EFFECT OF LIPOSOMES ON PLATELET FUNCTION


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 phosphatidylcholinelanthanolamine 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 phosphatidylcholinelanthanolamine. 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 liposome 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 phosphatidylcholinelanthanolamine 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.

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.