of FFBM is equivalent to and/or three to eight times higher than that of FBM, depending on the animal model, and the protective index (TD_{50}/ED_{50}) of FFBM is approximately 167 in the rat MES model (TABLE 2). Minimal toxicity of FFBM in this series of animal seizure models was identified in mice using the rotorod test and in rats by observing overt evidence of ataxia and abnormal gait and stance.

NEUROPROTECTIVE ACTIVITY

Fluorofelbamate protects against chemically (sodium cyanide) induced ischemia in cultured hippocampal neurons, provides dose-dependent protection against hypoxic CA1 injury in hippocampal slices, reduces infarct volume against *in vivo* hypoxia in rat pups, and protects against subicular CA1 damage associated with transient global ischemia in gerbils.[8]

MECHANISM OF ACTION

Initial studies indicate that 100 μM FFBM causes a slight decrease in GABA-mediated responses and a slight but statistically significant decrease in responses to kainate- and NMDA-receptor activation using whole-cell current measures in mouse cortical neurons. At a

TABLE 1. *Anticonvulsant Activity in Mice – ED_{50} (mg/kg)*

	Electrical Seizure Models		Chemical and Genetic Seizure Models			
Test Article	MES	6 Hz (32mA)	scPTZ	scPIC	AGS	TD_{50}
Fluorofelbamate	20	76	>160	79.3	10.9	115
Felbamate	35.5	171	126	108	10	220

MES (maximum electric shock) stimulus = 50 mA, 60 Hz for 0.2 s using corneal electrodes; 6 Hz stimulus = 32 mA for 3 s using corneal electrodes; chemoconvulsants = scPTZ (subcutaneous pentylentetrazol), scPIC (picrotoxin); audiogenic seizures (AGS) = (Frings mouse); stimulus = sound (110 decibels for 20 s); minimal toxicity = rotorod test – inability to maintain equilibrium in three trials during one minute on the rotating rod (6 rpm)TD_{50}.

TABLE 2. *Anticonvulsant Activity in Rats – ED_{50} (mg/kg)*

Test Article	MES	scPTZ	TD_{50}	Protective Index for MES (TD_{50}/ED_{50})
Fluorofelbamate	3.0	>250	>500	>167
Felbamate	25.3	>250	>500	>20

MES (maximum electric shock) = 50 mA, 60 Hz for 0.2 s using corneal electrodes; Chemoconvulsants = scPTZ (subcutaneous pentylentetrazol), scPIC (picrotoxin); Minimal toxicity = assessed by observations of gait and ataxia.

100 μM concentration, FFBM caused a slight but significant decrease in voltage-dependent sodium currents in NIE-115 neuroblastoma cells, down to 81 ± 4% of control values at a holding potential of −60 mV. This decrease was higher with FFBM than an equivalent amount of felbamate. The mechanism of action of FFBM, however, cannot be completely explained by either interactions at glutamate receptors sites or sodium channels.

PHARMACOKINETICS

In pharmacokinetic studies, C_{max} and area under the curve (AUC) increased proportionally with single dose in rats and dogs. In rats, C_{max} was similar for males and females, while AUC was 20–30% higher in males in single dose studies. Repeated dosing in rats and dogs for 28 consecutive days demonstrated mean plasma concentration–time profiles for FFBM to be qualitatively similar on days 1, 14, and 28 between male and female animals at each dose level. The increase in C_{max} was generally proportional to dose in males and females on each day for both species. Bioavailability in rats was high, in the range of 82–125% at all doses tested. In dogs, C_{max} was reached in two to six h and along with AUC was similar between males and females. Half-lives ranged from 9 to 14 h in the rat and from 4 to 10 h in dogs.

TOXICOLOGY

Using a functional observational battery designed to assess neurological toxicities, doses of FFBM less than 500 mg/kg in rats produced no effects. At higher doses (500 to 1000 mg/kg) some deficits in hindlimb grip strength were noted. Tests for QTc prolongation using the Purkinje fiber assay and genotoxicity screening tests in standard models were negative. Telemetry studies in dogs did not detect any QTc prolongation or adverse effects on respiratory assessment with FFBM.

Acute toxicity studies in rats and dogs identified a no-observed-adverse-effect-level (NOAEL) of 500 mg/kg and 75 mg/kg, respectively, with FFBM. Definitive, 28-day GLP oral repeat dose studies demonstrated that dose levels of 50 and 100 mg/kg body weight were well tolerated in rats. Reversible weight loss (8.9%) was observed in female rats at 100 mg/kg; FFBM appeared to accumulate in the plasma over the initial 14 days of dosing with a roughly two- to threefold increase from day 1 to day 14. Ultimately, a NOAEL of 50 mg/kg body weight/day was established for male and female Sprague-Dawley rats.

The 28-day study in dogs revealed transient, but significant decreases in body weight in animals receiving 25–50 mg/kg body weight/day. No significant effects on serum liver enzymes were observed for any treated animals. The mean half-lives ranged from 3.0 to 14.3 h and the results for AUC_{last} suggested that FFBM does not accumulate after daily administration to dogs. In summary, FFBM was well tolerated by rats and dogs at oral doses up to 200 mg/kg (rats) and 50 mg/kg (dogs). All signs of toxicity were reversible in the recovery groups.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Plasma AUC values in rats were comparable for males and females indicating similar bioavailability of [^{14}C]-FFBM derived radioactivity. Tissue C_{max} and AUCs were highest for the gastrointestinal tract and lowest for fat and bone. Plasma elimination half-lives ($t_{1/2}$) of 40 h for males and 80 h for females were reported and tissue half-lives ranged from 40 to 50 h in males and 60 to 70 h in female tissues. Excretion was rapid with the majority excreted within 24 h. Urinary excretion was the primary route of elimination with ninefold higher radioactivity than feces.

METABOLISM (FIG. 2)

In the case of FBM, a reactive aldehyde metabolite, atropaldehyde (ATPAL) is produced from 3-carbamoyl-2-phenylprionaldeyde (CBMA) and is thought to be responsible for the rare cases of aplastic anemia and liver toxicity. The CBMA is in dynamic equilibrium with 4-hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one (CCMF). Incubation of CBMA and CCMF with glutathione (GSH) leads to complete formation of adducts over a 24-hour period.[9]

TABLE 3. *Fluorofelbamate Dose Response in Hippocampal Kindled Rats*

Dose (mg/kg)	No. Protected/ No. Tested	Seizure Score ±S.E.M.	After Discharge Duration (s) ±S.E.M.
25	0/7	3.9 ± 0.7	59.29 ± 9.62
50	3/7	2.9 ± 0.83	68.57 ± 20.14
100	7/8	1.5 ± 0.57	29.00 ± 10.86

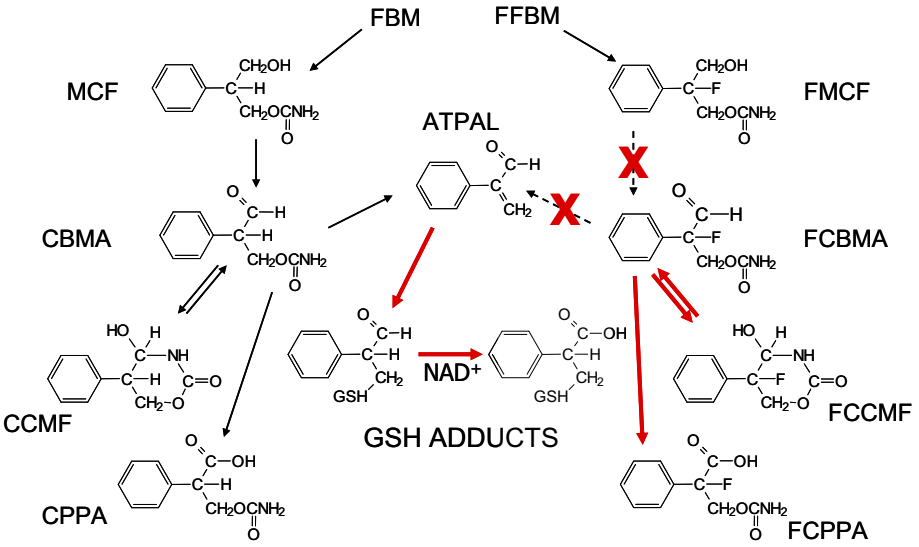


FIG. 2. Felbamate (FBM) metabolic pathway leading to the formation of atropaldehyde-glutathione (ATPAL-GSH and ATPA-GSH), a reactive aldehyde. The left hand side of the scheme outlines the established metabolic pathway for FBM. In contrast, the right hand scheme is a putative metabolic pathway if one assumes that fluorofelbamate (FFBM) hydrolyzes to FMCF (fluorinated 2-phenyl-1,3-propanediol monocarbamates). Results from the in vitro studies demonstrate that fluorofelbamate metabolism is blocked at the oxidation of FMCF preventing the formation of fluorinated 3-carbamoyl-2-phenylprionaldeyde (FCBMA) These results confirm that the addition of the fluorine atom prevents formation of reactive intermediates at two steps; FMCF to 3-carbamoyl-2-phenylprionaldeyde (CBMA) is blocked and if small quantities of FCBMA is generated, β-elimination does not occur to form ATPAL. (F-CCMF = fluorinated 4-hydroxy-5-phenyltetrahydro-1,3-oxazin-2-one; FCPPA = fluorinated 3-carbamoyl-2-phenylpropionic acid; NAD^+ = nicotinamide adenine dinucleotide.)

In contrast, this investigation demonstrated that when radiolabeled GSH is incubated under similar conditions, with the fluorinated derivative of CCMF (F-CCMF), spontaneous formation of adducts was not detected in the absence of cellular constituents.[10]

Experiments were carried out using pooled human liver S9 fractions with and without cofactors. Analysis of S9 supernatants that contained GSH, using a HPLC-atmospheric pressure chemical ionization-mass spectrometer, indicated that no ATPAL was produced from F-CCMF. Conversely an ATPAL-GSH adduct was detected when experiments were conducted with CCMF. When nicotinamide adenine dinucleotide (NAD^+) was added, CPPA (3-carbamoyl-2-phenylpropionic acid and the acid form of the ATPAL adduct were generated from CCMF. When F-CCMF was used, only F-CPPA was detected and once again there were no detectable adducts and no detectable nonfluorinated CPPA or CCMF.

Similar studies with MCF (2-phenyl-1,3-propanediol monocarbamate) and F-MCF demonstrated additional differences in metabolism. While CCMF and CPPA were generated from MCF, when F-MCF was used there was no production of F-CCMF or F-CPPA. Furthermore, no defluorinated compounds, CPPA or CCMF, were detected. More importantly, no adducts were identified, suggesting that no reactive metabolites were generated.

Overall, these results suggest that F-MCF does not enter the pathway that can generate reactive metabolites from the nonfluorinated analogue. Additionally, even if small quantities of F-CCMF or F-CBMA were generated, these metabolites are not cytotoxic and, as demonstrated above, will not form ATPAL adducts. Combined, these data indicate that FFBM utilizes different metabolic pathways than FBM and does not form reactive intermediates (e.g., ATPAL) from human liver preparations.[10]

CLINICAL STUDY IN HUMANS

A phase I single dose ascending study was recently performed in male volunteers. Doses ranged from 7.5 mg to 180 mg and were based on the NOAEL of 5 mg/kg from dogs with a maximum dose of approximately 3 mg/kg. Blood sample collection went out to 72 h. No clinical signs of toxicity were observed at any dose. The exposure was dose-proportional and initial evaluation indicated linear pharmacokinetics. Though the numbers were small, there were no apparent differences among races and/or ethnicities. The half-life was approximately 16.7 h, slightly lower than the 22–23 h observed for felbamate and a T_{max} on 1.10 h.

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