suggest that an important mechanism for the actions of Fluosol is through alteration of various neutrophil functions. Although we cannot completely eliminate a role for hyperoxic reperfusion or other actions in the beneficial effects of Fluosol (8), the current study does not support the finding that hyperoxic reperfusion is the primary mechanism or that the effects of Fluosol are not mediated through neutrophils.

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Reply

Murray et al. raise several questions concerning the methodologies used in our assessment of polymorphonuclear leukocyte (PMN) function (1). As Murray et al. point out, previous in vitro evidence supports the premise that the detergent component of Fluosol-DA, poloxamer 188, directly activates PMNs through phagocytosis (2). In light of these findings, these investigators propose that either Fluosol-DA or poloxamer 188 activate PMNs while in circulation. Activated PMNs would thus be refractory to subsequent stimulation and lose their ability to promote infarct extension upon reperfusion (2). Murray et al. take issue with our study because we exposed PMNs to cytochalasin B (an agent that blocks phagocytosis) before assessment of their function. Cytochalasin B is commonly used in PMN superoxide assays because it enhances both the rate and amount of superoxide anion ($\cdot O_2^-$) production. This effect most likely occurs by inhibiting phagocytic vacuole formation, which prevents the access of cell surface-generated superoxide anion to cytoplasmic superoxide dismutase. Therefore, more superoxide anion is exposed to the detector chromogen ferricytochrome C (3).

In our study, PMNs were pretreated with cytochalasin B for superoxide anion and lysozyme release assays. The PMNs were isolated at 3 h of reperfusion only after exposing the ischemic reperfused animal to Fluosol-DA or poloxamer 188 in vivo. For chemotaxis assays, PMNs were not pretreated with cytochalasin B because it prevents the polymerization of actin and therefore would impair chemotactic responses (4). If the hypothesis proposed by Murray et al. is correct, one would expect that PMNs isolated after treating rabbits with Fluosol-DA and poloxamer in vivo would exhibit "burnout" and resistance to stimulation. This was clearly not the case; PMNs from Fluosol-DA– and poloxamer 188–treated rabbits responded equally as well or better than control or sham operated (noninfarcted) animals (unpublished observations) to receptor-mediated activation. Furthermore, the myocardial myeloperoxidase content between groups was similar, suggesting that the degree of PMN infiltration in the ischemic region was not influenced by treatment.

We disagree with the comment by Murray et al. that the dose of Fluosol-DA used in the study (20 mg/ml) was not high enough to demonstrate changes in PMN function. The efficacy of this concentration of Fluosol-DA to reduce infarct size was demonstrated both in the present study in question and in a previous study in our laboratory (5). With regard to the poloxamer infarct size data, a recent study by Schaer et al. (6) utilizing the canine infarct model failed to demonstrate infarct size reduction with poloxamer 188 in normoxic reperfused hearts when it was administered as an intravenous bolus followed by a 4-h infusion, which is consistent with the finding in the present study (1). However, infarct size reduction was significantly reduced after a prolonged 48-h infusion of poloxamer 188 (6). Although in vitro treatment of PMNs with poloxamer 188 enhanced superoxide anion production and suppressed chemotaxis (6), this agent also has hemorheologic properties separate from neutrophil effects that may affect infarct size after reperfusion.

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