Ginkgo biloba Extract Facilitates Recovery from Penetrating Brain Injury in Adult Male Rats

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Adult, male Sprague–Dawley rats received 100 mg/kg Ginkgo biloba extract (GBE) intraperitoneally for 30 days. GBE reduced overall activity and decreased sensitivity to light in the open field maze. The rats were also less responsive to noxious stimuli after 13 days of treatment with GBE. After the last injection, all subjects were trained on a delayed-spatial alternation task. Subsequent to acquisition of the spatial task, the rats received either sham operations and saline or bilateral frontal cortex lesions treated with either saline or GBE. Thirty additional days of treatment began on the day of injury, and open field behavior, analgesia, and metabolic activity measurements were again measured. The rats with lesions treated with saline were more active than their GBE-treated counterparts and sham controls but there were no differences in response to illumination or noxious stimuli. Retention of the delayed-spatial alternation indicated that rats with lesions treated with GBE were less impaired than brain-injured subjects receiving saline treatment. Histological examination showed that GBE reduced the extent of brain swelling in response to the injury.

INTRODUCTION

Several studies show that systemic administration of Ginkgo biloba extract (GBE) has beneficial effects in the treatment of closed-head injury. For example, it was reported that GBE decreases the swelling or edema which occurs with cerebral microembolization (15) and protects the central nervous system (CNS) against hypoxia (12). With respect to the deficits that sometimes accompany the aging process, Gessner et al. (9) claimed that GBE reduces abnormal electrical brain activity in elderly human subjects. While these findings are interesting from a clinical perspective, there is no work being done to assess the effects of GBE on penetrating, traumatic injuries to the cortex.

In the present study, we examine the effects of chronic GBE treatment on a number of behavioral and metabolic measures before and after removal of the frontal cortex in young adult rats. We also examined neuronal degeneration, brain edema, and astrocyte proliferation in response to injury and subsequent to GBE treatment.

METHOD

Subjects

Thirty-two male, Sprague–Dawley CD strain rats 70–80 days of age were obtained from the Charles River Breeding Laboratory. Upon arrival, the animals were housed individually, given food and water ad lib. (except where noted below), and subjected to a 12/12 h light/dark cycle. Each subject's weight was recorded for 2 consecutive days prior to the initiation of the treatment schedule.

Extract Preparation

Concentrated GBE powder was supplied by Ipsen (Paris). This form of GBE contains terpenes such as bilobalide and ginkolides as well as proanthocyanidines, flavoglycosides and organic acids (22).

The solution for injection (100 mg/kg) was prepared in a vehicle of 0.9% isotonic saline. Because of the extract’s near insolubility in water, a 30 mg/ml suspension of GBE powder was formed using a 50-cc glass hand homogenizer (Wheaton). The mixture was prepared daily to ensure freshness and vortexed for approximately 30 s between injections.

Preoperative Treatment Regime

After 2 days of acclimation to the laboratory, subjects were randomly separated into three treatment groups. The Blank Injection group (N = 10) received blank injections to control for the introduction of saline injections in the postsurgical phase of the experiment. The Saline group (N = 10) was administered the vehicle alone in a volume equivalent to the 100 mg/kg dose of GBE. The Ginkgo group (N = 12) was given the 100 mg/kg dose of GBE. Subjects were treated for a period of 30 days and received their daily intraperitoneal injections during the midmorning hours. Each animal was weighed.
to the nearest gram. After injections, the rats were returned to their home cage.

**Open Field Activity (Pre- and Postoperative)**

**Apparatus.** The open field maze was constructed of a wooden square platform measuring 61 by 61 cm and divided into a total of 36 squares. The platform was painted flat gray with the squares delineated by 1.3-cm stripes. A 30.5-cm flat gray wall surrounded the platform on all four sides. The maze was placed on a table and positioned so that the normal room lighting illuminated only two-thirds of the platform (24 squares), while the wall surrounding the maze cast a shadow on the remaining one-third of the platform (i.e., 12 “dark” squares).

**Testing procedure.** Subjects were placed in the open field maze on the 7th, the 14th, and the 21st day of the 30-day treatment period. There was one 3-min trial at each time period given 4-5 h after injections. The rats were put in the center of the maze at the beginning of each trial and the number of blocks entered by the subject, the number of rears, grooming behaviors, and fecal bolae excreted were recorded by one experimenter who did not know group identities. Between the testing of each rat, the platform was wiped with 95% ethanol.

**Metabolic Activity (Pre- and Postoperative)**

**Apparatus.** Each metabolic cage (Nalgene) was equipped with a calibrated water bottle, a calibrated urine catch, and a uniformly weighted food hopper and feces catch.

**Data collection.** The rats were placed in the metabolic cages on the 5th, the 15th, the 20th, and the 30th day of the 30-day treatment period. On their scheduled day of entry, subjects were placed in the metabolic cages just after their injections. Approximately 60 g of food and 100 cc of tap water were provided. Data were recorded every 6 h with food and water replenished at the time of the readings. Treatments were administered in the usual manner as described above. After remaining 48 h in the metabolic cages, the subjects were injected and then returned to their home cages.

Measures consisted of food and water consumption and fecal and urine output. Using a percentage savings formula, we also calculated the percentage of food converted into solid waste. A similar method was employed to determine the conversion of water into urine:

\[
\text{consumed food (or water) - feces (or urine) \times 100} \]

\[
\text{consumed food (or water)} = \% \text{ savings.}
\]

**Analgesia Assessment (Pre- and Postoperative)**

**Apparatus.** The analgesic effect of the GBE was determined using a modified tail flick procedure as described in Green and Lee (10). The apparatus consisted of a light (Valtec Model 300) that produced a range of variable wattage. We used the highest setting available (150 W) and the smaller aperture of the two present on the lamp.

**Testing procedure.** Tail-flick to heat was recorded on the 13th and the 28th day of treatment during the 30-day treatment period. Testing consisted of three trials spaced by a 5-min delay between each to prevent habituation to the heat stimulus. The trial began when the restrained subject's tail was placed under the light bulb and the timer was started. The time was recorded at the first sign of the tail's movement by one experimenter who did not know the group identities. After the 6 s in the goal arm, the animals were removed from the maze and given a 30-s intertrial interval and the procedure was repeated twice again. A maximum stimulation time of 120 s was used to prevent tissue damage. Subjects not responding to the heat stimulus during this time interval were excluded from further analgesia testing.

**Preoperative Acquisition Training**

When the 30-day injection period was completed, the animals began a 23-h 55 min water deprivation schedule which was maintained throughout acquisition training. The procedure consisted of three phases of shaping the rats to perform 35-s delayed-spatial alternation in a T-maze for water reward (1). Upon reaching pretraining criterion, the subjects were trained to alternate their choice in the goal arms. The rats were weighed and then placed in the start box facing the experimenter, a 5-s interval elapsed and the doors were opened. The doors were then closed after a choice was made and 6 s were given for reward consumption. The first choice was always rewarded, while only alternate choices were rewarded thereafter. If an incorrect choice was made by repeating the previously selected goal arm, the subjects received no water reward and remained in the goal box for 6 s. Choosing the previously selected goal arm constituted an error. If the rat chose the incorrect goal arm two or more times, this was scored as a “perseveration.” After the 6 s in the goal arm, the animals were removed from the maze and given a 30-s intertrial interval and an additional 5 s in the start box before beginning the next trial. The floors and walls of the maze were wiped with 95% ethanol between subjects to eliminate potential olfactory cues. Ten trials per day were given and the criterion for learning was set at 10 of 10 (10/10) successively correct trials.

**Surgery and Postoperative Treatment**

After reaching acquisition criterion on the delayed-spatial alternation task, the rats were given either bilateral aspiration lesions of the medial frontal cortex (21) or sham operations in which the animals were anes-
tized and the scalp was cut and then resutured to resemble the operated group.

Just prior to surgery, the groups were redefined. Including mortality resulting from the operation, the Blank Injection group became the Sham + Saline group (S + Saline; N = 9), the Saline group became the Lesion + Saline group (L + Saline; N = 10), and the GBE group became the Lesion + Ginkgo group (L + Ginkgo; N = 10). The subjects received their treatments 3-4 h before surgery and continued to receive them for the next 29 days according to the regime specified above.

Postoperative Retention Testing

Thirty days after the injury and subsequent treatments, the 23-h 55 min water deprivation schedule was reinitiated. Retention testing then began on the same spatial alternation task used preoperatively. Testing continued until the rats attained a score of 10/10 successively correct alternation responses or until the subjects exceeded the number of trials they took to learn the task under the preoperative condition.

Histology

After reaching criterion on the retention task, the rats were killed with a lethal overdose of Nembutal (1.0 cc) and then perfused intracardially with Karnovsky’s fixative. The tissue was then cut on a freezing microtome into 40-μm sections and every sixth section was saved for lesion reconstruction and cell counting. Sections were stored in 0.1 M phosphate buffer solution (PB) for immunohistochemistry.

Lesion and Ventricle Size

Lesion size was calculated in square millimeters using an overhead microprojector to project serial brain sections stained with cresyl-echt violet onto a Summagraphics digitizing tablet. Perimeters of the lesions were then traced and quantified for statistical analyses. In order to determine whether GBE had an effect on brain edema, we calculated the combined size of the lateral and third ventricles at approximately 70 days postinjury (1). The ventricle sites chosen for quantification were selected using reference points taken from the atlas of Paxinos and Watson (16). The reference points consisted of the anterior commissure (bregma, 0.7) and the fimbria of the hippocampus (bregma, −4.3).

Degeneration Assessment

The medial dorsal nucleus of the thalamus (MDN), a major subcortical area projecting to the frontal cortex, was used to quantify the number of surviving neurons at 70 days postinjury. Sections of tissue stained with cresyl-echt violet were examined using light microscopy (×40). The specific method and criteria used in identifying neurons were based on the size and apparent health of the neuron. Three anterior sections which included the MDN proper were counted in each subject. Tissues from all subjects were used in this evaluation.

Astrocyte Proliferation

Glial fibrillary acidic protein (GFAP) technique (6) was used to assess the extent of astrocyte proliferation in response to the injury. Brain sections were first given three 15-min washes in PBS (pH 7.6) and then soaked in a 0.30% Triton X-100 (Sigma)/PBS solution for 1 h. The three washes were repeated before soaking the sections in a solution of 0.10% Triton X-100/PBS and 3% swine serum (Dako; diluent) for 1 h. Following another three 15-min washes in PBS, the sections were then incubated free-floating for 24 h in a solution consisting of the diluent and diluted rabbit anti-GFAP antiserum (dilution, 1:1000, generously donated by Dr. Lawrence Eng of Stanford University).

Subsequent to overnight incubation, the tissue was washed as above and then soaked in the diluent and 1:100 swine anti-rabbit immunoglobulins (Dako) for 1 h. After another three washes in PBS, the tissue was soaked for 1 h in a 1:100 soluble complex of horseradish peroxidase and rabbit anti-horseradish peroxidase (PAP, Dako) in diluent. After three 15-min washes in PBS, the sections were incubated in a 0.25% solution of 3,3′-diaminobenzidine (Sigma) in Tris buffer (pH 7.6) containing 0.05% H2O2 for 7 min and the reaction was stopped in PBS. All washes and incubations took place at room temperature. Sections were then mounted, air-dried, and coverslipped. To count astrocytes, the most posterior extent of the lesion was identified (×40) in each subject. The grid reticule was then centered upon the cingulum just dorsal to the corpus calosum of the next caudal section. Every other block of the 25-block reticule was used.

RESULTS

Behavioral

Preoperative open field activity. A repeated measures MANOVA performed on the data collected at the 7th, the 14th, and the 21st day of the 30-day treatment period indicated a significant effect of the treatment (F(2,54) = 5.81, P < 0.05). Further statistical analyses (one-way ANOVAs at each time period) showed a significant difference between the three treatment groups after 7 days of treatment (P < 0.05).

With respect to the number of dark blocks entered, the Scheffé multiple range test of means indicated that subjects receiving the blank injection were not significantly different from rats given 100 mg/kg of saline. However, subjects administered GBE entered fewer blocks than the controls (P < 0.05). Other measures such
as grooming or rearing behavior and mean feces excretion proved nonsignificant.

Despite the decreased activity exhibited by the rats treated with GBE, we found that these subjects were significantly more active in the illuminated area of the open field than those subjects given a blank injection or saline (one-way ANOVA; $F(2,27) = 7.90$, $P < 0.01$; Fig. 1A).

**Preoperative metabolism.** A repeated measures ANOVA was performed on the metabolic data. Potential differences were examined with respect to food and water intake, feces and urine excretion, or solid and fluid percentage savings. The ANOVA indicated that the treatment did not affect these measures of metabolic activity ($P < 0.05$).

**Preoperative analgesia.** A repeated measures MANOVA performed on the mean response latency to heat from a light source focused on the tail was significant at 13 days of treatment ($F(2,40) = 9.29$, $P < 0.001$). To prevent skin burns, two subjects of the GBE group not responding to the heat stimulus were excluded from testing. The Scheffé conducted on the data from the first trial revealed that subjects treated with GBE took longer to respond than those rats receiving either an equivalent amount of saline or a blank injection ($P < 0.05$) (Fig. 1B). The data of the remaining two trials, however, suggested that the subjects had habituated to the heat stimulus. No significant differences between any of the groups were found at 28 days of treatment ($P > 0.05$).

**Delayed-spatial alternation acquisition.** Our analyses showed that subjects receiving the blank injection, saline, or 100 mg/kg of GBE did not differ in the acquisition of the spatial alternation task ($P > 0.05$). ANOVAs conducted on the number of days, errors, and perseverative errors to reach 10 of 10 correct alternations (10/10 criterion) indicated no significant effect of the treatment. Similar results were found in the analyses of the number of days, errors, and perseverative errors to the less stringent criterion of 9 of 10 correct alternations (9/10).

**Postoperative open field activity.** Repeated measures MANOVA performed on the activity data showed that the groups were significantly different in the mean dark blocks traversed in the open field maze ($F(2,23) = 7.31$, $P < 0.01$). A one-way ANOVA was conducted at each time period (i.e., 7, 14, and 21 days into treatment) to isolate these differences. These results showed that the
treatment groups were significantly different only at Day 7 postinjury (F(2,26) = 7.85, P < 0.01). The Scheffé multiple range test at this time period revealed that subjects with frontal cortex lesions treated with saline were significantly more active than their GBE treated counterparts and sham operates (P < 0.05).

As in the preinjury assessment, we investigated the percentage of illuminated blocks traversed in the light-divided, open field maze. A repeated measures MANOVA revealed a significant main effect of the treatment (F(2,23) = 5.06, P < 0.01). One-way ANOVAs at the three time periods revealed a difference between the groups at 7 days postinjury. The Scheffe test of means showed that both the saline-treated and GBE-treated lesion groups occupied more blocks in the illuminated area of the open field than the saline-treated sham operates (P < 0.05) (Fig. 1C). No significant differences were found in additional measures recorded such as grooming behavior, rearing behavior, or fecal deposits.

Postoperative metabolism. Repeated measures ANOVA was performed on the metabolic data collected on the 5th, the 15th, the 20th, and the 30th day of the 30-day postinjury treatment period. Statistical examination showed no significant treatment effect with respect to food and water intake, fecal and urine output, and solid or fluid percentage savings (P > 0.05).

Postoperative analgesia. At 13 days postinjury treatment, a repeated measures MANOVA indicated that the mean response latency to heat from a light source focused on the tail was significant (F(2,20) = 3.82, P < 0.05). Post-hoc tests of the three successive trials revealed that brain-injured subjects treated with either saline or GBE took longer to respond than sham operates treated with saline (P < 0.05) (Fig. 1D). This significant effect, however, was only seen in the first of the three trials, while the data of the remaining two trials suggested that the subjects had habituated to the heat stimulus. An identical assessment at 28 days postinjury showed no significant differences between any of the groups (P > 0.05).

Delayed spatial alternation retention. ANOVA indicated that the three groups were significantly different with respect to the number of days to attain 10/10 correct alternations (F(2,25) = 10.67, P < 0.01). A Scheffé test of the group means showed that brain-injured subjects receiving either saline or GBE took significantly more days to attain this criterion than the sham-operated controls (P < 0.05).

Similar results were found in mean errors to the criterion of 10/10 correct alternations. A one-way ANOVA revealed that a significant difference existed between the three groups (F(2,25) = 10.37, P < 0.001). The Scheffé procedure revealed that sham operates treated with saline made significantly fewer errors than those subjects with frontal cortex lesions treated with either saline or GBE (P < 0.05). However, a beneficial treatment effect was found in the mean repeated, incorrect choices to this criterion.

One-way ANOVA performed on the mean perseverations to 10/10 criterion revealed that the groups differed significantly (F(2,25) = 7.26, P < 0.01). Subsequently, the multiple range test indicated that the brain-injured subjects receiving saline made significantly more perseverative errors than both sham operates and GBE-treated animals with lesions (P < 0.05). Figure 2A illustrates the mean days, errors, and perseverations to the 10/10 criterion.

A less stringent criterion of 9/10 was also examined. ANOVAs were performed on the mean days, errors, and perseverative errors to reach the 9/10 criterion. Each of these statistical analyses showed a significant difference between the groups (days: F(2,25) = 7.47, P < 0.01; errors: F(2,25) = 6.87, P < 0.01; perseverations: F(2,25) = 7.39, P < 0.01). The Scheffé multiple range test was performed after finding these differences.

Subjects with frontal cortex lesions treated with saline took significantly more days to attain the 9/10 criterion than those brain-injured animals treated with GBE or sham operates (P < 0.05). Additionally, the saline-treated lesion animals made more errors and perseverative errors than their GBE-treated counterparts and sham operates in reaching this criterion (P < 0.05). Figure 2B shows the mean days, errors, and perseverations to the 9/10 criterion.

Histological

Lesion size. Light microscopic examination of the brain tissue revealed damage to the genu of the corpus callosum in two cases. Three subjects received damage to the dorsal surface of the olfactory bulbs. The forceps minor and anterior cingulate cortex were injured in seven cases. Four subjects received minor damage to the frontal-parietal motor area. One-way ANOVA of the lesion size measurements revealed no significant difference between the saline-treated lesion group and counterparts treated with the GBE (P > 0.05; means respectively = 6.37 and 5.91 mm², N = 9 and 10). Figure 3 shows the minimal and maximal extent of damage in the two groups.

Ventricle size. One-way ANOVA indicated that the mean ventricle size was significantly different (F(2,18) = 5.60, P < 0.05). Scheffé post hoc test of means conducted at the 0.05 level revealed that the ventricle size of the sham operates was significantly smaller than that of brain-injured subjects treated with saline (P < 0.05). However, brain-injured animals treated with GBE did not exhibit ventricles significantly larger than those of shams. These analyses indicated an intermediate effect in that the ventricle size of the brain-injured rats treated with GBE did not differ from that of their saline-treated
counterparts (means in mm$^2$; S + Saline = 4.87, L + Saline = 9.22, L + Ginkgo = 7.57; Fig. 4).

**Degeneration.** Two factor ANOVA indicated a significant difference between the groups in mean neurons counted in the MDN ($F(2,82) = 5.31, P < 0.01$). However, the repeated measures factor as well as the interaction between repeated sampling and treatment were not significant ($P > 0.05$). Treatment effects were analyzed using one-way ANOVA. The results indicated that the number of neurons in the sham group (mean and standard error = 7.25 ± 0.688) was significantly greater than that found in either the brain-injured animals treated with saline (mean and standard error = 4.93 ± 0.518) or their GBE-treated counterparts (mean and standard error = 5.34 ± 0.300; $P < 0.05$). We found no significant differences between brain-injured rats given GBE or saline on this measure.

**Astrocyte proliferation.** As above, only a significant effect of the injury was found in the number of GFAP-reacted astrocytes counted in the cingulum cortex just posterior to the lesion. Sham operates were significantly lower than both brain-injured groups treated with either GBE or saline ($P < 0.05$). However, no significant differences were found between subjects with lesions treated with either saline or GBE ($P > 0.05$). Figure 5a shows few astrocytes in the cingulate area of a representative sham operate. With respect to brain-injured animals, astrocytes become clearly visible (Fig. 5b).

**DISCUSSION**

As indicated earlier in this paper, previous investigators have demonstrated that GBE reduces the morphological deficits associated with ischemia (15) and hypoxia (12). GBE has also been shown to reduce some of the cognitive impairments accompanying old age (9). In the present study, 100 mg/kg of GBE was administered daily for 30 days before and then after brain injury.

With respect to preoperative treatment, GBE did not disrupt metabolic activity during the 1-month period of injections. Also, GBE did not impair or facilitate subsequent acquisition of a complex spatial task. This latter finding is in agreement with Gessner et al. (9) who showed that GBE did not affect cognitive functioning in healthy human subjects.

Although learned performance was not affected, GBE decreased spontaneous activity in the open field maze under the preoperative condition. Although less active overall, those subjects treated with GBE exhibited more movement in the bright quadrants than controls and did not prefer to occupy the shadowed area of the open field. These data suggest that GBE decreased the avoidance response to illumination normally displayed by nocturnal animals (3). We attribute this decline to similar pharmacological characteristics of GBE and chlorpromazine, a potent psychotropic drug. Chlorpromazine as well as other tranquilizers are known to alter cell membrane resistance (19). Etienne et al. (7) demonstrated that GBE also significantly increased cell membrane resistance but to a lesser extent than chlorpromazine.

Analgesia testing showed that GBE reduced withdrawal from noxious stimuli after 13 days of treatment under the preoperative condition. It may be possible that the same component of GBE that induced a tranquilized state may have also produced analgesia. Similar data are offered by Bauer (2) who demonstrated that GBE increased the pain-free walking distance of patients suffering from peripheral arterial disease, although Bauer contends that the reduction in pain is due to improved circulation.

With respect to recovery from severe and acute brain injury, GBE significantly reduces the behavioral deficits occurring after frontal cortex injury. Postoperative open
field activity showed a beneficial effect of GBE treatment. Brain-injured subjects treated with GBE were as active as sham-operated controls in the total number of blocks traversed, while subjects with lesions and treated with saline exhibited the hyperactive behavior which typically occurs after frontal cortex injury (13).

With our preoperative results in mind, the similar performance between sham and brain-injured subjects treated with GBE may be explained by a different hypothesis. Habituation to the open field may have resulted from the repeated exposure during pre- and post-injury testing. This would cause less activity in our sham-operated controls and, of course, they would not differ significantly from the sedated, GBE-treated animals with lesions. However, the sedative and analgesic effects of GBE observed in the preoperative condition were not present after injury. For example, regardless of treatment, subjects with lesions were more hyperactive in the illuminated quadrants of the platform than sham operates. Also, the postinjury analgesia measurement indicated that animals with lesions and treated with saline did not differ from their GBE-treated counterparts on the 13th and the 28th day of treatment. Brain-injured subjects receiving either GBE or saline were slower to withdraw from heat focused on the tail than sham operates.

We believe that the psychotropic action of GBE may have played a protective role in the behavioral and morphological recovery we observed. Safar (18) has shown that, in comparison to ether, barbiturates administered for anesthesia at the time of injury reduce the mortality rate resulting from cerebral ischemia. This protective function is thought to be a property of all CNS depressants in that the need for oxygen is decreased. Although pentobarbital was used in all of the subjects for surgical anesthesia, GBE may have decreased blood-borne oxygen consumption during the period immediately following injury.

The open field, metabolic activity and analgesia measures were employed to provide a profile of the extract's effect on natural and reflexive behavior. However, it may also be possible that GBE helps "consolidate" behavior acquired prior to brain injury, perhaps by reducing responses to potentially disruptive stimuli. Retention of the delayed-spatial alternation task was significantly improved with pre- and postinjury GBE treatment. Despite the impairment in reaching the most stringent criterion of 10 of 10 correct alternations in rats with lesions treated with saline or GBE, the GBE group displayed superior performance over their saline-treated counterparts in the number of days, errors, and perseverations to score 9 of 10 correct choices.

With respect to anatomical measures of recovery, GBE did not appear to alter the reactive astrocytic response to injury. Our results indicate that the recovery we observed was not mediated by glial cell activity. In addition, we did not find sparing of neurons that project from the thalamus to the frontal cortex. Thus, there was no evidence to suggest that GBE has "neurotrophic" properties such as those reported for the nerve growth factor (11) or gangliosides (17). Nonetheless, the more rapid recovery from the frontal cortex injuries may have been the result of GBE's effects on ventricular dilation that is usually associated with the penetrating injury we induced in our animals. We found that GBE considerably reduced ventricular swelling or edema in response to the injury. The lateral and third ventricles were significantly smaller in brain-injured subjects treated with GBE than in their saline-treated counterparts.

A probable mechanism for this protective characteristic of GBE is that this substance is known to act as a free-radical scavenger (5). Neuronal damage occurs be-
FIG. 4. Photomicrographs of representative coronal sections from each treatment group illustrating the reduction of ventricular dilation in brain-injured subjects treated with GBE (approximately 70 days postinjury).
FIG. 5. Photomicrographs of astrocyte proliferation in the cingulate cortex of a sham-operated subject (a) and a brain-injured subject treated with saline (b) (approximately 70 days postinjury).
cause torn or occluded vessels prevent oxygen-rich blood from reaching intact tissue adjacent to the lesion. This impaired blood delivery causes increased amounts of cyclooxygenase, enhanced arachidonate metabolism, and phospholipase and lactate levels associated with free-radical activity (14). Although ventricular enlargement may be considered an indirect measure of brain swelling, our results agree with those of Le Poncin Laffitte et al. (15) who demonstrated that pretreatment with GBE (100 mg/kg for 21 days) normalized this cellular metabolism and oxygen delivery to injured CNS tissue.

The putative neurochemical effects of chronic GBE administration may also explain the behavioral recovery we observed. Brunello et al. (4) have demonstrated that 100 mg/kg/day of GBE given for 21 days resulted in enhanced cortical normetanephrine content. Feeney and Sutton (8) have shown that improving neurotransmitter release via catecholaminergic agonists reduces behavioral deficits following large cortical lesions.

In conclusion, our results demonstrate that GBE may reduce some of the impairment caused by severe and acute penetration injury to the frontal cortex. Recent work from our laboratory demonstrates that optimal recovery is achieved with combinations of treatments sometimes shown to be beneficial when administered alone. Slavin et al. (20) administered gangliosides to brain-injured subjects with fetal transplants and found that behavioral deficits were reduced in comparison to those animals receiving either treatment alone. GBE may enhance recovery from brain injury because the compound contains two or more “treatments” as discussed above.

ACKNOWLEDGMENTS

The authors thank Dr. Laurent Lescaudron and Christopher Palatucci for their help in histological preparations and Michele Pilotte and Tamara Parvizi for their technical assistance. We also thank Dr. John E. Taylor (Biomeasure, Inc.) for his comments on the manuscript. This research was funded by IPSEN (Paris) and performed as partial fulfillment of the Masters Degree (M.J.A.).

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