ACTION OF CONTRAST MEDIA ON SPONTANEOUS ERYTHROCYTE AGGREGATION

P. V. Sergeev, A. N. Usenko, N. N. Firsov, N. L. Shimanovskii, and V. O. Panov

UDC 615.45.03:616-073.755.4/.015.4:612.111

KEY WORDS: contrast media, erythrocyte aggregation.

Artificial contrasting by means of contrast media (CM) is currently one of the most widely used diagnostic methods. However, after administration of CM serious side effects and complications frequently develop and are associated with disturbances of the microcirculation due to changes produced in the rheologic properties of the blood. These changes depend mainly on the structural and functional properties of the erythrocytes. One of the central microrheologic stages determining the magnitude and character of the macrorheologic changes is the process of erythrocyte aggregation.

The aim of this investigation was to study the effect of CM of different structure and organ-affinity on the aggregation properties of erythrocytes.

A considerable influence of CM on aggregation has been demonstrated in a number of investigations [4, 5, 8]. However, these data characterize changes in aggregating properties of the cells only qualitatively. The method we have used, in conjunction with microscopy, enables changes in erythrocyte aggregation to be described not only qualitatively, but quantitatively.

EXPERIMENTAL METHOD

The most widely used method of studying aggregation properties of erythrocytes is that of recording the scattering of light during reflection or during transmission of a beam of light through a layer of blood [3].

In this investigation we used an aggregometer, constructed on the basis of a Couette viscosimeter with a transparent outer cylinder and blackened inner cylinder, rotated by a motor through reducing gear. The dynamics of light scattering was recorded by means of a photodiode, connected into the circuit of the amplifier, and recorded on an automatic writer. The working cell of the aggregometer, with a volume of 5.5 ml and blood layer thickness of 1 mm, was kept at a constant temperature of 25°C. The CM were introduced directly into the cell and the measurements made after mixing.

Analysis of the curve of spontaneous erythrocyte aggregation (signal amplitude as a function of time) by the Tobolski–Murakami method [7] exponential functions enables the aggregation process to be resolved into two exponential functions described by the equations: $A = A_0 e^{t/T1}$ and $A = A_0 e^{t/T2}$, where A_0 and A denote the amplitude of aggregation at zero time and at time t; T_1 and T_2 denote aggregation time. This time corresponds to the different structural formations of erythrocytes: T_1 characterizes the formation of small aggregates, T_2 their fusion into larger structures.

In the region of Casson flow the amplitude of aggregation and sliding velocity are connected by the equation $A = A_0 e^{B\dot{\gamma}}$, where $\dot{\gamma}$ is the sliding velocity and B a coefficient of proportionality, which can be used as a measure of the strength of the aggregates, for it indicates the sliding velocity at which the number of aggregates is reduced by *e* times.

The action of CM on erythrocyte morphology was studied by microscopy. Films were prepared from samples of normal human blood containing CM, dried in air, and then fixed for 15 min in 96° ethanol. Microscopy was then carried out and the number of different forms of erythrocytes (discocytes, echinocytes, spheroechinocytes) counted.

Department of Molecular Pharmacology and Radiobiology, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 274-276, March, 1990. Original article submitted September 12, 1990.

 0007-4888/91/0003-0340$12.50 ©1991 Plenum Publishing Corporation

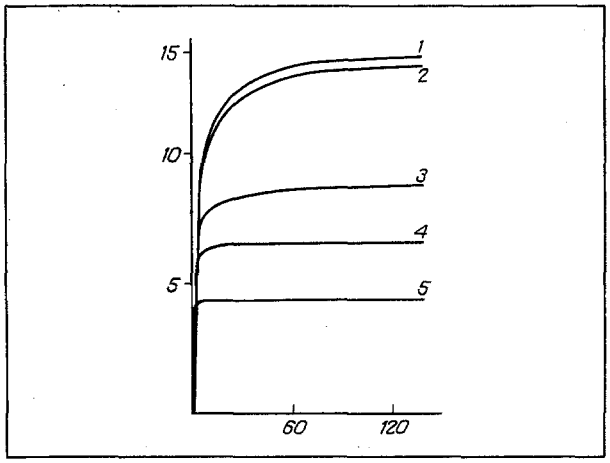

Fig. 1. Time course of spontaneous erythrocyte aggregation in the presence of bilignost 50%. Abscissa, time (in sec); ordinate, amplitude of aggregation (in cm). 1) Normal; 2) 10^{-3} M, 3) $5 \cdot 10^{-3}$ M; 4) 10^{-2} M; 5) 10^{-1} M.

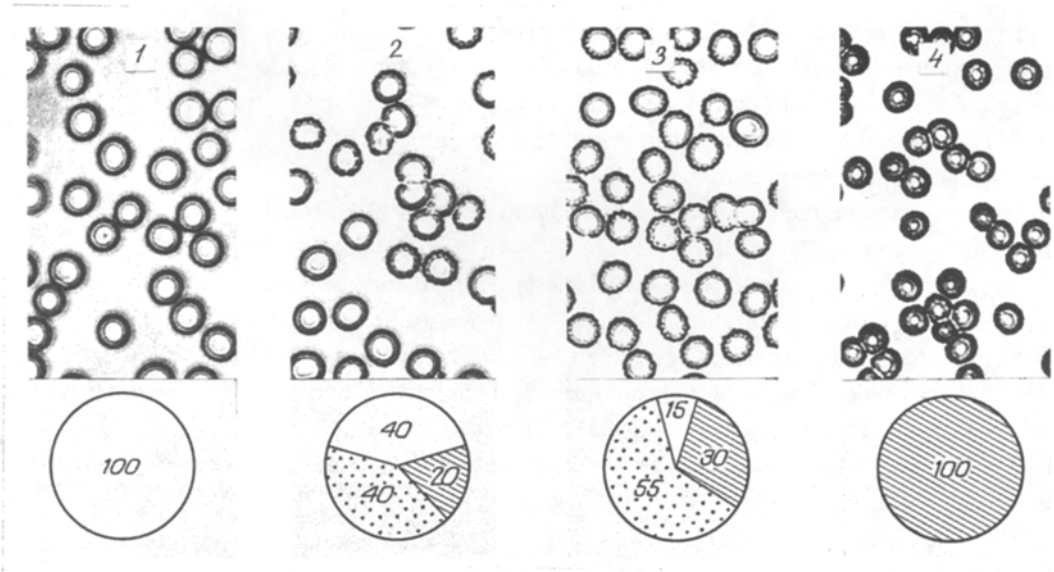

Fig. 2. Relative percentages of different forms of erythrocytes in film of blood containing bilignost 50%. 1) Normal;2) $5 \cdot 10^{-3}$ 3; 3) 10^{-2}; 4) 10^{-1}; unshaded segments represent discocytes; dotted segments — echinocytes; obiquely shaded — spheroechinocytes. Numbers in segments are percentages.

The following CM were studied: for cholecystography — bilignost (iodipamide) 50%, bilignost 20%, and bilimin; for angiourography — iodamide 380, triombrast 76%, and metrizamide; for hepatosplenography — lipotrast (an oily emulsion of triombrast). CM intended for intravascular injection were tested within the concentration range from $5 \cdot 10^{-4}$ to $5 \cdot 10^{-1}$ M, whereas bilimin, administered per os, was given within the concentration range from 10^{-5} to 10^{-2} M. Experiments were carried out in vitro on blood from healthy donors aged 18-50 years. The blood was stabilized with a 5% solution of Trilon B in the ratio of 1:30.

The data were subjected to statistical analysis by Wilcoxon's paired T test [1].

EXPERIMENTAL RESULTS

The results show that an increase in concentration of solutions of CM is accompanied, first, by a decrease in amplitude of erythrocyte aggregation and, second, deformation of the exponential shape of the aggregation curve. The latter effect was par-

TABLE 1. Effect of Contrast Media on Parameters of Spontaneous Erythrocyte Aggregation (in percent of control)

CM	Concentration, M	T_1	T_2	B
Bilignost 50%	$5 \cdot 10^{-3}$	21,4* (14,0—29,7)	50,8* (30,0—74,2)	369,8* (351,4—388,9)
Bilignost 20%	$5 \cdot 10^{-3}$	27,5* (18,8—38,0)	69,5* (63,1—82,6)	475,0* (330,8—708,0)
Bilimin	$5 \cdot 10^{-3}$	49,6* (48,5—71,3)	69,4* (59,4—79,9)	360,0* (277,0—592,0)
Iodamide	10^{-1}	45,5* (38,5—56,4)	56,2* (50,5—61,7)	228,0* (137,2—350,2)
Triombrast	10^{-1}	65,4* (51,6—70,8)	73,2* (54,2—85,2)	255,6* (155,1—405,4)
Metrizamide	$5 \cdot 10^{-2}$	70,9* (56,2—94,8)	108,7 (96,4—124,5)	689,3* (303,4—1034)
Lipotrast	$5 \cdot 10^{-2}$	61,6* (54,6—74,2)	117,0 (98,5—129,0)	113,0 (95,4—131,2)

Legend. Asterisk indicates significance of differences from control for $p_T \leq 0.05$ (number of experiments was 6). Lowest concentration of CM at which a significant effect appears are shown. Extreme values of parameters given between parentheses.

ticularly marked in the action of CM for cholecystography: bilignost 50%, bilignost 20%, and bilimin. Under the influence of these CM in a concentration as low as $5 \cdot 10^{-3}$ M the amplitude of aggregation was reduced almost threefold, and the character of the curve was considerably modified (Fig. 1). The angiourographic CM iodamide, triombrast, and metrizamide and the hepatosplenographic CM lipotrast had this type of action only in a concentration of 10^{-1} M, although under these circumstances the changes in the aggregation curve were more marked.

As the experiments showed, the process of spontaneous aggregation of healthy human erythrocytes is characterized by the following values of its parameters: $T_1 = 9.11 \pm 0.34$ sec, $T_2 = 40.75 \pm 1.54$ sec, $B = 35.84 \pm 2.89$ sec^{-1}. Addition of CM to the blood was accompanied by a change in these values (Table 1). The cholecystographic CM were found to have the strongest action on aggregation, starting with a concentration of $5 \cdot 10^{-4}$ M, reducing the aggregation time T_1 by 4-5 times, and T_2 by half. The strength of the aggregates in this case was increased by 3.5-4.5 times. A further increase in the concentration of the solution of CM led to considerable changes in the aggregation curve, preventing the time T_2 from being determined, because the aggregation process was over in a few seconds and stopped at the stage of formation of small aggregates, without the formation of a "rouleaux" type of structure. This conclusion is supported by the decrease in amplitude of aggregation. Meanwhile, with an increase in the concentration of CM, the strength of the aggregates amounted to 550-600% of the control value, when their concentration was 10^{-1} M.

The effect of the angiourographic CM triombrast and iodamide on aggregation was similar, but differed from the action of cholecystographic CM. Their effect on the time course of erythrocyte aggregation did not begin to manifest itself until the concentration reached $5 \cdot 10^{-2}$ M. An increase in its value to 10^{-1} M led to reduction of the aggregation time T_1 and T_2 on average by half and to an increase in strength of the aggregates by 2-2.5 times.

The action of metrizamide was rather different. In a concentration of $5 \cdot 10^{-2}$ M, T_1 was significantly reduced by 1.5 times, whereas with a subsequent rise of the concentration to 10^{-1} M it was reduced fivefold. In this case the time T_2 showed a tendency to decrease. The strength of the erythrocytic aggregates was increased by metrizamide, starting with a concentration of 10^{-2} M, and with a tenfold increase in its concentration it amounted to almost 770% of the control value, three times greater than in the presence of triombrast and iodamide.

Comparison of the action of triombrast and its liposomal form, lipotrast, on erythrocyte aggregation shows that both CM gave similar effects in a concentration of 10^{-1} M. It must be noted, however, that, first, the action of lipotrast begins to be manifested at a lower concentration and, second, lipotrast does not give rise to any increase in strength of the aggregates, which cannot be said about triombrast.

Microscopic analysis of films prepared from blood containing CM showed that an increase in concentration of the CM led to transformation of discocytes into echinocytes, and thence into spheroechinocytes (Fig. 2). This effect of CM decreased in the order: bilimin > bilignost 20% > bilignost 50% > metrizamide > lipotrast > iodamide — triombrast.

TABLE 2. Coefficients of Correlation between Microrheologic Parameters and Physicochemical Properties of CM

	T_1	T_2	B	def	% n	Lt	osm	C
T_1	1,00							
T_2	0,86	1,00						
B	−0,93	−0,79	1,00					
def	0,60	0,57	−0,65	1,00				
% n	0,84	0,68	−0,92	0,67	1,00			
Lt	−0,37	−0,57	0,37	−0,78	−0,34	1,00		
osm	−0,68	−0,49	0,74	−0,53	−0,69	0,06	1,00	
C	−0,54	−0,38	0,61	−0,50	−0,61	0,00	0,81	1,00

Legend. DEF) Deformability of erythrocytes, percent; n) number of normocytes in blood film, per cent; LT) lipotropism of CM; OSM) osmosis; C) concentration.

Comparison of these results with those obtained previously [2] suggests that the most probable causes of the considerable changes in erythrocyte aggregation under the influence of CM may be the appearance of echinocytes and a decrease in their deformability.

Cholecystographic CM act on erythrocyte aggregation in a lower concentration than angiourographic CM. In our view the main cause of these differences is the high membrane-toxicity of the cholecystographic CM, due to their comparatively high lipotropism which, together with high osmosis, causes the phenomena described above. The main factor in the action of the traditional angiourographic agents iodamide and triombrast is the osmotic activity of their solutions.

At the same time metrizamide, despite its nonionogenic nature, induces stacking of erythrocytes and changes in their aggregation parameters in a concentration only half as high as that of iodamide and triombrast. This reaction is probably due to the chemical structure of the molecule of the contrast medium, which contained 2-deoxy-D-glucose. According to data in [6] metrizamide is a competitive inhibitor of membrane carriers of glucose, blocking of which may probably lead to changes in shape of the cells also.

The action of lipotrast on erythrocyte aggregation is noteworthy. Despite the fact that the aggregation rate increases in the presence of lipotrast, the strength of the aggregates does not increase. It can be postulated that liposomes, by isolating the cells from each other, prevent the possibility of contact between them.

Correlation analysis of the data (Table 2) led to the conclusion that the high osmotic pressure of the solutions of CM, their lipotropism, and also some structural features of the molecules modify the structural and functional properties of erythrocytes. The appearance of echinocytes and spheroechinocytes, cells which cannot undergo deformation, leads to a more rapid aggregation process and to an increase in the hydrodynamic strength of the aggregates. These circumstances may be important in the genesis of the microcirculatory disturbances and associated complications arising during diagnostic roentgenography with the aid of CM.

LITERATURE CITED

1. E. Ch. Gubler, Computational Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
2. P. V. Sergeev, A. N. Usenko, N. N. Firsov, and N. L. Shimanovskii, Byull. Éksp. Biol. Med., No. 2, 194 (1989).
3. N. N. Firsov, Third All-Union Conference on Problems in Biomechanics, Proceedings [in Russian], Vol. 1, Riga (1983), pp. 262-264.
4. P. Aspelin and H. Schmid-Schönbein, Invest. Radiol., **23**, No. 2, 326 (1988).
5. P. Dawson, Invest. Radiol., **23**, No. 2, 310 (1988).
6. S. Ekholm, K. Reece, M. Foley, et al., Invest. Radiol., **19**, 574 (1984).
7. A. Tobolsky and K. Murakami, J. Polymer Sci., **40**, 443 (1959).
8. M. Zucker and E. Mauss, Invest. Radiol., **23**, No. 2, 340 (1988).