Effect of indomethacin pretreatment on acute mortality in experimental brain injury

HUN Joo KIM, M.D., JOSEPH E. LEVASSEUR, M.S., JOHN L. PATTERSON, JR., M.D., GEORGE F. JACKSON, B.S., GORDON E. MADGE, M.D., JOHN T. POWLISHOCK, PH.D., AND HERMES A. KONTOS, M.D., PH.D.

Departments of Medicine, Anatomy, and Pathology, Medical College of Virginia, Richmond, Virginia

The effect of indomethacin administration on the mortality rate of brain-injured rats was studied in four groups of animals subjected to a level of injury with a fluid-percussion apparatus predetermined to cause 50% mortality (50% lethal dose, or LD50). There were 24 animals in each of the following groups: 1) a control group, on which the LD50 was evaluated; 2) an ethanol-treated group with a mean blood serum level of 0.32 ± 0.03 gm% (± standard error of the mean); 3) an indomethacin-treated group at a dose level of 3 mg/kg body weight administered intraperitoneally 10 to 15 minutes before injury; and 4) an indomethacin/ethanol-treated group. Significant differences in mortality rates were found in these experimental groups; namely, 50%, 58%, 8.3% (p < 0.005), and 25% (p < 0.05), respectively. The predetermined LD50 level of a 2.5- to 2.6-atm peak pressure pulse produced immediate apnea in all animals, which was either sustained (Type III), followed by temporary respiratory recovery (Type II), or followed by permanent resumption of breathing (Type I). The most important effect of indomethacin on respiratory function was manifested by a much higher percentage of Type I respiratory responses and a much lower percentage of Type II and III responses (hence a lower mortality rate). There was also a more rapid return to normal breathing in the postapneic period of recovery. Suppression of prostaglandin synthesis and of superoxide anion production at the onset of trauma may explain, at least in part, these favorable effects of indomethacin.

Key Words: brain injury • ethyl alcohol • indomethacin • superoxide radical • rat

Although there have been major advances in understanding the complex events associated with central nervous system (CNS) trauma, treatment remains largely palliative, including measures to reduce intracranial pressure (ICP). In the past few years, however, research findings suggest avenues for more effective treatment; among these is the alteration of the role of oxygen free radicals in the overall pathophysiology of head injury. Brain injury, produced by a standardized fluid-percussion system, initiates accelerated arachidonic acid metabolism via cyclo-oxygenase with resulting production of oxygen free radicals. Anatomical changes follow such injury, in the form of multiple pial arteriolar lesions and increased permeability to protein, which can be substantially reduced or eliminated by cyclo-oxygenase inhibitors such as indomethacin or by free radical scavengers such as superoxide dismutase (SOD). Oxygen radicals are capable of inducing cell injury and ultimately cell death. Therefore, the potential for direct neuronal damage from oxygen radicals generated in brain injury is also present. The possibility of effective therapy after head injury is raised by the finding of accelerated arachidonic metabolism in CNS injuries, characterized by a marked increase in prostaglandin levels for a period of up to 1 hour after injury.

In view of the above findings, the present study addressed the question of whether the mortality rate of brain-injured, spontaneously breathing rats can be reduced by pretreatment with indomethacin which, by blocking cyclo-oxygenase activity, prevents the formation of superoxide radicals. In addition, since human head injuries on the highway and elsewhere are frequently associated with alcohol intoxication, a series of inebriated rats were studied, both with and without preadministered indomethacin.

Materials and Methods

The experiments were carried out on 96 Sprague-Dawley rats, each weighing between 250 and 350 gm.
Each animal was anesthetized with a stock solution containing 75 gm% urethane and 5 gm% α-chloralose at a dose of 0.5 ml/kg body weight given into the intraperitoneal cavity. A longitudinal incision, 2 cm long, was made on the ventral surface of the neck and the trachea was exposed. The endotracheal tube consisted of a 4-cm length of polyethylene (PE) 240 tubing and a 2.5-cm length of a plastic cone cut from an Eppendorf pipette tip (Fig. 1A). The short segment of tubing was pulled through the cone until an overall length of 6 cm was reached, thus minimizing the dead space. The endotracheal tube was secured in position with a 3-0 silk ligature, and the wound was closed with a 3-0 suture.

A craniotomy was performed with or without the use of a stereotactic device. A midline scalp incision was made, 3 cm long, and the bone surface was scraped clear of all tissues. A Bovie electrosurgical unit was used whenever necessary to dry the bone surface. A Dremel electric drill with a No. 4 round dental burr was used to cut a circular hole just caudal to the coronal suture line to accommodate the cranial connector (Fig. 1B). A slot, 1.5 mm wide, was also cut laterally on each side of the burr hole to place two retaining stainless steel screws, size 0-80. The screws were modified to minimize or prevent any trauma to the brain from impingement on its epidural surface (Fig. 1C). The dura was left intact in all preparations.

A retaining screw was placed in position by carefully sliding its head into each slot between the bone and the dura. The bent and denuded tip of a No. 28 Belden enamel-coated wire, 6 cm long, was placed in apposition to each screw for use as an electroencephalographic (EEG) electrode (Fig. 1D). Dental acrylic was applied in a ring around the burr hole to immobilize the retaining screws and EEG wires. The plastic cranial connector, 8 mm in length, was cut from the hub of a disposable hypodermic needle, placed over the burr hole, and fixed in place with a second application of dental acrylic. After the acrylic had been allowed to cure, the animal was cannulated with a 13-cm length of PE 10 tubing via one of the femoral arteries.

Arterial blood was analyzed for pCO2, pO2, pH, and hematocrit on samples taken before and 3 minutes after head injury. The arterial blood pressure was measured with a microvolume transducer system.14 The ICP was monitored with a standard pressure transducer* while an EEG recording was obtained through the two implanted epidural electrodes. Blood serum alcohol levels were analyzed on a gas chromatograph in a parallel series of 12 animals according to the method described by Curry, et al.4

All animals were placed in a prone position and connected by a hemicircular interconnector to a horizontally oriented fluid-percussion apparatus described in detail elsewhere.26 The level of percussion injury which produced a 50% mortality rate was determined

*Pressure transducer, Model 23Db, manufactured by Gould Inc., Cleveland, Ohio.
Indomethacin pretreatment in brain injury

First in a preliminary series and was found to be a housing pressure pulse of 2.5 to 2.6 atm with a duration of 20 to 22 msc. The animals were separated into four groups: 1) animals subjected to brain injury but no form of treatment and denoted as the control group; 2) animals given ethanol intraperitoneally 10 minutes before brain injury at a dose of 4 gm/kg body weight of 95% alcohol diluted to a 20% solution; 3) animals given indomethacin intraperitoneally 10 to 15 minutes before brain injury at a dose of 3 mg/kg body weight; and 4) animals given both ethanol and indomethacin treatments 10 to 15 minutes before brain injury.

Respiratory responses to the injury were evaluated on the basis of three general types (Fig. 2): animals with a single period of apnea followed by a sustained resumption of breathing (Type I); animals that showed two or more periods of apnea but that ultimately succumbed to a permanent state of respiratory arrest (Type II); and animals that never recovered from the initial apnea (Type III).

Sampling tracheal gas with a CO₂ analyzer† provides an uninterrupted record of respiration at the time of impact. Comparative trial recordings utilizing both the CO₂ signal from the Beckman analyzer and the displacement signal from a Whitney mercury gauge around the chest indicated that the Beckman system roughly approximated changes in thoracic circumference if the flow in the system was not excessive. On the other hand, since the respiratory rate of the rat is relatively high and the minimum effective sampling rate of the Beckman analyzer is greater than the rat’s tidal volume, no attempt was made to quantify the end-tidal pCO₂.

The Beckman system was used primarily as a respiratory rate indicator and a relative indicator of respiratory volume. In the later stages of the study, the intrathoracic pressure variations with respiration were recorded by means of a length of PE 10 tubing introduced percutaneously through the chest wall into the pleural cavity. This tubing was connected to a second microvolume pressure transducer unit. The time parallax for the Beckman system determined in this way was 0.3 seconds.

In a parallel series of experiments, the level of indomethacin in arterial blood plasma and in brain tissue was determined. A modification of the Ou and Frawley²¹ method was utilized for determining the concentration of indomethacin in the plasma. Fresh 100-μl arterial blood samples were immediately transferred to 1-ml tubes and stored in ice. The tubes were later gently rotated by hand and centrifuged for 10 minutes. The plasma was transferred to a second set of tubes and stored at −5°C for analysis.

The analysis was carried out with a liquid chromatograph‡ equipped with a gradient pump. This unit also had a Kratos Spectroflow 773 variable-wavelength detector, a Rheodyne injection valve fitted with a 20-μl sample loop, and a Phenomenex Octadecyl (C18) column, 150 mm long, containing 5-μm diffusion particles. The mobile phase consisted of 70% acetonitrile and 30% 0.1 mM sodium acetate (pH 3.6) pumped at a rate of 1 ml/min. The effluent was measured at 260-nm wavelength with a rise time of 2 seconds and a detector setting of 6 nm full scale. An integrator measured the areas under both peak deflections for indomethacin and the internal standard, prednisone.

A 50-μl plasma sample was dispensed into a 1.5-ml tube, and 10 μl of prednisone (2 μg/ml) was added. The tube was gently rotated by hand and made alkaline with the addition of 25 μl saturated borate solution. After brief rotation once again, 0.5 ml ethyl acetate was added, rotated for 1 minute more, then centrifuged for 5 minutes. The organic phase was transferred to a glass tube placed in a water bath at 40°C under a constant stream of air. The dried sample was reconstituted with 200 μl of acetonitrile, rotated for 20 seconds, then 20 μl was injected into a high-pressure liquid chromatography system for analysis. The retention time for indomethacin and prednisone was 2.96 and 1.88 minutes, respectively.

The unknown plasma samples were calculated from a standard curve generated from area ratios of indomethacin to prednisone for each chromatographic batch. This procedure was carried out on a daily basis. The coefficients of variance for 0.68, 2.06, 8.24, and 25.75 μg/ml plasma were 7.9%, 4.2%, 1.0%, and 1.9%, respectively. Analytical recovery was assessed by comparing the area ratios of plasma containing a known amount of indomethacin to that of an unextracted indomethacin sample at the same concentration. This ratio was 83%. Calibration was linear from 0.5 to 40 μg/ml.

The brain tissue was analyzed for ¹⁴C-labeled indomethacin by the combustion method of Jacobson and Gupta²⁶ and of Niimi and Burnison.³⁸ Tissue samples weighing 1 gm were dried at room temperature for 16 hours. The samples were then placed in a combustion

† CO₂ analyzer, Model LB2, manufactured by Beckman Instruments Inc., Fullerton, California.

‡ Liquid chromatograph, Series 400, manufactured by Perkin-Elmer Corp., Norwalk, Connecticut.
apparatus§ and completely oxidized. All tissue and standard, recovery, and memory samples were counted on a temperature-controlled liquid scintillation counter.‖ The brains were perfused in situ with normal saline and removed from the cranial cavity within 2 minutes following injury.

Experiments were monitored for 1 hour after brain injury if the animal survived for this period. All animals alive at the end of this observation period were declared survivors and were sacrificed. At necropsy, a visual in situ evaluation of the amount of subarachnoid bleeding over the dorsal aspect of the brains was made prior to their removal from the cranial vault. As the brains were carefully freed and lifted out of the skull, the extent of bleeding into the dorsal and ventral aspects of the brain stems was also noted. The overall degree of hemorrhage was assigned a graded value of 1+, 2+, or 3+ according to whether the severity was judged to be slight, moderate, or extensive, respectively. Next, the brains were transferred to fixative and then further macroscopically examined and photographed; any evidence of contusions, lacerations, or hemorrhage was recorded. Additionally, sagittal sections were obtained and again examined and photographed to confirm the presence of intraparenchymal hemorrhage. The lungs were also removed at necropsy and preserved for histological examination.

**Results**

Significant differences in mortality levels were found among the four experimental groups of animals (Fig. 3). The experiments were carried out in a random manner among the groups, for a total of 24 animals in each of the four categories. In each case, the intracranial injury was produced by a fluid pressure pulse of 2.55 ± 0.05 atm (mean ± standard error of the mean) with a duration of 20 to 22 msec. This percussion pulse produced, on average, a 50% mortality in the rats and this finding is reflected in the control results. Animals receiving ethanol, indomethacin, and the combination of ethanol and indomethacin had a mortality rate of 58%, 8.3%, and 25%, respectively. Statistical evaluation by the analysis of variance test showed a significant difference between the control and indomethacin-treated groups (p < 0.005) and between the ethanol and the ethanol/indomethacin-treated groups (p < 0.005). Despite the 50% reduction in mortality rate between the control and the ethanol/indomethacin groups, the difference was only marginally significant (p = 0.1).

The blood alcohol levels were determined in a parallel series of animals (Table 1). The ethanol dose, route of administration, and the time of sampling were identical to those utilized in the head-injury experiments. The results showed a mean blood serum ethanol value of 0.32 ± 0.03 gm% — a level of intoxication well into the range of impaired activity and CNS depression. The preinjury arterial blood samples taken from the femoral artery (Table 2) had mean pO2, pCO2, and pH values of 83.0 ± 1.1 mm Hg, 33.7 ± 0.8 mm Hg, and 7.422 ± 0.006, respectively, for the 96 experimental animals.

### Table 1

<table>
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<tr>
<td>total rats</td>
<td>12</td>
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<tr>
<td>body weight (kg)</td>
<td>0.315 ± 0.014</td>
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<tr>
<td>ethanol dose (ml)</td>
<td>6.29 ± 0.27</td>
</tr>
<tr>
<td>serum ethanol (gm%)</td>
<td>0.32 ± 0.03</td>
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* Serum ethanol samples were taken 10 minutes after injection. Values are means ± standard error of the means.

### Table 2

<table>
<thead>
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<tr>
<td>total rats</td>
<td>96</td>
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<tr>
<td>pO2 (mm Hg)</td>
<td>83.0 ± 1.1</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>33.7 ± 0.8</td>
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<tr>
<td>pH</td>
<td>7.422 ± 0.006</td>
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* Values are means ± standard error of the means. Samples were obtained approximately 10 minutes before brain injury.


‖ Liquid scintillation counter, Model LS-150, manufactured by Beckman Instruments Inc., Fullerton, California.

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Indomethacin pretreatment in brain injury

![Absorption profile from the intraperitoneal cavity to arterial blood for indomethacin. The time required for maximum drug concentration was about 10 minutes with an absorption rate constant of 0.195 min⁻¹. There was no significant difference between the control and the brain-injured groups.](image)

**Fig. 4.** Absorption profile from the intraperitoneal cavity to arterial blood for indomethacin. The time required for maximum drug concentration was about 10 minutes with an absorption rate constant of 0.195 min⁻¹. There was no significant difference between the control and the brain-injured groups.

The acute elevation in the arterial blood pressure commonly observed immediately following moderate CNS injury was most prominent in the control group, with a mean increment of 61.8 ± 6.0 mm Hg. The mean pressor response of 53.8 ± 5.5 mm Hg in the alcohol-treated animals was not significantly different from the control level. The pressor response was, however, significantly reduced to 39.3 ± 5.2 and 44.0 ± 7.0 mm Hg in the indomethacin-and ethanol/indomethacin-treated animals, respectively (p < 0.05 in both cases).

Virtually all percussion injuries at 2.5 to 2.6 atm induced apnea. The longest durations of the initially induced apnea associated with ultimate resumption of spontaneous breathing were in the range of 40 to 50 seconds after injury. The respiratory recovery was either sustained (Type I respiratory response) or temporary followed by a second period of apnea (Type II). In every experiment in which spontaneous breathing did not recur within this seemingly critical time limit, apnea was considered permanent (Type III). The initial period of apnea in the control group, when taken as a composite of Type I and Type II responses, had a mean value of 11.7 ± 2.6 seconds (Fig. 6). The comparative values for the ethanol-, indomethacin-, and ethanol/indomethacin-treated groups were 16.0 ± 3.1, 8.0 ± 1.3, and 12.7 ± 2.4 seconds, respectively. Whereas the general effect of indomethacin on respiratory function appeared to be a shortening of the initial period of apnea, the reduction was significant only between the ethanol-treated group and the indomethacin-treated group (p < 0.05). The values are means ± standard error of the means.

![Histogram of apneas showing combined Type I and II respiratory responses for each of the four groups: control, 11.7 ± 2.6 seconds; ethanol (EtOH), 16.0 ± 3.1 seconds; indomethacin, 8.0 ± 1.3 seconds; and ethanol/indomethacin, 12.7 ± 2.4 seconds. The differences are statistically significant only between the ethanol-treated group and the indomethacin-treated group (p < 0.05). The values are means ± standard error of the means.](image)

**Fig. 5.** ¹⁴C-labeled indomethacin content in brain normalized to arterial blood. The brain ¹⁴C content was significantly higher in the trauma group (p < 0.05).

![Lung histology showing pathological changes among the four experimental groups. The control group included one animal with extensive edema that died 3 minutes post-injury, one with pulmonary congestion, and one with pneumonia; the latter two both survived. The ethanol-treated group included one animal with intra-alveolar hemorrhage and marked congestion which died within 5 minutes after brain injury. The indomethacin-treated group included four animals with bronchial pneumonia which survived brain injury. The group receiving ethanol and indomethacin included two animals with pulmonary edema which survived, one animal with pulmonary congestion which survived, one with marked congestion associated with intra-alveolar hemorrhage which died within 3 minutes after brain injury, one with pneumonia which survived, and one with pneumonia which died 30 minutes after brain injury. The remaining 21 animals that died in these experiments did not manifest any microscopic pulmonary change to which death could reasonably be attributed.](image)

Examination of the brains at necropsy revealed no appreciable differences in the extent of subarachnoid bleeding between animals within the same group, while clear differences were observed between the four groups.
The indomethacin-treated rats showed the least amount of subarachnoid bleeding and all were rated as 1+. The control group and the animals treated with indomethacin and ethanol all suffered a moderate amount of bleeding and were assigned a 2+ rating. The alcohol-treated rats showed by far the most extensive subarachnoid hemorrhage and all were graded as 3+. Epidural hematomas at the site of impact were observed in about 50% of the animals in this study.

With the exception of the amounts of subarachnoid bleeding, macroscopic examination of both the whole and sagittally sectioned brain material revealed no other detectable differences in the pathological changes seen in the control versus the experimental groups. Although some heterogeneity in the degree of pathological change was seen within each experimental group, all showed a relatively consistent pattern of injury. Typically, portions of the frontoparietal and striate cortices were contused; in addition, varying amounts of subarachnoid blood were recognized within the superior and basal cisterns, in the cisterna magna, and over the convexities. At the site of contusion, hemorrhage occurred at the interface of the cortical gray and subcortical white matter and, on occasion, intraventricular hemorrhage was also recognized. With the exception of tissue tears directly beneath the injury connector, axonal damage, and hemorrhages as described, no histological changes in the brains in this injury model were visualized by conventional light microscopy 1 hour after injury.

Discussion

The principal aim of these experiments was to test the efficacy of indomethacin in reducing the mortality rate due to experimental brain injury. Additionally, in view of the high incidence of alcohol intoxication associated with brain injury in the United States and other countries, the effectiveness of this cyclo-oxygenase inhibitor was investigated in the presence of high blood levels of ethanol.

Indomethacin was chosen as the cyclo-oxygenase inhibitor in this study because of its well-known use as a nonsteroidal anti-inflammatory agent; hence, in the event of positive results, the drug could have immediate potential for therapeutic application in human head injury. It was, of course, realized that indomethacin might have other modes of action. The postulated protective mechanism of action, in this case reducing the extent of damage by blocking the production of superoxide anion radicals, could later be substantiated by substituting one of the action-specific scavenging enzymes, the SOD's. This indeed proved to be the case in our later demonstration of major reduction of mortality in this injury model in response to SOD.

The rationale for the use of indomethacin in the prevention of mortality from brain injury derives from earlier studies in which it was shown that the vascular abnormalities in this model of brain injury are mediated by oxygen radicals generated in association with accelerated arachidonate metabolism via cyclo-oxygenase. These studies showed that the following sequence of events takes place after fluid-percussion brain injury. Initially, there is activation of phospholipases, thereby leading to increased release and increased availability of free arachidonate, which in turn leads to accelerated prostaglandin synthesis via prostaglandin H synthase. In vitro experiments showed that this enzyme, in the presence of suitable reducing cosubstrates (such as the reduced form of nicotinamide adenine dinucleotide (NAD) or the reduced form of NAD phosphate) produces superoxide anion radicals via its hydroperoxidase action. Superoxide generation and its appearance in the cerebral extracellular space have been demonstrated after brain injury in cats as the SOD-inhibitable reduction of nitroblue tetrazolium. Consistent with this mechanism are the findings that inhibitors of cyclo-oxygenase or oxygen radical scavengers minimize or prevent the manifestations of vascular injury after brain injury.

The generation of oxygen free radicals can occur with extreme rapidity. For example, free radical concentration in the ischemic rabbit heart reaches a peak within 10 seconds after the onset of reperfusion with control perfusate. Reperfusion with r-h-SOD almost totally eliminated this burst of free radical generation.

The effectiveness of indomethacin in the present experiments is consistent with the view that the drug is active via inhibiting cyclo-oxygenase, thereby minimizing the production of oxygen radicals. The inhibition of oxygen radical generation by indomethacin may be exerting its beneficial effect either via the prevention of vascular damage which leads to edema or other forms of permeability change (thereby adversely affecting neural function) or via the prevention of direct damage by the radicals on neural tissue. The fivefold increase in 14C-labeled indomethacin in traumatized brain tissue conditionally meets the requirement for such direct action. The fact that the first breath after the injury pulse is incomplete suggests an initial biophysical effect on the respiratory center complex in the brain stem. Continuance of the apnea and its shortening by indomethacin are suggestive of a biochemical mechanism involving prostaglandins and oxygen free radicals.

Other possible mechanisms for the beneficial effect of indomethacin in these experiments should be considered. In the indomethacin-treated animals, the magnitude of the initial hypertensive episode after injury was less pronounced than in those not treated with indomethacin. The mechanism of this effect is not known. However, if one considers the alcohol- and indomethacin-treated groups, there is no clear relationship between mortality and the magnitude of the increase in blood pressure.

The incidence and severity of subarachnoid hemorrhage in the indomethacin-treated rats were less pronounced. In this respect, both indomethacin and vitamin E reduced the incidence of intraventricular and
Indomethacin pretreatment in brain injury

periventricular hemorrhage in premature infants. It is plausible but not proven that this effect of indomethacin is related to the reduction of oxygen radical generation and consequent reduction in vessel-wall injury.

Finally, since the animals died primarily because of apnea and since all brain material, irrespective of treatment, showed comparable pathological change, one should consider the possibility that indomethacin acts on the lungs in addition to its effects on the brain. This possibility is not supported in this study which showed the absence of significant lung pathology. Hence, it is more likely that apnea was the result of effects on brainstem respiratory center function rather than due to peripheral action on the lungs.

Our attention was focused on the respiratory, circulatory, and EEG variables. On the whole, the abnormalities in the lungs themselves were conspicuously absent in the 1st hour after brain injury. Edema, congestion, intra-alveolar hemorrhage, and/or bronchial pneumonia were confirmed histologically in only 13 animals, 10 of which were survivors. Hence, in 90% of the animals that died, respiratory arrest in the absence of appreciable lung pathology must have been induced by an early event of central origin, independent of any sequelae commonly attributed to the respiratory distress syndrome. The lack of pulmonary involvement does not minimize the importance of well-documented pulmonary complications, which may be latent as a result of the initial CNS injury. 1-13 It does, however, indicate a need to distinguish between the effects of a primary intraneuronal impairment of mechanisms that are responsible for the normal generation and/or flow of nerve impulses and the effects of secondary non-neurogenic (for instance, biochemical) modalities which subsequently come into play to alter system functions profusely.

High levels of acute blood alcohol have been shown to depress the cerebral metabolism despite an increase in cerebral blood flow and a decrease in cerebrovascular resistance. 5 More recently, ethanol was shown to slow the rate and decrease the extent of mitochondrial oxidation in response to direct cortical stimulation, suggesting that the reduced metabolism might be occurring, at least in part, via the graded inhibition of Na+, K+, and adenosine triphosphatase activity. 5 The specific processes by which ethanol (a lipophilic molecule) induces these metabolic alterations remain unidentified but, in general, the evidence points to a disturbance in the ionic equilibrium across cell membranes.

In no animal did routine gross or light microscopic examination of the brains and lungs reveal any morphological lesions to which death could be confidently attributed. Within the 1st hour after injury, conventional light microscopy did not disclose findings other than hemorrhage. These findings must be reconciled with the fact that all animals that died did so as a result of pulmonary arrest, manifested either as an inability to recover spontaneously from the initial (first) apnea or as an inability to maintain an effective tidal volume upon recovery from subsequent apneas. Clearly, the CNS insult was of sufficient intensity to cause at least an immediate electrophysiological breakdown in the processes that mediate respiratory movements. The next breath after the injury pulse is not taken. The immediacy of both the respiratory arrest in the end-expiratory phase and the disappearance of cortical EEG reflects initial devastating effects on nerve cell function on the part of the intracranially induced wave of volume displacement and/or the compression wave associated with the fluid-percussion pulse (these effects being often but not always reversible). These mechanical effects lead to an increased prostaglandin superoxide anion and hydroxyl anion formation, 29 the vascular effects of which have been documented by Wei and colleagues, 28 but not the effects on nerve cells.

The indomethacin-treated animals showed the least amount of subarachnoid bleeding while the ethanol-treated group of rats was observed to have suffered the greatest degree of hemorrhage. The augmented effect of ethanol on the trauma-induced hemorrhage has been linked to an adversely affected platelet function via the lipid solubility capabilities of the alcohol molecule on the membrane as well as to the pathopharmacological potentiation of free radical production by its principal metabolite, acetaldehyde. 38,9 The results support the view that the common link between mechanical and alcohol-induced biochemical traumas is the binding of free radicals onto unsaturated fatty acids. 7

The degree of intracranial bleeding correlated poorly with animal survival. It is, therefore, unlikely that indomethacin exerted any direct effect on mortality by reduction of intracranial hemorrhage. A finding, however, that did correlate well with survival was the reinitiation of spontaneous breathing following the first period of apnea. When the apnea was not interrupted by an effective inspiratory effort within 45 to 50 seconds after its onset, then a progressively worsening hypoxia and hypercapnia almost always resulted in death within 5 minutes. This determinant of eventual survival so early in the aftermath of CNS injury implicates an underlying disturbance upon which the hemorrhage was superimposed. Recent experiments have shown that superoxide radical generation can increase nearly threefold within 10 seconds after the initiation of myocardial reperfusion. 30 Additionally, the increase in phospholipase activity and in metabolites of arachidonic acid after fluid-percussion injury in brain parenchyma as well as in the vessel wall has been reported. 6,29 In a follow-up study in our laboratory in which the oxygen free radical scavenger SOD was substituted for indomethacin in 24 rats, the results showed a comparable reduction in mortality. 15 The evidence supports the existence of an oxygen free radical accumulation which indomethacin can prevent or minimize at the time of injury, with subsequent enhanced probability for survival.

On the whole, this study offers a new possibility in the pharmacological approach to the treatment of head
injury, with potential clinical significance. Pretreatment clearly is required to determine the maximum therapeu
tic potential of an agent in head injury. Studies with ad
ministration of the agent at different times postinjury
will be required to determine what fraction of this
maximum effect can be achieved by later administra
tion. Since superoxide anion production continues for
at least 1 hour after brain injury, our findings raise the
possibility of applicability of indomethacin, or other
compounds with similar effects, to the treatment of
human head injury. Our results with SOD clearly im
plicate the adverse role of oxygen free radicals in the
moribundity and mortality that follow brain trauma and
lend credence to the inhibitory role of indomethacin in
reducing the production of these radicals. Further stu
dies along these lines are needed to clarify more com
pletely the plurality of mechanisms that facilitate or
hinder the recovery of CNS function.

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Address for Dr. Kim: Department of Neurosurgery, Wonju
College of Medicine, Yonsei University, Wonju, Republic of
Korea.
Address reprint requests to: John L. Patterson, Jr., M.D.,
Division of Cardiopulmonary Laboratories and Research,
Department of Medicine, Medical College of Virginia, MCV
Station Box 282, Richmond, Virginia 23298.