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Effect of fluorocarbon blood substitute on neutrophil phagocytic function

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ABSTRACT Neutrophils were incubated at 37°C for 2 h with fluorocarbon blood substitute or its main components *in vitro*. Neutrophil phagocytosis was determined by the method of chemiluminescence (CL) and the concentration of intracellular cAMP and cGMP were assessed. The results showed that the CL was inhibited while the level of cAMP was elevated. The alteration of cAMP seemed to be correlated with the inhibition of CL. Only did the emulsifier Poloxamer F-68 (F-68) of fluorocarbon blood substitute have the same effects. It is suggested that fluorocarbon blood substitute can inhibit neutrophil phagocytic function and the emulsifier F-68 may be responsible for it. The mechanism may be associated with the elevation of intracellular cAMP concentration.

KEY WORDS fluorocarbons; neutrophils; luminescence

Fluorocarbon blood substitute (FCBS) is capable of carrying and delivering substantial amounts of oxygen. It can perform the role of red blood cells in transporting oxygen throughout the body and has been applied increasingly in clinical usage⁽¹⁾. However, FCBS had some adverse effects

on neutrophil phagocytosis, chemotaxis and even metabolism⁽²⁻⁴⁾. The present study was undertaken to investigate whether this effect was due to FCBS itself or its components.

MATERIALS AND METHODS

Fluorocarbons were obtained from Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, and emulsified with Poloxamer F-68 (F-68) in our laboratory. The emulsion contained (g/L): perfluorodecalin (FDC) 14, perfluorotripropylamine (FTP) 6, F-68 45, glycerol 10, glucose 1.5, hydroxyethyl starch 30, NaCl 3.0, KCl 0.27, CaCl₂ 0.22, MgCl₂ 0.15, NaHCO₃ 0.5, with pH 7.4-7.6. The average diameter of the emulsion particles was less than 0.1 μm.

Neutrophils were separated from heparinized blood of healthy donors using a modified method of gradient centrifugation⁽⁵⁾. Neutrophil suspensions were washed and then suspended in Hank's solution at a concentration of 2×10^8 neutrophils/ml prior to incubation. Over 90% of neutrophils was yielded by microscope screening

and the mean cell viability was 96.6% by the method of trypan blue dye exclusion. Neutrophil suspension was incubated with FCBS emulsion or its main components (FDC, FTPA and F-68) respectively at a volume ratio of 10:1 at 37°C for 2 h. Because FDC and FTPA are immiscible and so the suspension must be stirred during incubation. Control was treated with Hank's solution.

Neutrophil phagocytic function was determined by the method of chemiluminescence (CL)⁽⁶⁾. The intracellular cAMP and cGMP concentration were measured by radioimmunoassay^(7,8).

RESULTS

The CL of neutrophils was depressed when exposed to FCBS. Compared to control, it was decreased 34.0% ($32.3 \pm SD$ 2.6 vs 49.0 ± 5.1 mV, $P < 0.01$) at 1 h and 21.5% (41.6 ± 2.4 vs 52.9 ± 4.6 mV, $P < 0.05$) at 2 h of incubation but it was increased 28.5% more than the value at 1 h ($P < 0.05$). In order to investigate the critical cause affecting the CL, the main components of FCBS, F-68, FDC and FTPA were incubated separately with neutrophil suspension in the same condition as the FCBS. In this experiment, the CL (mV) of the neutrophils incubated with F-68, FDC or FTPA was 28.4 ± 2.3 , 52.0 ± 2.6 or 56.5 ± 3.6 respectively at 1 h. Compared to control (48.9 ± 3.6 mV), the CL of neutrophils exposed to F-68 was decreased 41.8% ($P < 0.01$) while those exposed to FDC or FTPA did not change. At 2 h, however, the CL of the neutrophils incubated with F-68 increased 34.5% (38.1 ± 3.1 vs 28.4 ± 2.3 mV, $P < 0.05$) but was still 34.1% lower ($P < 0.01$) than that of the control (57.9 ± 2.6 mV).

At 1 h of incubation with FCBS or F-68, the intracellular cAMP concentration increased significantly and was 15.0 or

15.5 times of the control, respectively. At 2 h, cAMP dropped but was still 3.9 or 3.7 times of the control, respectively. There was no difference between the results incubated with FCBS and F-68. The intracellular cGMP concentration did not change throughout the experiment (Tab 1).

Tab 1. Effect of fluorocarbon blood substitute and Poloxamer F-68 on neutrophil intracellular cAMP and cGMP concentration (pmol) at 1 and 2 h of incubation. $n = 9$, $\bar{x} \pm SD$, * $P > 0.05$, *** $P < 0.01$ vs control; † $P > 0.05$, †† $P < 0.01$ vs 1 h.

	Control	FCBS	F-68
cAMP			
1 h	0.6 ± 0.1	$9.1 \pm 1.1^{***}$	$9.4 \pm 1.1^{***}$
2 h	$0.9 \pm 0.2^{\dagger}$	$3.3 \pm 0.4^{***\dagger\dagger}$	$3.1 \pm 0.4^{***\dagger\dagger}$
cGMP			
1 h	0.6 ± 0.1	$0.7 \pm 0.1^*$	$0.8 \pm 0.1^*$
2 h	$0.4 \pm 0.1^{\dagger}$	$0.6 \pm 0.2^{\dagger}$	$0.5 \pm 0.1^{\dagger}$

DISCUSSION

The present study showed that FCBS could depress the neutrophil chemiluminescence, so it is confirmed that FCBS has an inhibitory effect on neutrophil phagocytic function, although it was not irreversible for the chemiluminescence of neutrophil with a significant increase at 2 h of incubation. On account for the analysis of inhibition effect of several main components of FCBS on neutrophil, we found that it was the emulsifier F-68 that inhibit the neutrophil phagocytic function.

The mechanism of the inhibition of neutrophil phagocytosis by FCBS and F-68 is not clear so far. It is known that the cAMP can inhibit leukocyte function and metabolism while cGMP enhance them⁽⁹⁾. This study showed that the intracellular cAMP concentration of the neutrophil incubated with FCBS or F-68 increased significantly while cGMP did not change. The results suggested that the inhibition of neutrophil phagocytosis caused by F-68 may be associated with the elevation of intracellular cAMP concentration, although

we were unable to carry on the experiment for much longer time.

Fluorocarbon blood substitute, as an oxygen carrier, is very valuable in clinical practice, for instance, in shock resuscitation and infusion instead of part of blood transfusion during operation. Since there are some adverse reaction in fluorocarbon usage^(10,11), further study is necessary to search for a new emulsifier better than F-68 with the same emulsifying result and less side effects in order to be benefit for the applications of FCBS.

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氟碳代血液对粒细胞吞噬功能的影响

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提要 将氟碳代血液(FCBS)或其成份与粒细胞在体外孵育2h, 粒细胞吞噬功能用化学发光(CL)法测定, 同时测定了细胞内cAMP和cGMP浓度。结果粒细胞CL显著受抑制, 细胞内cAMP明显升高而cGMP无改变, 乳化剂F-68与FCBS作用相同。表明

FCBS对粒细胞吞噬功能有抑制作用, 且与其成份F-68有关, 其机理可能是升高了细胞内cAMP之故。

关键词 氟碳化合物, 嗜中性白细胞, 发光

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