

NMDA Receptor Antagonist Felbamate Reduces Behavioral Deficits and Blood–Brain Barrier Permeability Changes after Experimental Subarachnoid Hemorrhage in the Rat

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ABSTRACT

Increased levels of glutamate and aspartate have been detected after subarachnoid hemorrhage (SAH) that correlate with neurological status. The NMDA receptor antagonist felbamate (FBM; 2-phenyl-1,3-propanediol dicarbamate) is an anti-epileptic drug that elicits neuroprotective effects in different experimental models of hypoxia-ischemia. The aim of this dose-response study was to evaluate the effect of FBM after experimental SAH in rats on (1) behavioral deficits (employing a battery of assessment tasks days 1–5 post-injury) and (2) blood–brain barrier (BBB) permeability changes (quantifying microvascular alterations according to the extravasation of protein-bound Evans Blue by a spectrophotofluorimetric technique 2 days post-injury). Animals were injected with 400 μ L of autologous blood into the cisterna magna. Within 5 min, rats received daily oral administration of FBM (15, 30, or 45 mg/kg) for 2 or 5 days. Results were compared with sham-injured controls treated with oral saline or FBM (15, 30, or 45 mg/kg). FBM administration significantly ameliorated SAH-related changes in Beam Balance scores on days 1 and 2 and Beam Balance time on days 1–3, Beam Walking performance on days 1 and 2, and Body Weight on days 3–5. FBM also decreased BBB permeability changes in frontal, temporal, parietal, occipital, and cerebellar cortices; subcortical and cerebellar gray matter; and brainstem. This study demonstrates that, in terms

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of behavioral and microvascular effects, FBM is beneficial in a dose-dependent manner after experimental SAH in rats. These results reinforce the concept that NMDA excitotoxicity is involved in the cerebral dysfunction that follows SAH.

Key words: behavioral deficits; blood–brain barrier; cognitive deficits; felbamate; NMDA receptor; subarachnoid hemorrhage

INTRODUCTION

DESPITE SIGNIFICANT IMPROVEMENTS in recent decades in the management of patients with aneurysmal subarachnoid hemorrhage (SAH), including early aneurysm surgery, endovascular techniques, and improved intensive care, the disease is still associated with significant mortality and long-term neurological morbidity and cognitive deficits (Germanò et al., 1997, 1998a; Saveland et al., 1992). These impairments have been ascribed mainly to ischemic brain injury occurring either during the initial bleeding episode or as a consequence of macro- and microvascular dysfunction and delayed ischemic deterioration (d'Avella et al., 1994, 1996; Delgado et al., 1985, 1986; Doczi, 1985; Doczi et al., 1986; Germanò et al., 1992, 2000; Jackowski et al., 1990; Johshita et al., 1990; Sasaki et al., 1985, 1986; Siesjo, 1992a,b; Zuccarello, 1989). Detailed knowledge about the pathological ischemic mechanisms in patients with SAH is critical to develop more efficient treatments. To this end, pathophysiological and experimental data together have significant clinical implications for the management of aneurysm patients and for investigating the rationale of new pharmacological approaches.

Substantial evidence has accumulated supporting the role of excitatory amino acid (EAA) neurotransmitters in the pathogenesis of the global brain dysfunction that follows SAH. Recently, bedside intracerebral microdialysis monitoring of patients with SAH exhibiting signs of delayed ischemia revealed dramatic changes in extracellular concentrations of excitatory amino acids, including glucose, piruvate, lactate, glycerol, and glutamate, confirming that these characteristic metabolic changes occur during vasospasm (Unterberg et al., 2001; Zouh et al., 1996). Moreover, a correlation between EAA concentrations and patient clinical status has been demonstrated (Enblad et al., 1996; Hutchinson et al., 2000; Inage et al., 2000; Nilsson et al., 1996, 1999; Persson et al., 1996; Sarrafzadeh et al., 1998, 2002; Saveland et al., 1996; Schulz et al., 2000; Staub et al., 2000).

Felbamate (FBM; 2-phenyl-1,3-propanediol dicarbamate) is a new, potent, nonsedative, anti-epileptic drug that elicits neuroprotective effects (*in vitro* and *in vivo*) in different experimental model of hypoxia-ischemia. In particular, FBM was protective against hypoxic damage induced in slices of rat hippocampus (Rekling, 2003;

Wallis et al., 1990, 1992). In neonatal rats, FBM reduced cortical infarct and hippocampal necrosis following bilateral cortical ligation and hypoxia (Arcadi et al., 1997; Sbuaiib, et al., 1996; Wasterlain et al., 1996). FBM administration reduced delayed hippocampal neuronal death induced by transient cerebral ischemia in the Mongolian gerbil (Wasterlain et al., 1992). Several mechanisms of action for FBM have been identified, including inhibition of voltage-sensitive sodium and calcium channels, potentiation of γ -amino-butyric acid (GABA)-mediated chloride currents, and interaction with the NMDA receptor complex through inhibition of the strychnine-insensitive glycine binding site (McCabe et al., 1993; Świader et al., 2003; Taylor et al., 1995). Specifically, FBM reduces excitatory glutamatergic neurotransmission and intracellular calcium concentrations (Soderpalm, 2002).

Our laboratory has developed an experimental rat model of SAH that has provided extensive information about changes induced by intracisternal blood injection on hemodynamic, angiographic, biochemical, pathophysiological, and acute and chronic behavioral parameters, and parallels those changes seen in humans after SAH (d'Avella et al., 1990, 1994, 1996; Germanò et al., 1992, 1994, 2000). This model has also enabled our laboratory to study the effects of systemic administration of different molecules on these parameters (d'Avella et al., 1993; Germanò et al., 1998b, 2002; Imperatore et al., 2000).

The purpose of the present study was to evaluate the effects of FBM administration on behavioral and microvascular changes in this *in vivo* rodent model of SAH. In these dose-response studies, we first evaluated the effect of FBM on the behavioral consequences of SAH by employing a battery of well-characterized assessment tests over a 5-day observation period. In the second experiment, we measured the effect of FBM on microvascular blood–brain barrier (BBB) permeability changes 2 days after SAH by quantifying the extravasation of protein-bound Evans Blue using a spectrophotofluorimetric technique in different areas of the brain parenchyma.

METHODS

The experimental protocol was approved by the Ethical Committee on the Care and Use of Laboratory Ani-

mals at our Institution, and conforms to the University of Messina guidelines for the care and the use of animals in research.

Experimental Design and Induction of Subarachnoid Hemorrhage

Studies were conducted using 96 male Albino Sprague-Dawley rats (Charles River Italia SpA, Calco, Lecco, Italy), weighing approximately 250 g. Animals were housed (four per cage, which measured 580 × 385 × 200 mm) at a constant temperature of 22°C, under a 12-h, light–dark cycle (light switched on at 6:00 a.m.), with free access to food and water.

Rats were divided into eight experimental groups ($n = 12$). Rats were randomly assigned to one of the eight groups. Groups I (sham-injured + saline) and V (SAH + saline) served as controls—the latter to evaluate the effects of SAH on the investigational parameters. Groups I–IV (sham-injured + saline, sham-injured + FBM 15 mg/kg, sham-injured + FBM 30 mg/kg, and sham-injured + FBM 45 mg/kg) were used to evaluate the possible effects of vehicle and FBM administration on the investigational parameters in sham-injured rats. Groups V–VIII (SAH + saline, SAH + FBM 15 mg/kg, SAH + FBM 30 mg/kg, and SAH + FBM 45 mg/kg) were necessary to compare the effects of FBM administration on SAH-induced changes in investigational parameters. The experiments were conducted as follows: on day –1, baseline pre-assessment for the behavioral tasks was performed. On day 0, animals underwent SAH or sham surgery. During days 1–5, rats were subjected to the behavioral tests. On day 2, six animals from each group were sacrificed to assess BBB permeability changes. These time points were chosen because we have shown that significant behavioral changes peak 24 h after SAH and last for up to 5 days (Germanò et al., 1994, 1998b, 2002; Imperatore et al., 2000). We choose to assess BBB permeability changes 2 days after injury because previous experiments conducted in our laboratory demonstrated that significant BBB alterations peaked at 48 h (Germanò et al., 1992, 1998b, 2000, 2002; Imperatore et al., 2000).

Surgical Procedures

All procedures were performed under intraperitoneal ketamine (Ketalar, Parke-Davis; 150 mg/kg in a volume of 3 mL/kg) anesthesia. Rectal temperatures were controlled at approximately 37°C using heat lamps. SAH was induced in 48 rats (Groups V–VIII) by administering autologous blood into the subarachnoid space via the cisterna magna. Details of the procedure have been previously published (d'Avella et al., 1990, 1993, 1994, 1996;

Germanò et al., 1992, 1994, 1998b, 2000, 2002; Imperatore et al., 2000). Briefly, the atlanto-occipital membrane was exposed through a midline occipital incision. For simulation of SAH, 400 μ L of autologous arterial non-heparinized blood was injected over a period of approximately 30 sec into the cisterna magna via a 30-gauge needle fitted to a 500- μ L Hamilton syringe.

Sham surgery was performed in 48 rats (Group I–IV). Specifically, the atlanto-occipital membrane was exposed through a midline occipital incision and punctured as described above. No intracisternal injection was made since previous studies performed in our laboratory using this rodent model demonstrated that mock CSF-injected rats (using Ringer's solution) did not display any enduring behavioral detriment. In addition, there was no appreciable change in BBB quantitative assessment in these animals as compared to control un-injected rats (Germanò et al., 1992).

Drug Administration

Within 5 min of sham-injury (Groups II–IV) or SAH (Groups VI–VIII), rats received oral administration of FBM (generously provided by Shering Plough, Milano, Italy) as a bolus at a dose of 15 mg/kg (Groups II and VI), 30 mg/kg (Groups III and VII), or 45 mg/kg (Groups IV and VIII). Rats in Group I and V received vehicle (sterile saline) as a bolus. Rats were given oral FBM or oral saline for either 2 or 5 consecutive days by gavage, according to the general treatment plan. This dose regimen was selected on the basis of previous clinical and experimental evidence demonstrating the pharmacodynamic, pharmacokinetic, and toxicological profile of FBM (Adusumalli et al., 1991; Graves et al., 1989; Lepik et al., 1993; Palmer et al., 1993; Wallace Laboratories, 2003).

Behavioral Assessment Protocol

Beam Balance Test, Beam Walking Test, and measure of body weight were used to characterize the enduring behavioral deficits over a 5-day period after SAH or sham surgery, and the effect of FBM on these deficits. Baseline pre-assessment of the behavioral tasks was performed on day –1 prior to SAH or sham surgery. These tests have been extensively used in our laboratory in this experimental rodent SAH model, and we have reported significant behavioral changes that peak 24 h after SAH that last for up to 5 days (Germanò et al., 1994, 1998b, 2002; Imperatore et al., 2000).

Beam balance. The Beam Balance Test is a task that assesses both motor and vestibular functioning by quantifying the animal's ability to balance on a narrow

TABLE 1. BEAM BALANCE RATING SCALE

Score	Behavior
1	Balances with steady posture
2	Grasps sides of beam and/or has shaky movements
3	Hugs the beam or slips or spins on the beam
4	Attempts to balance on the beam but falls off
5	Falls off: no attempt to balance or hang on the beam

T1 → wooden beam (1.0 cm wide) for up to 60 sec. For assessing Beam Balance capacity, rats received a score according to the Beam-Balance rating scale (Table 1). In addition, the duration of time the animals remained on the beam was recorded for up to 60 sec. Data for each daily session consisted of the mean \pm standard deviation (SD) of three consecutive trials.

Beam walking. The Beam Walking Test is a learned avoidance test similar to that used by Feeney et al. (1982). This task evaluates the somatomotor, motivational, and attentional functions together with memory and locomotor activities. Rats are trained with a negative reinforcement paradigm, in which termination of the adverse stimuli (loud white noise and bright light) serve as a reinforcement reward. During both training (day -1) and testing (days 1-5), animals were placed on one end of an elevated (1 m) narrow wooden beam (120 \times 5 cm) across from the source of the noise and light. The difficulty of the task was increased by placing four equally spaced pegs (5 cm in height) along the top of the beam. Noise and light were turned off immediately after the animal traversed the beam and entered a darkened goal box (30 \times 15 \times 18 cm) with its two forelegs. The time taken to traverse the beam was recorded. Data for each daily session represented the mean \pm SD of three consecutive trials.

Body weight. Body weight, a gross measure of food and water intake, is a parameter that assesses the appetite drive and the occurrence of motivational deficits. Rats were pre-weighed the day before the SAH or sham-injury (day -1), and weight was recorded daily for the following 5 days.

Blood-Brain Barrier Evaluation Protocol

The BBB assessment protocol was conducted 48 h after the SAH or sham surgery in six animals per group. We choose this time interval because previous experiments conducted in our laboratory have demonstrated significant BBB alterations beginning 36 h after SAH, peaking at 48 h, and lasting for up to 60 h (Germanò et al., 1992, 1998b, 2000, 2002; Imperatore et al., 2000).

We quantitatively evaluated the vascular permeation of Evans Blue with a fluorescence spectrophotometer technique according to the measurement protocol of Uyama et al. (1998) and the extraction technique of Rössner and Tempel (1996), which was modified in our laboratory. Briefly, 2% Evans-Blue in saline in a volume of 5 mL/kg was intravenously administered through the cannulated jugular vein and allowed to circulate for 60 min. The chest of the animal was opened and to remove the intravascular dye, the animals were perfused with saline through the left ventricle at 110 mm Hg pressure until colorless perfusion fluid was obtained from the right atrium. The whole brain was removed, and the following regions were dissected as defined by the Paxinos and Watson atlas (1982) and weighed: frontal, temporal, parietal, occipital, and cerebellar cortices; subcortical and cerebellar gray matter; and brain stem nuclei. Each brain region was homogenized in 1 mL of 50% trichloroacetic acid (w/v) and centrifuged (10,000 rpm) for 20 min. One milliliter of the supernatant was added to 1.5 mL of the solvent (50% trichloroacetic acid/ethanol, 1:3). An FP-920 fluorescence detector Jasco (Jasco Corp., Tokyo, Japan) was used at an excitation wavelength of 620 nm (bandwidth 10 nm) and an emission wavelength of 680 nm (bandwidth 10 nm). Calculations were based on external standards in the solvent (10-500 ng/mL). Data are expressed as mean \pm SD for each μ g of extravasated Evans Blue/gram of tissue.

Statistical Analysis

Differences among groups were analyzed using parametric and non-parametric methods using analysis of variance (ANOVA) followed by parametric Dunnett test (Beam Balance time, Beam Walking, Body Weight, and BBB permeability) and Steel test (nonparametric Dunnett type test: Beam Balance scores). Data were corrected with Bonferroni adjustments when needed. Statistical significance was set at a *p*-value < 0.05.

RESULTS

General observations and systemic physiologic evaluations concerning this experimental model (MABP, arterial blood gas levels, blood pH, plasma glucose levels, and body temperature as monitored throughout the experimental procedure) have been described in detail elsewhere (D'Avella et al., 1990, 1993, 1996; Germanò et al., 1992, 1994, 1998b, 2000, 2002; Imperatore et al., 2000). Briefly, rats tolerated the procedure well, and no signs of acute neurological dysfunction were noted. In rats sacrificed on the second day after SAH, a blood clot was still clearly identifiable in the cisterna magna and in

the basal cisterns. No extradural hemorrhages were found, while the presence of blood in the ventricles was detected in approximately 25% of cases. In rats sacrificed on day 5 post-SAH, no blood clot was visible in the cisterna magna or in other brain loci. Ketamine, vehicle, or FBM administration per se did not induce any significant alteration in the rodent physiologic parameters, neither in control animals nor in SAH rats.

Behavioral Assessment

None of the experimental groups differed significantly from one another in baseline pre-injection assessments (day -1) (Figs. 1–4).

scores ($p < 0.05$) and on days 1–3 for beam balance time ($p < 0.05$), as compared to sham-injured saline-treated animals. SAH-injured 45 mg/kg FBM-treated animals (Group VIII) exhibited a significantly improved beam balance score on days 1 ($p < 0.01$) and 2 ($p < 0.05$) as compared to SAH-injured saline-treated animals. SAH-injured 30 mg/kg FBM-treated animals (Group VII) exhibited a significantly improved beam balance score on day 2 ($p < 0.05$), compared to SAH-injured saline-treated animals (Fig. 1). SAH-injured animals who received 15, 30, or 45 mg/kg FBM (Groups VI–VIII) exhibited a significantly improved beam balance time on days 1–3 ($p < 0.05$) as compared to SAH-injured saline-treated animals (Fig. 2).

Beam walking. Vehicle and FBM administration did not induce any significant beam walking alteration in sham-injured animals (Fig. 3; groups I–IV). The SAH-injured saline-treated rats (Group V) exhibited signifi-

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Beam balance. Vehicle and FBM administration (Groups I–IV) did not induce any significant beam balance alteration in sham-injured animals (Figs. 1 and 2). The SAH-injured saline-treated rats (Group V) exhibited significant deficits on days 1 and 2 in beam balance

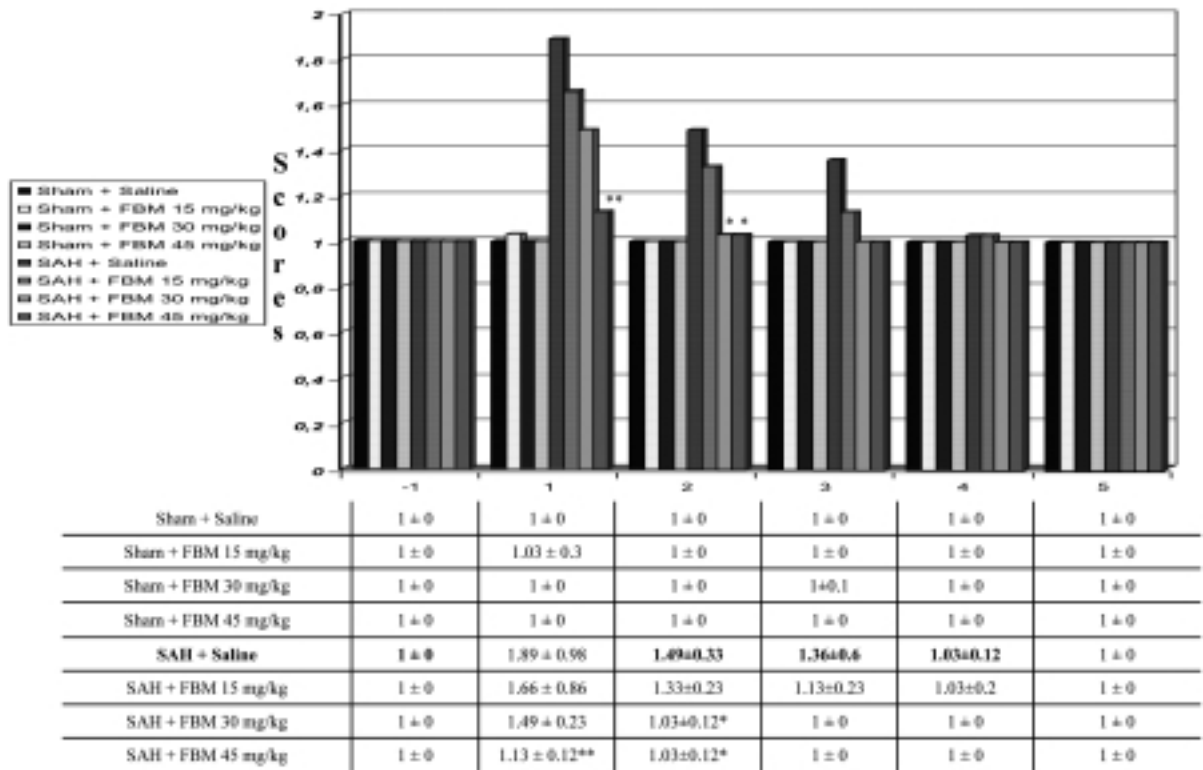


FIG. 1. Bar graph showing beam balance test scores for each daily time point (day -1 to day 5). The values shown are the means of three consecutive trials ± SD for each group. * $p < 0.05$ and ** $p < 0.01$ SAH + saline (Group V) vs. SAH + FBM 15, 30, and 45 mg/kg groups (Groups VI–VIII). Vehicle and FBM administration did not induce any significant change in beam balance score in sham-injured animals. The SAH-injured saline-treated rats had significant deficits in beam balance scores on days 1 and 2, compared to sham-injured saline-treated animals. SAH-injured 45 mg/kg FBM-treated animals had a significantly improved score on days 1 and 2, and SAH-injured 30 mg/kg FBM-treated animals had a significantly improved beam balance score on day 2, compared to SAH-injured saline-treated animals.

EFFECTS OF FBM AFTER EXPERIMENTAL SAH

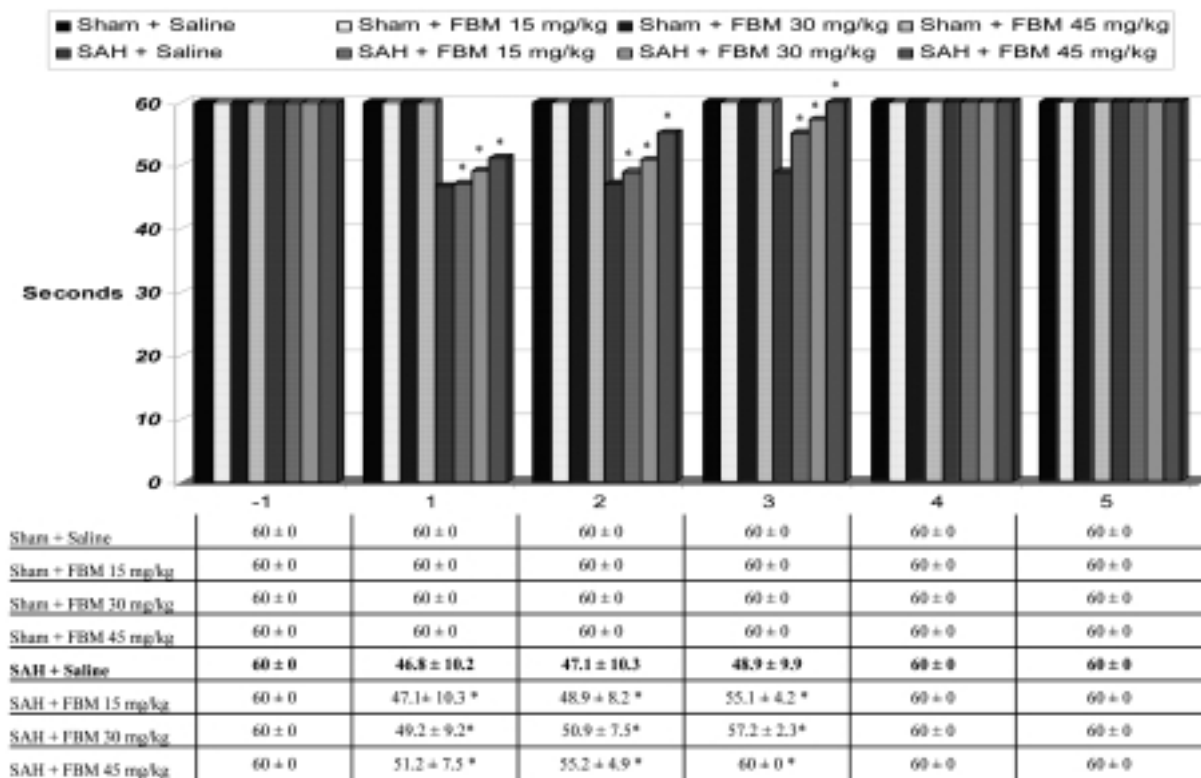


FIG. 2. Beam-balance test. Bar graph comparing the time (in seconds) animals remained on the beam between all groups of rats at daily time points (days -1 to 5). The values shown are means of three consecutive trials ± SD of the means for each group. * $p < 0.05$, SAH + saline vs. SAH + FBM 15, 30, and 45 mg/kg groups (Groups VI–VIII). Vehicle and FBM administration did not induce any significant change in beam balance time in sham-injured animals. The SAH-injured saline-treated rats exhibited significant deficits in beam balance time on days 1–3, compared to sham-injured saline-treated animals. SAH-injured animals who received 15, 30, or 45 mg/kg FBM (Groups VI–VIII) had significantly improved beam balance time on days 1–3, compared to SAH-injured saline-treated animals.

cantly increased latency to traverse the beam on days 1–4 (day 1, $p < 0.001$; day 2, $p < 0.01$; days 3–4, $p < 0.05$), compared to sham-injured saline-treated animals. In SAH-injured animals who received 15, 30, or 45 mg/kg FBM, latency to traverse the beam was significantly reduced on days 1 and 2 (day 1 FBM 45 and 30 mg/kg, $p < 0.05$; FBM 15 mg/kg, $p < 0.01$; day 2 $p < 0.05$), compared to SAH-injured saline-treated rats.

Body weight. Vehicle and FBM administration did not induce any significant body weight alteration in sham-injured animals (Groups I–IV). The SAH-injured saline-treated rats (Group V) exhibited significant decreased body weight on days 1–5 ($p < 0.05$), as compared to sham-injured saline-treated animals. Administration of 45 mg/kg FBM significantly reduced the SAH-related loss in body weight on days 3–5 ($p < 0.05$), as compared to SAH-vehicle treated rats. Conversely, administration of 15 or 30 mg/kg FBM did not affect the SAH-related

loss in body weight, as compared to SAH-vehicle treated rats (Fig. 4).

Blood–Brain Barrier Evaluations

Table 2 summarizes the mean concentration ± SD of extravasated Evans Blue dye (expressed as micrograms per gram of brain tissue) for all loci examined in the eight experimental groups. In sham-injured saline-treated rats, baseline levels of Evans Blue ranged from 2.075 ± 0.45 , to 2.787 ± 0.09 (Group I). Values obtained in sham-injured and SAH-injured saline-treated animals were consistent with those described in previous experiments performed in our laboratory (D’Avella et al., 1990, 1993, 1996; Germanò et al., 1992, 1994, 1998b, 2000, 2002; Imperatore et al., 2000). As compared with sham-injured saline-treated animals, in SAH-injured saline-treated rats (Group V), Evans Blue dye extravasation was significantly increased ($p < 0.001$) in the frontal, temporal, parietal, occipital, and cerebellar cortices, subcortical gray matter, cerebellar nuclei,

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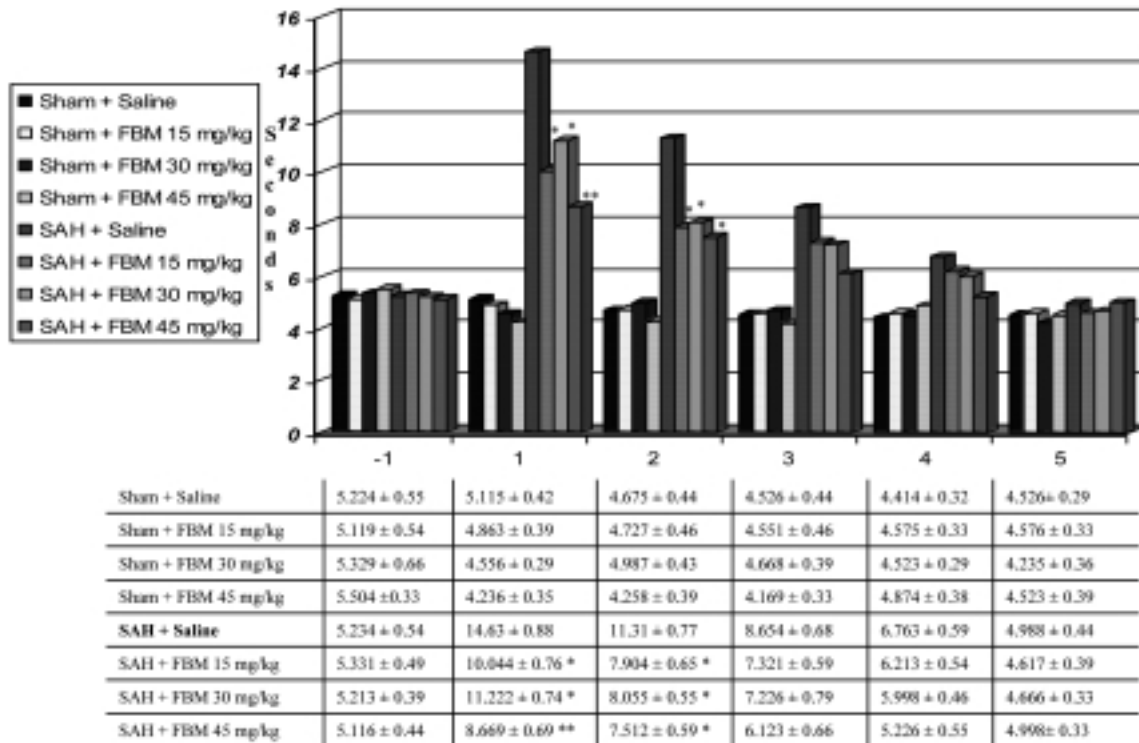


FIG. 3. Beam-walking test. Bar graph showing time (in seconds) taken to traverse the beam at daily time points (days -1 to 5). The values shown are means of three consecutive trials \pm SD of the means for each group. * $p < 0.05$ and ** $p < 0.01$ SAH + saline vs. SAH + FBM groups. Vehicle and FBM administration did not induce any significant beam walking alteration in sham-injured animals. The SAH-injured saline-treated rats had a significantly increased latency to traverse the beam on days 1-4, compared to sham-injured saline-treated animals. In SAH-injured animals who received 15, 30, or 45 mg/kg FBM, latency to traverse the beam was significantly reduced on days 1-2, as compared with SAH-injured saline-treated rats.

and brainstem (Group V). In sham-injured animals, FBM administration *per se* did not cause any significant change in Evans Blue dye extravasation (Groups II-IV). As compared to the SAH-injured saline-treated animals, 15 mg/kg FBM administration (Group VI) significantly decreased the SAH-induced BBB permeability changes in frontal, temporal, and parietal cortices, and brainstem ($p < 0.01$); 30 mg/kg FBM administration (Group VII) significantly decreased the SAH-induced BBB permeability changes in frontal, temporal, and cerebellar cortices, subcortical gray matter, and brainstem ($p < 0.01$), and parietal cortex ($p < 0.05$); and 45 mg/kg FBM administration (Group VIII) significantly decreased the SAH-induced BBB permeability changes in frontal, temporal, parietal, and occipital cortices, subcortical gray matter, cerebellar nuclei, brainstem ($p < 0.01$), and cerebellar cortex ($p < 0.01$).

DISCUSSION

In this study, we evaluated the effects of FBM in a rodent model of SAH by assessing the (1) behavioral

changes over a 5-day period and (2) BBB function 48 h after SAH. FBM significantly reduced SAH-related behavioral alterations and microvascular BBB breakdown in a dose-dependent manner. In sham-injured animals, FBM administration did not demonstrate any significant effect on the chosen investigational parameters. The data reported here may also have potential clinical implications for the management of SAH patients.

Considerable clinical and experimental work has shown that SAH induces focal and generalized disturbances of several brain functions, such as reduced cerebral blood flow (d'Avella et al, 1996; Delgado et al., 1986; Jackowski et al, 1990; Prunel et al, 2003, 2004), impaired control of vascular autoregulation (d'Avella et al., 1994; Delgado et al., 1985; Sasaki et al., 1982, 1985, 1986), BBB dysfunction (d'Avella et al., 1994; Doczi et al., 1985, 1986; Germanò et al., 1992, 1998b, 2000, 2002; Gules et al, 2003; Imperatore et al., 2000; Zuccarello et al., 1989), brain edema and increased intracranial pressure (ICP) (Jackowski et al, 1990), and free-radical generation associated with lipid peroxidation phenomena (d'Avella et al., 1990, 1994; Doczi, 1985; Doczi et al.,

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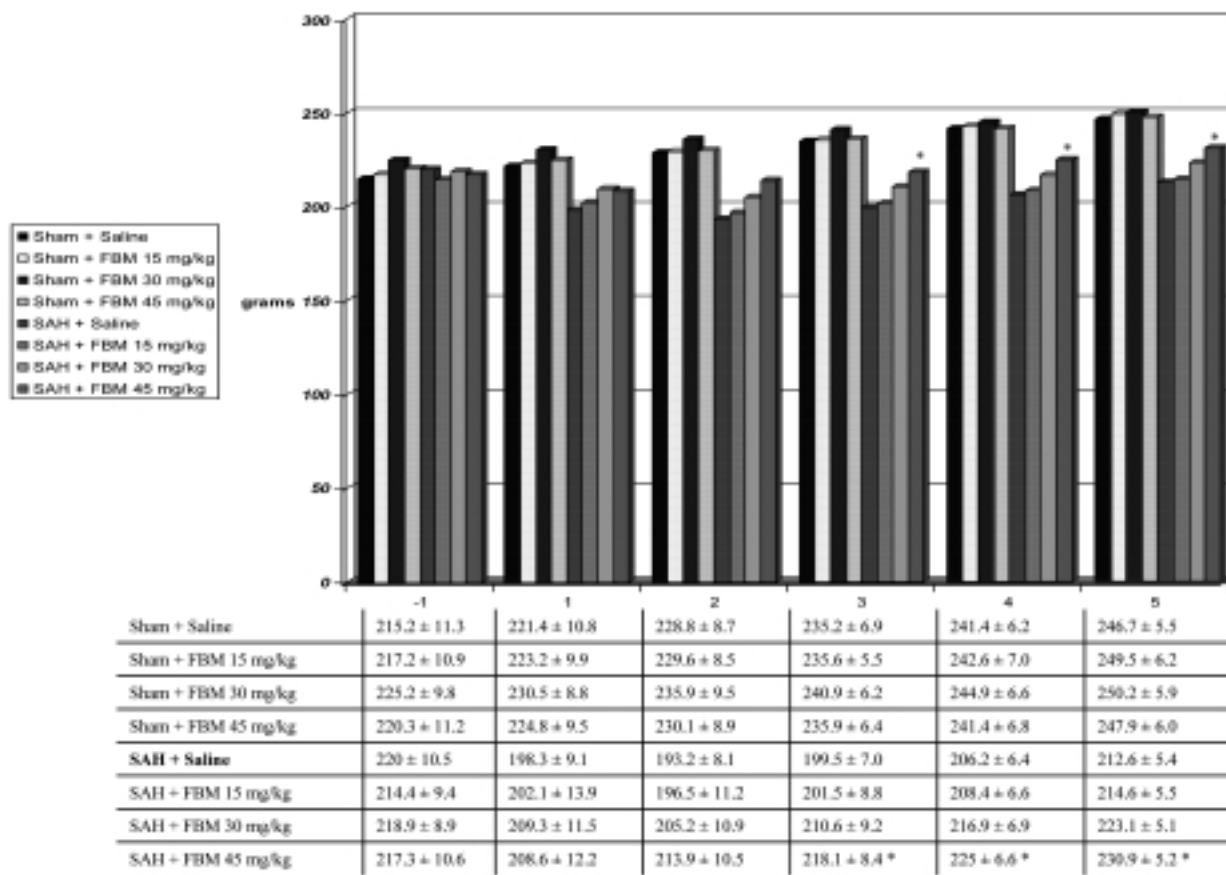


FIG. 4. Body weight. Bar graph showing changes in body weight (in grams) of body weight at daily time points (days -1 to 5). The values shown are means ± SD of the means for each group. **p* < 0.05 SAH + saline vs. SAH + FBM 45 mg/kg group. Vehicle and FBM administration did not induce any significant body weight alteration in sham-injured animals. The SAH-injured saline-treated rats exhibited significant decreased body weight on days 1–5, compared to sham-injured saline-treated animals. Administration of 45 mg/kg FBM significantly reduced the SAH-related loss in body weight on days 3–5, as compared with SAH-vehicle treated rats.

1986; Enblad et al., 1996; Germanò et al., 1992, 1998b, 2000, 2002; Imperatore et al., 2000; Jackowski et al., 1990; Kawamata et al., 1997; Koide et al., 1985; Nilsson et al., 1996, 1999; Persson et al., 1996; Siesjo et al., 1992a,b; Smith et al., 1996; Staub et al., 2000; Yoshida et al., 1980; Zuccarello et al., 1989).

Though mortality rates related to SAH have significantly decreased over the last decade, the issue of long-term cognitive and neuropsychological outcome is gaining increasing attention by the neurosurgical community (Germanò et al., 1997, 1998a; Saveland et al., 1992). The neurotoxic effects of widespread subarachnoid blood associated with transient cerebral ischemia cause diffuse cortical damage that occurs immediately after the hemorrhage (Doczi, 1985; Doczi et al. 1986; Enblad et al., 1996; Gotoh et al., 1984; Hutchinson et al., 2000; Jackowski et al., 1990; Nilsson et al., 1996, 1999; Persson et al., 1996; Sarrafzadeh et al., 1998, 2002; Saveland et al.,

1996; Schulz et al., 2000; Staub et al., 2000; Untenberg et al., 2001). This damage results from the interaction of numerous pathophysiologic factors where presumably, the blood itself and/or active substances derived in part from the degradation of the extravasated blood are centrally involved. Previous investigations conducted in our laboratory have demonstrated that this rodent model of SAH is associated with enduring measurable behavioral and neurologic alterations that may parallel those seen in humans after SAH (Germanò et al., 1994, 1997, 1998a,b, 2002; Imperatore et al., 2000). It should be emphasized that, because of the significant interspecies difference, a direct extrapolation of our results to humans may not be appropriate. However, these results further confirm the rat model of SAH as a viable laboratory instrument for the study of the pathophysiology of SAH and provide normative values for evaluation of new treatment modalities.

TABLE 2. RESULTS OF BBB PERMEABILITY CHANGE ASSESSMENTS IN ALL GROUPS OF RATS

Area	Evans Blue dye ($\mu\text{g/g}$ tissue)							
	Sham + vehicle	Sham + FBM 15 mg/kg	Sham + FBM 30 mg/kg	Sham + FBM 45 mg/kg	SAH + vehicle	SAH + FBM 15 mg/kg	SAH + FBM 30 mg/kg	SAH + FBM 45 mg/kg
Frontal cortex	2.272 \pm 0.36	2.366 \pm 0.66	2.554 \pm 0.44	2.874 \pm 0.55	8.619 \pm 1.83	6.219 \pm 1.33**	5.731 \pm 1.15**	5.65 \pm 0.75**0
Temporal cortex	2.075 \pm 0.45	2.587 \pm 0.26	2.498 \pm 0.49	2.567 \pm 0.42	7.359 \pm 1.11	6.340 \pm 0.30**	5.55 \pm 0.29**	5.058 \pm 0.35**
Parietal cortex	2.233 \pm 0.02	2.115 \pm 0.55	2.441 \pm 0.22	2.654 \pm 0.24	7.779 \pm 0.18	6.573 \pm 0.09**	7.634 \pm 0.04*	5.552 \pm 0.08**
Occipital cortex	2.749 \pm 0.44	2.441 \pm 0.44	2.544 \pm 0.15	2.887 \pm 0.24	8.111 \pm 0.71	6.978 \pm 0.967	6.952 \pm 0.86	5.362 \pm 0.48**
Subcortical GM (CPT)	2.214 \pm 0.04	2.156 \pm 0.32	2.874 \pm 0.24	2.245 \pm 0.35	7.076 \pm 0.29	6.975 \pm 0.4	5.534 \pm 0.11**	5.413 \pm 0.06**
Cerebellar cortex	2.787 \pm 0.09	2.145 \pm 0.44	2.552 \pm 0.65	2.335 \pm 0.45	6.861 \pm 0.6	6.436 \pm 0.43	5.141 \pm 0.44**	5.296 \pm 0.44*
Cerebellar nuclei	2.167 \pm 0.21	2.554 \pm 0.67	2.756 \pm 0.51	2.546 \pm 0.35	5.049 \pm 0.95	5.023 \pm 1.02	4.801 \pm 0.82	3.786 \pm 0.62**
Brain stem	2.661 \pm 0.13	2.658 \pm 0.55	2.687 \pm 0.57	2.879 \pm 0.66	5.652 \pm 0.27	4.996 \pm 0.52**	4.502 \pm 0.35**	4.279 \pm 0.4**

Changes in BBB permeability as evidenced by extravasated Evans Blue dye. The BBB permeability changes were measured 2 days after sham or SAH surgery in all groups of rats. Values are expressed as the means \pm SD for six animals in each group.

GM, gray matter.

* $p < 0.05$; ** $p < 0.01$ SAH \pm saline vs. SAH \pm FBM groups.

Early changes in BBB function have been suspected to be one of the major causative factors responsible for post-SAH cerebral dysfunctions (Doczi, 1985; Jackowski et al., 1990; Joshita et al., 1990; Zuccarello et al., 1989). An impairment of BBB following SAH has been demonstrated in humans, which develops after the acute stage of SAH, and has been correlated with the development of delayed cerebral ischemia and poor clinical outcome (Doczi, 1985; Doczi et al., 1986). This phenomenon is independent of raised ICP, hypertension, brain edema and cerebral swelling, reduced cerebral blood flow, and disrupted brain metabolism, which may also in turn, disrupt the BBB (d'Avella et al., 1990, 1993, 1994, 1996; Delgado et al., 1986; Doczi, 1985; Doczi et al., 1986; Germanò et al., 1992, 1994, 1998b, 2000, 2002; Gules et al., 2003; Imperatore et al., 2000; Jackowski et al., 1990; Joshita et al., 1990; Koide et al., 1985; Peterson et al., 1990; Prunell et al., 2003, 2004; Sasaki et al., 1985, 1986; Zuccarello et al., 1989). However, the state of the capillary system after experimental SAH remains controversial and there have been relatively few studies focused on post-SAH BBB alterations. Two models of barrier opening to water-soluble materials have been proposed. For one model, it is postulated that capillary wall deformation caused by endothelial shrinkage and/or capillary vasodilatation stretches and opens the interendothelial tight junctions (d'Avella et al., 1994). For the other model, it is hypothesized that increased vesicular activity augments transfer of material between blood and brain either by shuttling of microvesicles across the endothelium or by the coalescing of vesicles to form continuous intracellular channels through the endothelium (Fenstermacher et al., 1984). Recent qualitative and quantitative experimental investigations conducted in our laboratory employing this SAH rodent model consistently demonstrated that a marked capillary permeability increase occurs at the very acute stage after experimental SAH (Germanò et al., 2000).

Considerable evidence has accumulated supporting the role of EAA neurotransmitters not only as mediators of brain injury, but in the pathogenesis of global brain dysfunction that follows SAH. Data collected in both clinical and experimental settings have reported remarkable changes in levels of lactate, glycerol, glutamate, and aspartate, in addition to changes in the lactate/piruvate ratio. These levels are in turn, affected by ischemic deterioration, with changes in metabolite levels varying with respect to substance, location, and time (Enblad et al., 1996; Hutchinson et al., 2000; Inage et al., 2000; Nilsson et al., 1996, 1999; Persson et al., 1996; Sarrafzadeh et al., 1998, 2002; Saveland et al., 1996; Schulz et al., 2000; Staub et al., 2000; Untenberg et al., 2001).

Brain vasculature is affected by NMDA receptors. Dietrich et al. (1992) demonstrated that the intraventricular

injection of NMDA caused the opening of the BBB in rats, and pretreatment with the NMDA antagonists MK-801 (Yang et al., 1994), memantine (Gorgulu et al., 2000), and citicoline (Onal et al., 1997) reduced the occurrence of BBB breakdown in rodent models of focal cerebral ischemia. The administration of the anticonvulsant noncompetitive EAA antagonist, remacemide hydrochloride, significantly reduced the occurrence of post-SAH cerebral vasospasm (Zuccarello et al., 1994). Additional support for the vascular role of the EEAs is demonstrated by the presence of EAA receptors in cerebral endothelial cells (Krizbai et al., 1998; Sharp et al., 2003).

FBM is a novel anti-epileptic drug with a broad anticonvulsant profile (Arcadi et al., 1997; McCabe et al., 1993; Reckilin et al., 2003; Sbuai et al., 1996; Soderpalm et al., 2002; Swiader et al., 2003; Wallis et al., 1992; Wasterlain et al., 1992; Taylor et al., 1995). The dosage of FBM as an anticonvulsant is 1200 mg/day for adults and 15 mg/kg/day for pediatric patients. Dosage may be increased to a maximum of 3600 mg/day in adults and 45 mg/kg/day in pediatric patients, respectively (Palmer et al., 1993, Wallace Laboratories, 2003) FBM enters the central nervous system with a brain/plasma coefficient of approximately 0.9 (Leppik et al., 1993). The peak anticonvulsant response after oral administration of FBM is reached after 1–4 h (Graves et al., 1989). FBM inhibits voltage-sensitive sodium channels probably by prolonging their inactivation and decreasing the firing rate of neurons. Voltage-sensitive calcium channels are also blocked by FBM. At high concentrations of the drug, FBM can potentiate GABA-mediated chloride currents. Initially, FBM was thought to interact with the phencyclidine/MK-801 receptor sites acting as an antagonist. It had also been considered a non-competitive antagonist of glycine and glutamate. Moreover, recently published data have shown that FBM interacts with the NR1-2B subtype of NMDA receptors. FBM competitively inhibits glycine-enhanced NMDA-induced intracellular calcium currents in mice and induced the stimulation of receptor-gated calcium ion channels, which appear to be regulated by glycine (Swiader et al., 2003). This amino acid increases NMDA-evoked currents in different tissues by increasing the opening frequency of the NMDA channel. McCabe et al. (1993) showed that the neuroprotective effects of FBM are mediated by its binding to a strychnine-insensitive glycine receptor. It is generally accepted that neurotransmission mediated through the NMDA receptor complex is associated with ischemic neuronal injury. Thus, it could be theorised that reducing the magnitude of the effects strictly linked to SAH-induced transient cerebral ischemia may ameliorate the overall outcome of patients with aneurysm.

To date, no other study has examined the effects of FBM on experimental SAH-induced brain dysfunction. In the current study, FBM showed beneficial properties at doses tolerated by animals and humans. The reported effect of FBM in experimental SAH is not surprising considering that the alterations characterising this experimental model (and also the clinical picture of SAH) could be related in part, to secondary transient cerebral ischemia. These results support the protective properties of FBM and stress the need for a more profound understanding of the mechanisms underlying the SAH-induced injury. Moreover, these observations provide a theoretical pharmacological rationale for FBM's beneficial effect on the behavioral deficits and microvascular changes induced after SAH, which is based on intrinsic physiological, biochemical, and anatomical factors. Further efforts in understanding the mechanism of action that underlies the neuroprotective effects of FBM after SAH and the potential clinical benefits of this drug are warranted.

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