Control of Plasma Nitric Oxide Bioactivity by Perfluorocarbons
Physiological Mechanisms and Clinical Implications

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Background—Perfluorocarbons (PFCs) are promising blood substitutes because of their chemical inertness and unparalleled ability to transport and upload O₂ and CO₂. Here, we report that PFC emulsions also efficiently absorb and transport nitric oxide (NO).

Methods and Results—Accumulation of NO and O₂ in PFC micelles results in rapid NO oxidation and generation of reactive NOx species. Such micellar catalysis of NO oxidation leads to formation of vasoactive S-nitrosothiols (RSNO) in vitro and in vivo as detected electrochemically. The efficiency of PFC-mediated S-nitrosation depends on the amount of PFC in aqueous solution. The optimal PFC concentration that produced the maximum level of RSNO was 1% (vol/vol). Larger PFC amounts were progressively less efficient in generating RSNO and functioned simply as NO sink. These results explain the characteristic hemodynamic effects of PFCs. Intravenous bolus application of PFC (0.14 g/kg, 1% vol/vol) to Wistar-Kyoto rats decreased mean arterial pressure significantly (∼10 mm Hg over 40 minutes). PFC-induced hypotension could be further stimulated (∼17 mm Hg over 140 minutes) by exogenous thiols (cysteine and glutathione). In contrast, a larger amount of PFC (1 g/kg, ∼7% vol/vol) exhibited a strong hypertensive effect (11 mm Hg over 40 minutes).

Conclusions—The present study reveals a physiologically significant pool of endogenous plasma NO and underscores the crucial role of the circulating hydrophobic phase in modulating its bioactivity. The results also establish PFC as a conceptually new pharmacological tool for various cardiovascular complications associated with NO imbalance. (Circulation. 2004;110:3573-3580.)

Key Words: blood pressure ■ hemodynamics ■ nitric oxide ■ oxygen ■ fluorocarbons

Nitric oxide (NO) is enzymatically produced by various types of cells and represents a central mediator within the cardiovascular system.1 As a free radical, NO is highly unstable in vivo. Its primary targets include heme proteins such as guanylyl cyclase and free radical species, eg, O₂. Another physiologically significant aspect of NO biochemistry is the formation of thionitrite esters with cysteine (Cys) and Cys residues (S-nitrosothiols; RSNO). Small RSNOs (eg, S-nitrosoglutathione [GSNO]) and nitroso-derivatives of proteins such as albumin and hemoglobin are known to be generated in vivo and exert NO-like activity. They induce vasodilation, inhibit platelet aggregation, and participate in various signal transduction pathways.3-7 Because RSNOs are relatively stable and can release NO when required on reaction with various reducing agents,8,9 they may serve as a buffering system that magnifies the range of NO action along the vascular tree.10,11

Although the pharmacological potential of RSNO is well appreciated,12-14 the role of plasma NO and the mechanism of endogenous RSNO formation under physiological conditions are a source of considerable debate.15 The apparent limitation for plasma NO to exert its biological function is the constant presence of abundant hemoglobin (10 mmol/L) from red blood cells that converts NO into inactive metabolites at near-diffusion–limited rates.16 Moreover, NO is unable to react directly with thiols. It must be oxidized first to form nitrosative agents, such as N₂O₃. Because of the slow rate of the third-order reaction of NO with O₂ (k ∼4×10⁹ mol/L⁻² per second),17,18 the formation of N₂O₃ and eventually RSNO should depend primarily on the local concentration of NO and the molecular environment. Indeed, strong acceleration of NO oxidation occurs within lipid membranes19 and hydrophobic pockets of plasma proteins20 that effectively sequester NO from the aqueous phase. Recently, we provided experimental evidence suggesting that such micellar catalysis of NO oxidation and N₂O₃ formation by serum albumin accounts for the presence of vasoactive RSNO in the circulation under physiological conditions.21

In the present study, we explored a synthetic hydrophobic phase (perfluorocarbon [PFC]) to manipulate plasma NO
bioactivity and establish its physiological role. PFCs are highly hydrophobic, inert chemicals that are not miscible with water and are capable of dissolving large quantities of gases, including O₂ and CO₂. With sophisticated technology, it is possible to generate stable PFC emulsions of small, uniform particles that can be used as temporary intravascular oxygen carriers.22–24 Here, we show that by changing the volume and properties of the circulating hydrophobic phase with PFCs, one can rapidly and precisely modulate NO transport and delivery, thus controlling blood pressure, platelet aggregation, and other NO-dependent processes. The results demonstrate the existence of a physiologically significant pool of circulating plasma NO and the crucial role of nitrosative chemistry in its vascular activity. We also suggest a conceptually new pharmacological approach for treating disorders associated with NO disparity.

**Methods**

**Chemicals and Reagents**

One hundred milliliters of Perftoran emulsion (Perftoran Co) contains 13 g of perfluorodecalin (C₁₀F₁₈), 6.5 g of perfluoromethylcyclohexylpiperidine (C₆F₁₄N), 4.0 g of Proxanol 268, 0.6 g of NaCl, 39 mg of KCl, 19 mg of MgCl₂, 65 mg of NaHCO₃, 20 mg of NaH₂PO₄, 0.2 g of glucose, and H₂O. Physical properties of Perftoran include a particle size of 0.03 to 0.15 μm/L, osmolality of 280 to 340 mOsm, viscosity of 2.5 cP, pH 7.5, O₂ solubility of 7.0 vol% (pO₂=760 mm Hg, 20°C), and CO₂ solubility of 60 vol% (pCO₂=760 mm Hg, 20°C). Saline in control experiments contained all of the chemicals listed above except PFCs and Proxanol. Other chemicals were from Sigma unless specifically indicated.

**Determination of NO Partitioning (Q)**

NO/H₂O solution was prepared in an anoxic device by bubbling NO gas (Aldrich) that had been purified from higher oxides by passing it through a 1-mol/L solution of KOH into water until the concentration of dissolved NO reached 1 mmol/L. Water (Milli-Q grade) was deaerated by boiling and then cooling under argon (Praxair). The NO concentration was measured immediately before the reaction with an ISO-NO Mark II nitric oxide electrode (WPI). To determine Q₅₀ for PFC, the ice-cold Perftoran (10 mL) was degassed in the anoxic device by bubbling with argon for 6 hours. Next, 1.3 mL of Perftoran was added to 12.2 mL of NO solution (11.2 μmol/L) in the presence of argon. The temperature was adjusted to 20°C, and the ISO-NO Mark II electrode was inserted into the solution under argon and sealed. Q₅₀ is calculated as described in Figure 1.

**Determination of Thiols and RSNOs**

To prepare plasma samples (100 μL), 0.5 mL of arterial blood was collected into heparinized plastic tubes and centrifuged at 4500 rpm at 4°C for 3 minutes. Plasma was processed immediately after preparation. Plasma GSH and Cys were detected by high-performance liquid chromatography (Waters 600E pump) at 230 nm with a multiwavelength detector (Waters 490E) and C₁₈ column with a mobile phase of 99% 0.1 mol/L monochloroacetate buffer pH 3 and 1% methanol. In addition, thiols were detected with monobromo (trimethylammonio)bimane bromide as described previously.25

The total amount of RSNO in vitro or in plasma was determined electrochemically with 1.5 mmol/L CuCl₂ (Cu²⁺/Cu⁺) to rapidly decompose all RSNO. The released NO was detected with the ISO-NO Mark II electrode. GSNO and SNO-BSA were used as standards. They were prepared by incubation of GSH or BSA with a 2-fold molar excess of NaNO₂ in acidified water on ice. To estimate the fraction of low-molecular-weight (LMW) RSNO in total RSNO, the real-time kinetics of RSNO decomposition were traced described above except that 40 μmol/L CuCl₂ was used (Figure 5).

**Animals**

Adult male Wistar rats (300 to 400 g) were used. Both femoral arteries and the left femoral vein of anesthetized animals (urethane 1.2 g/kg IP) were cannulated with polyethylene tubing (PE-50, WPI). An arterial catheter was used for continuous monitoring of aorta blood pressure and heart rate and to withdraw blood samples. The venous catheter was used for drug administration. The same infusion rate of drugs or saline (0.2 mL/min) was applied for all animals. The drugs were used as follows: L-NAME 20 mg/kg, GSH 2 mg/kg, Cys 0.8 mg/kg, NaNO₂ 1 mg/kg, and Perftoran 136 mg/kg (low dose) or 1 g/kg (high dose). Before drug administration, mean arterial pressure (MAP) was recorded, and the initial level of RSNO was determined in plasma as described above. MAP and heart rate were detected with the pressure sensor connected to an amplifier. The signal was digitally converted at a PowerLab 200 workstation (ADInstruments) and transmitted to a desktop computer. All statistical calculations were performed with Origin 7.0 software (Origin-Lab Corp).

**Results**

**Dramatic Acceleration of NO Oxidation by PFC**

To study the effect of PFC on NO bioavailability, we chose Perftoran, the Russian brand of clinically approved blood substitute.26 Perftoran contains 13% (wt/vol) perfluorodecaline and 6.5% (wt/vol) perfluoromethylcyclohexylpiperidine, which forms ~0.07-μm micelles stabilized by a poloxamer-type surfactant (Figure 1). O₂ solubility by Perftoran is ~12
times greater than that in water or blood plasma. Unlike the chemical binding of O₂ to the porphyrin-iron sites of hemoglobin, O₂ dissolution in PFC is a simple, passive process in which hydrophobic gas molecules occupy PFC micelles. In contrast to the characteristic sigmoid binding curve of O₂ to hemoglobin, O₂ solubility in PFC emulsions increases linearly with partial pressure.

Because NO is hydrophobic, we reasoned that it should be concentrated by PFC micelles along with O₂ under aerobic conditions, implying that PFC would accelerate NO oxidation (Figure 1b). To estimate the maximum acceleration value (H) of this reaction, the partition coefficient (QNO) for a PFC/H₂O system was determined. To calculate QNO, which is described by the equation shown in Figure 1c, we used a Clark-type NO electrode attached to an airtight O₂-free gas/liquid mixing device to monitor changes in NO concentration on addition of PFC. We determined QNO(PFC/H₂O) to be ≈200 (Figures 1c and 1d). QNO(PFC/H₂O) has been shown to be ≈12.2 For the trimolecular reaction of N₂O₃ formation, the equation H=Q₂N₂O₃·QNO=200²·12·5×10⁵ is used. This estimation implies that even a small quantity of PFC in mammalian blood (e.g., 1% vol/vol) would sequester a large amount of circulating NO. At the same time, it should boost production of reactive N₂O₃ species hundreds of thousands of times, which in turn would result in increased nitrosation of external molecules such as thiols (Figure 1b).

Catalysis of RSNO Formation by PFC In Vitro

To investigate the stimulating effect of PFC on RSNO formation, we compared the rate of GSNO accumulation as a function of PFC volume in the probe. GSNO was detected with Cu²⁺ followed by electrochemical detection of released NO. A representative experiment (Figure 2a) shows that 1% (vol/vol) PFC that has been aerobically saturated with water solution of NO (0.5 μmol/L) increased the rate of GSNO formation ≈10-fold. A similar result was obtained with L-cysteine (data not shown). Importantly, the efficiency of PFC-mediated S-nitrosation depends on the amount of PFC in the probe (Figure 2b). The resonance-like maximum was observed at ≈1% (vol/vol) Perftoran. Larger or smaller amounts of PFC were progressively less efficient. This fact is readily explained in terms of micellar catalysis of NO oxidation, in which a relatively small “optimal” volume of the hydrophobic phase (in this case, PFC) generates maximum acceleration of the reaction. These results suggest that the use of various amounts of PFC can regulate the level of endogenous vasoactive LMW RSNO.

PFC as a Powerful NO Sink In Vivo

To evaluate the ability of PFC to absorb NO in vivo, we studied the effect of Perftoran on MAP in rats. Intravenous bolus application of PFC (1 g/kg, ≈7% vol/vol) was characterized by extensive hypertension (11 mm Hg) over the first 40 minutes, followed by mild hypotension (Figure 3a). Because the PFC solution was isoosmotic (see Methods) and the rate of its infusion was slow (0.2 mL/min), PFC-induced vasconstriction could not be attributed to the change in the osmotic status.

To confirm that the PFC-mediated increase in blood pressure was due to plasma NO sequestration, we administered a nonselective NO synthase inhibitor, N⁵-nitro-L-arginine methyl ester (L-NAME; 50 mg/kg IP) to deplete the vasculature of endogenous NO. This caused a characteristic steep increase in MAP (Figure 3b). Because PFC failed to increase MAP any further in this case, we concluded that intravascular NO was essential for PFC-induced vasoconstriction.

To further substantiate the link between PFC-mediated hypertension and plasma NO, we used sodium nitrite, a well-established NO source in vivo and a potent vasodilator. Reduction of nitrite to NO is catalyzed in vivo by xanthine oxidoreductase and deoxyhemoglobin and also can occur nonenzymatically. Moreover, a significant circulating arteriovenous plasma nitrite gradient has been reported, which argues in favor of intravascular nitrite being a stable natural source of NO. Infusion of NaNO₂ (1 mg/kg IP) 90 minutes after L-NAME decreased MAP sharply (Figure 3c). Subsequent PFC administration elevated MAP back to the original L-NAME level (Figure 3c). Because nitrite is slowly reduced to NO in vivo under physiological pH (hence its vasorelaxation effect), an ability of PFC to specifically counteract this effect confirms its mode of action as a powerful NO sink.

Taken together, these results demonstrate the existence of a sizable pool of circulating NO in mammalian blood that
serves as a systemic dilator. Limitation of its bioavailability by PFC leads to systemic vasoconstriction.

PFC-Mediated Catalysis of RSNOs In Vivo and Control of Blood Pressure

As discussed above (Figure 2), PFCs not only limit the concentration of free NO but also accelerate its oxidation and formation of RSNOs. Generation of vasoactive RSNOs can explain the delayed dilation effects of PFC in the experiments described in Figure 3. A modest but reproducible hypotension was observed 60 minutes after PFC infusion (Figure 3a). Furthermore, in the nitrite-treated rats, MAP dropped below the baseline level 30 minutes after PFC infusion (Figure 3c), whereas in the control group (without PFC), MAP remained 25 mm Hg above baseline (Figure 3c). We interpret these results to mean that during the first phase of action, PFCs rapidly absorb plasma NO, which causes the initial increase in MAP; however, because local concentration of NO and O₂ in PFC micelles becomes high, N₂O₃ forms at a much greater rate, which leads to accumulation of vasoactive RSNOs.

To test whether PFCs can indeed decrease vascular tone via generation of endogenous RSNOs, a series of in vivo studies that used 10 times less PFC than that used in the experiments reported in Figure 3 were performed. The reason for using a smaller amount of PFC derives from the graph in Figure 2b, which shows that 1% PFC in aqueous solution is the optimal amount that generates the maximum acceleration of NO oxidation and RSNO formation. Therefore, one would expect that at this concentration in vivo, PFC would convert plasma NO into vasoactive RSNO most efficiently. Indeed, the bolus infusion of the low dose of PFC (136 mg/kg IV, 1% vol/vol total blood) did not cause any increase in MAP (Figure 4a). Instead, it produced a more rapid and pronounced decrease in MAP (10 mm Hg over 40 minutes) than that observed with the high dose of PFC (compare Figures 4a and 3a).

To confirm that the observed PFC-mediated vasodilation was due to endogenous RSNO formation, we measured LMW RSNO in plasma before and after PFC administration (Figure 4b). Plasma LMW RSNOs were determined as described in Figure 5, using a concentration of CuCl₂ (40 μmol/L) selected to decompose predominantly LMW RSNO. More stable high-molecular-weight (HMW) RSNOs (e.g., S-NO-albumin) were largely resistant to such a low concentration of Cu²⁺ during the experiment (Figure 5). Using this approach, we found that the basal level of LMW RSNO in rat plasma is 10±6 nmol/L (n=6) and total RSNO is 200 nmol/L (Figure 4). These numbers are in good agreement with the level of RSNO previously detected in human plasma by a similar electrochemical method. Because of RSNO rapid decomposition during sample preparation, the detected values of RSNO reflect only a fraction of their actual amount in blood. This explains at least in part a wide range of RSNO values in the circulation determined by different methods. As Figure 4b shows, the level of plasma LMW RSNO increased

![Figure 3](https://circ.ahajournals.org/)

**Figure 3.** Relationship between PFC-mediated hemodynamic effects and NO. a, Effect of high dose of PFC on MAP of anesthetized rats. Infusion of bolus 1 g/kg PFC (7% vol/vol of total blood) provokes steep increase in blood pressure followed by gradual asymptotic recovery and mild hypotension. Bars represent extremes of MAP during time course of continuous monitoring. Mean±SE (n=12, P<0.05). ΔMAP indicates change in MAP. Arrow indicates time point of PFC or saline infusion. b, Inactivation of NOS abolishes hemodynamic effects of PFC. Infusion of L-NAME (50 mg/kg) causes prolonged and stable hypertension, which is not further affected by 1 g/kg PFC. Mean±SE (n=8, P<0.05). c, Delivery of NO donor resumes hemodynamic effects of PFC under L-NAME conditions. Infusion of NaNO₂ 1 mg/kg IP 110 minutes after L-NAME administration leads to partial recovery of MAP (24 mm Hg). In this case, subsequent infusion of PFC (1 g/kg) fully restored hypertensive effect of L-NAME (ΔMAP=28 mm Hg; n=10; P<0.05). d, Schematic interpretation of experimental results. Artery is shown with endothelial cells producing NO that diffuses in all directions. In a, PFC micelles (gray circle) rapidly absorb plasma NO, thus causing vasoconstriction and hypertension. In b, NOS is inactivated, which limits the amount of plasma NO and prevents further potential constriction by PFC. In c, reducing components of blood convert exogenous NO₂⁻ to NO, thus causing partial relaxation from L-NAME. However, PFC micelles sequester exogenous NO and thus constrict vessel back to L-NAME position.
5-fold 10 minutes after PFC injection (1% vol/vol) and then gradually decreased over the next hour. The burst of plasma RSNO induced by PFC explains the drop in MAP in Figure 4a. Significantly, despite the marked increase in the level of LMW RSNO in response to PFC, the amount of total RSNO undergoes only a slight change, which indicates that PFC-mediated NO oxidation targets primarily the pool of LMW RSH in the circulation. Indeed, only small thiols are capable of penetrating PFC micelles efficiently. This result implies that LMW RSNO has a much greater specific vasoactivity than the more abundant HMW RSNO. The latter can be considered as a relatively constant NO pool for storage and transport purposes.10 A slight drop of total plasma RSNO 10 minutes after PFC administration (the time of maximal LMW RSNO increase) reflects the process of PFC-mediated redistribution of NO from its HMW depot to actively used LMW RSNO.

Figure 4. Effect of low dose of PFC on MAP and RSNO formation in anesthetized rats. a, Systemic vasodilation response to intravenous bolus of 136 mg/kg PFC (1% vol/vol of total blood). Bars represent extremes of MAP during time course of continuous monitoring. Mean±SE (n=12, P<0.05). Time of PFC or saline infusion is indicated by arrow. ΔMAP indicates change in MAP. b, Stimulation of plasma LMW RSNO formation by PFC. ΔRSNO indicates change in plasma LMW RSNO. LMW RSNO in plasma was detected as described in Figure 5. Total RSNO concentration in control (PFC) group was 210±25 (180±20), 205±20 (160±15), and 195±25 (195±20) nmol/L at 0, 10, and 25 minutes, respectively (n=10, P<0.05). c, Schematic explaining vasodilating effect of PFC. At 1% vol/vol concentration, PFC micelles (gray circle) absorb NO and O2 to generate reactive N2O3 species via micellar catalysis of NO oxidation. N2O3 then nitrosates plasma small thiols to form vasoactive RSNOs that cause vasorelaxation.
RSNO formation (87% increase) and vasodilation (-17 mm Hg, 140 minutes) was observed when L-Cys (0.8 mg/kg IV) was used instead of GSH (Figure 6b). Without PFC, neither glutathione nor cysteine had any effect on plasma RSNO or blood pressure at these concentrations. Obviously, the combined hemodynamic effect of PFC and thiols was much greater than that of PFC alone (compare Figures 4 and 6b), which provides direct evidence that PFC-induced vasodilation was due to endogenous RSNO formation.

Because the half-life of Perftoran in human and animal blood is >24 hours,26 the eventual recovery of MAP after a single dose of RSH was likely due to rapid RSH/RSNO uptake by tissues rather than PFC inactivation. The data of Figure 6b confirm this conclusion. Receiving a second dose of l-Cys (0.8 mg/kg) ~2 hours after the first thiol infusion, PFC-treated animals responded by a second wave of marked vasodilation that was virtually identical to the first one. This result demonstrates that one can maintain the steady level of circulating RSNO and control blood pressure for a long period of time simply by supplying small amounts of natural thiols to PFC-treated animals.

Discussion
The physiological and pharmacological significance of the present study is several fold. First, it establishes the crucial role of plasma NO in systemic hemodynamics. Second, it directly implicates the intravascular hydrophobic phase in regulation of plasma NO bioactivity via the micellar catalytic mechanism of endogenous RSNO formation. Third, it establishes intrinsic plasma LMW RSNO as a potent relaxing factor under noninflammatory conditions. Fourth, it explains various hemodynamic effects associated with the use of PFC in the clinic. Fifth, it provides a conceptionally new pharmacological approach to manipulate NO bioactivity.

Physiological Role of Plasma NO and the Mechanism of Endogenous RSNO Formation
It has been widely assumed that a fraction of endothelium-derived NO that does not diffuse into the vessel wall gets rapidly trapped and inactivated by hemoglobin from red blood cells. Recently, we challenged this view by showing that under physiological conditions, a significant amount of NO accumulates in the hydrophobic compartments of serum albumin.21 The high local concentration of NO within the protein interior leads to accelerated NO oxidation and formation of nitrosative N2O3 species, which in turn are responsible for maintaining the basal level of circulating vasoactive RSNO.21 In support of this model, a number of studies, including the present one, detected the basal level of RSNO in mammalian plasma in the 20 to 200 nmol/L range.34 Moreover, intravenous application of NO was shown to exert a
systemic hemodynamic effect via RSNO in humans. However, it has remained unclear whether intrinsic plasma NO or RSNO played any significant role in cardiovascular processes. In the present study, we addressed this important issue by devising an approach to specifically modulate the pool of plasma NO and RSNO without boosting NO production or delivering any exogenous NO into the system.

The approach is based on unique physical and chemical properties of PFC emulsions. By utilizing a commercial PFC product, Perftoran (Figure 1a), we demonstrate that PFC micelles adsorb NO from the aqueous phase, greatly accelerating NO oxidation and RSNO formation under aerobic conditions (Figures 1 and 2). In accord with the theory of micellar catalysis, the rate of NO oxidation and RSNO formation depends on the volume of the hydrophobic phase, with a resonance-like maximum at ~1% vol/vol PFC (Figure 2b). Larger or smaller amounts of PFC were progressively less effective. Next, we applied PFC as a tool for modulating plasma NO bioavailability and RSNO formation in the mammalian vasculature. Administration of a large dose of PFC (7% vol/vol) caused extensive vasoconstriction due to rapid sequestration of plasma NO (Figure 3). Alternatively, a small dose of PFC (1% vol/vol) induced long-lasting systemic dilation (Figure 4). In this case, most of PFC-sequestered NO was efficiently converted into N₂O₃ to elicit endogenous vasoactive LMW RSNO (Figures 4 and 6). Because the basal level of endogenous LMW RSNO can be increased dozens of fold simply by changing the property of the circulating hydrophobic phase, there must be a substantial amount of available NO in mammalian plasma at any given moment. Moreover, the level of plasma LMW RSNO can be elevated further by supplementing PFC with exogenous LMW RSH (Figure 6), which indicates that RSNO formation is limited by circulating thiols rather than by plasma NO. Importantly, the fate and physiological significance of plasma NO are determined by the local hydrophobic environment. Taken together, the results of the present study directly implicate intrinsic plasma NO in maintaining vascular tone despite the presence of red blood cells or other potential NO scavengers. Furthermore, our results underscore the physiological importance of nitrosative chemistry in plasma, establishing endogenous RSNO as a significant transport and delivery system of bioactive NO in the mammalian circulation.

Pharmacological Applications of PFC as a Potent Modulator of NO Bioactivity

PFC emulsions have been clinically evaluated in many countries as artificial O₂ carriers to reduce allogeneic blood transfusion and improve tissue oxygenation. They have been successfully used to augment acute hemodilution after trauma or surgery and to treat conditions such as cerebral or myocardial ischemia. Although PFCs are well tolerated in animals and humans, their unexpected pulmonary and systemic hemodynamic effects have limited their clinical use. In particular, the higher doses of PFC have been associated with progressive hypertension and decreased heart rate. However, unexpected beneficial anti-inflammatory and antithrombotic effects of PFCs have been noticed that could not be explained merely by the ability of PFC to transport and upload O₂ or change blood viscosity.

The present study establishes PFC as a potent modulator of NO bioactivity, thus explaining, at least in part, its dose-dependent systemic effects. Most importantly, the present findings raise an attractive possibility of using PFC as a pharmacological tool to influence vascular tone and platelet function by modulating plasma NO activity via endogenous blood-borne NO carriers. In this regard, PFCs represent a conceptually new drug for the treatment of cardiovascular disorders associated with compromised and excessive NO bioavailability. Indeed, depending on the dose, PFC functions either as a potent NO stimulator or suppressor. The stimulating effect occurs at lower doses owing to the boosting of endogenous RSNO production (Figures 4 and 6). At higher doses, PFC suppresses NO activity by decreasing its available concentration (Figure 3a).

Vasoregulation With Natural Thiols and PFC: A Safe Alternative to Exogenous RSNO

RSNOs have a wide variety of therapeutic effects and may be particularly useful alternatives to organic nitrates because they do not elicit tolerance. As potent systemic vasodilators and platelet inhibitors, RSNOs have been evaluated in the treatment of various cardiovascular complications, including thrombosis, vasospasm, preeclampsia, and ischemia/reperfusion injury. Furthermore, because of their bronchodilating properties and the fact that the airway level of RSNOs is reduced in asthma and cystic fibrosis patients, RSNOs are considered as promising therapeutics for pulmonary conditions. Finally, RSNOs may be used as antiviral and antimicrobial agents, because they inhibit viral protease enzymes and possess a strong bacteriostatic effect. However, despite growing interest in the pharmacological potential of RSNOs, a number of general problems limit their clinical use. The main shortcoming of RSNOs as a potential drug is an unpredictable rate of decomposition in vivo and instability in physiological vehicles. Excess RSNOs can lead to hypotension and hemorrhage due to impaired platelet function. Furthermore, synthetic drugs that have been S-nitrosylated retain their own side effects.

In this regard, the use of PFC and PFC/RSH combinations provides a unique pharmacological solution for safe delivery of RSNOs. Because PFC-mediated generation of RSNOs occurs in vivo and can be precisely controlled by PFC and exogenous RSH concentrations, concerns about RSNO overdose and storage problems are eliminated. Furthermore, chemically inert PFC emulsions (eg, clinically safe Perftoran) and natural thiols, such as l-Cys or GSH, should have minimal immunologic side effects. Finally, because PFC uncouples RSNO formation from overall NO synthesis, it creates a unique opportunity to relieve nitrosative stress, ie, excessive nitration of macromolecules and tissue damage during inflammation, while maintaining control over vascular tone, blood clotting, and organ perfusion.

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References

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