Effect of phenytoin and corticosteroids on seizures and lipid peroxidation in experimental posttraumatic epilepsy

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Posttraumatic epilepsy occurs in correlation with the severity of head trauma. \textsuperscript{10,26,53} It is estimated that 7\% of head-injured patients in a civilian population,\textsuperscript{3} and 34\% of patients injured in combat,\textsuperscript{4} will develop persistent seizures. Although brain injury responses are major factors in the initiation of posttraumatic epilepsy, the expectation that the prophylactic administration of anticonvulsant drugs to head-injured patients to prevent the development of posttraumatic epilepsy is efficacious. In this study, rats received a 10-\mu l injection of 100 mM FeCl\textsubscript{2} at a depth of 1.8 mm into the isocortex, or an equal volume of saline. Rats were then treated with 30 mg/kg methylprednisolone (MPS), 90 mg/kg MPS, 100 mg/kg phenytoin, or with an equal volume of propylene glycol. Behavioral or electroencephalographic (EEG) seizures occurred in all control-treated iron-injected rats within 93 ± 6 minutes of injection. Brain injury responses as measured by the occurrence of fluorescent product formation from iron-induced lipid peroxidation showed 6.6 ± 0.8 units/gm in the saline-injected animals, and 16.7 ± 2.5 units/gm in the control-treated iron-injected rats. Of the 90-mg/kg MPS-treated rats, 8\% had seizures; fluorescence in those animals was 5.7 ± 0.5 units/gm. Phenytoin treatment prevented the occurrence of convulsive and EEG seizures; however, lipid peroxidation was unaffected (16.5 ± 4.1 units/gm).

If posttraumatic epilepsy develops because of RBC extravasation, hemolysis, parenchymal deposition of heme compounds, and initiation of lipid peroxidation, then treatments designed to prevent peroxidation may be more effective for epilepsy prophylaxis than administration of anticonvulsant drugs that mask convulsive seizures while biochemical brain injury continues.

**KEY WORDS** · posttraumatic epilepsy · lipid peroxidation · phenytoin · steroids · iron salts
After the addition of 3 ml of HzO, each tube was agitated, and placed on ice. The tissue was homogenized where killed by decapitation. Platinum animals in each group underwent tracheotomy, and immediately, and placed in glass centrifuge tubes, to which was added. Each tube was capped, gently vortexed for 1 minute. The homogenates were placed on ice for 15 minutes, then centrifuged at 1200 G at 0°C for 5 minutes.

Fluorescence was measured using an Aminco SPF-125 spectrophotofluorometer with a 1-cm light path, and a Varian Eimac 150 W xenon arc lamp. The fluorometer was coupled to a Houston Instruments chartstrip recorder. Slit widths were set at 4, 1, 0.5, and 0.5 mm at the 1 to 4 positions, respectively, and the metermultiplier was set at 1. The sensitivity was adjusted such that a freshly prepared quinine sulfate standard exhibited a fluorescence ranging between 11 and 16 units. Then 1 ml of the chloroform layer was pipetted into a quartz cuvette and 0.1 ml of methanol was added. Fluorescence, measured at excitation of 370 nm and emission at 430 nm, was expressed as units of fluorescence per gram wet weight per ml of extraction read. Significance was determined using the Student t-test.

Approximately 90 minutes after intracortical injection, while still under anesthesia, at least half of the animals in each group underwent tracheotomy, and were mechanically ventilated on room air. Platinum needle electrodes were placed in the scalp of each animal, and electroencephalogram (EEG) activity was recorded on a Grass Model 6 electroencephalograph for 30 minutes.† The onset or absence of either behavioral or EEG seizures was recorded, after which the animals were killed by decapitation. Fluorescent product formation was measured by a modification of the chloroform/methanol extraction procedure of Fletcher, et al.18 After removal of the cerebellum, brains were bisected through the interhemispheric fissure. The tissue samples were weighed immediately, and placed in glass centrifuge tubes, to which 2 ml chloroform (spectroscopy grade) followed by 1 ml methanol was added. Each tube was capped, gently agitated, and placed on ice. The tissue was homogenized for 15 seconds using a homogenizer with a 2-cm blade. After the addition of 3 ml of H2O, each tube was vortexed for 1 minute. The homogenates were placed on ice for 15 minutes, then centrifuged at 1200 G at 0°C for 5 minutes.

Fluorescence was measured using an Aminco SPF-125 spectrophotofluorometer with a 1-cm light path, and a Varian Eimac 150 W xenon arc lamp.‡ The fluorometer was coupled to a Houston Instruments chartstrip recorder. Slit widths were set at 4, 1, 0.5, and 0.5 mm at the 1 to 4 positions, respectively, and the metermultiplier was set at 1. The sensitivity was adjusted such that a freshly prepared quinine sulfate standard containing 0.1 μg of quinine sulfate/ml 0.05 M H2SO4 exhibited a fluorescence ranging between 11 and 16 units. Then 1 ml of the chloroform layer was pipetted into a quartz cuvette and 0.1 ml of methanol was added. Fluorescence, measured at excitation of 370 nm and emission at 430 nm, was expressed as units of fluorescence per gram wet weight per ml of extraction read. Significance was determined using the Student t-test.

† Model 6 electroencephalograph manufactured by Grass Instruments Co., Quincy, Massachusetts.
‡ Varian Eimac 150 W xenon arc lamp manufactured by Image Intensifier Group, LSE Division, Varian Enterprises, Palo Alto, California.

### Results

Figure 1 shows an EEG recorded from a paralyzed, ventilated rat, injected with 10 μl of 100 mM FeCl2 and treated with PG. Low-amplitude multiple spikes beginning at the site of injection became generalized and increased in amplitude over 2 seconds, and were followed in this continuous record by high-amplitude multiple sharp waves and spike and wave discharges. In comparable animals observed but not subjected to EEG, the generalized polyspikes corresponded to generalized tonic seizures; followed by tonic-clonic convulsive seizures corresponding to the spike and wave discharges. Latency to seizure onset in all untreated iron-injected animals was 93 ± 6 minutes after injection.

Figure 2A is an EEG that was recorded from an animal treated with 90 mg/kg of MPS. None of the animals injected with high-dose MPS experienced behavioral or EEG seizures. The EEG in this animal shows anesthetic effect from the uninjected right hemisphere, while suppression of normal background was recorded from the iron-salts injected hemisphere. The EEG in Fig. 2B was recorded from an animal treated with 100 mg/kg phenytoin. None of the phenytoin-treated animals had behavioral convulsions or generalized electrographic epileptiform discharges (Table 1). However, 73% of those animals had brief episodes of vibrissa twitching or rare bursts of polyspike discharges on the EEG. Ninety-two percent of the animals treated with low-dose MPS had either behavioral convulsions or EEG seizures; however, only 8% of animals treated with high-dose MPS had convulsive seizures (Table 1). None of the saline-injected animals had seizures, while all of the control-treated animals had behavioral or EEG seizures.

Figure 3 shows the data obtained from measurement of fluorescence from the injected hemisphere. Untreated (PG) animals showed fluorescence of 16.7 ± 2.5 units/gm. Treatment with MPS at a dose of 30 mg/kg caused modest decrease in peroxidation (10.2 ± 2.0

### Table 1

<table>
<thead>
<tr>
<th>Cortical Injection</th>
<th>Treatment</th>
<th>No. of Rats</th>
<th>Seizures No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>none</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FeCl2 (10 μl, 100 mM)</td>
<td>PG (1 ml)</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>MPS (30 mg/kg)</td>
<td>25</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>MPS (90 mg/kg)</td>
<td>23</td>
<td>2</td>
<td>8†</td>
</tr>
<tr>
<td></td>
<td>PT (100 mg/kg)</td>
<td>18</td>
<td>0</td>
<td>0‡</td>
</tr>
</tbody>
</table>

*PG = propylene glycol; MPS = methylprednisolone; PT = phenytoin.
† Difference significant by Student’s t-test (p < 0.001).
‡ Although none of the PT-treated animals had convulsive seizures, 73% had vibrissa twitching or brief bursts of spikes on the electroencephalogram.
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FIG. 1. Electroencephalograms recorded from a control rat treated with 1 ml of propylene glycol at the time of intracortical injection of 10 μl of 100 mM FeCl₂ into the left isocortex. A: Focal epileptiform discharges begin within the injected hemisphere, and rapidly generalize, with high-amplitude polyspike discharges recorded from both hemispheres. B: The polyspike activity evolves to a pattern of spikes and waves.

FIG. 2. A: Illustrative electroencephalogram (EEG) from an animal treated with 90 mg/kg of methylprednisolone at the time of 100 mM FeCl₂ injection showing decreased amplitude in the background activity recorded from the injected hemisphere. The EEG from the contralateral hemisphere showed an anesthetic effect. B: Representative EEG from an animal treated with 100 mg/kg of phenytoin at the time of iron salts injection showing rare brief bursts of sharp waves.
macrophages,\(^{59}\) and the occurrence of acute\(^{15,39,56}\) and then recurrent epileptiform discharges and behavioral seizures.\(^{22,49,59,60}\) The association of the development of epilepsy with hemorrhage into the cortex and the experimental observations of iron-induced epileptogenesis have suggested the hypothesis that deposition of iron, or iron-containing compounds derived from extravasated RBC's following head trauma, may be of critical importance in the development of posttraumatic epilepsy.\(^{24,25,59}\)

The addition of heme compounds\(^{24}\) or ionic iron to suspensions of subcellular organelles or to polysaturated fatty acids results in the formation of highly reactive free radical oxidants.\(^{1,5,43,46,54,61}\) Perferryl ions,\(^5\) singlet oxygen,\(^{46}\) and hydroxyl radicals,\(^{19}\) thought to form by iron-catalyzed Haber-Weiss reactions,\(^{4,28}\) cause hydrogen abstraction at carbonyl bonds of polysaturated fatty acids and lipids within membranes,\(^{19}\) thus initiating and propagating peroxidation reactions. The process of lipid peroxidation disrupts membranes of subcellular organelles,\(^{43,51}\) and yields diene conjugates, malonylaldehyde (MDA),\(^7\) gases, and fluorescent chromophores.\(^{13}\) A nonvolatile, fluorescent Schiff base derivative has been synthesized from MDA and amino acids,\(^{13}\) forming 1-amino-3-iminopropene; phospholipid peroxidation yields fluorescent chromophores with excitation and emission maxima similar to this compound.\(^{8,12}\) Nonvolatile lipid peroxidation products are extractable in chloroform-methanol, allowing assay of the rate and degree of iron-induced peroxidation in tissue by spectrofluorometry.\(^{5,34,63}\)

Several uncontrolled prospective and retrospective studies have suggested that the administration of anti-convulsant drugs to head-injured patients would prevent the development of posttraumatic epilepsy.\(^{37,42,61,64}\) However, randomized placebo-controlled double-blind investigations have failed to document the efficacy of the use of phenytoin in preventing epilepsy after head trauma.\(^{11,31,65,66}\) Repetitive electrical stimulation of cortical and subcortical structures through electrodes implanted in experimental animals causes the development of seizures.\(^{52}\) This process of kindling can be prevented by the prophylactic administration of phenobarbital.\(^{2,50,52}\)

The prophylactic administration of phenytoin\(^{32}\) to cats will prevent induction of EEG seizures following the cortical implantation of metallic cobalt.\(^{17}\) However, the transient epileptogenic properties of cobalt\(^{41}\) appear to depend upon diffusion of the cations of cobalt from the implantation site,\(^{8,55}\) with subsequent penetration of the metal through calcium channels in the neuronal plasma membrane.\(^{3,40}\) If a portion of the action of phenytoin within the brain depends upon an effect on calcium channels,\(^{21,44}\) then its purported prophylactic effect in the cobalt model of epilepsy may have been due to the prevention of the chemical effect of the cations on neurons, rather than upon an action assumed to be related to prevention of true epileptogenesis. However, pretreatment of rats prior to intracortical

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Fig. 3. Lipid-soluble fluorescence measured in chloroform-methanol extracted homogenates 120 minutes after intracortical injection of 0.9% saline (open bar) or 10 \(\mu\)l of 100 mM FeCl\(_3\) (shaded bar). Although treatment with 30 mg/kg of methylprednisolone (MPS) caused significant inhibition of lipid peroxidation, a 90-mg/kg dose of MPS caused peroxidation inhibition to equal values in the saline-injected brains. Treatment with 100 mg/kg of phenytoin (PT) failed to inhibit fluorescence formation, with values equal to units of activity found in control iron-injected animals that received propylene glycol (PG). Data are means ± standard deviation for four to six rats. Asterisks = \(p < 0.001\).
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injection of iron salts with \(\alpha\)-tocopherol and selenium, agents known to scavenge free radicals and prevent lipid peroxidation, will prevent the seizures and the histopathological changes usually caused by iron.\(^7\)

In this experiment, treatment of rats with corticosteroids prevented lipid peroxidation and the development of acute iron-induced seizures. Corticosteroids may affect cortical lipid peroxidation either by intercalation within the hydrophobic portion of plasma membranes, protecting unsaturated double bonds from propagation of peroxidation reactions initiated by free radicals, or by a direct antioxidant effect.\(^5\) However, phenytoin treatment prevented seizures by its anticonvulsant action, but had no effect on lipid peroxidation. If post-traumatic epilepsy develops because of deposition of heme compounds or iron within the cortex, then treatment designed to prevent peroxidation may be more effective in epilepsy prophylaxis than the administration of anticonvulsant drugs that mask convulsive seizures while biochemical brain injury continues.

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References


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