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Molecular mechanism underlying impaired platelet responsiveness in liver cirrhosis

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We have studied platelet function in 10 patients with severe liver cirrhosis, compared to healthy subjects. Using washed platelets, we have investigated the molecular mechanism underlying the defect in platelet aggregation frequently observed in these patients. We have found that platelets from cirrhotic patients have a reduced responsiveness to thrombin and collagen in terms of aggregation, and receptor-dependent activation of phospholipase C, A_2 and cyclooxygenase/thromboxane synthetase. We thus suggest that this impairment in transmembrane signalling is responsible for the defective platelet function observed in cirrhosis.

Cirrhosis; Transmembrane signaling; (Human platelet, Liver)

1. INTRODUCTION

Liver cirrhosis is commonly associated with hemorrhagic disorders. A number of factors may contribute to the bleeding tendency that constitutes one of the main causes of death in this condition [1]. One of these factors is defective platelet response to various agonists, and the degree of this impairment has been related to the severity of liver disease [2]. However, it is still unclear whether the reduced platelet responsiveness is due to a primary defect in platelets or is due to abnormal circulating factors specifically related to cirrhosis. In order to answer this question, and to focus our investigation solely on platelet function, we used washed platelets, thus avoiding any interference by plasmatic alterations. We examined platelet aggregation and the underlying molecular mechanism in response to exogenous thrombin and collagen, which are two major platelet physiological agonists.

The action of these agonists on human platelets is associated with shape change, aggregation and release reaction, which occur in parallel with fibrinogen receptor expression, formation of inositol polyphosphates and 1,2-diacyglycerol, its phosphorylated product phosphatidic acid, formation of arachidonic acid metabolites, and phosphorylation of specific proteins [3,4]. Sequential activation of phospholipase C and phospholipase A_2 leads to the production of arachidonic acid and its metabolites (endoperoxides and thromboxane) which further amplify the response through receptor-mediated activation of phospholipase C.

In this study we have monitored agonist-induced platelet aggregation, phospholipase C and A_2 activation, and thromboxane B_2 formation in washed platelets from healthy subjects and cirrhotic patients in order to define the molecular mechanism underlying the platelet defect in cirrhosis.

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2. EXPERIMENTAL

Ten cirrhotic patients, 7 males and 3 females, were included in this study. The aetiology of cirrhosis was as follows: chronic alcohol abuse in 6 cases; chronic hepatitis B virus infection in 3 cases; unknown origin in 1 case. The age range of the patients was 35–63, mean age 53. Diagnosis of liver disease was based on clinical and biochemical features and liver biopsy. All patients belonged to class B or C, according to a modified Child classification [5]. Neither patients nor healthy controls had taken any medication known to interfere with platelet function for at least 2 weeks prior to the study.

Preparation of washed platelets, labelling and platelet aggregation determinations were performed as described [6,7]. Formation of [1-^{14}C] phosphatidic acid, [1-^{14}C]arachidonic acid, and 12-hydroxy[1-^{14}C]heptadecatrienoic acid from platelets prelabelled with [1-^{14}C]arachidonic acid was measured by separating these compounds by thin-layer chromatography [7]. Thromboxane B_2 formation was measured by radioimmunoassay [8].

Materials: Thrombin (human) and collagen were from Sigma, USA. [1-^{14}C]Arachidonic acid was from Amersham, USA. All other reagents were of analytical grade.

3. RESULTS AND DISCUSSION

Table 1 shows that aggregation induced by thrombin (0.3 units/ml) or collagen (20 μg/ml), in washed platelets obtained from cirrhotic patients with severe liver disease, is greatly reduced compared to healthy subjects. [1-^{14}C]Phosphatidic acid formation is also impaired in cirrhotic patients, in response to thrombin and collagen. This might represent a defect in receptor-dependent phospholipase C activation in cirrhosis. Although we know that measurement of phosphatidic acid formation is only an indirect way to investigate phospholipase C activation, we have chosen this method in order to obtain the greatest amount of information on each patient; as a matter of fact, common thrombocytopenia of cirrhotic patients is a major obstacle in obtaining an adequate number of platelets to perform a detailed study on [^{3}H]inositol phosphate formation.

Table 1

Platelet responses to thrombin and collagen in cirrhotic patients and healthy subjects

	Thrombin		Collagen	
	Patients	Controls	Patients	Controls
Aggregation	22 ± 3	85 ± 5	12 ± 2	67 ± 4
[1-^{14}C]PA	165 ± 8	369 ± 11	135 ± 3	260 ± 5
[1-^{14}C]AA	148 ± 5	1355 ± 10	118 ± 4	357 ± 8
[1-^{14}C]HHT	187 ± 15	778 ± 80	120 ± 13	387 ± 41
TxB_2 (RIA)	22 ± 6	115 ± 12	55 ± 4	112 ± 7

Samples (0.5 ml) of washed platelets that had been prelabelled with [1-^{14}C]arachidonic acid were incubated at a final concentration of 4×10^8/ml in aggregometer tubes at 37°C. Thrombin (0.3 units/ml) or collagen (20 μg/ml) were then added and the reaction was stopped 2 min after the addition of the agonists. Light transmission was recorded throughout the experiment and displayed on a chart recorder. Platelet aggregation is expressed as % increase in light transmission. Phosphatidic acid (PA), arachidonic acid (AA), and 12-hydroxyheptadecatrienoic acid (HHT) were extracted by adding 1.88 ml of chloroform/methanol (100:200, v/v). These compounds were then separated by thin-layer chromatography, as described [7]. Data are represented as % increase over basal (unstimulated) values. Basal values of representative experiments were: [1-^{14}C]PA, 157 ± 17 cpm; [1-^{14}C]AA, 1671 ± 85 cpm; [1-^{14}C]HHT, 125 ± 18 cpm (means ± SE of quadruplicate samples). TxB_2 was extracted and measured by radioimmunoassay (RIA), as described in [8]; data are expressed as ng/ml. All the results are shown as means ± SE from 10 separate experiments (i.e., one cirrhotic patient and one healthy subject in each experiment) each performed in quadruplicate

Agonist-induced activation of phospholipase A_2 is also greatly impaired in cirrhosis, as reflected by decreased liberation of [1-^{14}C]arachidonic acid in cirrhotic patients compared to healthy controls. It is worth noting that in these experiments labelling with [1-^{14}C]arachidonic acid was performed in washed platelets, where the number of platelets derived from cirrhotic patients and healthy volunteers was adjusted to the same concentration. By so doing, we have been able to determine that platelets of both groups incorporate approximately the same amount of [1-^{14}C]arachidonic acid. Therefore, we believe that the observed impairment in agonist-induced arachidonic acid liberation is actually due to a defect in the

transmembrane signalling machinery, specifically related to the severity of liver disease.

Consistently, production of cyclooxygenase/thromboxane synthetase metabolites is also impaired in cirrhotic patients, as shown in table 1. In this case we have measured 12-hydroxy[1-^{14}C]heptadecatrienoic acid by thin-layer chromatography analysis, and thromboxane B_2 by radioimmunoassay. The consistency of the results leads us to think that a reduced production of cyclooxygenase/thromboxane synthetase actually occurs in platelets from cirrhotic patients stimulated by thrombin or collagen.

We think that the impairment in transmembrane signalling that we have observed in platelets from cirrhotic patients is responsible for the reduced platelet responsiveness to agonists in terms of aggregation. We suggest that changes in the lipid composition and in the fluidity of platelet plasma membrane in cirrhosis are responsible for this impairment. Several findings support this hypothesis: it has been demonstrated that alterations in plasma membrane phospholipid composition, similar to those observed in cirrhosis, greatly reduce phospholipase C activation [9]. A decrease in platelet plasma membrane fluidity reduces thrombin-stimulated thromboxane synthesis [10], and it has been demonstrated that patients with liver disease have a lower membrane fluidity [11].

In conclusion, we propose that platelet impairment in cirrhosis is a primary platelet defect that involves a reduced responsiveness of the enzymes related to agonist-stimulated transmembrane signalling, i.e., phospholipase C, A_2, and cyclooxygenase/thromboxane synthetase.

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