Original Article

Experimental Study of the Relationship between Perfluoro-Octyl Bromide Emulsion and Norepinephrine Release in Reperfusion Arrhythmia: Isolated Guinea Pig Heart Model

Mitsuhiro Isaka, DVM, PhD,[1,2] Ichiro Sakuma, MD, PhD,[3] Norihiko Shiiya, MD, PhD,[1]
Shoji Fukushima, PhD,[4] Kunihiko Nakai, DVM, PhD,[5] Akira Kitabatake, MD, PhD,[3] and
Keishu Yasuda, MD, PhD[2]

Background: **Perfluoro-octyl bromide (PFOB), one of the perfluorochemical oxygen transporters, improved postischemic cardiac dysfunctions. Also norepinephrine (NE) is one of the important inducible factors on reperfusion arrhythmias (ventricular fibrillation [VF]). We used these methods to evaluate the relationship between PFOB emulsion and NE release on reperfusion arrhythmias.**

Materials and Methods: **The perfusion of isolated guinea pig hearts was employed: each of four groups of 6–7 hearts were used with Krebs-Henseleit solution (KHS) as control, and KHS with 5%, 15%, or 30% PFOB emulsion. The hearts were perfused in a constant pressure Langendorff model, stabilized for 30 min, followed by 30 min preischemia, then 30 min ischemia and 45 min reperfusion at normothermia.**

Results: **PFOB emulsion dose-dependently limited VF and inhibited NE release in reperfusion. Only 30% PFOB emulsion showed the significant improvement of VF ($p = 0.05$). In hemodynamic parameters, only 5% PFOB emulsion showed a significant decrease in reperfusion, but there was no difference in coronary flow rate (CFR). No differences among the four groups were demonstrated in cardiac oxygen metabolic parameters.**

Conclusions: **It was most likely that a high concentration of PFOB emulsion attenuated reperfusion arrhythmia by decreasing NE release. (Ann Thorac Cardiovasc Surg 2008; 14: 363–368)**

Key words: perfluoro-octyl bromide, norepinephrine, reperfusion arrhythmia, ventricular fibrillation

Introduction

Ventricular arrhythmias, such as ventricular fibrillation (VF), elicits during the reperfusion of ischemic myocardium, leading to cardiac sudden death.[1)] Many investigators have thought norepinephrine (NE) to be a closely related and crucial factor of reperfusion arrhythmia in the experimental and clinical settings.[1)]

NE release has been proposed mainly by two mechanisms: Ca^{2+}-dependent exocytotic release and Ca^{2+}-

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From Departments of [1]Cardiovascular Surgery and [3]Cardiovascular Medicine, Hokkaido University School of Medicine, Sapporo, Japan; [2]Pediatric Cardiac Surgery, Arkansas Children's Hospital, Little Rock, USA; [4]Department of Pharmaceutics, Kobe Gakuin University, Kobe, Japan; and [5]Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, Japan

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Address reprint requests to Mitsuhiro Isaka, DVM, PhD: Department of Cardiovascular Surgery, Hokkaido University School of Medicine, N14 W5, Kita-ku, Sapporo, Hokkaido 060–8648, Japan / Marble Veterinary Medical Center, 3–675 Ishikawa, Fujisawa, Kanagawa 252–0815, Japan.

independent carrier-mediated release associated with the activation of NE transporter in the outward direction.[1,2] In protracted ischemia, intraneuronal acidosis caused by failure of the H^+adenosine triphosphate (ATP) (H^+-ATP) pump due to ischemia activates the Na^+-H^+ exchanger leading to accumulation of intracellular Na^+. This phenomenon, combined with increased axoplasmic NE that causes a reversal of the NE transporter, was in an outward direction, eliciting a carrier-mediated NE release.[1] The carrier-mediated NE release strongly associates with reperfusion arrhythmias, especially VF (duration), in human, guinea pig, and rat hearts.[1,3–5]

Perfluorochemical (PFC), one of the artificial oxygen transporters, is hydrocarbons in which most or all of the hydrogen atoms have been replaced with fluorine.[6,7] Perfluoro-octyl bromide (PFOB) emulsion was developed by a new base and emulsification technique from second-generation PFCs.[8] We introduced a new emulsifying technology and improved the stability and oxygen transport capacity.[9] Furthermore, this PFOB emulsion showed cardioprotective effects after 6 hours of heart cold storage in guinea pigs[10] and benefical effects during cardiopulmonary bypass with hemodilution.[11] Under myocardial ischemia reperfusion condition, some researchers reported that PFC showed the improvement of myocardium during transient ischemia in angioplasty[12,13] and cardiac function after heart transplantation.[14,15] However, there are few concerns about the relationship between PFOB emulsion and NE release in reperfusion arrhythmias.

The purpose of this study was to assess the relationship between PFOB emulsion and NE release in the reperfusion arrhythmias after protracted global ischemia in isolated guinea pig hearts.

Materials and Methods

Isolated heart perfusion

Twenty-six male Hartley guinea pigs (Sankyo Co., Ltd., Tokyo, Japan) weighing 400 to 500 g each were divided by four groups: Krebs-Henseleit solution (KHS), 5% PFOB emulsion, 15% PFOB emulsion, and 30% PFOB emulsion (n = 6–7 in each group). All animals have received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals,” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

The hearts were removed after cervical dislocation performed under light anesthesia with CO_2 vapor. They were rapidly perfused at an aortic pressure of 29.6 ± 0.3 mmHg in a constant pressure Langendorff model. This model consisted of double reservoirs with filters, including a perfusion solution with or without drugs, pump, and heart chamber, with a KHS composed of NaCl 118.2 mmol/L, $NaHCO_3$ 25 mmol/L, KH_2PO_4 1.0 mmol/L, KCl 4.83 mmol/L, $MgSO_4$ 2.37 mmol/L, $CaCl_2$ 2.5 mmol/L, and glucose 11.1 mmol/L, with or without PFOB emulsion (v/v; 5%, 15%, or 30%) with bovine serum albumin (BSA) saturated with 95% O_2, 5% CO_2 (pH 7.52 ± 0.02). BSA (3% for 15%, 30% PFOB emulsion, and 1% for 5% PFOB emulsion) was added to the perfusion buffer containing PFOB emulsion to prevent the formation of crystals with calcium. BSA concentration was determined by a preliminary study (data not shown). Also, in the preliminary study there were no significant effects on NE amount, reperfusion arrhythmias, and others (data not shown). Electrocardiograms were continuously recorded with surface electrodes from the right atrium and the left ventricle to measure the heart rate (HR) and to recognize arrhythmias. Cardiac temperature was measured by attaching a thermoprobe on the surface of the heart. The values of cardiac surface temperature were 37.2ºC ± 0.1ºC in preischemia, 36.9ºC ± 0.1ºC in ischemia, 36.9ºC ± 0.2ºC in reperfusion, respectively. The cardiac pressure was determined from the left peak systolic pressure via the left ventricule, using a fluid-filled latex balloon connected to a transducer (Baxter International Inc., Deerfield, IL) for the measurement of left ventricular developed pressure (LVDP). The volume of the balloon was initially adjusted to achieve left ventricle diastolic pressure of 10 mmHg, and this volume was maintained throughout the experiments.

Experimental protocol

Initially, isolated guinea pig hearts were perfused to stabilize for 30 min with KHS, followed by 30 min perfusion as a preischemia with or without PFOB emulsion, 30 min ischemia induced by global stop perfusion, and 45 min reperfusion with or without PFOB emulsion. HR was evaluated at R-R intervals by electrocardiography. The coronary effluent was collected every 5 min for 30 min preischemia. In the first 10 min of reperfusion, sampling tubes were replaced every 2 min and during the final 35 min every 5 min. The volume of

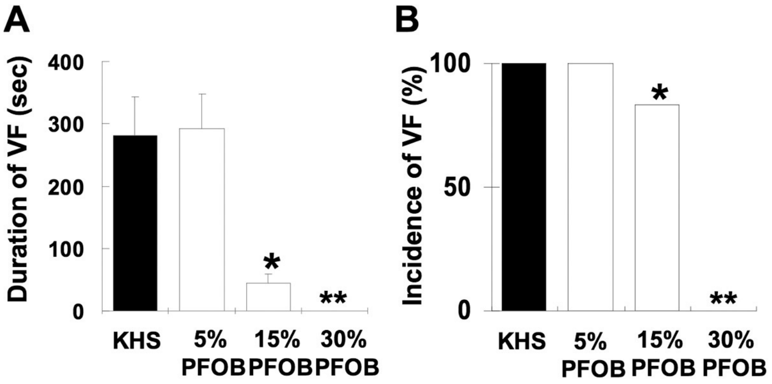

Fig. 1. Duration and incidence of VF in 45 min reperfusion.
A: Duration.
B: Incidence.
Bars represent mean ± SEM (n = 6–7 in each group).
**p* <0.05 vs. KHS.
***p* <0.05 vs. 15% PFOB, derived by one-way ANOVA, and the Bonferroni *t* test was used for post hoc analysis.

coronary effluent collected in each period was measured for coronary flow rate (CFR) and analyzed for amount of NE. The CFRs are expressed in ml per min per wet heart weight (ml/min/g).

Artificial blood substitute

We used a previously reported PFOB emulsion.[10,11] In brief, the PFOB emulsion (100 ml) used in this study was 28% PFOB with about a 210 nm average particle size as a base, 12% of perfluoroalcohol esters with oleic acid (FO-9982), 2.4% yolk lecithin, and 0.12% polyethyleneglycol (PEG), 1, 2-distearoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-PEG (DSPE-50H).

Evaluation of VF

Arrhythmias were analyzed from continuously recorded electrocardiogram tracings, according to the guidelines defined by the Lambeth Conventions.[16] VF was the most common and persistent type of reperfusion arrhythmia. Also the duration of VF showed the strong correlation with the amount of NE release in reperfusion.[3,5] Thus, we used only VF as an index of reperfusion arrhythmia.

NE measurements

The amount of NE was assayed in the coronary perfusate by high-performance liquid chromatography coupled to electrochemical detection followed by a previously described method.[5] Values are expressed in picomoles per gram of wet heart weight (pmol/g).

Cardiac oxygen metabolic variances

Oxygen concentration of perfusate was used as arterial PO_2, and oxygen concentration of coronary effluent was used as venous PO_2. O_2 extraction was calculated as follows: perfusate O_2–coronary effluent O_2. Myocardial oxygen consumption (MVO_2) was calculated as coronary flow volume/g × (perfusate PO_2–coronary effluent PO_2) × 24 μl O_2/ml at 760 mmHg.

Statistic analyses

Experimental values were expressed as mean ± standard error of mean (SEM) for 6–7 hearts in each group (data normalized to 1 g of wet heart weight). Comparisons of more than two groups were performed by one-way analysis of variance (ANOVA), and the Bonferroni *t* test was used for post hoc analysis. Yates' corrected χ^2 test was used to analyze the differences in the incidence of VFs. A *p* value of <0.05 was considered statistically significant.

Results

Duration and incidence of VF

PFOB emulsion prevented the duration of VF in a dose-dependent manner; 30% (0 ± 0 sec) and 15% (44 ± 15.2 sec) PFOB emulsion significantly inhibited the duration of VF compared to KHS (281.6 ± 62.1 sec) and 5% PFC (292.3 ± 56.1 sec) (Fig. 1A). These VFs were an onset of reperfusion and naturally stopped. Furthermore, although 30% PFOB emulsion completely limited the incidence of VF (0%), 5% (100%), and 15% (83.3%). PFOB emulsion did not show the significant inhibition of the incidence of VF (Fig. 1B).

NE release

In Fig. 2, PFOB emulsion (5%, 594.4 ± 71.3 pmol/g; 15%, 349.58 ± 16.1 pmol/g; and 30%, 198.9 ± 24.8 pmol/g) showed dose-dependent inhibition of NE release compared to KHS (1,069.7 ± 177.3 pmol/g) during 45 min reperfusion, following 30 min normothermic global cardiac ischemia.

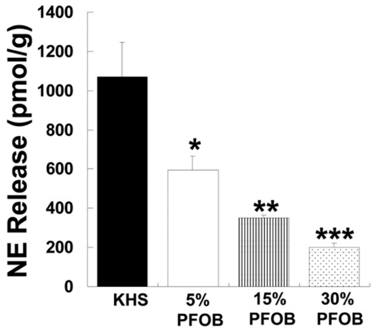

Fig. 2. NE release during 45 min reperfusion followed by 30 min normothermic global ischemia.
Bars represent mean ± SEM (n = 6–7 in each group).
*p <0.05 vs. KHS.
**p <0.05 vs. 5% PFOB.
***p <0.05 vs. 15% PFOB, derived by one-way ANOVA, and the Bonferroni t test was used for post hoc analysis.

Table 1. HR, CFR, and LVDP in all experiments

		Preischemia	10 min reperfusion	45 min reperfusion
	KHS	233.3 ± 6.3	238.8 ± 18.7	205.3 ± 10.8
HR	5% PFOB	223.5 ± 16.7	242.1 ± 22.3	155.0 ± 9.6*
(beats/min)	15% PFOB	205.2 ± 13.6	200.2 ± 9.6	190.0 ± 11.5
	30% PFOB	234.7 ± 15.5	213.3 ± 16.9	206.1 ± 14.3
	KHS	5.1 ± 0.7	3.4 ± 0.9	2.9 ± 0.7
CFR	5% PFOB	5.7 ± 0.6	2.9 ± 0.8	2.2 ± 0.5
(ml/g/min)	15% PFOB	5.8 ± 0.5	4.8 ± 1.1	3.6 ± 0.8
	30% PFOB	5.1 ± 0.7	3.4 ± 0.9	2.9 ± 0.7
	KHS	49.1 ± 7.1	23.1 ± 4.3	21.0 ± 3.5
LVDP	5% PFOB	37.9 ± 5.7	5.5 ± 2.9*	4.8 ± 1.3*
(mmHg)	15% PFOB	40.7 ± 7.5	20.5 ± 3.9	25.5 ± 5.2
	30% PFOB	35.4 ± 3.5	36.0 ± 6.0	27.0 ± 4.6

Each value shows mean ± SEM (n = 6–7 in each group).
*p <0.05 vs. KHS, derived by one-way ANOVA, and the Bonferroni t test was used for post hoc analysis.
HR, heart rate; CFR, coronary flow rate; LVDP, left ventricular developed pressure; KHS, Krebs-Henseleit solustion; PFOB, perfluoro-octyl bromide; SEM, standard error of mean; ANOVA, analysis of variance.

Table 2. Oxygen extraction and MVO_2 in all experiments

		Preischemia	10 min reperfusion	45 min reperfusion
	KHS	67.3 ± 1.6	56.2 ± 5.1	59.8 ± 3.5
Oxygen	5% PFOB	71.3 ± 1.1	50.6 ± 3.4	50.6 ± 2.2
extraction	15% PFOB	66.8 ± 2.2	65.6 ± 0.9	61.6 ± 3.6
	30% PFOB	66.1 ± 2.0	63.1 ± 3.8	62.0 ± 6.7
	KHS	26.5 ± 3.6	24.3 ± 5	23.2 ± 3.6
MVO_2	5% PFOB	30.3 ± 5.2	26.2 ± 4.7	16.9 ± 3.3
	15% PFOB	40.7 ± 6.5	30.0 ± 7.9	27.3 ± 10.6
	30% PFOB	55.8 ± 25.7	48.2 ± 27.7	33.1 ± 12.8

Each value shows mean ± SEM (n = 6–7 in each group).
*p <0.05 vs. KHS, derived by one-way ANOVA, and the Bonferroni t test was used for post hoc analysis.
MVO_2, myocardial oxygen consumption; KHS, Krebs-Henseleit solustion; PFOB, perfluoro-octyl bromide; SEM, standard error of mean; ANOVA, analysis of variance.

Hemodynamics

Only 5% PFOB emulsion showed significant decreases at 45 min reperfusion, compared to KHS in HR and LVDP (Table 1). However, we could detect no differences among the four experimental groups (Table 1).

Cardiac oxygen metabolic variances

As shown in Table 2, no significant differences were demonstrated among four experimental groups in O_2 extraction and MVO_2 in preischemia and reperfusion.

Discussion

In our study, the result of incidence showed a nondose-dependent manner because the number of each experimental group might be small**.** However, PFOB emulsion showed the inhibition of NE release and reperfusion arrhythmias in a dose-dependent manner (Figs. 1 and 2). In the protracted ischemia, NE release is carrier mediated, which has a strong correlation with reperfusion arrhythmia.[1,2,5] To address the fashion of NE release in this study, we used the NE transporter inhibitor (desipramine hydrochloride; DMI, 10 nM). This compound significantly inhibited NE release ischemia and completely abolished reperfusion arrhythmias (data not shown), suggesting the carrier-mediated NE release in this study, supported by previous studies.[1,2,5] Furthermore, PFC delivered sufficient oxygen to allow ATP production within submerged organs during ischemia.[17,18] Collectively, PFOB emulsion might possibly prevent hypoxia with the inhibition of the failure of the H^+-ATP pump, leading to the attenuation of a carrier-mediated NE release associated with reperfusion arrhythmias. However, further studies are required to clarify the inhibitory mechanism of PFOB emulsion on reperfusion arrhythmias because PFCs have the potential to attenuate the production of reactive oxygen species,[19] which induces reperfusion arrhythmias.[20]

In hemodynamic parameters, high concentrations (15% and 30%) of PFOB emulsion did not change these parameters (Table 1). Some researchers demonstrated that PFC itself impairs cardiac contractile functions after ischemia and 6 hours cold storage.[10,12,13,21] The reason for this discrepancy is not totally clear. However, the ischemic period might partly be associated because in this study it might be for only a short time compared to previous studies. On the other hand, a low concentration (5%) of PFOB emulsion showed only significant decreases at 45 min reperfusion compared to KHS in HR and LVDP (Table 1). Perfluorotributylamine (FC)-43 (FC-43), one of the PFCs, had cytotoxic effects involved in oxygen-free radical production[22] in the heart during long-term hypothermic exposure.[23] To the contrary, PFCs also attenuated the production of reactive oxygen species.[19] Al least there might have been a relationship between the concentration of PFCs and the production.

PFC emulsions have been evaluated as artificial oxygen carriers to reduce allogeneic blood transfusion and to improve tissue oxygenation in clinical setting. Also, it augmented acute hemodilution after trauma or surgery and treated the conditions, such as myocardial ischemia.[7,24,25] The results of this study might reflect possible clinical benefits of new PFOB emulsion during cardiac surgery by method of preconditioning.

In conclusion, the preconditioning with high concentrations of PFOB emulsion might prevent the occurrence of reperfusion arrhythmias, at least in part, due to the inhibition of NE release.

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References

1. Schömig A. Catecholamines in myocardial ischemia. Systemic and cardiac release. *Circulation* 1990; **82** (3 Suppl): II13–22.
2. Imamura M, Poli E, Omoniyi AT, Levi R. Unmasking of activated histamine H_3-receptors in myocardial ischemia: their role as regulators of exocytotic norepinephrine release. *J Pharmacol Exp Ther* 1994; **271**: 1259–66.
3. Imamura M, Lander HM, Levi R. Activation of histamine H_3 receptors inhibits carrier-mediated norepinephrine release during protracted myocardial ischemia. Comparison with adenosine A_1-receptors and α_2-adrenoceptors. *Circ Res* 1996; **78**: 475–81.
4. Kurtz T, Richardt G, Hagl S, Seyfarth M, Schömig A. Two different mechanisms of noradrenaline release during normoxia and stimulated ischemia in human cardiac tissue. *J Mol Cell Cardiol* 1995; **27**: 1161–72.
5. Oka J, Imamura M, Hatta E, Maruyama R, Isaka M, et al. Carrier-mediated norepinephrine release and reperfusion arrhythmias induced by protracted ischemia in isolated perfused guinea pig hearts: effect of presynaptic modulation by α_2-adrenoceptor in mild hypothermic ischemia. *J Pharmacol Exp Ther* 2002; **303**: 681–7.
6. Lowe KC, Davey MR, Power JB. Perfluorochemicals: their applications and benefits to cell culture. *Trends Biotechnol* 1998; **16**: 272–7.
7. Lowe KC. Perfluorinated blood substitutes and artificial oxygen carriers. *Blood Rev* 1999; **13**: 171–84.
8. Faithfull NE. Second generation fluorocarbons. *Adv Exp Med Biol* 1992; **317**: 441–52.
9. Sakanoue J, Tamura M, Fukushima S, Takeuchi Y,

Sakuma I, et al. Assessment of newly developed perfuorocarbon emulsion: oxygen carrying capacity as the blood substitute in vivo. *Artif Cells Blood Substit Immobil Biotechnol* 2001; **29**: 389–97.

10. Isaka M, Imamura M, Sakuma I, Shiiya N, Fukushima S, et al. Cardioprotective effect of perfluorochemical emulsion for cardiac preservation after six-hour cold storage. *ASAIO J* 2005; **51**: 434–9.
11. Isaka M, Sakuma I, Imamura M, Makino Y, Fukushima S, et al. Experimental studies on artificial blood usage for hemodilution during cardiopulmonary bypass. *Ann Thorac Cardiovasc Surg* 2005; **11**: 238–44.
12. Cleman M, Jaffee CC, Wohlgelernter D. Preservation of ischemic during percutaneous transminal coronary angioplasty by transcatheter infusion of oxygenated Fluosol DA 20%. C*irculation* 1986; **74**: 555–62.
13. Tokioka H, Miyazaki A, Fung P, Rajagopalan RE, Kar S, et al. Effects of intracoronary infusion of arterial blood or Fluosol-DA 20% on regional myocardial metabolism and function during brief coronary artery occlusions. *Circulation* 1987; **75**: 473–81.
14. Segel LD, Follette DM, Iguidbashian JP, Contino JP, Castellanos LM, et al. Posttransplantation function of hearts preserved with fluorochemical emulsion. *J Heart Lung Transplant* 1994; **13**: 669–80.
15. Ueda K, Genda T, Hirata I, Shimada M, Shibata T, et al. Beneficial effect of fluorocarbon reperfusion on postoperative cardiac dysfunction of transplanted heart. *J Heart Lung Transplant* 1992; **11** (4 Pt 1): 646–55.
16. Walker MJ, Curtis MJ, Hearse DJ, Campbell RW, Janse MJ, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovasc Res* 1988; **22**: 447–55.
17. Sakai T, Kuroda Y, Suzuki Y, Hamano M, Matsumoto S, et al. Adenine nucleotide metabolism of the small bowel during preservation by the cavitary two-layer method. *Transplant Proc* 1996; **28**: 2615–9.
18. Fujino Y, Suzuki Y, Kakinoki K, Tanioka Y, Ku Y, et al. Protection against experimental small intestinal ischaemia-reperfusion injury with oxygenated perfluorochemical. *Br J Surg* 2003; **90**: 1015–20.
19. Smitsh TM, Steinhorn DM, Thusu K, Fuhrman BP, Dandona P. A liquid perfluorochemical decreases the in vitro production of reactive oxygen species by alveolar macrophages. *Crit Care Med* 1995; **23**: 1533–9.
20. Bernier M, Hearse DJ, Manning AS. Reperfusion-induced arrhythmias and oxygen-derived free radicals. Studies with “anti-free radical” interventions and a free radical-generating system in the isolated perfused rat heart. *Circ Res* 1986; **58**: 331–40.
21. Martin SM, Laks H, Drinkwater DC, Stein DG, Barthel SW, et al. Perfluorochemical reperfusion limits myocardial reperfusion injury after prolonged hypothermic global ischemia. *Biomater Artif Cells Immobilization Biotechnol* 1992; **20**: 985–9.
22. Lane TA, Krukonis V. Reduction in the toxicity of a component of an artificial blood substitute by supercritical fluid fractionation. *Transfusion* 1988; **28**: 375–8.
23. McCoy LE, Becker CA, Goodin TH, Barnhart MI. Endothelial response to perfluorochemical perfusion. *Scan Electron Microsc* 1984; **16** (Pt 1): 311–9.
24. Lane TA. Perfluorochemical-based artificial oxygen carrying red cell substitutes. *Transfus Sci* 1995; **16**: 19–31.
25. Spahn DR. Blood substitutes. Artificial oxygen carriers: perfluorocarbon emulsions. *Crit Care* 1999; **3**: R93–7.