The Effect of Fluosol-DA® on the Reticuloendothelial System in Surgical Patients

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The effect of Fluosol-DA® (Perfluorochemicals), an oxygen carrying blood substitute, on the function of the reticuloendothelial system (RES) was investigated by means of Ferrichondroitin sulfate in a series of patients undergoing gastrectomy for cancer of the stomach. In 20 patients, 500 ml of blood were replaced with the same amount of either Fluosol-DA® or hydroxyethylstarch (HES) prior to surgery. Changes of perioperative cellular immunity were studied by PHA-stimulated T-lymphocyte transformation. It was found that the RES functions were significantly depressed postoperatively and fairly recovered by the sixth postoperative day in the Fluosol-DA® group. A prolongation of the suppression of cellular immunity with sustained fever was noted in this gastrectomized series. In analyzing the decay of infused Perfluolochemicals in blood, the half life of Fluorodecalin (FDC) was 4.9±0.1 hr, for Perfluorotripropylamine (FTPA) it was 5.1±0.1 hr. The prolonged depression of the RES function might be caused by saturation of RES with particles of Perfluorochemicals. (Key words: ferrichondroitin sulfate, colloidal carbon, perfluorochemicals, PHA-P, phagocytic index)

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The research for a blood substitute that would be capable to carry and deliver oxygen and to remove carbon dioxide in an emergency has been going on for many years. The clinical applicability of Fluosol-DA®, a mixture of Perfluorochemicals (PFCs), has been investigated in Japan by Mitsuno et al.[1] as an oxygen transporting blood substitute.

Infused Fluosol-DA® is supposed to be expired into alveolar air. In vitro, the mean particular size of Fluosol-DA® is 0.1 micron and no particles are larger than 0.6 micron. As particles larger than 0.4 micron would cause microemboli and would be captured by monocytes, no Fluosol-DA® particles should be captured by them. However, this has not been confirmed in vivo clinical cases, as the effect of surfactants, pluronic F-69 and yolk phospholipid which are used to keep the particles in emulsion, may be altered in the blood stream resulting in larger particles by coadhesion. The possibility of depression of the reticuloendothelial system (RES) function remains if agglomerated particles of infused Fluosol-DA® are captured by the RES, resulting in RES saturation.

The RES plays important roles in phagocytosis, opsonification and antibody production. RES suppression due to shock or trauma is known to negatively affects survival rates.[2] To ascertain the safety of Fluosol-DA® infusion in patients in hemorrhagic shock, its effect on the RES function must be studied. In the present study, the effect of Fluosol-DA® in surgical patients

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who received Fluosol-DA® as a blood substitute to induce a mild autohemodilution was assessed.

Methods

(1) Experimental protocol

Twenty patients undergoing gastrectomy for cancer of the stomach were subjected to this study. Written, informed consent was obtained from all participants according to the Ethical Committee. All were younger than 60 years, their expected blood loss was less than 1,000 ml, their Hb values were over 11.5 g/dl and their ASA physical status was 1 or 2.

The patients were anesthetized with 2.5% thiopental and their trachea were intubated with succinylcholine chloride. General anesthesia was maintained with 66% N_2O and 33% oxygen supplemented with 0.5–1% halothane. Optimum doses of pancuronium were used as muscle relaxant during surgery. Intraoperatively, normocapneic ventilation was maintained.

Before surgery, the 20 patients were randomly divided into two equal groups. In 10 patients, 500 ml of blood were replaced with the same amount of either 20% Fluosol-DA® or 6% HES (hydroxyethyl starch which degree of saturation by hydroxyethyl on starch, DS 0.55. Actually 3% HES with DS 0.65 has been used as the solvent for 20% Fluosol-DA®, however, 6% HES with DS 0.55 in clinical use has the same colloidal osmolarity). The drawn blood was kept at room temperature in Red Cross blood bank reservoir bags containing ACD-A anticoagulant and re-transfused either when intraoperative blood loss reached 500 ml or at the end of surgery.

Re-transfusion was performed within 3 hours of blood removal. None of the patients required supplemental intraoperative blood transfusions, however, there were 3 patients who received transfusion in 48 hours postoperatively.

The vital signs (BP and HR), Hb and Hct values were examined and blood gas analysis on hemodilution was carried out. Changes in serum transaminase, GOT and GPT levels (Karmen IU/l) were followed by UV method[3] for two weeks postoperatively. The body temperature was recorded at six o'clock in the morning for two weeks postopertively. The concentration of PFCs in blood and urine was determined by gas-chromatography[4] for one week postoperatively.

The RES functions were assessed by phagocytic indices on iron[5,6]. Colloidal ferrichondroitin sulfate (Blutal®, Dainihon Pharmaceuticals Co.) 0.8 mg/kg was administered intravenously as an indicator, and 2 ml of plasma were obtained 10, 20 and 30 min thereafter. Deferrization of indicator in plasma was performed by adding 2 ml of 0.8 N HCl. Subsequently, the samples were deproteinized for 2 min in a water bath (80–95°C) and centrifuged with 2 ml of 16% TCA. To the resulting 3 ml of supernatant was added 1 ml of sodium bathophenanthroline sulfonate (40 ml/dl) for colorization.

The phagocytic index (K-value) was obtained spectrophotometrically at 535 nm according to the formula:

$$K = \frac{\log C_1 - \log C_2}{t_2 - t_1}$$

values were determined before, and 1 hr, 2 and 5 days after surgery.

In two patients of each group, the cellular immunocapability was investigated by evaluating the rate of transformation of patient T-lymphocytes stimulated by purified phyto-hemoagglutinin (PHA-P) in micromethod[7]. Peripheral venous blood (1 ml) was mixed with 10 unit of heparin, 0.1 ml of this mixture was suspended in 0.9 ml TC-medium 199 and incubated for 24 hr at 37°C in humidified air with 5% CO_2. After adding 1 μCi of ^{3}H-thymidine, incubation was continued for an additional 24 hr. Cells were harvested, washed with RLB (red cell lysis buffer) solution to induce rapid lysis of the erythrocytes and 10 ml of 5% TCA was added. This was followed by filtration through a millipore filter (pore size 1.2 micron) and the radio-activity remaining in the filter was measured in a liquid scintillation counter (scintisol AL-I®; Wako).

(2) Reproducibility of the phagocytic index

The reproducibility of the technic applied in the present study regarding the RES function was confirmed using three healthy male volunteers. Four consecutive tests were run on each volunteer, employing the same experimental time schedule as in the clinical series.

(3) Accuracy of the colloidal ferrichondroi-

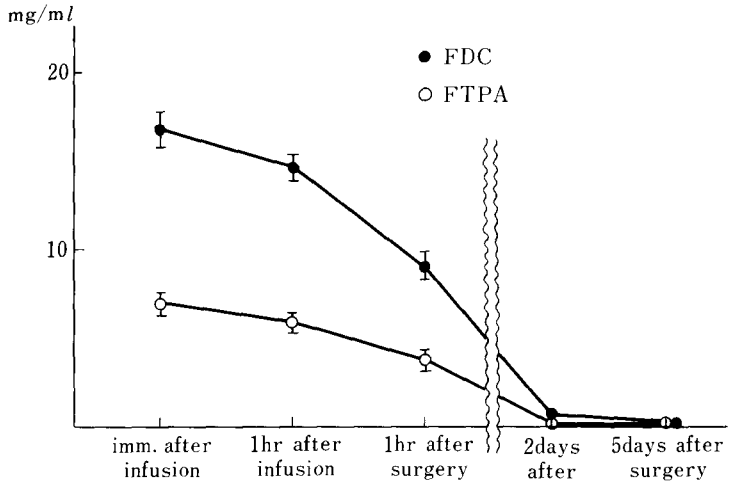

Fig. 1. Decay of PFCs in blood after infusion of Fluosol-DA®. The half-life of FDC and FTPA was 4.9±0.1 and 5.1±0.1 hr, respectively. Values are mean±SEM.

Table 1. Data on the two patient groups

	HES Group (n=10)	Fluosol-DA® Group (n=10)
Age (years)	50.4±3.2	50.8±2.8
Duration of surgery (min)	216±16.3	211±16.2
Blood loss (ml)	730±129.5	662±92.9
Administered volume (ml/kg)	9.4±0.4	9.2±0.5

Values are mean ± SEM.

tin sulfate method

The correlation between the technic with colloidal iron and an established colloidal carbon method reported by Biozzi et al.[8] for determing the RES function was examined in dogs.

Four mongrel dogs, weighing 8 to 10 kg were anesthetized with pentobarbiturate, and intravenously infused with both colloidal carbon (40 mg/kg; Pelikan C-11/143 la®) and colloidal ferrichondroitin sulfate (0.8 mg/kg; Blutal®). Measurement was carried out 11 times in each animal.

(4) Statistical analysis

The data obtained were represented by mean and SEM. The differences between two groups were assessed by independent t-test. The changes of values in the same group were analysed using paired t-test with the preoperative values as controls.

Results

No death was reported in this series of patients. There was no statistical difference in the values between two operated groups (table 1).

Due to mild hemodilution, the Hb values fell from 13.7 to 11.3 g/dl, however, the vital signs (BP and HR) were well maintained with no significant difference between the two groups. The oxygen content of blood, corrected by fluorocrit[9], exhibited no remarkable change upon Fluosol-DA®-induced hemodilution. The infusion of HES, however, resulted in a transient but significant reduction of the oxygen content by 2 ml/dl immediately after hemodilution. No changes in the acid-base balance in either group.

In analyzing the intervascular decacy of infused PFCs, perfluorodecalin (FDC) and perfluorotripropylamine (FTPA) were separately measured, as Fluosol-DA® consists of 14% FDC and 6% FTPA. Each half life was 4.9±0.1 hr, for FDC and 5.1±0.1 hr for FTPA (fig. 1). On the 3rd postoperative day, FDC and FTPA levels were hardly detectable. There was no urinary excretion of PFCs.

In Fluosol-DA®-infused patients, a sustained and progressive increase in GOT and GPT though within a limit of clinical significance (table 2) was noted. The significant elevation of body temperature compared to preoperative value continued for longer days in Fluosol-DA®-infused patients than in HES-infused patients

Table 2. Postoperative changes in serum transaminases (Karmen Units)

		Before Surgery	1 week after Surgery	2 weeks after Surgery
HES Group	GOT (IU/L)	19.9±2.7	29.8±5.1	30.3±6.3
	GPT (IU/L)	19.0±4.0	33.1±7.2	37.2±9.6
Fluosol-DA® Group	GOT (IU/L)	18.2±1.4	33.8±5.5*	44.7±7.9**
	GPT (IU/L)	18.5±4.4	29.0±4.8	49.4±6.8**

Table footnotes indicate significant differences with respect to before surgery (*; $p<0.05$, **$p<0.01$).
Values are mean ± SEM.

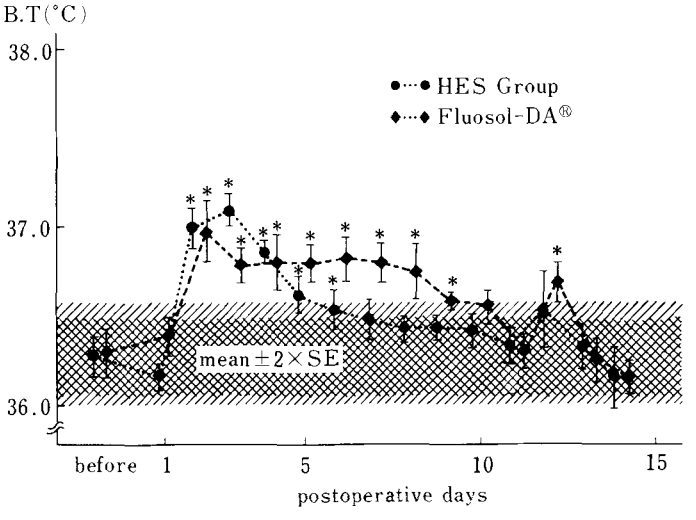

Fig. 2. Changes in body temperature measured at 6 o'clock in the morning. The zone painted with oblique lines show 95% reliable ranges of preoperative values; single line HES group and double line Fluosol-DA®. Foot notes (*) indicate significant differences ($p<0.05$) between preoperative values. Values are mean ± SEM.

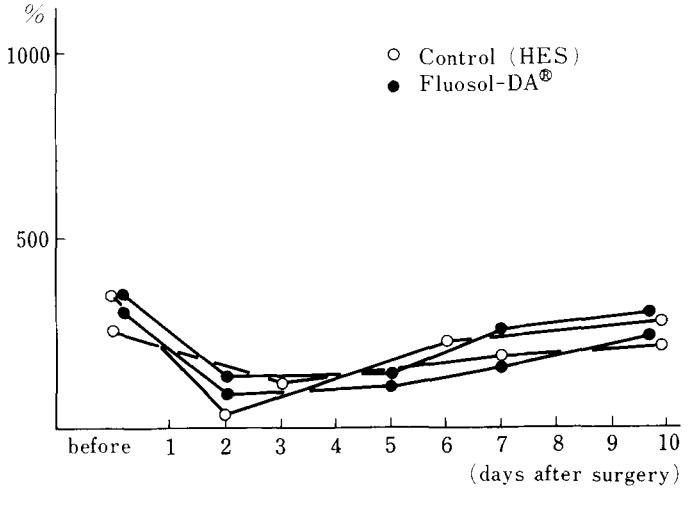

Fig. 3. Effect of Fluosol-DA® infusion on PHA-P stimulated lyomphocyte transformation

(fig. 2).

Perioperative changes in RES function, expressed as K-value and reproducibility in healthy volunteers, are presented in table 3. In statistical analyses of indivisual and sequence variants on reproducibility, our technical error was 7.99%. RES function was severely depressed at one hour after surgery in both operated groups. In the HES group, this depression recovered by the 3rd postoperative day. In the Fluosol-DA® group, on the other hand, the depression of RES function was prolonged; in

Table 3. RES function expressed in K-values

	Before Surgery	After Surgery		
		one hour	2 days	5 days
Volunteer (n=3)	0.0217 ±0.0006	0.0210 ±0.0013	0.0207 ±0.0015	0.0218 ±0.0013
HES Group (n=10)	0.0215 ±0.0020	0.0052** ±0.0016	0.0240 ±0.0028	0.0231 ±0.0028
Fluosol-DA® Group (n=10)	0.0211 ±0.0020	0.0026** ±0.0012	0.0125* ±0.0021	0.0163 ±0.0021

Table footnotes indicate significant differences with respect to before surgery (*;$p<0.05$, **;$p<0.01$). Values are mean ± SEM.

2 patients it was still severely depressed on the 6th postoperative day. In the other 8 Fluosol-DA®-infused patients, the RES function had recovered by this time.

A high correlation ($r = 0.856 \pm 0.316$, $p<0.01$) was obtained between the technic with colloidal ferrichondroitin sulfate and the colloidal carbon method when RES function in dogs measured.

The effect of major gastric surgery on cellular immunity is shown in fig. 3. There was marked immunosuppression in both groups on the second postoperative day. This suppression lasted for a week.

Discussion

The phagocytic activity of the RES can be assessed quantitatively in experimental animals by injecting various types of inert indicator particles, colloidal carbon, ^{131}I-heat-denatured human serum albumin[10,11] and ^{131}I-micro-aggregates of denatured human serum albumin[12]. In humans, however, the use of colloidal carbon should be avoided, and the use of labeled radioisotopes involves radiation-hazards, particularly if frequent perioperative assessments of the RES function are performed. Colloidal ferrichondroitin sulfate (Blutal®) has been used clinically for iron deficient anemia. Thus, the technic is safer and facilitates the repeated assessments of RES function in surgical patients.

Lutz et al.[13] reported that in rats, the RES function was depressed to less than half of the control values immidiately after the intravenous administration of PFC (4.4 g/kg). After full recovery, these workers noted a secondary depression lasting until the 4th day after PFC administration. Ohyanagi et al.[14] who used the colloidal carbon method after injecting rats with 10–20 ml/kg Fluosol-DA® observed a biphasic change in RES function. They reported hyperfunction followed by suppression at 24 to 72 hr. Using colloidal carbon method in rats anesthetized with halothane, similar results were previously obtained in my laboratory.

As 85% of the RES exists in the liver, the RES function depends on hepatic blood flow and on hepatic production of ATP. Anesthetics have been reported to significantly impair the RES function[15] and surgical trauma could further suppress the RES function in man[16]. This suppression is transient during the immidiate postoperative period which is followed by a secondary hyperfunction[17].

In this patient series, the infusion of 9.2 ml/kg Fluosol-DA®, was equivalent to a similar dose administrated to rats by Ohyanagi[14] resulted in depression of the RES function. During the 6 day postoperative observation period, no rebound hyperfunction of the RES was noted. It must be kept in mind that gastric cancer patients were already immunosuppressed and that they subjected to major surgical trauma. Species differences as well as differences in the detection methods must also be taken into account in explaining the absence of secondary hyperfunction of the RES in this clinical study.

Bruce et al.[18] suggested that the depression of lymphocyte transformation after major surgery is related primarily to tissue trauma rather than anesthetics or anesthetic technique. In order to evaluate the extent of surgical trauma negatively affecting the RES function, the cellular immunosuppression was studied in the present study. There was obvious immunosuppression which was prolonged by the surgical trauma. The degree and duration of this suppression was not affected by administration of HES or Fluosol-DA®.

The obtained results in the present study on the similar half life of FDC (vapor pressure 12.7 torrs at 37°C) and FTPA (vapor pressure 17.5 torrs at 37°C) conflicted with the reported longer half life of these agents[19]. The shorter half-lives in this series and the absence of urinary

excretion of PFCs confirms their uptake by the RES. Theoretically, if the particle size of PFCs is 0.1 micron, they have a longer half-life as they are too small to be captured by RES.

HES, which is used as the solvent for PFCs in Fluosol-DA®, is phagocytosed by the RES[20]. The perliminary studies performed in my laboratory on hemodiluted rats showed that differences in the molecular weight and DS of HES did not affect the K-value. This confirmed me the RES function was suppressed in Fluosol-DA® by saturation of the RES with PFC particles. A fact of prolonged febrile period postoperatively more in Fluosol-DA® group than HES one may be caused by RES suppression. There was, however, no significant difference in morbidities except fever between two groups.

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