EFFECT OF LIPOSOMES ON PLATELET FUNCTION

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UDC 612.017.1-085.2

The effect of different types of liposomes on the dynamic functions of platelets was studied. Liposomes of different composition (containing cholesterol and phosphatidylcholinethanolamine or not) and charge (containing negatively charged dicetyl phosphate or not) were shown to cause qualitatively similar but quantitatively slightly different effects and to modify the properties of the platelets: to reduce their powers of aggregation and their spherulation. The tendency for the platelets to aggregate was reduced by an increase in the concentration of liposomes, by an increase in the duration of incubation of the platelets with liposomes, and by the change from ordinary lecithin liposomes to liposomes containing phosphatidylcholinethanolamine. Suggestions are put forward regarding the mechanism of the observed phenomena and the absence of an unfavorable effect of various liposomes on platelet function is noted.

KEY WORDS: liposomes; platelets; aggregating power; spherulation.

In recent years liposomes – artificial phospholipid vesicles – have begun to be regarded with good justification as promising agents for in vivo drug transportation [6, 7]. If a drug, usually of protein nature, is securely introduced into the lipsome and, as a result, it does not come into contact with the blood, it will not undergo biodegradation, it will not be eliminated prematurely, and it will not give rise to undesirable toxic or immunologic reactions [4, 13]. The possibility of controlled transportation of drugs by means of liposomes to the outer surface of which a molecule possessing increased affinity for a characteristic component of the target organ is attached, has also been discussed [7, 15]. Meanwhile, intensive research into the mechanisms of interaction between liposomes and various cells [8, 10] is in progress for, despite their high degree of biological compatibility, liposomes themselves are foreign bodies and, because of this, their effect on different organs cannot be predicted beforehand.

It will be evident that the first stage of interaction between liposomes and the systems of the body if injected intravenously will be their interaction with blood. There is already evidence to show that liposomes interact with lymphocytes [2], but no really significant data on the effect of liposomes on the functions and behavior of platelets – these important formed elements of the blood – are yet available.

The object of this investigation was to study the effect of liposomes of different composition and with different surface charge on dynamic properties of the platelets such as their ability to aggregate and to change their shape.

EXPERIMENTAL METHODS

Egg lecithin, cholesterol, and phosphatidylcholinethanolamine and dicetyl phosphate (from Sigma) were used. Liposomes were obtained by the standard method [1] by preparing solutions of lecithin, of lecithin and cholesterol in molar proportions of 8:2 and 5:5, and of lecithin, cholesterol and a charged phospholipid in molar proportions of 6:2:2 in chloroform. The solution was then evaporated to dryness on a rotary evaporator, the resulting film of lipids was treated with phosphate buffer, pH 7.4, in the ratio of 1 ml buffer to 10 mg lipid, and the resulting emulsion was sonicated on a UZDN-2 disintegrator at 25°C for 15 min with portions of ultrasound (frequency 35 kHz), each 30 sec in duration, with intervals of 1 min to allow the mixture to cool. According to the writers' previous observations [12], under these conditions multilamellar liposomes with a mean diameter of 800 Å are obtained.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 141-143, August, 1979. Original article submitted September 19, 1978.

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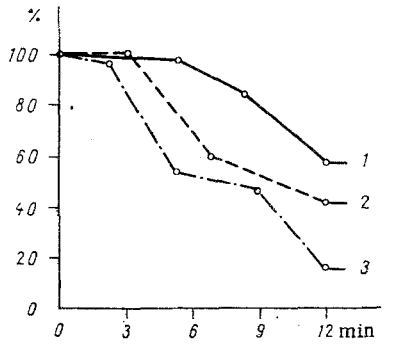

Fig. 1. Dependence of degree of ADP-induced aggregation of platelets on duration of incubation with liposomes (37°C). Abscissa, duration of preincubation (in min); ordinate, aggregation (in %). Final concentration of liposomes composed of lecithin : cholesterol in molar proportions of 10 : 0 (curve 1, 2 mg lipid/ml), 8 : 2 (curve 2, 2 mg lipid/ml); 5 : 5 (curve 3, 1 mg lipid/ml). Points on graph represent mean results of four measurements; scatter did not exceed 10%.

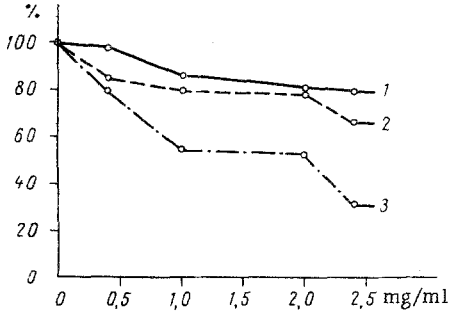

Fig. 2. Dependence of degree of ADP-induced aggregation of platelets on liposome concentration (37°C, 5 min). Abscissa, concentration of lipids (in mg/ml); ordinate, aggregation (in %). 1, 2, 3) Liposomes composed of lecithin : cholesterol in molar proportions of 10 : 0, 8 : 2, and 5 : 5 respectively. Points on graphs correspond to mean values of three measurements; scatter did not exceed 10%.

Platelet-enriched plasma (300,000–500,000 cells/μl), obtained from citrated (9 : 1) rabbit blood by centrifugation (280g, 12 min), was used. Aggregation of the platelets was measured by the standard nephelometric method [3] and changes in the shape of the platelets by the method in [9], based on recording changes in the light transmission of plasma containing oriented and disoriented platelets. Aggregation was induced by the addition of ADP in a final concentration of 10 μM. Aggregation of platelet-enriched plasma without the addition of liposomes was taken as 100% aggregation.

EXPERIMENTAL RESULTS

It is well known that platelets react specifically to foreign bodies by a change in shape from disk-like to spherical (spherulation), and they also change their ability to aggregate [5]. Since these parameters are of essential importance for the assessment of platelet function, changes in them during contact between platelets and liposomes were studied.

TABLE 1. Aggregation of Platelets at Different Temperatures and in the Presence of Liposomes of Different Composition (1 mg lipid/ml, incubation for 10 min)

Composition of liposomes, lecithin : cholesterol	Temperature, °C	Aggregation, %
8:2	25	92
	37	57
5:5	25	93
	37	32

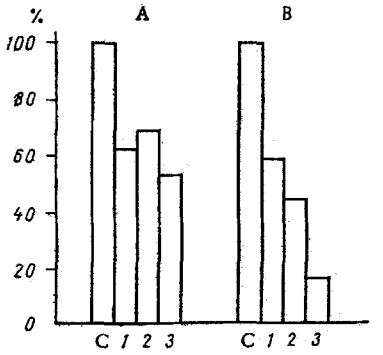

Fig. 3. Effect of charge of liposome on degree of ADP-induced platelet aggregation (37°C, 5 min). Abscissa: C) control (platelets without addition of liposomes, 1) platelets and liposomes composed of lecithin : cholesterol 5 : 5, 2) platelets and liposomes composed of lecithin : cholesterol : dicetyl phosphate 6 : 2 : 2, 3) platelets and liposomes composed of lecithin : cholesterol : phosphatidylcholinethanolamine 6 : 2 : 2. A) 2 mg lipid/ml, B) 3 mg lipid/ml.

Addition of liposomes in a concentration of 1-2 mg (as lipid)/ml was found to cause appreciable inhibition of ADP-induced platelet aggregation. The duration of preliminary incubation of the platelets with liposomes was reflected in the magnitude of the effects observed (Fig. 1). A further factor which influenced the magnitude of the effect was the concentration of liposomes in the sample. If the liposome concentration was increased from 0.5 to 2.5 mg/ml platelet aggregation was reduced by 33-67%.

The composition of the liposomes also was important. When uncharged liposomes (not containing dicetyl phosphate, which is negatively charged) were used in the experiments, an increase in the cholesterol concentration in the liposomal membrane (or, in other words, an increase in the rigidity of the membrane) led to increased inhibition of aggregation (Fig. 2).

The temperature at which the experiments were carried out also had a marked effect on the inhibition of aggregation. Incubation of liposomes and platelets at 37°C led to a greater decrease of aggregation than at 25°C (Table 1).

This effect was evidently due to temperature-dependent changes in platelet function, for the properties of liposomal membranes can only undergo insignificant changes within this narrow range of temperatures, for the phase transition temperature for most of the lipids contained in them is below 20°C [14].

Comparative analysis of the action of neutral liposomes of different composition and of negatively charged liposomes showed that the degree of the inhibitory effect on platelet aggregation increases in the following order: neutral liposomes of lecithin and cholesterol (molar proportions 5 : 5), negatively charged liposomes of lecithin, cholesterol, and dicetyl phosphate (molar proportions 6 : 2 : 2), liposomes containing lecithin, cholesterol, and phosphatidylcholinethanolamine (molar proportions 6 : 2 : 2). With an increase in the

concentration of the liposomes the differences in their inhibitory action became more noticeable. The results of one typical experiment are illustrated in Fig. 3.

In every case the liposomes affected the change in shape of the platelets. After addition of liposomes to the platelet-enriched plasma a decrease was observed in the difference between light transmission when the platelets were oriented and disoriented (by 30-50%), evidence that the platelets had become more spherical in shape. This spherulation was largely reversible (5-10 min).

The results indicate that interaction between liposomes and platelets is determined both by the type of the liposomes themselves and also by the functional state of the platelets.

It was thus shown that liposomes of different types and with different surface properties (charge) give rise to qualitatively similar but quantitatively somewhat different effects on the dynamic functions of platelets. One possible explanation of the observed effects may be a small change in the composition of the platelet membrane as a result of the well-known [11] exchange of components between membranes of various cells and liposomes. This hypothesis is supported by the fact that the effects depend on the duration of incubation of the platelets with liposomes although, of course, this requires further verification.

A very important result of this investigation is the discovery that aggregation of platelets is inhibited during their contact with liposomes of widely different types. This leads to the hope that the clinical use of various liposomes as carriers of drugs will not be accompanied by an undesirable increase in platelet aggregation and it is evidence in support of the high degree of biological compatibility of liposomes.

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