Recent experiments in brain ischemia have demonstrated the formation of both cyclooxygenase- and lipoxygenase-derived metabolites of arachidonic acid during early postischemic reperfusion. We have demonstrated the accumulation of granulocytes in ischemic areas following incremental air embolism. In the same model, we have shown that indomethacin, prostacyclin, and heparin improved the outcome of neuronal function and blood flow, acting presumably by reducing the production of thromboxane and prostacyclin, with replacement of prostacyclin to achieve reduced platelet aggregation and vasodilation. Previous work indicated that heparin is necessary for enhanced recovery as well. However, we have demonstrated that indomethacin may increase leukocyte accumulation in models of inflammation and have postulated that this may be due to increased metabolism of arachidonic acid via lipoxygenase when the cyclooxygenase pathway is blocked. We expected, therefore, that our therapeutic combination might produce increased granulocyte accumulation after ischemia. If this were true, it would indirectly suggest that leukotrienes are mediators of leukocyte chemotaxis after ischemia and that inhibition of lipoxygenase might improve the outcome after ischemia by reducing leukocyte-induced damage.

As a test of this hypothesis, we compared the cortical somatosensory evoked response (CSER), brain blood flow, and accumulation of 111In-labelled leukocytes in dogs subjected to 1 hour of multifocal ischemia induced by air embolism and treated with prostacyclin, heparin, and indomethacin with untreated ischemic animals.

Materials and Methods

The techniques for incremental air embolism, as well as the collection, labelling, and detection of leukocytes in brain tissue, have been described in detail. For this experiment, 21 conditioned, male mongrel dogs (Canis familiaris) weighing 9-15 kg were premedicated with 1.1 mg/kg xylazine and 0.05 mg/kg atropine s.c.; surgical anesthesia was induced with 80 mg/kg α-chloralose and maintained with doses of 20 mg/kg as needed. The dogs were intubated and mechanically ventilated. Femoral arterial and venous catheters were inserted for monitoring of blood pressure, administration of drugs, and intermittent monitoring of blood gases, pH, and hematocrit. The right internal carotid artery was catheterized for administration of air, and electrodes were mounted in the skull for recording the response evoked by left median nerve stimulation, using a Nicolet CA-1000 evoked response system.

These experiments were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Resources, National Research Council, Department of
Prior to ischemia, 102 ml of blood was collected from a femoral artery catheterer and placed in 18 ml of anticoagulant citrate dextrose solution (ACD-Formula A, Fenwall Laboratories, Deerfield, Ill.). One hundred milliliters of Ringer's lactate solution was injected immediately after blood sampling. A Beckman JE-6B elutriator was used to collect a sample of granulocytes with 95% purity and viability as assessed by microscopy and trypan blue exclusion. The granulocyte suspension was incubated with 1 mCi of $^{111}$Inoxine and then centrifuged and washed to assure that the radioactivity was present only in the granulocytes and was not bound to plasma protein. The cells were then resuspended in saline and reinfused 5 minutes prior to ischemia.

Five CSERs were measured prior to ischemia, and the average P1–N1 amplitude of these 5 responses was calculated as a baseline. Ischemia began with the infusion of 50 μl of air into the right internal carotid artery. CSERs were obtained every 2 minutes during the next 60 minutes, and repeated doses of 20–50 μl of air were used according to a predetermined algorithm to maintain the P1–N1 amplitude of the CSER at 10–20% of baseline. After ischemia, dogs received either no therapy (control) or 4 mg/kg indomethacin (Merck Sharp & Dohme, West Point, Penn.) i.v. in Tris buffer, 300 IU/kg heparin i.v., and a continuous infusion of prostacyclin (PGI2, Upjohn Co., Kalamazoo, Mich.) in Tris buffer at a rate of 90–140 ng/kg/min. CSER was measured every 10 minutes for 4 hours following ischemia. Recovery was calculated as percent of the baseline amplitude.

At the conclusion of the 4-hour recovery period, 60 μCi/kg of $^{14}$Ciodoantipyrine was infused over 1 minute, and arterial blood samples were collected from a previously placed femoral shunt for later calculation of cerebral blood flow by autoradiography.4 The dog previously placed femoral shunt for later calculation of cerebral blood flow was killed rapidly with KCl injected into the right carotid catheter. An unexplained electrical artifact in 1 treatment group suffered an extubation accident during the recovery phase, and a single dog in each group experienced significant hemorrhage from the carotid catheter. An unexplained electrical artifact in 1 treatment dog made interpretation of the CSER impossible.

Single comparisons between the control and treatment groups were made using Wilcoxon’s rank sum test, and estimates of the Type II error were based on the F distribution. We considered results significant at p < 0.05.

### Results

Hematocrit, pH, PaCO2, PaO2, rectal temperature, and mean arterial pressure were not different in the control and treatment groups during the baseline, ischemia, and therapy sampling times. We attempted to ensure that the duration and severity of ischemia in both groups was similar by comparing the amount of air injected during the ischemic period (0.22 ± 0.11 ml, control; 0.15 ± 0.07 ml, treated), the proportion of CSER readings <10% of baseline P1–N1 amplitude during ischemia (32.9 ± 16.5%, control; 35.5 ± 18.5%, treated) and the proportion of CSER readings <20% of baseline (71.7 ± 11.9%, control; 71.2 ± 14.6%, treated) in both groups. There were no significant differences between the groups for any index. Treatment with indomethacin, PGI2, and heparin did not produce significant recovery of the CSER amplitude 4 hours after ischemia when compared with control (38.0 ± 13.6% vs. 21.0 ± 4.6%, mean ± SEM). However, this negative conclusion is not statistically powerful, having a Type II error probability of <0.03.

The percent of injected granulocyte suspension $^{111}$In activity that was not in cells was 1.7 ± 1.0% for all 13 experimental dogs. The $t_0$, for clearance of labelled cells from the blood was determined in 7 animals, and did not differ from published values.11 The mean right–left hemispheric differences in $^{111}$In activity after 4 hours of posts ischemic reperfusion were the same in both groups. The values were (mean ± SEM) 3,298 ± 763 cpm/g in the control group and 3,807 ± 665 in the treated group. Based on the sample size and SD of our samples, a 50% enhancement of granulocyte accumulation could have been shown with a power of 0.50.

Our method of preparing sections for autoradiography yielded sections that allowed identification of 9 gray matter and 5 white matter structures for each dog. Blood flows were calculated for each area and averaged for the gray and white matter in each hemisphere. Average flows were not different in the two groups, probably because of the multifocal nature of the insult. The number of dogs with very low flows (<15 ml/100
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**Discussion**

Despite the elimination of areas of very low blood flow with therapeutic manipulation of the cyclooxygenase pathway of arachidonic metabolism, we demonstrated no change in leukocyte accumulation 4 hours after ischemia induced by air embolism. These results appear to reject our initial hypothesis and suggest either very little effect on lipoxygenase product formation by indomethacin in this model or only a minor role for lipoxygenase products (leukotrienes and hydroxy acids) as granulocyte chemotaxins in the early postischemic reperfusion period.

It is not possible to use the results of this experiment to completely reject a role for lipoxygenase products in the postischemic accumulation of leukocytes. Heparin may have an effect on granulocyte accumulation although this is variable, and Higgs et al demonstrated in vivo inhibition of granulocyte adherence using PGI₂ in doses similar to those employed in our study. The net result of the competing effects of PGI₂, heparin, and indomethacin may therefore result in no change in leukocyte accumulation. In addition, the effectiveness with which indomethacin increases lipoxygenase product formation varies depending on dose and species used, and therefore we cannot be certain that the dose of indomethacin we used would have been effective. Finally, air embolism causes extensive endothelial injury, which may promote granulocyte adherence without the need for chemotaxins. Other models of ischemia may not cause such extensive endothelial damage and may therefore be more sensitive to changes in chemotactic mediators.

Although we did not influence granulocyte accumulation with the therapy used, we were able to show a correlation of leukocyte accumulation with both the severity of ischemia and with the number of areas of very low blood flows. The presence of granulocytes in ischemic areas within 4 hours of injury supports but does not prove a role for granulocyte participation in postischemic reperfusion injury. Work by Romson et
aPl in leukopenic dogs, and Mullane et al12 using a lipoygenase inhibitor, suggest that granulocytes worsen damage in models of myocardial infarction. Granulocytes produce arachidonic acid metabolites such as prostaglandins, leukotrienes, and hydroxy acids, capable of producing local vasoconstriction and increasing microvascular permeability. 8,9,23 The potent granulocyte chemotactic properties of the leukotrienes and hydroxy acids allow granulocytes to function in a self-amplifying fashion. Granulocytes can also produce cellular injury through superoxide anion and other free radical products, hydrolytic enzyme release from intracellular granules, and by the synthesis of platelet activating factor. 23-27 These theoretical considerations, as well as our data, suggest that a search for therapies that modify granulocyte accumulation may yield important benefits in postischemic neuronal recovery.

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References


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P M Kochanek, A J Dutka and J M Hallenbeck

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