(5) Metabolic and Kinetic Study of Platelets in Thrombogenesis

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Platelets, the third components of blood cells, are important in the plug formation in hemostasis. Recent advance in this field revealed that these particles derived from the cytoplasm of megakaryocytes in the bone marrow, possess all cell components; mitochondria, ribosome, granules, vesicle systems, glycogen microtubulus, except nucleus. These small cells are active in metabolism to maintain their shape and functions; active transport of bioactive substances, pseudopod formation, release reaction, adhesion, aggregation, retraction, and phagocytosis. Their main energy sources are glucose in plasma and glycogen in the cells. They are active in de novo synthesis of glycogen, protein, and fatty acid.

In the process of thrombogenesis, adhesion of platelet to collagen or other subendothelial tissues, or aggregation of primary step, induce release reaction of mainly nucleotides: ADP, which induces further aggregation of secondary. We presented the results that these primary important step was deeply energy dependent and closely related to the oxidative phosphorylation in the mitochondria via citric acid cycle (CAC). To elucidate the metabolic and kinetic basis of thrombogenesis it is important to predict episodes and establish the early prophylactic therapy in thrombogenic diseases, which are increasing in number; cardiovascular and cerebral thrombosis and implantation of artificial thrombogenic organs. We conducted in vitro experiment of glucose metabolism of human platelets and in

vivo study of ^{51}Cr labeled platelet survival on the patients with thrombogenic diseases. The platelets were obtained from healthy volunteer and the patients with marked thrombocytotic disorders; chronic myelogenous leukemia (CML), myelofibrosis with myeloid metaplasia (MMM) and primary thrombocythemia and vascular and heart valvular diseases. Glucose metabolism was studied by measuring the radioactivity of produced $^{14}\text{CO}_2$ and ^{14}C -lactate from ^{14}C -glucose, with scintillation counter. Lactate and glycogen were determined as was described (Ser. Haemat 4:98, 1971). ADP, collagen, epinephrine, ristocetin and Latex particles of 0.234μ in size were used as aggregating agents. Kinetic study was performed using $^{51}\text{Cr}(\text{Na}_2\text{CrO}_4)$ to label separated platelets from ABO compatible donors or autologous blood of 200 to 450 ml in volume. Procedures were as described previously (Acta Haemat Jap 29:664, 1966). Body surface scanning were also done to detect organ distribution of tagged platelets. In vitro study of platelet function of aggregation and adhesion was done according to Born's method and modified Salzman's method.

RESULTS

To study the energy metabolism in aggregating platelets, we used differently labeled glucose as the substrate. In platelets, aerobic and anaerobic glycolysis are operating. In the event of aggregation with release reaction increased glucose consumption took place

especially in CAC. CO_2 production by non-aggregating platelets were 26.7 nmoles from ^{14}C -1-glucose, 2-3 nmoles from ^{14}C -2-glucose, 1.5-1.9 nmoles from ^{14}C -3.4-glucose and 1.5-1.7 nmoles from ^{14}C -6-glucose and 1.21-1.55 μ moles lactate per 1×10^9 platelets for 2 hours. ADP, thrombin, collagen and Latex particles did stimulate glucose consumption of ^{14}C -6-glucose indicating product increment of twice by collagen and epinephrine and 8 to 10 times increase by Latex particles. No net increase of hexose monophosphate shunt (HMS) was observed by these agents. In contrast, methylene blue stimulated HMS around 10 times, but not of citric acid cycle.

These responses to metabolic stimulators, were inhibited by metabolic inhibitors such as deoxyglucose, iodoacetate, KCN, Amytal 1 mM respectively and prostaglandin E_1 (10^{-4} - 10^{-6} M). Latex particles are the most potent stimulators via phagocytic stimulation of lysosomes (BBA 201: 471, 1970). In contrast to phagocytic neutrophils, platelets phagocytosis accompanied citric acid cycle stimulation in place of HMS. Prostaglandins, long chain unsaturated fatty acid have been closed up as important mediators in cell function. In platelets, PGE_1 acts as an activator of adenylate cyclase in the platelet membrane, bringing about increase of cyclic AMP production. PGE_1 of 10^{-4} to 10^{-6} M concentration inhibited platelet aggregation induced by ADP, pinephrine, collagen, by inhibiting release reaction. Addition of PGE_1 of these concentrations did not alter the basal production of CO_2 via CAC but rather increased lactate production from anaerobic glycolysis. PGE_1 of 10^{-8} M concentration did not act as an inhibitor any more. PGE_2 which was produced from arachidonic acid, behaves differently in platelets. Recently, arachidonic acid and their intermediates; cyclic endoperoxides and PGE_2 are proved as active substance to sensitize platelets aggregability. The effect of PGE_2 on platelet function and

metabolism were studied. To evaluate the risk of thrombosis in increased number of platelets in CML, MMM and primary thrombocythemia, metabolic and kinetic study were done as well as adhesion and aggregation. Those platelets which responded abnormally to epinephrine, sometimes to collagen, but reacted to ADP, ristocetin normally, were incubated with PGE_2 , resulting in partial or total improvement of abnormal aggregation pattern by epinephrine to the normal. In CML, basal production of 1.27 n moles increased to 1.88 ($\times 1.5$) by epinephrine, to 2.43 ($\times 1.9$) by epinephrine plus PGE_2 . In MMM, basal level of 2.51 n moles did not increase by epinephrine alone but some increase by preincubation with PGE_2 to 3.18 n moles ($\times 1.3$). These findings suggested abnormality in platelets not only quantitative; marked increase in number, but also qualitative. These abnormalities, however, might be effective for self control mechanism to reduce high risk of aggregability due to increased number. Kinetic study on those cases with this abnormality, was done on a case of MMM who showed short survival ($T\ 1/2=1.75$ d.) with increased turnover and decreased recovery. A case of CML and a case of primary thrombocythemia exhibited normal life span.

Similar abnormalities in aggregation were seen in cases of long term administration of anti-platelets agents; dipyridamole and aspirin, after operation of artificial valve replacement. To predict an episodes of thrombosis as early as possible is most important problems in their aftercare. On 11 cases of those patients, short survivals ($T\ 1/2$); 3.64 ± 0.70 days in mirtal valve replacement (MVR), and 3.30 ± 0.67 days in aortic valve replacement (AVR): with decreased recovery more pronounced in AVR than in MVR, were proved suggesting increased sequestration and consumption. Turnover of platelets also increased slightly in MVR ($4.65 \pm 2.30 \times 10^4/\text{cmm}/\text{day}$)

but marked in AVR ($7.55 \pm 2.73 \times 10^4$), suggesting compensated active thrombopoiesis.

Conclusion

Among those parameters, short survival less than 3 days, was helpful evidence to predict thrombotic episodes in two cases, indicating increased consumption. It was also suggested that intrinsic change due to drugs could be involved and prompted us metabolic study on those platelets (Thromb Res Suppl

2, 1976 in press). Thus in conclusion, we presented in this study, essential process of platelet plug formation; release reaction and aggregation are energy dependent. Therapeutic trials and analysis of self control mechanism of this process have been effective and promising for prophylaxis. Aspirin and other antipyretics have been proved potent inhibitors of PGE_2 biosynthesis in platelets, and establishment of isolation and determination of these fatty acid or metabolites are in progress.