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Effect of FC43se on Endotoxin-induced Disseminated Intravascular Coagulation in Rats

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ABSTRACT

Perfluorotributylamine/Pluronic F68 Stem-Emulsion (FC43se), which is a blood substitute, was assessed for its effectiveness on disseminated intravascular coagulation (DIC) in the rat model. Rats were infused intravenously with 2.5 mg/kg of *Escherichia coli* lipopolysaccharide (*Escherichia coli* 055:B5 lipopolysaccharide B) for four hours. At the same time, FC43se or normal physiological saline was infused at 2.5 ml/kg/hr. The white blood cell and platelet counts, prothrombin time (PT), activated partial thromboplastin time (APTT), and the plasma levels of interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor alpha (TNFα) were determined at 4hr. The infusion of FC43se markedly prevented a decrease in platelet counts ($p=0.0004$) and a prolongation of both PT and APTT ($p<0.05$ and $p<0.03$ each). The serum level of IL-1β and IL-4 showed no significant change. The serum level of IL-6, IL-10 and TNFα increased significantly ($p=0.0007$, $p=0.0004$ and $p<0.05$ each) with infusion of FC43se in rats treated with bacterial endotoxin. FC43se has beneficial effects on endotoxin-induced DIC as an anticoagulant and anti-inflammatory cytokine induced agent.

Key words: *Perfluorocarbons, Disseminated intravascular coagulation, Cytokine, Endotoxin*

Disseminated intravascular coagulation (DIC) is a syndrome characterized by a tendency toward diffuse hemorrhage. This tendency to hemorrhage results from exhaustion of the platelets and coagulation factors due to multiple thrombi which occur in various organs as a result of intravascular activation of the coagulation cascade in various pathologic conditions[13]. The mortality rate of patients with septic shock who develop DIC is significantly higher than that of patients without DIC[8]. The principal aims of treatment for DIC are to treat the causative disease and to prevent the formation and development of fine thrombi caused by stimulation of coagulation.

Table 1. Composition of FC43se, structural formulas of FC43 and Pluronic F68

Composition of FC43se	
FC43 (perfluorotributylamine)	20 w/v%
Pluronic F-68 (polyxypropylene-polyxyethylene-copolymer)	2.56 w/v%
Structual formula of FC43 (perfluorotributylamine)	
CF3-CF2-CF2-CF2-N-CF2-CF2-CF2-CF3 CF3-CF2-CF2-CF2	
Structual formula of Pluronic F-68	
HO(CH2CH2O)a(CHCHH20)b(CH2CH2O)CH CH3	

Perfluorochemicals (PFCs) have been widely investigated as blood cell substitutes (RCS) in the medical literature[25]. Perfluorotributylamine/Pluronic F-68 Stem-Emulsion is one such perfluorocarbon which was developed as an artificial blood substitute in 1970 (Table 1). Due to side effects such as a long-term accumulation in the reticuloendothelial system and the necessity of maintaining a high partial oxygen pressure over 550 mmHg to transport sufficient oxygen, its clinical applications have been abandoned. However, we previously reported the effectiveness of FC43se for the prevention of hyperacute rejection in a heart and lung model, using guinea pig to rat xenodiscordant transplantation, and in reperfusion injury in rabbit lung[17,20,23]. FC43se is thought to inhibit thrombus formation and a decrease in the number of platelets. For these reasons, the anticoagulation properties of FC43se were studied in endotoxin-induced DIC in rats. The influence of FC43se in the release of cytokines, which are important mediators in the septic state and DIC, was studied simultaneously.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River Japan) weighing 286±4.9 g (mean±SE) were housed for at least 7

days in our animal facility and allowed a standard laboratory diet and water prior to the experiments. All of the animals were maintained in accordance with the guidelines of the National Institute of Health (NIH publication No. 86–23, revised 1985).

Drugs

FC43se (Perfluorotributylamine/Pluronic F-68 Stem-Emulsion) was obtained from Green Cross, Tokyo. Endotoxin (*Escherichia coli* 055:B5 lipopolysaccharide B) was obtained from Difco Lab. Detroit, Mich. and dissolved in pyrogen-free physiological saline at 2.5 mg/ml.

Animal Experimental Design

Experimental DIC was induced by a 4hr sustained infusion of endotoxin according to the methods of Yoshikawa et al[26]. The rats were anesthetized by giving an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Both the left and right femoral veins were exposed and cannulated with a venous needle. Endotoxin was infused into the right femoral vein for 4hr (2.5 ml/kg/hr). At the same time, FC43se (2.5 ml/kg) and physiological saline, or physiological saline only, were infused into the left femoral vein for 4hr (2.5 ml/kg/hr), using a syringe pump (Terufusion STC-531, TERUMO Co. Ltd. Japan). To examine the effectiveness of FC43se on DIC, after 4hr of infusing, the blood of the rats used in the experiment was withdrawn from the abdominal aorta into plastic syringes and carefully mixed with 1 part sodium citrate solution (0.1 mol/liter) to 9 parts blood, avoiding blood coagulation. Plasma was obtained by centrifuging at 3,000 r.p.m. for 10 min. The severity of the DIC and septic condition were determined by measuring various parameters, including platelet count and white blood cell count (WBC), prothrombin time (PT), activated partial thromboplastin time (APTT) (Hoechst Behring, Japan), and the concentrations of the following cytokines, Interleukin-1beta (IL-1β), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Tumor necrosis factor alpha (TNFα) (TFB Co. Ltd. Tokyo, Japan).

Platelet and WBC Counts

Platelet and WBC counts were determined automatically by a Celltac α Counter (Nihon Kohden, Japan).

PT (Prothrombin Time)

The coagulation process was triggered by incubation of rat plasma with an optical amount of thromboplastin and calcium and the time to form a fibrin clot was measured, using a Behring Fibrin timer (Hoechst Behring, Japan).

APTT (Activated Partial Thromboplastin Time)

The incubation of rat plasma with an optical amount of phospholipids and an activator leads to the activation of factors in the intrinsic coagulation pathway. The addition of calcium ions triggers coagulation and the time taken for a fibrin clot to form was measured, using the fibrin timer.

Cytokines (IL-1β, IL-4, IL-6, IL-10, TNFα)

The rat cytokine ELISA kit is a solid phase sandwich Enzyme Linked-Immunosorbent Assay (ELISA). An antibody specific for rat cytokine has been coated onto the wells of the microtiter strips provided. Samples, including standards of known rat cytokine concentration, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated second antibody. During the first incubation, the rat cytokine antigen binds simultaneously to the immobilized (capture) antibody at one site, and to the solution phase biotinylated antibody at a second site. After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four layered sandwich. After a second incubation and washing to remove the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of rat cytokines present in the original specimen.

Experimental Group

The rats were divided into the following four groups. Each group contained ten rats.

Group 1 (Normal group): Physiological saline was infused into both femoral veins.

Group 2 (FC43se control group): Physiological saline was infused into the right femoral vein, and FC43se mixed with physiological saline (2.5 ml/kg) was infused into the left femoral vein.

Group 3 (Control group): Endotoxin was infused into the right femoral vein, and physiological saline was infused into the left femoral vein.

Group 4 (FC43se group): Endotoxin was infused into the right femoral vein, and FC43se mixed with physiological saline (2.5 ml/kg) was infused into the left femoral vein.

Statistical Analysis

Differences in all groups were compared using the Mann-Whitney U test, and were considered to be statistically significant if p values were less than 0.05. Each data point was expressed as the mean±SE.

RESULTS

Laboratory analysis was performed in all groups.

Changes in WBC Counts

The WBC counts were 4,450±517 /μl in Group 1 (Normal group), and 3,080±433 /μl in Group 2 (FC43se control group). The counts were reduced to 1,270±165 /μl in Group 3 (Control group) which was significantly different ($p<0.001$) from Group 1 and from 1,010±221 /μl in Group 4 (FC43se group). No significant difference was noted between Group 1 and Group 2, or between Group 3 and Group 4 (Fig. 1).

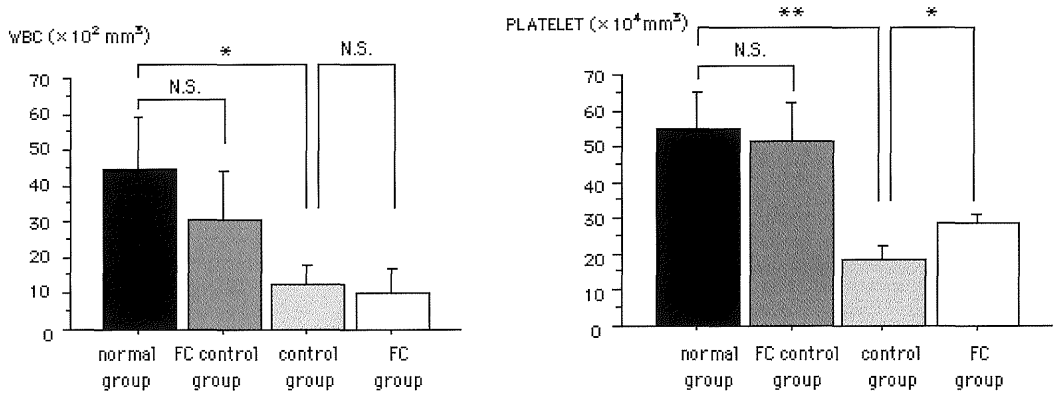

Fig. 1. Changes in WBC and Platelet Counts
Effect of FC43se on leukopemia and thrombocytopenia induced by lipopolysaccharide infusion in rats. Lipopolysaccharide at 25 mg/kg or saline was infused continuously for 4hr and simultaneously with FC43se or not. Blood samples were collected at the end of the infusion. Platelet and WBC counts were determined automatically by a Celltacα Counter. The results are expressed as means±S.E.M. of ten experiments per group. No significant difference was noted between Group 1 and Group 2, or between Group 3 and Group 4 in the WBC counts. A decrease in platelet count was significantly inhibited by infusion of FC43se. (* $p<0.001$ ** $p<0.0001$)

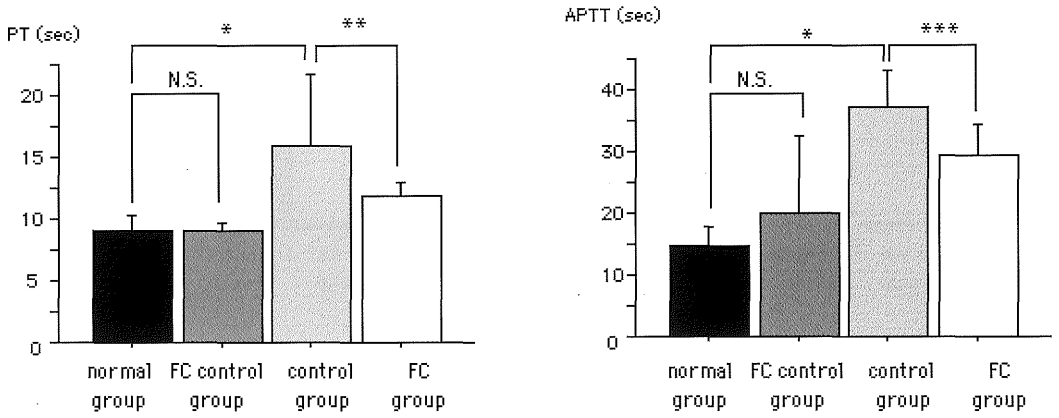

Fig. 2. Changes in PT and APTT
Effect of FC43se on coagulation function prothrombin time and activated partial thrombin time in rats treated with lipopolysaccharide. Blood samples were carefully mixed 1 part sodium citrate solution (0.1 mol/liter) with 9 parts blood, avoiding the formation form. After the plasma was obtained by centrifuging at 3,000 r.p.m. for 10 min, PT and APTT were measured with a fibrin timer. The results are expressed as means±S.E.M. of ten experiments per group. Administration of FC43se inhibited the prolongation of PT and APTT. (* $p<0.001$,** $p<0.01$, ***$p<0.05$)

Changes in Platelet Counts

The platelet counts were 54.9±3.5×104 /μl in Group 1 (Normal group) and 51.9±3.3×104 /μl in Group 2 (FC43se control group). The counts were reduced to 18.4±1.3×104 /μl in Group 3 (Control group) and 28.6±0.8×104 /μl in Group 4 (FC43se group), and these values were significantly different ($p<0.0001$). A decrease in the platelet count was significantly inhibited by the infusion of FC43se ($p=0.0004$) (Fig. 1).

Changes in Prothrombin Time

Prothrombin time (PT) was 9.1±0.5 sec in Group 1 (Normal group) and 9.1±0.2 sec in Group 2 (FC43se control group) with no significant difference. PT was prolonged to 16.0±1.8 sec in Group 3 (Control group) with a significant difference from Group 1 ($p<0.001$). The prolongation of PT, which was 12.5±0.6 sec in Group 4 (FC43se group), was significantly inhibited by infusing FC43se ($p<0.05$) (Fig. 2).

Changes in Activated Partial Thromboplastin Time

Activated partial thromboplastin time (APTT) was 14.7±1.0 sec in Group 1 (Normal group), and 19.9±4.2 sec in Group 2 (FC43se control group) with no significant change. APPT was prolonged to 37.2±1.9 sec in Group 3 (Control group), which was significantly different from Group 1 ($p<0.001$), and to 29.5±1.6 in Group 4 (FC43se group). The prolongation of activated partial thromboplastin time was significantly inhibited by the infusion of FC43se ($p<0.03$) (Fig. 2).

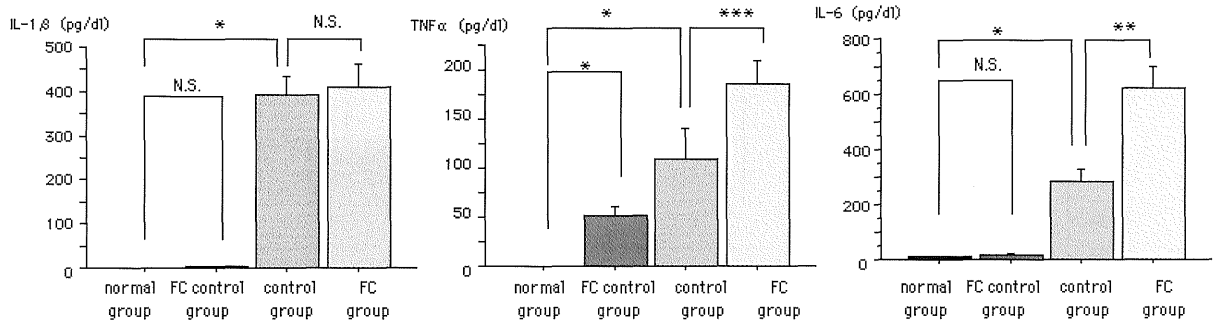

Fig. 3. Changes in IL-1β, TNFα and IL-6
Effect of FC43se on the concentrations of plasma pro-imflammatory cytokines IL-1β, IL-6 and TNFα concentration in rats treated with lipopolysaccharide. IL-1β, IL-6 and TNFα were measured by ELISA. The results are expressed as means±S.E.M. of ten experiments per group. In the non septic state FC43se stimulated macrophages and evoked increased levels of TNFα. In the septic state the levels of TNFα and IL-6 were increased significantly by administration of FC43se. (* $p<0.001$, ** $p<0.01$, *** $p<0.05$)

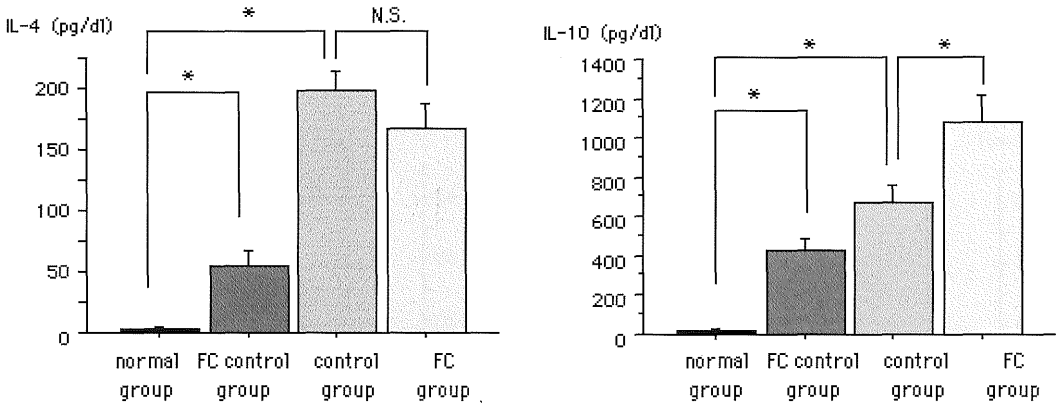

Fig. 4. Changes in IL-4 and IL-10
Effect of FC43se on the concentraions of plasma pro-inflammatory cytokines IL-4 and IL-10 in rats treated with lipopolysaccharide. IL-4 and IL-10 were measured by ELISA. The results are expressed as means±S.E.M. of ten experiments per group. In the non septic state the levels of IL-4 and IL-10 were increased significantly by infusion with FC43se, and in the septic state IL-10 levels were higher than in the control group. (* $p<0.001$)

Changes in IL-1β

IL-1β was 0 pg/ml in Group 1 (Normal group), and 2.9±2.3 pg/ml in Group 2 (FC43se control group), with no significant difference between them. IL-1β was increased to 390.8±62.6 pg/ml in Group 3 (Control group), which was significantly different ($p=0.0002$) from Group 1, and to 420.9±89.9 pg/ml in Group 4 (FC43se group). No significant difference was noted between Group 3 and Group 4 (Fig. 3).

Changes in IL-4

IL-4 was 3.4±1.7 pg/ml in Group 1 (Normal group), and 54.8±34.4 pg/ml in Group 2 (FC43se control group), with a significant difference between Group 1 and Group 2 ($p=0.0003$). IL-4 was increased to 198.9±62.8 pg/ml in Group 3 (Control group), which was significantly different ($p=0.0003$) from Group 1, and to 167.7±29.7 pg/ml in Group 4 (FC43se group). No significant difference was noted between Group 3 and Group 4 (Fig. 4).

Changes in IL-6

IL-6 was 9.0±4.6 pg/ml in Group 1 (Normal group), and 14.6±7.7 pg/ml in Group 2 (FC43se control group), with no significant difference between them. IL-6 was increased to 251.4±49.5 pg/ml in Group 3 (Control group), which was significantly different ($p=0.0007$) from Group 1, and to 619.7±125.9 pg/ml in Group 4 (FC43se group). A significant difference was noted between Group 3 and Group 4 ($p=0.0029$) (Fig. 3).

Changes in IL-10

IL-10 was 21.9±6.7 pg/ml in Group 1 (Normal group), and 428.6±76.7 pg/ml in Group 2 (FC43se control group), with a significant difference between them ($p=0.0004$). IL-10 was increased to 674.3±131.1 pg/ml in Group 3 (Control group), which was significantly different ($p=0.0004$) from Group 1, and to 1,077.8±213.4 pg/ml in Group 4 (FC43se group). A significant difference was noted between Group 3 and Group 4 ($p=0.0004$) (Fig. 4).

Changes in TNFα

TNFα was 0 pg/ml in Group 1 (Normal group) and 51.2±21.0 pg/ml in Group 2 (FC43se control group), with a significant difference between them ($p=0.0006$). TNFα was increased to 110.1±30.9 pg/ml in Group 3 (Control group), which was significantly different ($p=0.0003$) from Group 1, and to 186.6±48.6 pg/ml in Group 4 (FC43se group). A significant difference was noted between Group 3 and Group 4 ($p<0.05$) (Fig. 3).

DISCUSSION

Bacterial endotoxin frequently induces coagulation disorders and abnormalities in fibrinolysis[8]. Widespread microvascular thrombosis in various organs is a common feature of lethal septic shock and may considerably contribute to organ dysfunction. The most pronounced feature is DIC, which is characterized by microvascular thrombosis, consumption of platelets and stimulation of fibrinolysis. The symptoms of septic coagulation disorders are mediated by the release of cytokines such as IL-1β and TNFα, pro-inflammatory agents which activate the coagulation cascade[22]. Pro-inflammatory cytokine levels in patients with DIC have been shown to be higher than those of patients without DIC. To overcome the high mortality rate, a large quantity of anti DIC and anti septic agents have been developed and reported[5,7,24].

One perfluorochemical, Perfluorotributylamine/Pluronic F-68 Stem-Emulsion, is a biochemical inert fluoride in liquid form which has been studied as a potential red cell substitute (RCS)[3,14]. It is necessary to emulsify FC43se to use hydrophobic fluid that is Perfluorochemical as a blood substitute. FC43se was emulsified with non-ionic surfactant pluronic F-68 by the high pressure method using a Manton Goring type homogenizer. Drawbacks such as its immunosuppressive effects and its long retention time in the reticuloendothelial system have caused PFC-based agents to become less popular as RCS[10]. In preliminary reports we described the effectiveness of these agents in the prevention of hyperacute rejection in the heart and lung model of xenodiscordant transplantation from guinea pig to rat and in reperfusion injury in rabbit lung[17,20,23]. FC43se is thought to prevent thrombus formation and platelet adhesion to endothelial cells.

In the present study, we examined the effect of FC43se in rats on the onset of a DIC like syndrome induced by continuous lipopolysaccaride infusion and on the normal state, by measuring hemostatic function, coagulation systems and cytokine concentrations.

The FC43se control group, with FC43se administration alone, tended to have a reduced WBC count and platelet count, but differences were not significant compared with the Normal group, infused with saline. Similar results were obtained in measurements of PT and APTT. The FC43se group increased the level of pro-inflammatory cytokines such as TNFα, but other pro-inflammatory cytokines such as IL-β and IL-6 were not increased. The increases in IL-4 and IL-10 were statistically significant. TNFα is produced from activated macrophages stimulated by various invasions, and pivotally activates neutrophils with other pro-inflammatory cytokines[5]. Immunosuppression of FC43se was recognized by cytokine hyposecretion and macrophage hypofunction[10,15]. In our study increased TNFα levels were the evidence of macrophage stimulation by FC43se, evoking increased levels of IL-4 and IL-10. Continuous hyper anti-inflammatory cytokines has been considered part of a compensatory anti-inflammatory response syndrome, which has a profound effect on monocyte function and inhibits T- and B-lymphocyte activity[1,3].

The control group showed significant changes in all experiments compared with the normal group. The control group demonstrated the septic state and DIC. In this state, neutrophils and macrophages are stimulated by LPS, and adhere to the endotherial cells with subsequent release of cytokines and activation of the coagulation cascade in response to an increase in tissue factor levels[12]. In the FC43se group, infusion of FC43se inhibited the decrease in platelet counts and the prolongation of PT and APTT, with significant differences from the control group. The levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα were increased compared with the control group. On the other hand, the levels of IL-4 and IL-10, which are anti-inflammatory cytokines, were increased in both groups, although IL-4 increased more in the control group, but there was no significant difference between levels in the control group and the FC43se group. Infusion with FC43se, however, prompted IL-10 release, and levels were higher than in the control group. IL-10 is produced in various situations involving immune inactivation by the TH2 subsets of helper T cells, as well as by monocytes, macrophages and B cells[9]. It exerts a wide variety of immunostimulatory effects on B cells together with a number of immunosuppressive effects on monocytes and macrophages. IL-10 protects against the detrimental effects of pro-inflammatory cytokines by deactivation of macrophages[6]. IL-10 has also been shown to prevent the secretion of a number of cytokines by inhibiting the cytokine-specific mRNA synthesis induced by LPS and Tissue Factor (TF) production at the mRNA level[2,16]. In the septic state FC43se may suppress acceleration of the coagulation cascade by increasing IL-10. IL-6 was also increased in our experiments. IL-6 plays an important role as an alarm signal in response to various stresses, and in the production of hepatic acute phase proteins (APP)[22]. The pref-

erential induction of IL-1 receptor antagonist (IL-1Ra) by APP may contribute to these anti-inflammatory effects and provide an important regulatory signal in the acute phase response[19]. Thomassen reported that Perflubron, a perfluorochemical which is the same as FC43se and which can be used for liquid ventilation, decreased cytokine secretion (TNF, IL-1, and IL-6) from lipopolysaccaride-stimulated human alveolar macrophages[18]. Human umbilical vein endothelial cells (HUVEC) were activated by IL-1, TNF or *E. coli* endotoxins (LPS) in the presence or absence of Perflubron. HUVEC activation was monitored by the extent of up-regulation of intercellular adhesion molecule-1 (ICAM) and endothelial-leukocyte adhesion molecule-1 (ELAM). Perflubron neither activates HUVEC nor interferes with HUVEC activation by IL-1 or TNF, and prevented HUVEC activation by LPS in a dose and time-dependent manner[11]. These results differed from our observation but similarly suggest that perfluorochemicals act as agents of anti-inflammation and preservation on endothelial cells.

In conclusion, our study demonstrated that in the DIC and septic state induced by LPS 4 hours after endotoxin administration in rats, FC43se inhibited a decrease in platelet count and the prolongation both PT and APTT, in addition to increasing levels of the anti-inflammatory cytokines in the DIC state. FC43se is thus potentially an agent against DIC.

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REFERENCES

1. **Abraham, E.** 1991. Physiologic stress and cellular ischemia: Relationship to immunosuppression and susceptibility to infection. Crit. Care. Med. **19:** 613–618.
2. **Bogdan, C., Paik, J., Vodovotz, Y. and Nathan, C.** 1992. Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-beta and interleukin-10. J. Biol. Chem. **32:** 23301–23308.
3. **Bone, R.C.** 1996. Sir Isaac Newton, sepsis, SIRS and CARS. Crit. Care. Med. **24:** 1125–1128.
4. **Cannon, J.G., Tompkins, R.G., Gelfand, J.A., Michie, H.R., Stanford, G.G., van Der Meer, J.W., Endres, S., Lonnemann, G., Corsetti, J. and Chernow, B.** 1990. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. J. Infect. Dis. **161:** 79–84.
5. **Emerson, T.E. Jr.** 1994. Antithrombin III replacement in animal models of acquired antithrombin III deficiency. Blood Coagul. Fibrinolysis **5 suppl. 1:** 37–45.
6. **Ertel, W., Keel, M., Steckholzer, U., Ungethum, U. and Trentz, O.** 1996. Interleukin-10 attenuates the release of proinflammatory cytokines but depresses splenocyte functions in murine endotoxemia. Arch. Surg. **131:** 51–56.
7. **Eskandari, M.K., Bolgos, G., Miller, C., Nguyen, D.T., DeForge, L.E. and Remick, D.G.** 1992. Anti-tumor necrosis factor antibody therapy fails to prevent lethality after cecal ligation and puncture or endotoxemia. J. Immunol. **9:** 2724–2730.
8. **Fourrier, F., Chopin, C., Goudemand, J., Hendrycx, S., Caron, C., Rime, A., Marey, A. and Lestavel, P.** 1992. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Chest **101:** 816–823.
9. **Howard, M. and O`Garra, A.** 1992. Biological properties of interleukin 10. Immunol. Today **13:** 198–200.
10. **Lane, T.A. and Lamkin, G.E.** 1984. Paralysis of phagocyte migration due to an artificial blood substitute. Blood **64:** 400–405.
11. **Lane, T.A., Smith, D., Wancewicz, E. and Funk, R.** 1993. Inhibition of endotoxin-mediated activation of endothelial cells by a perfluorocarbon emulsion. Biomater. Artif. Cells Immobilization Biotechnol. **21:** 163–172.
12. **Levi, M., ten Cate, H., van der Poll, T. and van Deventer, S.J.** 1993. Pathogenesis of disseminated intravascular coagulation in sepsis. JAMA. **270:** 975–979.
13. **Minna, J.D., Robboy, S.J. and Colman, R.W.** 1974. Disseminated intravascular coagulation. Heart-Lung. **3:** 786–796.
14. **Mitsuno, T., Ohyanagi, H. and Yokoyama, K.** 1984. Development of a perfluorochemical emulsion as a blood gas carrier. Artif. Organs. **8:** 25–33.
15. **Miura, J.** 1985. Immunosupressive effects of perfluorochemical emulsions and its mechanism. Fukushima J. Med. Sci. **35:** 345–353.
16. **Ramani, M., Ollivier, V., Khechai, F., Vu, T., Ternisien, C., Bridey, F. and de Prost, D.** 1993. Interleukin-10 inhibits endotoxin-induced tissue factor mRNA production by human monocytes. FEBS. Lett. **334:** 114–116.
17. **Sugawara, Y., Matsuura, Y., Sueda, T., Wada, S., Ochikubo, H. and Kajihara, H.** 1998. Efficacy of Perfluorotributilamine/Pluoronic F-68 Stem-Emulsion (FC43se) against hyperacute rejection in discordant lung xenografts. Transplant. Proc. **30:** 3826–3830.
18. **Thomassen, M.J., Buhrow, L.T. and Wiedemann, H.P.** 1997. Perflubron decreases inflammatory cytokine production by human alveolar macrophages. Crit. Care. Med. **25:** 2045–2047.
19. **Tilg, H., Vannier, E., Vachino, G., Dinarello, C.A. and Mier, J.W.** 1993. Antiinflammatory properties of hepatic acute phase proteins: preferential induction of interleukin 1 (IL-1) receptor antagonist over IL-1 beta synthesis by human peripheral blood mononuclear cells. J. Exp. Med. **5:** 1629–1636.
20. **Ueno, T., Matsuura, Y., Wada, S., Murakami, H. and Kajihara, H.** 1997. Efficacy of perfluorotributylamine/pluronic F-68 stem-emulsion (FC43se) against reperfusion injury in ischemic rabbit lungs. Transplant. Proc. **29:** 1349–1353.
21. **Wada, H., Tanigawa, M., Wakita, Y., Nakase, T.,**

Minamikawa, K., Kaneko, T., Ohiwa, M., Kageyama, S., Kobayashi, T. and Noguchi, T. 1993 Increased plasma level of interleukin-6 in disseminated intravascular coagulation. Blood. Coagul. Fibrinolysis. **4:** 583–590.

22. **Wada, H., Tamaki, S., Tanigawa, M., Takagi, M., Mori, Y., Deguchi, A., Katayama, N., Yamamoto, T., Deguchi, K. and Shirakawa, S.** 1991. Plasma level of IL-1 beta in disseminated intravascular coagulation. Thromb. Haemost. **4:** 364–368.
23. **Wada, S., Kajihara, H., Murakami, H., Sueda, T. and Matsuura, Y.** 1996. Effects of perfluorochemical-based artificial blood compounds in discordant xenotransplantation. Artif-Organs. **20:** 930–935.
24. **Yamamoto, N., Sakai, F., Yamazaki, H., Nakahara, K. and Okuhara, M.** 1996. Effect of FR167653, a cytokine suppressive agent, on endotoxin-induced disseminated intravascular coagulation. Eur. J. Pharmacol. **314:** 137–142.
25. **Yokoyama, K., Yamanouchi, K., Watanabe, M., Matsumoto, T., Murashima, R., Daimoto, T., Hamano, T., Okamoto, H., Suyama, T., Watanabe, R. and Naito, R.** 1975. Preparation of perfluorodecalin emulsion, an approach to the red cells substitute. Fed. Proc. **34:** 1478–1483.
26. **Yoshikawa, T., Furukawa, Y., Murakami, M., Takemura, S. and Kondo, M.** 1981. Experimental model of disseminated intravascular coagulation induced by sustained infusion of endotoxin. Res. Exp. Med. Berl. **179:** 223–228.