Fluorocarbons Reduce Myocardial Ischemic Damage After Coronary Occlusion

Abstract. *Open-chest, anesthetized dogs with occlusions of the left anterior descending coronary artery breathed 100 percent oxygen while they were bled to a hematocrit of 25 percent and infused with an approximately equal volume (40 milliliters per kilogram) of fluorocarbon preparation or Ringer solution. Dogs breathing room air and receiving no treatment served as controls. After undergoing 6 hours of coronary occlusion, animals bled and treated with fluorocarbons developed smaller infarctions than those receiving Ringer solution or no treatment.*

Most of the deaths in patients hospitalized with acute myocardial infarction result from destruction of an excessive amount of myocardial tissue (*1*). Several types of interventions modify the extent of necrosis in experimentally induced coronary occlusion (*2*) through various mechanisms of action (*3*).

Synthetic fluorocarbons (*4*) could have a beneficial effect on acutely ischemic myocardium because fluorocarbons have a high affinity for oxygen and a potential to carry and deliver oxygen to tissue (*5*). They have been tested extensively as experimental substitutes for red blood cells and as breathing liquids (*5*, *6*) and have been successfully used as blood substitutes in patients who, because of religious convictions, refused blood transfusions (*7*). The oxygen content of fluorocarbons increases linearly with oxygen tension (PO_2) and thus can be increased substantially by respiration with 100 percent oxygen (F_IO_2 = 100 percent) (*8*). We now report on the effect of fluorocarbons on the size of experimental myocardial infarctions. To minimize the variability in infarct size that is due to variable collateral flow, we assessed the myocardium at risk of developing infarction by injecting animals with human albumin microspheres labeled with technetium-99m and subsequently examining autoradiographs of slices of the left ventricle (*9*).

Thirty-eight dogs, anesthetized with sodium pentobarbital (30 mg/kg, intravenously), were intubated and ventilated with a Harvard respirator at 20 percent F_IO_2. Lead AVL of an electrocardiogram and aortic pressure were monitored continuously. The chest was opened and the left anterior descending coronary artery was isolated just proximal to the first major diagonal branch. A polyvinyl catheter was inserted into the left atrium for continuous monitoring of pressure and for injection of microspheres. The myocardium at risk of developing infarction was evaluated before treatment by occluding the coronary artery with a Schwartz vascular clamp, then injecting technetium-99m–labeled human albumin microspheres (0.4 mCi/kg) into the left atrial line (*9*) and flushing with saline. Thirty seconds later the clamp was released.

Thirteen dogs were treated with a preparation of fluorocarbons and then respirated with pure oxygen (F_IO_2 = 100 percent). Fourteen dogs received an equal volume of Ringer solution and were respirated with pure oxygen. Eleven dogs receiving no intervention were respirated with room air (F_IO_2 = 20 percent) and served as controls. In order to prevent volume overload and allow the infusion of large quantities of fluorocarbons to maximize possible therapeutic effects, we withdrew blood (30 to 40 ml/kg) for 30 minutes to attain a hematocrit averaging 25 percent in the dogs that were to be treated with fluorocarbons or Ringer solution; controls were not bled. The groups receiving treatment were then given fluorocarbon emulsion (40 ml/kg) or Ringer solution (40 ml/kg). The fluorocarbon preparation consisted of 15 volumes of Decamine 60 (*10*) with 85 volumes of 10 percent Pluronic F68. To ensure similar electrolyte concentrations in fluorocarbon- and Ringer-treated dogs, we added 30 ml of Eri-lyte 8360 salt concentrate (*11*) to each liter of fluorocarbon emulsion immediately before infusion. Respiration with 100 percent oxygen was begun 15 minutes before permanent coronary occlusion in both fluorocarbon- and Ringer-treated dogs; controls were ventilated with 20 percent oxygen throughout the experiment.

The coronary artery was then permanently occluded with the Schwartz clamp. During the next 6 hours, fluid balance was maintained in all groups by administering 0.9 percent saline in a quantity sufficient to maintain left atrial pressure between 4 and 8 mm-Hg. Six hours after coronary occlusion the dogs were killed with an overdose of barbiturate, and the heart was excised. The left ventricle was isolated, frozen in Freon or liquid nitrogen, and sectioned parallel to

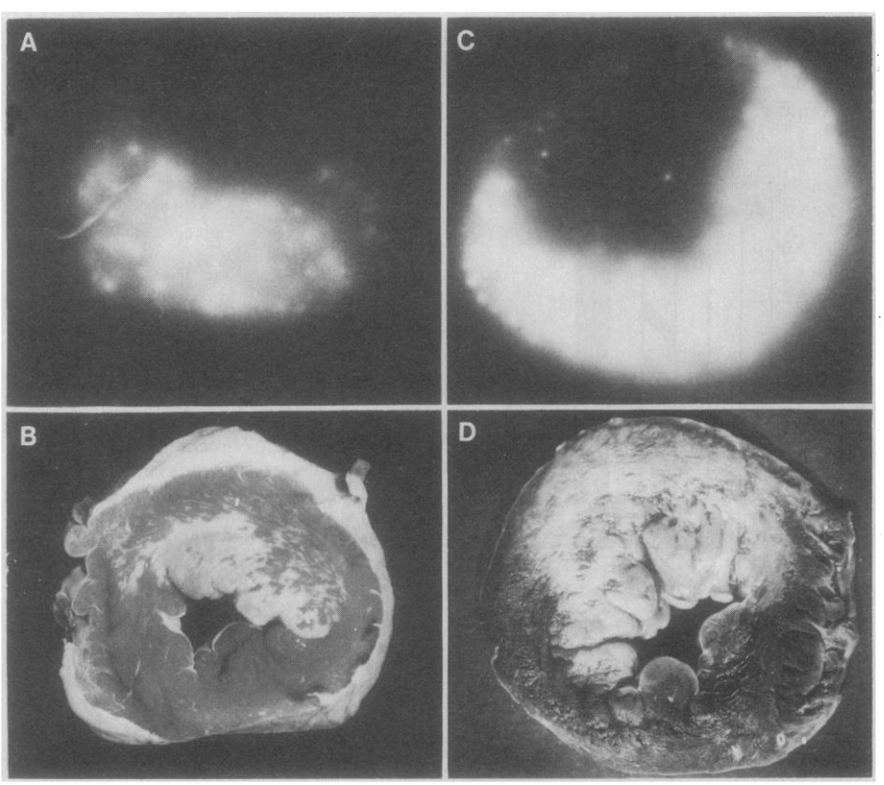

Fig. 1. Representative heart slices from (A and B) a dog treated with fluorocarbon and (C and D) a dog treated with Ringer solution. The area at risk of necrosis as determined by autoradiography (A and C) is shown as the portion of myocardium with low radiographic density (black) compared to the nonischemic tissue (white) labeled by human albumin microspheres (*9*). The area of necrosis (B and D) is shown as the myocardium unstained by TTC (pale area) compared to the noninfarcted myocardium (darkly stained). In the Ringer-treated dog the area of necrosis (D) is essentially identical to the area at risk (C). In the fluorocarbon-treated dog the area of necrosis (B) is smaller than the area at risk (A).

0036-8075/81/0327-1439$00.50/0

the atrioventricular groove into 4-mm-thick transverse slices. The slices were incubated for 15 minutes in triphenyltetrazolium chloride (TTC) at 37°C; the area of infarction appeared as a distinct pale gray area in contrast to the noninfarcted tissue, which was brick red (Fig. 1, B and D), as previously described (*12*). Subsequently, autoradiographs were made for visualization of the nonperfused myocardium at risk of developing infarction before the intervention. The heart slices were placed, apical surface down, on high-speed x-ray film and exposed for 18 hours. Pilot studies showed that autoradiographic patterns were identical if the slice was placed apex down or basal surface down on the x-ray film; that is, the slices were thin enough to reflect blood flow through the entire slice of tissue. Ischemic areas appeared as clearly demarcated zones of absent radiographic density (Fig. 1, A and C) corresponding to areas of absent or reduced numbers of the radiolabeled microspheres (*9*). Each heart slice was analyzed for the distribution of myocardium at risk and the distribution of infarction as assessed by TTC. Slices and specific areas at risk or areas of necrosis were traced on clear acetate paper under a magnifying lens and planimetered. Both apical and basal surfaces of each slice were analyzed for necrosis and averaged to obtain an overall mean area of infarction of each slice. Each area was converted to a weight for each slice and expressed as a percentage of the total left ventricular weight. For statistical evaluation, analysis of variance and Fischer's multiple comparison methods were used. Data are presented as group means ± standard errors.

Of the 38 dogs in the study, nine dogs (three in the fluorocarbon group, four in the Ringer group, and two controls) died during the experiment; one dog in each group died as a result of ventricular fibrillation during the first brief occlusion, and the others had arrhythmias during the first 30 minutes of the permanent coronary occlusion. The remaining fluorocarbon-treated (N = 10), Ringer-treated (N = 10), and control dogs (N = 9) were similar in weight (17.8 ± 2.3, 17.3 ± 2.5, and 18.1 ± 3.0 kg, respectively) and site of occlusion, measured postmortem as the distance from the coronary ostium (2.1 ± 0.2, 2.2 ± 0.2, and 2.2 ± 0.3 cm, respectively). The hematocrit was reduced similarly to 25.3 ± 0.6 percent in fluorocarbon-treated and 24.7 ± 0.5 percent in Ringer-treated dogs, and both groups received similar amounts of volume replacement (850 ± 35 ml of fluorocarbon preparation and 800 ± 46 ml of Ringer solution). Mean arterial PO_2 was not significantly different after 15 minutes of respiration with 100 percent oxygen immediately before permanent coronary occlusion (477 ± 27 mm-Hg in fluorocarbon-treated and 452 ± 21 mm-Hg in Ringer-treated dogs). Control dogs, which were not bled, had a mean hematocrit of 44 ± 3 percent (P < .001, compared to each of the treated groups) and mean arterial PO_2 of 115 ± 5 mm-Hg (P < .001, compared to each of the treated groups.

No significant differences were found among the three groups in mean arterial pressures (108 ± 7 mm-Hg in fluorocarbon-treated, 100 ± 6 mm-Hg in Ringer-treated, and 112 ± 6 mm-Hg in control dogs, respectively) or heart rates (134 ± 15, 126 ± 14, and 135 ± 7 beats per minute, respectively) immediately before the permanent coronary occlusion.

The areas of myocardium at risk of developing necrosis were similar, as assessed by autoradiography. In dogs receiving fluorocarbons, 32.3 ± 1.4 percent of the left ventricle below the occlusion was not perfused and hence at risk of becoming infarcted; the values were 31.7 ± 1.6 percent in dogs receiving Ringer solution and 30.8 ± 2.2 percent in control dogs—neither value was significantly different from that in fluorocarbon-treated dogs (Fig. 2). However, the quantity of myocardium infarcted after 6 hours of occlusion was significantly smaller in the fluorocarbon-treated dogs (22.8 ± 2.1 percent) than in the Ringer-treated dogs (32.8 ± 1.7 percent, P < .01) and controls (28.8 ± 2.0 percent, P < .01) (Fig. 2). Only 70.0 ± 5.4 percent of the area at risk went on to necrosis in fluorocarbon-treated animals compared to 103.6 ± 2.6 percent in dogs receiving Ringer solution (P < .01) and 97.0 ± 2.0 percent in untreated controls (P < .01) (Fig. 2). Representative heart slices obtained from a fluorocarbon-treated dog and a Ringer-treated dog are shown in Fig. 1.

Thus, treatment with fluorocarbon compounds decreases the extent of myocardial damage after 6 hours of experimental coronary occlusion. Since treatment with fluorocarbons requires the administration of large volumes of emulsion, we replaced a portion of the blood volume with the emulsion. Since anemia has been shown to influence the distribution of the myocardium at risk of necrosis and infarct size (*13*), one treatment group consisted of dogs that were bled to a similar hematocrit but received Ringer solution as volume replacement. A control group was not bled and received no therapeutic intervention. Fluorocarbons decreased the extent of myocardial damage after 6 hours of coronary occlusion in comparison with both the anemic Ringer-treated dogs and the untreated controls. This could be shown

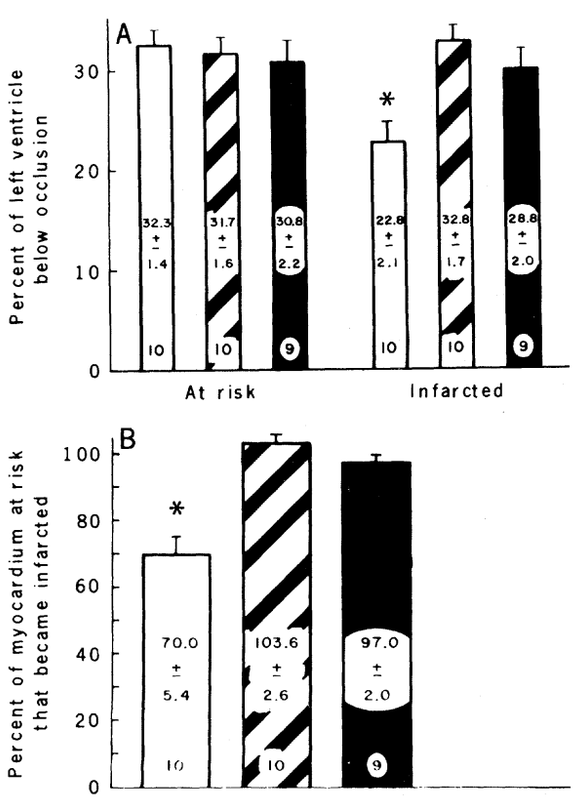

Fig. 2. (A) Bar graph showing the percentage of the left ventricle at risk and the percentage of the left ventricle that became infarcted for fluorocarbon-treated dogs (open bars), Ringer-treated dogs (striped bars), and controls (closed bars). (B) Percentage of the myocardium at risk that became infarcted in fluorocarbon-treated dogs (open bar). Ringer-treated dogs (striped bar), and controls (closed bar). Asterisk indicates P < .01.

by comparing infarct size alone ($P < .01$) or by expressing infarct size as a percentage of the area at risk of developing necrosis ($P < .01$).

The mechanism by which fluorocarbons limit infarct size is not clear. Fluorocarbons may increase oxygen delivery because their oxygen-carrying capacity at high PO_2 is actually better than that of blood (*8*) or by increasing collateral blood flow to the ischemic myocardium, perhaps because the viscosity of fluorocarbons is less than that of blood (*14*). In addition, the small size (less than 0.1 μm) of fluorocarbon particles (*10*), in comparison with the much larger red blood cells, would improve the distribution of oxygen to the myocardium at risk. That increased oxygen delivery may be the mechanism of action was suggested by a recent study by Rude *et al.* (*15*), who showed that the decrease in intramyocardial PO_2 as measured by mass spectrometry during 5-minute coronary occlusions was considerably blunted by the administration of fluorocarbons with oxygen, but not by oxygen alone. Bleeding to a low hematocrit and the resultant anemia cannot explain the protection of ischemic myocardium in this investigation, since bleeding followed by volume replacement with Ringer solution appeared to have no significant effect on the size of the infarct in comparison with untreated controls.

The autoradiographic method used to determine the portion of myocardium at risk of developing infarction can predict the extent of myocardial damage (*9, 16*). The advantage of the technique is that, before an intervention, a well-defined region at risk of developing necrosis is identified. The percent of the myocardium at risk that became infarcted in the control group was similar to that which we had previously found in control animals that were not bled (*9, 17*).

A significant amount of ischemic myocardium can be salvaged by use of fluorocarbons. These agents, which have already been safely administered to humans, deserve further study for their potential benefits in patients with acute myocardial infarction.

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4. The term fluorocarbon refers to the class of highly fluorinated liquids which are inert oxygen solvents. The term perfluoro is often used to describe the same class of compounds, but strictly speaking refers to the replacement of all hydrogen atoms attached to carbon atoms by fluorine atoms. The prefix F is used to designate perfluoro. F-Decalin exists in a *cis-* and *trans-* form and is $C_{10}F_{18}$. F-tributylamine is a mixture of several closely related isomers and is $(C_4F_9)_3N$.
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8. The solubility of oxygen in Decamine 60 is 39.4 ml per 100 ml of liquid at 25°C under exposure to 100 percent oxygen at atmospheric pressure (*6*); under similar conditions human blood carries only 20 ml of oxygen.
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10. Decamine 60 is a mixture of six parts of F-Decalin, purified by spinning-band distillation, and four parts of F-tributylamine. Particle size distribution is under study; at present it appears that it may range from 0.1 μm down to large molecular dimensions with a mean of about 0.08.
11. Eri-lyte 8360 hemodialysis concentrate (Erika, Rockleigh, N.J.) contains sodium, potassium, calcium, magnesium, acetate, chloride, and dextrose.
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18. We thank E. W. Inscho, Jr., and J. Tumas for technical assistance and N. Watterson for manuscript preparation. Supported in part by grants R01-HL-23140, R01-HL-23526, and SCOR HL-26215 from the National Heart, Lung, and Blood Institute. D.H.G. is a recipient of a research fellowship from the Max Kade Foundation, 100 Church Street, New York 10077.
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17 August 1980; revised 22 October 1980

F-Cell Production in Sickle Cell Anemia:

Regulation by Genes Linked to β-Hemoglobin Locus

Abstract. *Strong correlation of F-reticulocyte levels within sib pairs with sickle cell (SS) anemia suggests that the wide-ranging levels found in the SS population are governed by genes linked to the β^s-site. Correlations between F-cell levels in parents and F-reticulocyte levels in their children indicates that these same genes regulate F-cell production in nonanemic persons. Comparison of outcrossed and inbred SS populations suggests that relative well-being arises from homozygosity for alleles dictating high F-reticulocyte response to anemia.*

Fetal hemoglobin (HbF)–bearing red blood cells (F cells) are resistant to sickling in most patients with sickle cell (SS) anemia (*1*). In turn, severity of SS disease is governed in part by numbers of F cells produced, that is, by the level of F reticulocytes (*2*). Those uncommon persons who synthesize HbF in every cell (*3*) or in a large fraction of cells (*4*) are comparatively free of disease. We supposed that the signs and symptoms of SS anemia might be attenuated in others were it possible to increase the relative abundance of their F reticulocytes. With this goal in mind, one of the first questions we attempted to answer was whether the capacity to produce F reticulocytes in SS anemia is inherited and, if so, where the gene or genes are located.

Genes for the production of F reticulocytes exist in other species. DeSimone (*5*) found that the capacity to produce HbF and F cells in baboons with phenylhydrazine-induced hemolytic anemia is inherited. Phenotypically, anemic animals fall into three clusters with respect to the maximum HbF level attained: low (1 to 8 percent), intermediate (12 to 24 percent), and high (31 to 59 percent). Progeny of any given sire exhibit either low to intermediate responses or intermediate to high ones. No sires produce other combinations of progeny. In addition, correlation between nonanemic parents and anemic offspring, as well as correlation between levels of HbF attained before and after induction of hemolysis, suggest that the genes governing HbF production in anemia also regulate the lower, but individually distinctive, amounts of HbF produced in nonanemic animals.

Past attempts (*6*) to apply equivalent genetic analysis to SS anemia have been confounded by the fact that HbF and F cells may be augmented by preferential survival of F cells in SS disease. Since preferential F-cell survival varies widely between SS individuals (*1*), estimates of HbF and F cells are relatively poor indices of F-cell production. In the study reported here we avoided this problem by measuring F reticulocytes directly (*2*) and adducing three lines of evidence to support the idea that F-reticulocyte responsiveness in SS anemia is inherited.

First, the percentages of F reticulo-

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