Original Article

Platelet Activation in Patients After Splenectomy with Total Gastrectomy for Gastric Cancer

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OBJECTIVE: We investigated change in platelet activation using flow cytometry in patients before and after splenectomy with total gastrectomy for gastric cancer.
METHODS: Six patients who underwent splenectomy for lymphadenectomy with total gastrectomy for gastric cancer were the subjects in this study. In the patients, platelet count and platelet activation were evaluated before the operation, 1 week after the operation, and 1 month after the operation. Expression of CD62P (P-selectin) was analysed as a marker of platelet activation using flow cytometry.
RESULTS: Although platelet count significantly increased 1 week after the operation, the platelet count 1 month after the operation did not increase significantly. Expression of CD62P (P-selectin) significantly decreased at 1 week and 1 month after the operation, compared with the level before the operation. No postoperative complications occurred in any patient.
CONCLUSION: In the present study, platelet activation did not progress after the operation. The results mean that the risk of thrombosis after splenectomy does not increase. [*Asian J Surg* 2007;30(2):93–5]

Key Words: CD62P (P-selectin), gastric cancer, platelet activation, splenectomy, total gastrectomy

Introduction

Splenectomy for lymphadenectomy has often been performed with total gastrectomy for gastric cancer. Since the spleen is concerned with platelet count and platelet function, increases in platelet count and abnormalities in platelet function are a cause of worry in almost all patients after splenectomy.[1] However, we could not find any reports on the difference in platelet count and function before and after splenectomy.

Platelet activation is useful for the clinical evaluation of platelet function. It is important to detect platelet activation *in vivo* because platelet activation is concerned not only with normal haemostasis but also with formation and progress of various diseases, especially pathogenic thrombosis. Although the standard clinical test of platelet activation is platelet aggregometry, there are methodological problems such as the influence of centrifugation or the impossibility of quantitative analysis. The detection of substances in plasma released by platelets is another test for platelet activation. But the major problem with these assays is the progress of release during blood collection and plasma separation.[2,3] Recently, antibodies for activation-specific antigen on the platelet surface can be used for detecting platelet activation by flow cytometry.[3,4] This method has many advantages, such as preventing the

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influence of centrifugation by fixing whole blood and to quantify activation-specific antigens on the platelet surface and it is more useful in detecting platelet activation *in vivo*,[5,6] it has detected platelet activation in various diseases. In this pilot study, we investigated the change in platelet activation by using the flow cytometry method before and after splenectomy with total gastrectomy for gastric cancer in patients.

Patients and methods

Between April 2004 and April 2005, six patients who underwent splenectomy for lymphadenectomy with total gastrectomy for gastric cancer were enrolled.

Five of the six patients were males. The median age was 61 years (range, 42–80 years). All of them underwent D2 lymphadenectomy and Roux-en-Y reconstruction.

Blood sample was obtained before operation, 1 week after operation, and 1 month after operation. We measured both the platelet activation and platelet count.

A Becton Dickinson FACScan was used for flow cytometry to measure platelet activation. We used a monoclonal antibody (MAb) for an activation-specific antigen, which is an α-granule membrane protein CD62P (P-selectin) on the platelet surface for measuring the platelet surface expression of CD62P (P-selectin). Values were represented as the proportion of CD62P (P-selectin) antigen expression to that before operation.

Blood was carefully drawn from an antecubital vein through a 19-gauge needle and collected into a plastic syringe containing 1.5 mg/mL disodium ethylenediaminetetraacetate (EDTA-2Na). The blood sample was then fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS) for 2 hours at 4°C. After fixation, platelet-rich plasma (PRP) was obtained by centrifugation at 100*g* for 10 minutes at room temperature. The samples were diluted 40 times with PBS. This diluted PRP (250 μL) was incubated with 2 μL of phycoerythrin (PE)-conjugated anti-CD62P (P-selectin) MAb (BD Biosciences) for 15 minutes at room temperature. After incubation, 10,000 platelets from each sample were analysed using a FACScan for platelet surface expression of CD62P (P-selectin).

Analysis of variance and Dunnett's test were used for the statistical analysis, with a risk ratio of less than 0.05 considered significant.

This study was performed following the Declaration of Helsinki; all the patients were informed and consented to participate in this study, and the study protocol was approved by our hospital's ethics committee.

Results

Platelet count increased significantly 1 week after operation compared with the level before operation ($p = 0.01$). On the other hand, it was still higher than the level before operation, but the increase in platelet count 1 month after operation was not significant (Figure 1). Expression of CD62P (P-selectin) was significantly lower 1 week after operation ($p < 0.001$) and 1 month after operation ($p = 0.03$) than the level before operation. But it tended to return to the level before operation 1 month after operation (Figure 2).

No postoperative complications including thrombosis occurred in any of the patients.

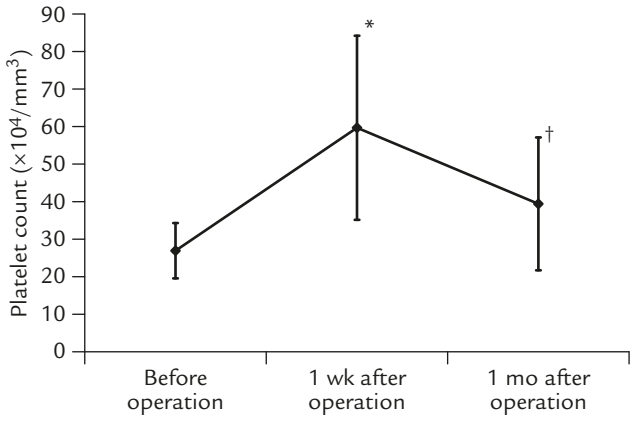

Figure 1. Platelet count. Bars represent the mean ± standard deviation. *$p = 0.01$, †$p < 0.05$ versus before the operation.

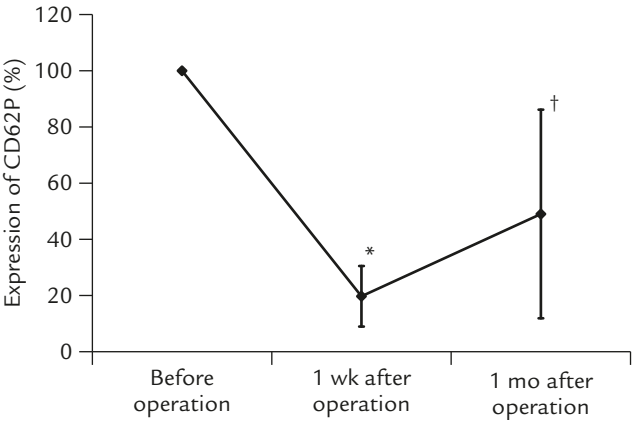

Figure 2. Expression of CD62P (P-selectin). Bars represent the mean ± standard deviation. Values are represented as CD62P (P-selectin) expression in proportion to that before operation. *$p < 0.001$, †$p = 0.003$ versus before operation.

Discussion

The increase in expression of CD62P (P-selectin) in patients with cerebral infarction and ischaemic heart disease has been reported,[7,8] and CD62P (P-selectin) expression was significantly higher in patients with cerebral infarction than in patients without cerebral infarction after heart valve replacement.[9] It was also reported that though the expression of activation-specific protein increased significantly, there was no significant difference in the platelet counts or levels of substances released from platelets in the diseases related to abnormalities of platelet function. In short, detecting substances in plasma released from platelets and investigating platelet count are insufficient to evaluate platelet activation. Murakami et al reported that flow cytometric analysis of antigen on the platelet surface may be more valid for the detection of platelet activation.[8]

The flow cytometry method measuring activation-specific antigens on the platelet surface as a marker of platelet activation was applied to determine platelet activation after stimulation by various platelet agonists. It was reported that an increase in the expression of platelet surface antigens was observed when platelets were activated.[4,10] Further, expression of platelet surface antigens increased in disease related to platelet activation.[11] In this study, CD62P (P-selectin) expression decreased and platelet activation was suppressed after splenectomy.

Platelet count increased in almost all patients after splenectomy with total gastrectomy in the perioperative period. In the present study, platelet count increased significantly 1 week after operation and tended to return to the level before operation 1 month after operation. But it remained higher than the level before operation.

Although an increase in platelet count was observed, the expression of an activation-specific antigen decreased. This showed that platelet activation was suppressed after operation. From these results, increases in platelet count and abnormalities of platelet function might not be a cause for worry in almost all patients after splenectomy.

Though we have investigated only platelet count in the perioperative period of splenectomy with total gastrectomy, the detection of platelet activation is important as well. In all patients, in spite of a tendency for the platelet count to return to the level before operation, the platelet activation was recovered later. In future, we intend to observe the progress until the condition returns to that before operation and make a comparative study between patients with and without splenectomy.

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