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EFFECT OF PHOSPHOLIPID VESICLES IN THE BLOOD COAGULATION PROCESS IN VITRO

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The effect of phospholipids on blood coagulation was studied using sonicated vesicles containing either phosphatidylserine or phosphatidylcholine or both. It is suggested that in the blood coagulation process the mixing of the two kind of phospholipids occurs in the reaction field.

KEYWORDS —— phospholipid; vesicle; blood coagulation; clotting time; mixing of phospholipid

It is well-known that phospholipids play an important role in blood coagulation.[1] But a lack of systematic investigation in this field and impurities in the phospholipid preparations in early works have prevented understanding molecular mechanism. For example, although both phosphatidylserine and phosphatidylethanolamine have high clot-promoting activity,[2,3] the former is also inhibitory under certain conditions.[4] Thus, many questions remain about phospholipid participation in blood coagulation.

We have studied the effect of phospholipids on blood coagulation using sonicated phospholipid vesicles and find that in the coagulation process there may be a mixing of the phospholipids of the phosphatidylserine and phosphatidylcholine vesicles.

Sonicated phospholipid vesicles were prepared by drying their chloroform solutions and then dispersing them in water by sonication for one hour to form a lipid suspension. Blood clotting time was determined according to the standard a-PTT method[5] using "Ci-TROL" (DADE Co. Ltd.) as a standard plasma and the sonicated vesicle suspensions instead of using "Actin" (DADE Co. Ltd.). Phosphatidylcholine from egg yolk and phosphatidylserine from bovine brain were obtained from Sigma Chemical Co. Ltd. and were 98-99% pure. All reagents used in the clotting time measurement were from DADE Co.Ltd.

The clotting time for the vesicles with different proportions of phosphatidylserine and phosphatidylcholine are shown in Fig. 1. The results are summerized as follows: The vesicles composed of phosphatidylcholine alone did not promote clotting at the concentration ranges examined; the vesicles composed of phosphatidylserine alone not only had no clot-promoting effect, but was even inhibitory at concentrations above 1×10^{-5}M; the optimal molar ratio of phosphatidylserine to phosphatidylcholine in vesicles for clot-promoting activity was 3/7 and the maximal effect was attained at a total lipid concentration of 2.5×10^{-5}M. The effect on clotting of mixing the two kinds of vesicles is shown in Fig. 2. One of the vesilces was composed of phosphatidylserine only and the other of phosphatidylcholine only. The phosphatidylserine

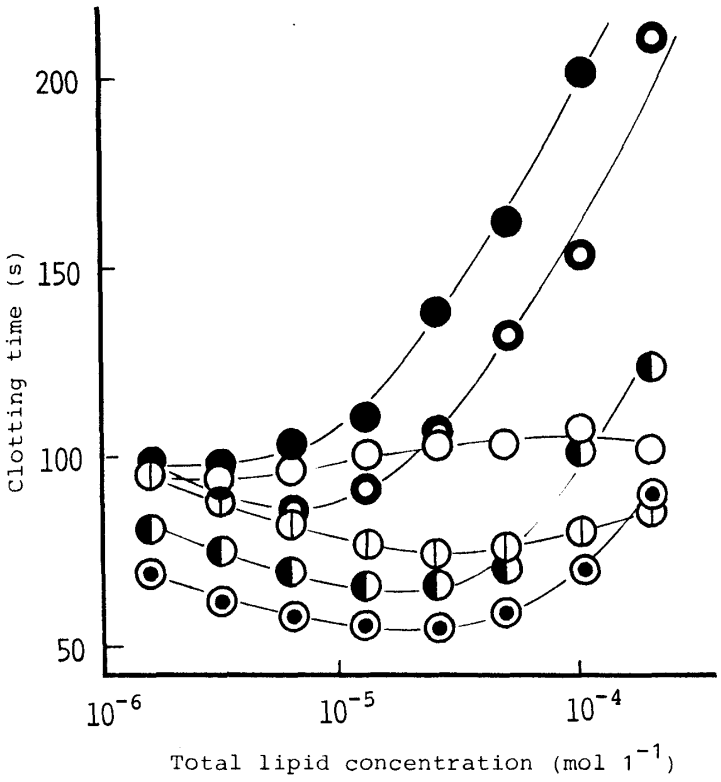

Fig. 1. Clotting times for different mixtures of phosphatydilserine / phosphatidylcholine vesicles as a function of the total lipid concentration. The different plots represent different mole fractions of phosphatidylserine (○) 0/100; (◑) 10/100; (◉) 30/100; (◐) 50/100; (●) 90/100; (●) 100 mol/100 mol.

vesicle and the phosphatidylcholine vesicle were mixed at a molar ratio of 3/7. Note that vesicles composed of phosphatidylserine only or phosphatidylcholine only did not facilitate clotting. However, Fig. 2 shows that the clotting time was shortened considerably by adding the vesicle mixture. This observation is easily explained by assuming that the exchange of phospholipids between the vesicles or vesicle fusion occurs under these conditions. It is known that fusion between acidic phospholipid vesicles can be induced by the presence of Ca^{++}.[6] However, fusion of acidic phosphatidylserine and neutral phosphatidylcholine vesicles may not occur spontaneously unless other factors are involved. The clotting time observed in the presence of a mixture of phosphatidylserine and phosphatidylcholine vesicles (Fig. 2) roughly corresponds to the average clotting times observed in the presence of a vesicle composed of phosphatidylserine and phosphatidylcholine in 9/1 molar ratio (Fig. 1). This suggests that the exchange of 10-20% of phospholipids occured between the phosphatidylserine vesicle and phosphatidylcholine vesicle in the experimental time interval, that is, in several minutes. But according to a recent report, the half-time for the spontaneous transfer of phospholipids between vesicles is 13-24 hours at 36°C.[7] Therefore, the spontaneous exchange of 10-20% of the phospholipids should take more

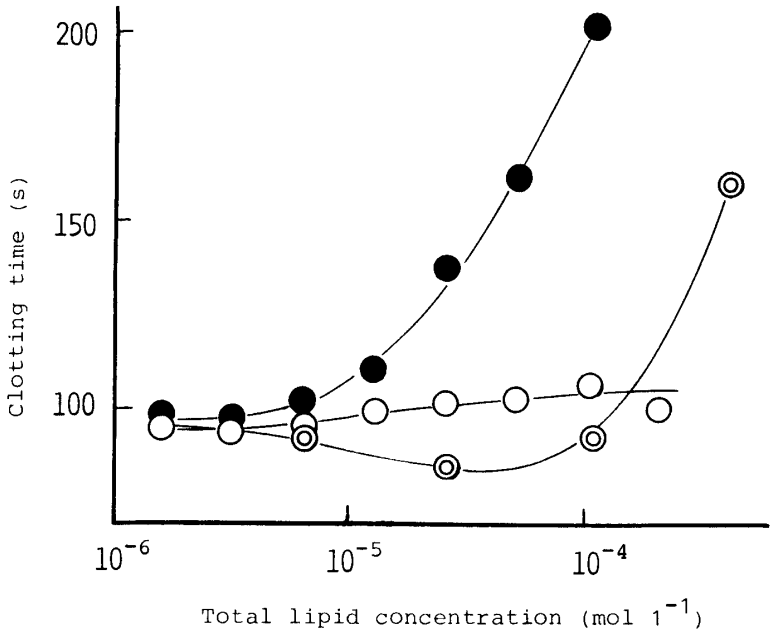

Fig. 2. Clotting time for phosphatidylserine vesicles (●), phosphatidylcholine vesicles (○), and the mixture of the vesicles (◎) containing 30% phosphatidylserine vesicles and 70% phosphatidylcholine vesicles.

than several hours. Futhermore, if the exchange occurred spontaneously, the clotting time measured after pre-incubation of the vesicle mixtures should be shortened as compared with that measured immidiately after the preparation of the vesicle mixtures. However, the pre-incubation of the vesicle mixtures for up to one hour did not affect the clotting time (data not shown). These results suggest that the mixing of phospholipids between phosphatidylserine vesicles and phosphatidylcholine vesicles may be facilitated by some plasma factors, such as plasma proteins. Also it has not been established whether the mixing can be attributed to the exchange of phospholipids between the vesicles or to vesicle fusion. Another explanation for the clot-promoting activity of the mixture of phosphatidylserine and phosphatidylcholine vesicles deserving consideration is that the mixing of the phospholipids does not occur between vesicles but they are transferred independently from the respective vesicles to the site of their action where they are mixed to give an appropriate molar ratio.

REFERENCES

1) R.F.A. Zwall, Biochim. Biophys. Acta 515, 163 (1978).
2) D.L. Turner, R.R. Holburn, M.J. Silver, and L.M. Tocantins, J. Lipid Res., 4, 52 (1963).
3) A.J. Marucus, Adv. Lipid Res., 4, 1 (1966).
4) D.L. Turner, M.J. Silver, E. Baczynski, N. Giordano, and I. Rodalewicz, J. Lipid Res., 5, 616 (1964).
5) I. Kanai and M. Kanai, "Rinsho Kensa- Taiyo" 28th ed, Kinbara Press, Tokyo (1978).
6) S.T. Sun, C.C. Hasng, E.P. Day, and J.T. Ho, Biochim. Biophys. Acta, 557, 45 (1979).
7) M.A. Rosenman and T. E. Thompson, Biochemistry, 19, 439 (1980).

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