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Prevention of Neutrophil-mediated Injury to Endothelial Cells by Perfluorochemical

David G. Babbitt, Mervyn B. Forman, Rhoda Jones, Ashok K. Bajaj, and Richard L. Hoover

From the Department of Medicine, Division of Cardiology, and Department of Pathology, Vanderbilt University, Nashville, Tennessee

Myocardial salvage after reperfusion may be limited by neutrophil-mediated microvascular damage. The effect of the perfluorochemical, Fluosol-DA, and its various components on neutrophil adherence, cytotoxicity, and proteolytic enzyme release was examined on sheep large and small vessel endothelial cells in vitro. ***Cells were studied under normoxic (N) and anoxic conditions (A). Various concentrations of Fluosol (10%, 25%, and 50%) significantly reduced neutrophil adherence under both experimental conditions*** [***mean 22 ± 3.25%*** versus ***7 ± 0.8% (N) and 20 ± 3.2%*** versus ***7.5 ± 0.9% (A)***; ***P < 0.01***]. ***The perfluorocarbons, perfluorodecalin (PFD), and perfluoro-tripropylamine (PFTP) in a 50 volume/percent concentration exhibited profound effects on adherence, particularly on cells subjected to anoxia (51% and 69% reduction in adherence, respectively; P < 0.01). No effect on adherence was observed with other components, including the detergent, pluronic F68. A 25% reduction (P < 0.02) in endothelial cytotoxicity was noted when neutrophils were preincubated with Fluosol. However, pretreatment of endothelial cells with Fluosol did not inhibit neutrophil adherence. Neutrophils stimulated with cytochalasin B and FMLP showed a significant reduction in lysozyme release after incubation with Fluosol (28 ± 5%*** versus ***17 ± 4%; P < 0.01). This study demonstrates that Fluosol significantly attenuates neutrophil adherence, cytotoxicity, and enzyme release in an*** in vitro ***model of microvascular injury. It also suggests that prevention of neutrophil-mediated microvascular damage may be an important mechanism whereby Fluosol enhances myocardial salvage after ischemia and reperfusion. (Am J Pathol 1990, 136:451–459)***

Endothelial cells are metabolically very active and are known to secrete a variety of compounds that act to maintain flow in the microcirculation. These include substances that regulate vascular smooth muscle tone and prevent mechanical plugging of vessels by cellular elements, especially the neutrophil.[1–5] Ischemic damage to vascular endothelial cells associated with complement deposition and neutrophil activation sets the stage for accelerated vascular damage at the time of reperfusion with the introduction of cellular elements.[6,7] Activated neutrophils produce a variety of substances that are cytotoxic and that amplify the inflammatory response. These include reactive oxygen species, numerous potent proteolytic enzymes, arachidonic acid metabolites, and platelet-activating factor.[8–11]

The perfluorochemical Fluosol-DA is a substance with a small particle size and high oxygen-carrying capacity. When reconstituted it consists of an emulsion of two perfluorochemicals, a detergent (pluronic—F68), glycerol, phospholipids, and various electrolytes. We have previously shown that administration of the compound after reperfusion significantly reduces infarct size in a canine preparation of reperfusion and this is associated with both structural and functional preservation of the endothelium in the ischemic microcirculatory bed.[12–14] A subsequent study showed that Fluosol significantly inhibited neutrophil chemotaxis into the ischemic myocardium.[15]

The effect of the perfluorochemical Fluosol-DA on neutrophil degranulation remains controversial.[15,16] Previous studies from our laboratory have demonstrated that neutrophils exposed *in vivo* to perfluorochemical manifest a significant reduction in lysozyme release when stimulated *ex vivo* with phorbol myristate acetate (PMA) or opsonized zymosan.[15] Lane and Lamkin did not observe suppression of β-glucuronidase release by Fluosol-DA *in vitro*.[16] The reasons for these discrepancies are unclear but may be related to technical variations among studies, including washing techniques, duration of exposure to the drug, and use of different neutrophil stimuli. In light of these find-

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Address reprint requests to Mervyn B. Forman, MD, Division of Cardiology, CC-2218 Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232-2170.

ings, we performed experiments in this study to clarify the effect of Fluosol and its detergent component, Pluronic F68, on neutrophil degranulation using both a physiologic neutrophil stimulant, FMLP, and a clinically relevant concentration of Fluosol and its detergent component. In an attempt to simulate the *in vivo* situation, the effect of Fluosol and its various components on neutrophil adherence and cytotoxicity was evaluated using cultured endothelial cells obtained from large and small blood vessels under both normoxic and anoxic conditions. These results suggest that Fluosol may mediate its protective effect in reperfusion injury by suppressing neutrophil adherence and neutrophil-mediated endothelial damage.

Materials and Methods

Endothelial Cell Isolation

Large vessel endothelial cells were obtained from isolated sheep pulmonary arteries by the methods of Booyse et al.[17] Coronary endothelial cells were obtained from the proximal epicardial arteries of normal canines and isolated using the same method. The pulmonary arteries and coronary arteries were treated with collagenase (1 mg/ml Hanks balanced salt solution buffered with 10 mM Hepes, pH 7.4 (buffered HBSS) for 20 minutes at 37 C. The endothelial cells were stripped off by perfusion with fresh HBSS, and the cells were washed twice in growth medium consisting of RPMI-1640 medium supplemented with 20% fetal calf serum (FCS) plus penicillin (100 μg/ml), streptomycin (100 mg/ml), and amphotericin (0.25 mg/ml). Cells were used either as primary cultures or as clones selected from primary cultures up to a maximum of eight passages.

Small vessel endothelium was isolated from sheep lung tissue. The methods were essentially those of Wagner and Matthews,[18] which we had used before.[19] Brief, a small piece (1 cm^2) of peripheral lung tissue was excised, teased apart with forceps, rinsed with buffered HBSS, and incubated with collagenase (1 mg/ml) for 45 minutes at 37 C. The cell mixture was triturated briefly with a Pasteur pipette and centrifuged at 150*g* for 5 minutes at room temperature. The pellet was washed once in buffered HBSS and plated out in a medium consisting of a 1:1 mixture containing one part RPMI-1640 medium plus 20% FCS and one part K1, a defined medium described by Taub and Sato.[20] Endothelial origin was confirmed by the typical cobblestone morphology,[21] anti-Factor VIII staining,[22] presence of angiotensin- converting enzyme activity,[23] and binding and uptake of acetylated low-density lipoprotein.[24] To be considered endothelial, the cells must possess at least three of the above characteristics.

Human Neutrophil Isolation

Venous blood was collected from health volunteers into 10 ml (250 U heparin/tube). Human neutrophils were used due to their ready availability. We have previously demonstrated that cross species similarity exists in adhesion of neutrophils and cultured endothelial cells of various species.[25] Polymorphonuclear leukocytes (PMNs) were separated by mixing whole blood with 25% of a 6% dextran (500,000 molecular weight) solution in buffered HBSS. After the erythrocytes had sedimented (approximately 45 minutes at room temperature), the leukocyte-rich upper phase was removed and centrifuged at 150*g* for 7 minutes at 4 C. Any remaining red blood cells in the pellet were removed by hypotonic lysis for 30 seconds with a small volume of distilled water. Subsequent addition of a large volume of buffered HBSS rapidly returned these cells to isotonicity. The suspension was centrifuged and the preceding step repeated if the pellet contained noticeable erythrocytes. The PMNs were counted and resuspended in buffered HBSS at the desired concentration. This technique yielded 90% to 95% neutrophils by Giemsa staining and electron microscopy. Viability was assessed by trypan blue exclusion, resulting in > 95% viability. Experiments involving the PMNs were completed within 5 hours to ensure the use of healthy cells.

Adhesion Assays

PMN adhesion was monitored using a monolayer collection assay as described by Walther et al[26] using both coronary and sheep endothelial cells. The endothelial cells were plated in 24-well cluster dishes and allowed to grow to confluency. Adhesion to endothelial monolayers was determined under two experimental conditions; normoxia (20% O_2, 5% CO_2) and 2 hours of anoxia (95% N_2, 5% CO_2). PMNs were first labeled with ^{51}Cr (10 $\mu Ci/10^7$ cells; sodium chromate, sp. act. 330 mCi/mg, New England Nuclear, Boston, MA) for 1 hour at 4 C. The lower temperature prevented aggregation of the PMNs and their adherence to the sides of the incubation tube. The cells were then rinsed in excess buffered HBSS and resuspended at the appropriate concentration in buffered HBSS. In all experiments the PMNs were stimulated with phorbol myristate acetate (PMA) (15 ng/ml) immediately before the assay. The PMNs (2×10^6/ml) were then added (0.5 ml/well) on top of the confluent monolayers of endothelial cells in the absence or presence of Fluosol or its components. The cells were incubated together at 37 C under normoxic conditions in both the anoxic and normoxic cell groups. After 15 and 30 minutes the unattached PMNs were aspirated off and the monolayers were rinsed once with buffered HBSS. The cells were disrupted with 0.5 ml

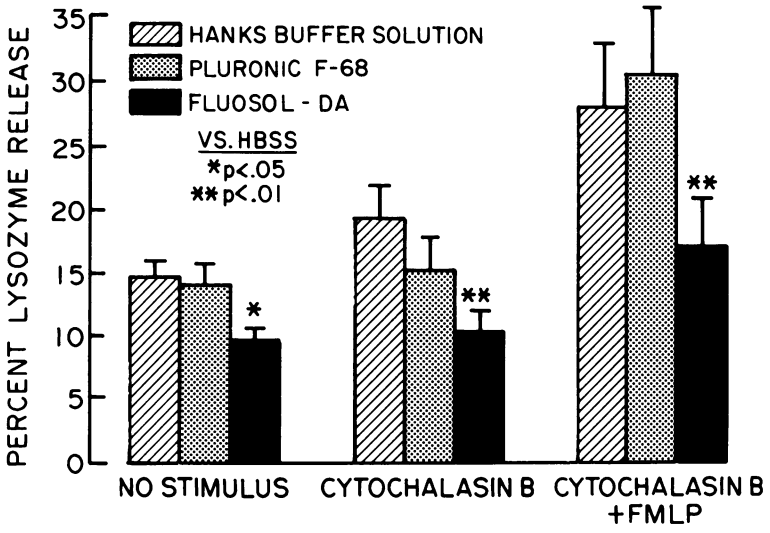

Figure 1. *The effect of Fluosol on percentage lysozyme release from unstimulated and stimulated neutrophils is shown. A significant reduction in enzyme release, particularly with stimulated cells, was observed on cells incubated with Fluosol. Pluronic F-68 had no significant effect on neutrophil-lysozyme release. FMLP = Formyl-methionyl leucine-phenylalanine. Results are expressed as mean ± SD.*

of 1N NH4OH, added to scintillation vials to which was added 4.5 ml Aquasol (New England Nuclear), and counted in a liquid scintillation counter. Adherence was expressed as the percentage of cpms left in the wells as compared to the number of cpms initially added. Due to the daily and donor variability in PMNs, adhesion measurements were normalized to controls by dividing the percentage adherence of experimental samples by the percentage adherence of control samples for the same set of experiments. Control values ranged between 5.5% to 32.5%.

To determine which cell was affected by the perfluorochemical, adhesion experiments were carried out in which either the PMNs stimulated by PMA or endothelial cells were pretreated before carrying out the assay. The endothelial cells were pretreated with the perfluorochemical for 15, 30, or 60 minutes under normoxia immediately after incubation of the cultures under normoxic or anoxic conditions. After this incubation, the cells were rinsed twice with buffered HBSS, and untreated ^{51}Cr-labeled PMNs stimulated with PMA were added to the monolayers. The stimulated PMNs were treated for 5 minutes with the perfluorochemical emulsion before incubation with endothelial cells. Adhesion was monitored as described above. Similar experiments were carried out with stimulated PMNs that were pretreated for 5 minutes with the various combinations of Fluosol and its components. Adherence was expressed in terms of an adherence ratio normalized to control values.

Endothelial Cell Cytotoxicity

The cytotoxic effects of PMA-stimulated PMNs on endothelial monolayers (normoxic and anoxic) from sheep vasculature were determined using a ^{51}Cr-release assay as previously described.[27,28] Confluent monolayers of endothelial cells grown in 24-well cluster dishes were prelabeled with ^{51}Cr (10 μCi/ml) for 2 hours at 37 C. The cells were then rinsed twice with buffered HBSS and studied under normoxic and anoxic conditions. Anoxia alone did not affect the viability of the endothelial cultures as measured by chromium release, at least for the time intervals that we studied (maximum, 24 hours) (normoxia: 20.8 ± 3.1% *vs.* anoxia: 19.6 ± 4.7%; n = 4). One milliliter of PMNs with and without Fluosol was added to cultures of endothelial cells at a concentration of 10^7 cells/ml, approximately 10 PMNs/endothelial cell, after the cells had been incubated for 2 hours under anoxic or normoxic conditions. At the end of 2 hours of incubation, the media was removed and 0.5 ml of buffered HBSS was used to rinse each well. The rinse solution for each well was combined with the corresponding incubation media for each well and counted in a liquid scintillation counter. The cells in the wells were lysed with 1N NH4OH and similarly counted. Cytotoxicity is directly proportional to the amount of ^{51}Cr released by the cells. Percentage release was calculated as cpm in the rinse and media divided by cpm in rinse and media plus cpm in remaining cells. Values were compared to control monolayers with no addition of PMNs. Detachment of endothelial cell monolayers by anoxia or perfluorochemical treatment was excluded by phase microscopy.

Lysozyme Release

Lysozyme enzyme release was analyzed by measuring absorbance changes of Micrococcus lysodeikticus bacterial suspension as previously described.[29] Measurements were made on neutrophils (2×10^6/ml) exposed to either buffered HBSS, 20% solution of reconstructed

Table 1. *Normalized Adherence (%) of Stimulated Neutrophils to Cultured Canine Coronary Artery Endothelial Cells*

	Coronary artery endothelial cells			
	Anoxia		Normoxia	
Treatment group	15 Minutes	30 Minutes	15 Minutes	30 Minutes
Control	1.0	1.0	1.0	1.0
FDA 10%	0.44 ± 0.07*	0.54 ± 0.07*	0.55 ± 0.07*	0.55 ± 0.09*
FDA 25%	0.34 ± 0.03*	0.44 ± 0.04*	0.37 ± 0.03*	0.42 ± 0.03*
FDA 50%	0.08 ± 0.01*	0.09 ± 0.02*	0.08 ± 0.01*	0.10 ± 0.01*

Results are normalized to controls; mean ± SD.
* $P < 0.01$; Control *vs.* FDA (perfluorochemical).

Fluosol and 20% solution of pluronic F68 (2.7 weight/volume). In each experiment cells were stimulated with either cytochalasin B (1×10^5), formyl-methionyl-leucyl-phenylalanine (10^{-7}M), or a combination of the two. Each experiment had internal controls to determine total lysozyme content after treatment with Triton-X and sonication. The results are expressed as a percentage of the total intracellular lysozyme content. We have previously validated that Fluosol does not interfere with the assay.[15]

Statistical Analysis

All experiments were carried out in triplicate using at least two different endothelial clones and three different donors of PMNs. Student's *t*-test for unpaired and paired data was used with a standard statistical package. Values were considered different if the *P* value was < 0.05. The Bonferroni correction was used when multiple comparisons were made.

Results

Neutrophil Proteolytic Enzyme Release (Figure 1)

Endothelial cytotoxicity by stimulated PMN may be mediated by proteolytic enzyme release. Figure 1 shows the effect on lysozyme release by Fluosol on nonstimulated PMNs and after incubation with cytochalasin B alone and a combination of cytochalasin B and FMLP. A significant reduction in neutrophil proteolytic enzyme release was observed, particularly on stimulated cells. No significant alteration in lysozyme release was noted with pluronic F-68 incubation.

Effect of Fluosol on PMN-Endothelial Adhesion (Tables 1 and 2)

Tables 1 and 2 illustrate the effect of 10%, 25%, and 50% concentration of perfluorochemical [Fluosol DA] on neutrophil adherence to canine coronary and sheep lung microvasculature endothelial cells determined after 15 and 30 minutes. A marked reduction in neutrophil adherence is observed with all three concentrations of Fluosol at both incubation times. Neutrophil adhesion to cultured canine coronary artery cells showed the greatest reduction with Fluosol and was dose dependent.

Fluosol Inhibits Adherence Via an Effect on Neutrophil (Figure 2)

Fluosol could affect PMN adherence by affecting either the endothelial cell or the neutrophil. Therefore we determined if preincubation of endothelial cells would alter PMN adherence. While treatment of PMN with a 25% solution of Fluosol for 5 minutes significantly reduced neutro-

Table 2. *Normalized Adherence of Stimulated PMNs to Sheep Lung Microvascular Endothelial Cells*

	Anoxia		Normoxia	
Treatment group	15 Minutes	30 Minutes	15 Minutes	30 Minutes
Control	1.0	1.0	1.0	1.0
FDA 10%	0.69 ± 0.93*	0.84 ± 0.02*	0.73 ± 0.04*	0.88 ± 0.08*
FDA 25%	0.72 ± 0.05*	0.66 ± 0.07*	0.64 ± 0.09*	0.85 ± 0.04*
FDA 50%	0.64 ± 0.08*	0.62 ± 0.04*	0.59 ± 0.11*	0.85 ± 0.93*

Results are normalized to controls; mean ± SD.
* $P < 0.01$; Control *vs.* FDA (perfluorochemical).

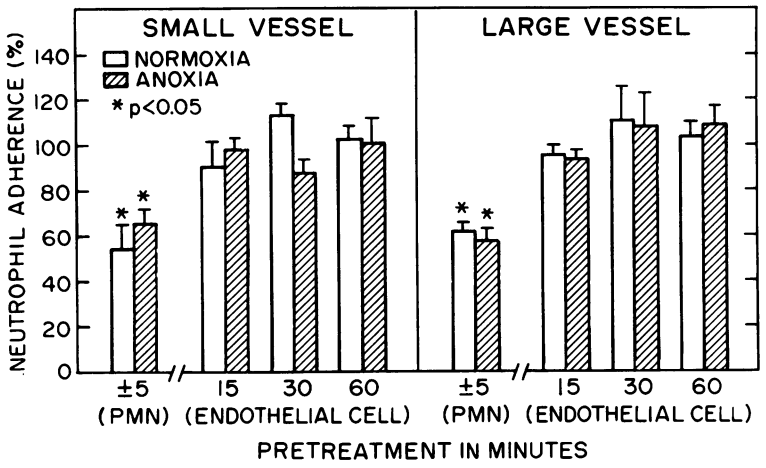

Figure 2. *Neutrophil adherence expressed as normalized values on small- and large-vessel sheep endothelial cells is shown. Either neutrophils (PMN's) or endothelial cells were pretreated with a 25% concentration of Fluosol. Note that decreased adherence was only observed when stimulated neutrophils were exposed to Fluosol, suggesting that the predominant effect of perfluorochemical is on the neutrophil. Results are expressed as mean ± SD.*

phil adherence (1.0% *vs.* 0.61% ± 0.07%; $P < 0.05$), preincubation of endothelial cell with a similar concentration for 15, 30, and 60 minutes failed to alter adhesion. These results suggest that the reduced adherence of neutrophils to endothelial cells by Fluosol is mediated primarily through its action on the neutrophil.

Effect of Perfluorochemical Components on PMN-Endothelial Adhesion

Figure 3 demonstrates the effects of the specific components and/or combinations of Fluosol in a concentration analogous to a 10 and 50 volume/percentage solution on neutrophil–endothelial interaction. All components were evaluated under conditions of normoxia and anoxia as defined above. No effects were seen with glycerol, phospholipids, or pluronic F68. A significant reduction in adherence was found with both perfluorocarbons, perfluorotripropylamine and perfluorodecalin, which was most striking with the 50 volume/percentage Fluosol concentration.

Endothelial Cell Cytotoxicity

The effects of a 10%, 25%, and 50% concentrations of Fluosol on endothelial cytotoxicity under conditions of normoxia and anoxia with stimulated neutrophils are shown in Figure 4. Endothelial cytotoxicity was only observed when stimulated PMNs were added to endothelial cells that had been exposed to a 90-minute period of hypoxia. All concentrations of Fluosol significantly reduced endothelial cytotoxicity under anoxic conditions for both large- and small-vessel endothelial cells.

Discussion

Present Study

This study demonstrates that the perfluorochemical Fluosol-DA significantly reduces adherence of stimulated neutrophils to endothelial cells under both normoxic and anoxic conditions. A discrepancy was observed between the percentage adherence of neutrophils to canine and sheep endothelial cells. This may represent an increased sensitivity of canine endothelial cells or may be related to a difference between cells based on the size of the vessel from which they were isolated. Stimulated neutrophils pretreated with Fluosol were less cytotoxic to endothelial cells that had been primed by 2 hours of anoxia. The failure to observe endothelial cell injury under normoxia is in accordance with previous observations from our laboratory that have demonstrated that endothelial cytotoxicity occurs under normoxic conditions only when large numbers of neutrophils are added or when the defense mechanisms of the endothelial cell have been compromised.[19]

Previous studies have demonstrated that a 50% concentration of Fluosol significantly reduced neutrophil adherence to plastic surfaces and nylon wool columns.[16,30] Because these substances are unphysiologic, this adhesion would be considered a nonspecific reaction. In addition, a 50% concentration of Fluosol is considerably higher than could be expected to have been achieved in our *in vivo* canine study.[15] Experiments performed in this study provide further insights into the mechanism of action of perfluorochemical in modifying neutrophil-endothelial interactions. We have previously shown that Fluosol failed to scavenge reactive oxygen species using a xanthine–xanthine oxidase system.[31] Preincubation of endothelial cells with various concentrations of Fluosol did not reduce neutrophil adherence. These findings, in association with a significant reduction in lysozyme release by

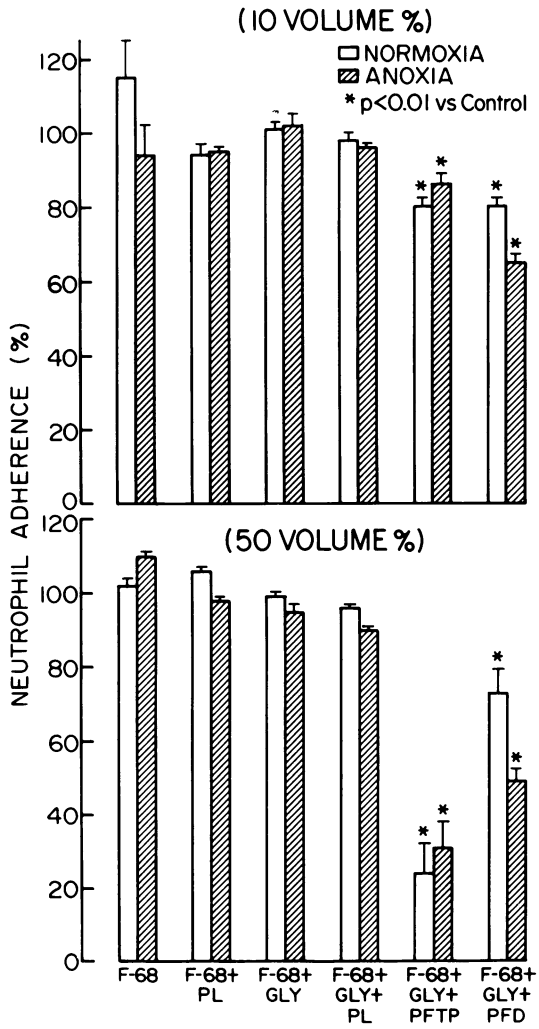

Figure 3. *Adherence of neutrophils to cultured endothelial cells treated with various perfluorochemical components in a 10 volume % (upper panel) and 50 volume % (lower panel) concentration is illustrated. Adherence was studied under both normoxia and anoxia. A significant reduction in adherence is observed only when neutrophils stimulated with PMA were treated with the perfluorocarbons, perfluorodecalin, and perfluorotripropylamine, and this was observed under both experimental conditions. Adherence was more strikingly reduced with the higher Fluosol concentration. F68 = Pluronic F68; GLY = Glycerol; PL = yolk phospholipids; PFD = Perfluorocdecalin; PFTP = Perfluorotripropylamine. Results are expressed as mean ± SD.*

neutrophils preincubated with Fluosol, supports the hypothesis that its primary effect is modulation of various neutrophil functions. Therefore, Fluosol may not only prevent neutrophils from accumulating at sites of ischemic myocardium but would also inhibit the release of proteolytic enzymes and reactive oxygen species by cells already present in the area.[15,30] Because perfluorochemical is a complex emulsion, we investigated the effects of its various components on neutrophil adherence. The two perfluorochemicals, perfluorodecalin and perfluorotripropylamine, demonstrated the most profound effect on neutrophil adherence, especially under anoxic conditions. No effects were seen with any of the other components, including the detergent pluronic F68.

Metabolic Function of Endothelium

The high metabolic activity and the large surface area occupied by endothelial cells in the heart suggests that they play an important role under various physiologic and pathologic conditions.[1,2] Endothelial cells regulate vascular smooth muscle tone by secreting a number of vasodilatory compounds such as endothelial-derived relaxation factor (EDRF), adenosine, and prostacyclin.[1-5] The endothelium also prevents mechanical plugging of the microcirculation by producing anticoagulants (tissue plasminogen activator, heparinoids) and substances that inhibit platelet aggregation and thromboxane release (adenosine, prostacyclin).[1-3,32] Recent evidence highlights the importance of the endothelium in modulating local inflammatory and immune responses.[9] The plasmalemma of these cells contains a negative glycocalyx that limits neutrophil adherence.[9] Coronary endothelial cells contain large quantities of adenosine, which both reduces superoxide anion production by stimulated neutrophils and inhibits neutrophil adherence and cytotoxicity to cultured cells.[2,33,34] Prostacyclin and cyclic AMP also may modulate neutrophil–endothelial interaction under inflammatory conditions.[1,22] Therefore depletion of endogenous anti-inflammatory mediators produced by endothelial cells by ischemia may contribute to increased neutrophil accumulation after reperfusion.

Mechanisms of Endothelial Cell Injury

Myocardial ischemia probably induces changes in cellular membranes that result in amplification of neutrophil–endothelial interactions after reperfusion.[35,36] Neutrophil adherence, the initial essential step in the inflammatory response, is augmented by hypoxic endothelial cell injury *in vitro*.[37] The importance of neutrophil adhesion in the inflammatory response after reperfusion is suggested by the observation that infarct size is significantly reduced in animals infused with a monoclonal antibody to neutrophil adhesive antigen.[38] Monokines induce the expression of antigens on endothelial cell surface, which increase neutrophil adherence.[39] Neutrophils become activated during ischemia by exposure to various chemotactic stimuli such as complement, leukotrienes, platelet-activating factor, and reactive oxygen species.[6,9] Depletion of anti-inflammatory mediators and free radical scavenging enzymes during ischemia would be exacerbated by washout at reperfusion, further enhancing neutrophil accumulation. Ac-

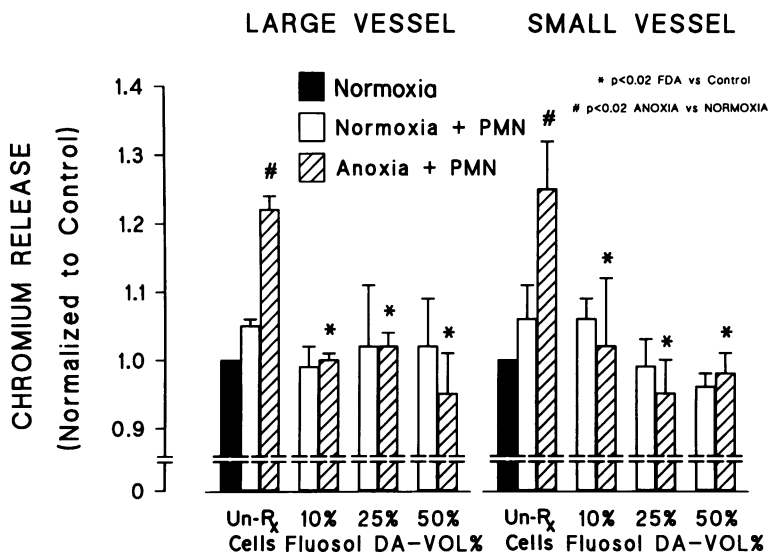

Figure 4. *Endothelial cytotoxicity determined by chromium release was measured on endothelial cells under normoxic conditions and under normoxic and anoxic conditions with the addition of PMN-stimulated neutrophils. Note that endothelial cytotoxicity was only observed when stimulated neutrophils were added to untreated (Un-Rx) cells that had been exposed to anoxia. All concentrations of Fluosol prevented neutrophil-mediated damage to cultures exposed to anoxia. Results are expressed as mean ± SD.*

tivated neutrophils produce a number of compounds capable of producing vascular injury, including hypochlorous acid formed by myeloperoxidase, various proteolytic enzymes, and reactive oxygen species.[9,10,27,40] Both elastase and reactive oxygen species induce endothelial cytotoxicity *in vitro*.[27,40] Leukotrienes released by neutrophils would enhance chemotaxis and endothelial adhesion of other neutrophils and increase the inflammatory response in an exponential manner.[8,41]

Comparison with In Vivo *Studies*

Histologic findings in animal models of ischemia and reperfusion support the hypothesis that neutrophils play a role in mediating microvascular damage.[4,35,36] In contrast to permanent occlusion, reperfusion results in a rapid influx of neutrophils into the ischemic zone, associated with accelerated endothelial and neutrophil plugging and damage of capillary lumens.[14,35] We have demonstrated that both intracoronary and intravenous administration of Fluosol significantly reduces neutrophil infiltration and endothelial cell structural changes in a canine preparation of reperfusion and this was associated with significant myocardial salvage compared to control animals.[12–15] Endothelial-dependent vasodilatory reserve was significantly preserved in treated animals 1 hour after reperfusion both *in vivo* and *in vitro*.[14] This laboratory and others have previously shown that Fluosol reduces neutrophil chemotaxis, adherence, and superoxide production *in vitro*.[16,30] Neutrophils removed from animals treated with intravenous perfluorochemical before reperfusion manifest a marked suppression of chemotaxis and lysozyme release *ex vivo*.[15]

Anoxia was used in this study to simulate the *in vivo* experimental studies. Previously we have shown that various durations of anoxia resulted in a continual decline in endothelial production of tissue plasminogen activator and enhanced production of plasminogen activator inhibitor (PAI) compared to normoxic conditions.[42] However, 48 hours of anoxia did not affect cell viability as measured by chromium release. Neutrophil-mediated endothelial injury occurred in the present study when cells had been exposed to a period of anoxia. These findings suggest that depletion of endogenous anti-inflammatory mediators such as adenosine, reduction in antioxidant enzymes, and/or expression of endothelial–leukocyte adhesion molecules may play a role in the pathogenesis of vascular injury. The present study provides further evidence that perfluorochemicals prevent endothelial cytotoxicity *via* their anti-neutrophil actions. Marked inhibition of neutrophil adherence was observed even with low concentrations of Fluosol and this was associated with reduced proteolytic enzyme degranulation. Because other cellular elements and plasma components that modify neutrophil-endothelial interactions were absent in this system, further studies are required to determine the exact mechanism through which Fluosol affects vascular interactions.

Implications

Uncontrolled neutrophil activation has been implicated in the pathogenesis of numerous clinical processes, including myocardial reperfusion injury. We and other researchers previously have shown that anti-neutrophil agents significantly enhance myocardial salvage after reperfusion. This study provides further evidence that Fluosol inhibits

vascular damage by reducing both adherence of neutrophils to damaged endothelial cells and inhibiting degranulation. Infusion of perfluorochemical may be a useful pharmacologic agent for reducing inappropriate neutrophil activation after successful myocardial reperfusion.

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