APPEARANCE OF BIOLOGICALLY ACTIVE SUBSTANCES DURING OXIDATION OF CHOLESTEROL ON THE SURFACE OF FLUOROCARBON EMULSIONS

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Preparations based on fluorocarbon emulsions are widely used nowadays in medical practice. Since they are chemically inert compounds [3, 4], not metabolized in the body, the idea has evolved that these substances possess no biological activity. However, this is not so. The inducing action of fluorocarbons on cytochrome P-450 and on the monooxygenase system of the liver as a whole was described previously [1]. It must likewise not be forgotten that emulsions constitute a phase boundary with a very large area: an emulsion of 10 vols. % with particle diameter of 0.1 μ has a surface area of 60 m^2/ml. A phase boundary provides favorable conditions for a number of chemical reactions to take place, more especially if the emulsion is initially saturated with oxygen. Thus the question arises of the possibility of active oxidative processes taking place on the surface of the emulsion. After intravenous injection of an emulsion into animals, intensive sorption of components of the blood plasma takes place on the surface of particles of the fluorocarbon phase [2]. It can be expected that cholesterol, contained in lipoproteins and membranes, will also undergo sorption on the surface of an emulsion, followed by the formation of oxidized derivatives, some of which possess high biological activity [6-8].

The aim of this investigation was to study the possibility of oxidative modification of cholesterol on the surface of fluorocarbon emulsions in the presence of oxygen.

EXPERIMENTAL METHOD

Perfluorodecalin (PFD) and perfluoro-*p*-methylcyclohexylpipezidine (PFMCP) were obtained from the Institute of Organoelementary Compounds, Academy of Sciences of the USSR. Their degree of purity was 98 and 95.2% respectively, Phospholipids were obtained from egg yolks (Khar'kov Bacterial Preparations factory), and chromatographically pure phosphatidylcholine was obtained from soy beans (Nattermann, West Germany) and diethyl phosphate from Serva (West Germany). Cholesterol (from Sigma, USA) was twice recrystallized from hot ethanol. Absence of contamination by cholesterol oxidation products was verified by thin-layer chromatography.

The buffer was prepared from Tris-buffer (Reanal, Hungary).

The fluorocarbon emulsion contained 10 vols. % of a mixture of PFD and PFMCP (2:1 by volume) in distilled water. A mixture of egg yolk phospholipids and cholesterol (4:1 by weight) with a final concentration of 2.5% was used as the emulsifier.

The phospholipids (a 5% solution in ethyl alcohol) were mixed with cholesterol (4:1 by weight). Alcohol was removed from the mixture by evaporation in a jet of argon at 60°C. The dried mixture was suspended in distilled water until a homogenous suspension was formed with a lipid concentration of 4% (by weight). The suspension was mixed with the fluorocarbon phase. The fluorocarbon emulsion was prepared on a high-pressure extrusion homogenizer ("Donor-1," Design and Construction Department, Academy of Sciences of the USSR, Chernogolovka). The volume of emulsion prepared was 300 ml, the treatment

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TABLE 1. Immunodepressive Action of Cholesterol Oxidation Products

Preparation	Number of rosettes per 100 lymphocytes	Inhibition of rosette formation, per cent
Buffer	44±2	0
Cholesterol	42±2	3±5
7-Peroxycholesterol	24±2	46±4
7-Hydroxycholesterol	32±1	27±3
7-Ketocholesterol	40±1	8±3

time 1.5 h, temperature 26°C, and pressure 400 atm. The mean particle diameter of the emulsion, measured by means of a "Coulter N4" Nanosizez (Coultronix, France), was 230 ± 54 nm.

Cholesterol oxidation products were analyzed by thin-layer chromatography on Silufol plates (Czechoslovakia) in a solvent system of chloroform—methanol—water (75:15:1 by volume). The plates were developed with 10% H_2SO_4, with controlled heating to 80°C, and also with a 1% solution of N,N,N′,N′-tetramethylphenylenediamine dihydrochloride.

The action of cholesterol oxidation products on activity of immunocompetent cells was assessed by their effect on rosette formation by human T lymphocytes with sheep's red blood cells [10]. Oxidized cholesterol was incorporated into lipid micelles consisting of triolein and phosphatidylcholine. The medium and lipid micelles composed of phosphatidylcholine, cholesterol, and triolein (1:1:1, moles) were used as controls.

The micellar preparation (0.5 ml) with a concentration of 10 mg lipid/ml, was mixed with 5 ml of normal donor's blood and incubated for 1 h at 37°C. Each sample was then mixed and, after 15 min, the upper part was taken for the rosette formation test [10].

EXPERIMENTAL RESULTS

Oxidation of lipids in the composition of the fluorocarbon emulsion was carried out at 37°C for 5 days. Oxygen was passed through the fluorocarbon emulsion for 10 min twice a day. The emulsion was then centrifuged at 25,000*g* for 30 min. The residue was treated with 20 ml of diethyl ether. The ethereal phase was separated and dried in vacuo on a rotary evaporator. The dry residue was dissolved in the minimal quantity of hexane and mixed with 200 ml of cold acetone. The solution of sterols in acetone was filtered to remove phospholipids precipitated on a Buchner funnel. The resulting solution was concentrated on a rotary evaporator and analyzed by thin-layer chromatography. During development a deep blue (at 65°C, R_f = 0.54) and a bright red (at 70°C, R_f = 0.72) spot were formed. A spot with a blue color can be formed only by 7-peroxycholesterol and 7-hydroxycholesterol [9]. On development of the plates with a solution of N,N,N′,N′-tetramethylphenylenediamine dihydrochloride solution, which is a qualitative reagent for peroxy compounds, a violet spot was formed (R_f = 0.54), corresponding to 7-peroxycholesterol.

The cholesterol oxidation product was isolated by preparative thin-layer chromatography and verified by chromatomass analysis, which confirmed the structure of the product. In this 7-peroxycholesterol was obtained, and other cholesterol derivatives in the C_7 position can easily be obtained from it. The yield of the product, as shown by thin-layer chromatography, was about 60%.

The results of testing the immunodepressive action of the cholesterol oxidation product isolated from the fluorocarbon emulsion, and incorporated into the micellar preparation composed of phosphatidylcholine—oxidized cholesterol—triolein (1:1:1 by molarity), are given in Table 1. By contrast with the original buffer and micelles with pure cholesterol, used as the control, preparations with oxidized cholesterol caused considerable inhibition of rosette formation.

In experiments of cultures of prokaryotic and eukaryotic cells (data not given) the oxidized derivatives of cholesterol in the C_7 position also were found to have cytostatic activity. The experiments thus showed that effective oxidation of cholesterol can take place on the surface of fluorocarbon emulsions, with the appearance of oxidized C_7 derivatives. The C_7 derivatives obtained in this way possess marked biological activity, for they exert a cytostatic and immunomodulating action.

These results indicate that the possibility of oxidative modification of substances contained in blood plasma may take place on the surface of particles of fluorocarbon emulsions, with the appearance of highly active compounds, must be taken into consideration.

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