Alteration of release and role of adenosine diphosphate and thromboxane A\(_2\) during collagen-induced aggregation of platelets from cattle with Chediak-Higashi syndrome

Naofumi Honda, DVM; Katsuyuki Ohnishi, DVM; Tsuyoshi Fujishiro, PhD; Masahiro Ikeda, DVM, PhD; Katsuaki Ito, DVM, PhD

Objective—To compare the interaction of endogenous ADP with collagen and thromboxane A\(_2\) (TXA\(_2\)) during collagen-induced platelet aggregation between platelets from healthy cattle and those with Chediak-Higashi syndrome (CHS).

Population Sample—Platelets harvested from blood samples from healthy Japanese Black cattle and those with CHS.

Procedures—Aggregation of gel-filtered platelets; release of ATP-ADP; and generation of thromboxane B\(_2\) (TXB\(_2\)), a metabolite of TXA\(_2\), were measured.

Results—The potency of collagen to induce aggregation in platelets of cattle with CHS (ie, CHS platelets) was less than a tenth of that in platelets of healthy cattle (ie, control platelets). Platelet aggregation induced by collagen at an intermediate concentration depended on the coexistence of ADP and TXA\(_2\), suggesting that released ADP cannot cause platelet aggregation by itself. Collagen-induced ADP release was markedly decreased, whereas TXB\(_2\) production was slightly low in CHS platelets, compared with that in control platelets. A combination of subthreshold amounts of ADP and 9,11-dideoxy-9\(\alpha\),11\(\alpha\)-methanoepoxy-prostaglandin F\(_{1\alpha}\) (U46619), a TXA\(_2\) analogue, caused platelet aggregation. Similarly, a combination of subthreshold amounts of collagen and ADP caused platelet aggregation, whereas collagen and U46619 were not synergistic.

Conclusions and Clinical Relevance—Deficient ADP release ensuing from the \(\delta\)-storage pool deficiency in platelets from cattle with CHS resulted in reduction of collagen-induced platelet aggregation, through attenuation of synergism between TXA\(_2\) and ADP and between ADP and collagen. Furthermore, results of the study reported here indicated that TXA\(_2\) was important for aggregation of bovine platelets. (Am J Vet Res 2007;68:1399–1406)

Chediak-Higashi syndrome is a recessively inherited disorder, which is found in humans, cattle, mice, rats, cats, mink, and foxes and is characterized by giant intracellular granules in leukocytes and melanocytes and by variable degrees of oculocutaneous albinism. Increased susceptibility to infection and enhanced bleeding tendencies are commonly observed symptoms in people and clinical signs in animals with CHS. Ogawa et al reported that 54 of 56 Japanese Black cattle with CHS had a bleeding episode, including umbilical bleeding and bleeding during or after castration or parturition, epistaxis, and superficial or abdominal hematomas, and that 4 of them died of hemorrhage or infection.

Aggregation to collagen is specifically decreased in platelets from cattle with CHS, and this disorder is a cause of bleeding tendency. Dense granules are deficient in platelets of cattle with CHS (ie, CHS platelets), and the ADP content in platelets of affected cattle is much less than that in platelets of healthy cattle (ie, control platelets). Because dense granules are deficient in CHS platelets, the insufficiency of platelet aggregation is referred to as a \(\delta\)-storage pool deficiency.

Because platelets release ADP, ATP, or 5-hydroxytryptamine from dense granules upon stimulation with collagen and because ADP is a potent agonist for platelet activation, it is believed that a decrease in ADP release is responsible for the insufficient aggregation response of CHS platelets to collagen. However, few studies are found in which ADP release (or ATP) in CHS platelets...
has been measured. In a study\(^1\) in cats, ATP secretion was inhibited in platelets of cats with CHS. Furthermore, although general agreement exists that ADP released from dense granules is critical for collagen-induced platelet aggregation, it is not known whether collagen-releases ADP in amounts sufficient to cause platelet aggregation by itself or whether ADP exerts some supportive effect on the action of collagen.

Collagen also releases the other endogenous agonist TXA\(_2\), through activation of the arachidonic acid cascade. It is not known whether production and action of TXA\(_2\), are altered during collagen-induced aggregation of CHS platelets. In a study\(^3\) on mice with CHS (beige mice), TXA\(_2\) production was normal or decreased depending on collagen concentration. Results of our previous study\(^4\) revealed that collagen-induced increases in cytosolic Ca\(^{2+}\) concentration are low in CHS platelets, indicating a possibility that the signal that stimulates the arachidonic acid cascade is weak in these platelets. The role of TXA\(_2\), in collagen-induced aggregation of bovine platelets is not known; results of several studies\(^5,6,7\) indicate that bovine platelets have a minimal response to TXA\(_2\).

Investigation of the function of CHS platelets would provide useful information about the role of endogenous ADP in collagen-induced aggregation. The purpose of the study reported here was to compare the interaction of endogenous ADP with collagen and TXA\(_2\), during collagen-induced platelet aggregation between platelets from healthy cattle and those with CHS. Collagen-induced ADP and TXA\(_2\), release was compared during collagen-induced aggregation, and the roles of these agonists in the aggregation of platelets from healthy cattle and those with CHS were also compared.

**Materials and Methods**

**Animals**—Experiments were conducted according to the Guide for the Care and Use of Laboratory Animals, University of Miyazaki. Healthy Japanese Black cattle were maintained in Sumiyoshi Ranch, University of Miyazaki, Japan. Japanese Black cattle with CHS, as determined by a genetic test (PCR–restriction fragment length polymorphism analysis of the bovine LYST gene\(^8\)), were brought from Kagoshima Prefecture to the Veterinary Hospital of the University of Miyazaki. None of the cattle had any clinical signs of acute infection at the time of the experiment. For all cattle included in the study, platelet counts were within reference range limits.

**Reagents**—The following drugs were used: native fibrillar collagen (prepared from horse Achilles tendon),\(^9\) AR-C66096,\(^10\) U46619,\(^11\) and thrombin (from bovine plasma).\(^12\) Other reagents, including apyrase (adenosine-5′-triphosphatase diphosphohydrolase, grade VI),\(^13\) A3P5PS,\(^4\) and fibrinogen (from bovine plasma), were also used.\(^4\)

**Preparation of platelets**—Blood (approx 60 mL) was collected by jugular venepuncture and mixed with 0.15 volumes of acid-citrate-dextrose (70mM citric acid, 85mM trisodium citrate, and 110mM dextrose). Platelet-rich plasma was obtained by centrifuging blood at 150 × g for 15 minutes at 20°C and by collecting the supernatant. Platelet-rich plasma was centrifuged at 745 × g for 10 minutes at 20°C, and the pellet was re-suspended in HEPES–Tyrode’s solution (136mM NaCl, 2.7mM KCl, 0.42mM NaH\(_2\)PO\(_4\), 1.0mM MgCl\(_2\), 12.0mM NaHCO\(_3\), 10.0mM HEPES, and 5.5mM glucose [pH, 7.4]) containing 0.35% bovine serum albumin. Gel-filtered platelets that were recovered from the column were adjusted to a concentration of 3 × 10\(^9\)/mL.

**Platelet aggregation**—Aggregation response of gel-filtered platelets was determined in the presence of fibrinogen (1 mg/mL) and CaCl\(_2\) (1mM) by light transmission through a platelet suspension in an aggregometer\(^6\) with constant stirring (1,080 revolutions /min) at 37°C. For each experiment, the aggregometer was calibrated with HEPES–Tyrode’s solution (100% transmission) and the gel-filtered platelet suspension (0% transmission). The extent of aggregation was expressed as percentage transmission.

**ATP-ADP release**—Adenosine triphosphate released into the medium was measured with the luciferin-luciferase assay. Gel-filtered platelets (6 × 10\(^5\) in 200 µL) were incubated with luciferin-luciferase reagent\(^6\) at 37°C, while being stirred in a luminescence reader.\(^6\) The extent of ATP release was quantitatively estimated from the maximum curve height above baseline value during the 5 minutes following addition of collagen. Adenosine diphosphate was measured after conversion of ADP to ATP by use of pyruvate kinase and phosphoenolpyruvate.\(^17\)

**TXB\(_2\), generation**—Thromboxane B\(_2\) (a metabolite of TXA\(_2\)) generated from platelets was measured as an index of TXA\(_2\), production. Platelets were stimulated by collagen at 37°C in an aggregometer. After 5 minutes, the reaction was stopped by adding citrate buffer (pH, 4.0) containing 1mM aspirin and the sample was quickly placed on ice. The sample was centrifuged at 9,300 × g for 10 minutes at 4°C. The supernatant was diluted (1:100) with buffer (HEPES–Tyrode’s solution to a citrate ratio of 1:1), and the sample was stored in a deep freezer (−80°C) until just before analysis. Thromboxane B\(_2\), was measured by use of an ELISA according to the instructions of the manufacturer.\(^17\)

**Statistical analysis**—Results are expressed as mean ± SEM of at least 3 independent experiments. Multiple means were subjected to the Tukey-Kramer test after performing an ANOVA. Comparisons between mean values were performed by use of a Student t-test. Values of P < 0.05 were considered significant.

**Results**

**Collagen-induced aggregation of platelets**—Collagen-induced platelet aggregation in a concentration-dependent manner, but the response in CHS platelets was considerably decreased, compared with control platelets (Figure 1). The threshold concentration of collagen to induce aggregation was 0.1 and 1 µg/mL in control and CHS platelets, respectively, and the concentration of collagen for maximum aggregation was 3 to 10 µg/mL and 30 µg/mL, respectively. The concentration of
collagen required to induce 50% aggregation was 0.70 ± 0.18 µg/mL (n = 8) and 9.21 ± 1.21 µg/mL (9) in control and CHS platelets, respectively, indicating that CHS platelets were much less sensitive to collagen than were control platelets.

Dependence of collagen-induced platelet aggregation on ADP and TXA₂.—To evaluate the involvement of endogenous ADP or TXA₂ in collagen-induced aggregation, influences of ADP and TXA₂ were pharmacologically blocked. Two major ADP receptors, P2Y1 and P2Y12, are found in platelets. To block the effect of ADP, gel-filtered platelets were treated with a mixture of ADP receptor antagonists A3P5PS (a P2Y1 antagonist, 100µM) and AR-C66096 (a P2Y12 antagonist, 100nM). Use of A3P5PS (100µM) and AR-C66096 (100nM) were sufficient to block the action of endogenous ADP; treatment with these drugs abolished 10µM ADP-induced platelet aggregation (data not shown). To exclude the influence of TXA₂, aspirin (an inhibitor of cyclooxygenase; 1mM) was added 45 minutes before application of collagen. To compare the involvement of endogenous agonists for a similar response, concentrations of collagen were chosen that induced a similar degree (approx 70%) of aggregation for control and CHS platelets. A collagen concentration of 1 and 10 µg/mL was used as intermediate concentrations for control and CHS platelets, respectively.

Either ADP receptor antagonists or aspirin greatly inhibited control platelet aggregation that was induced by collagen at 1 µg/mL (Figure 1). When all blockers (aspirin, A3P5PS, and AR-C66096) were combined, the inhibition was similar to that induced by a single treatment with ADP receptor antagonists or that with aspirin.

Although the response of control platelets to collagen at 10 µg/mL was comparable to that of control platelets to collagen at 1 µg/mL, aggregation of CHS platelets was more resistant to ADP receptor antagonists or aspirin than that of control platelets (Figure 1). However, the inhibition by a single use of ADP receptor antagonists or aspirin was similar to that when all blockers were combined. Thus, in either control or CHS platelets, the combination of ADP receptor antagonists and aspirin did not exert an additive effect on the response to collagen at an intermediate concentration.

Resistance to ADP receptor antagonists and aspirin in CHS platelets was 25.4 ± 1.8% on the basis of aggregation in the presence of collagen at 10 µg/mL (n = 4; Figure 1). Resistance to ADP receptor antagonists and aspirin in control platelets stimulated by the same concentration of collagen was 44.3 ± 6.9% (n = 4, data not shown). Hence, aggregation of CHS platelets as a direct action of collagen, which was independent of ADP and TXA₂, was significantly (P = 0.038) less in CHS platelets than in control platelets.

ATP-ADP release in control and CHS platelets—Release pattern of ATP-ADP in control or CHS platelets that were stimulated by collagen at 10 µg/mL was determined (Figure 2). Adenosine diphosphate was measured as ATP after conversion in the presence of pyruvate kinase and phosphoenolpyruvate. In CHS platelets, collagen induced ADP and ATP release in a biphasic manner, whereas the release appeared monophasic in control platelets. This biphasic pattern in CHS platelets was detected throughout the entire concentration range (ie, collagen at 3 to 30 µg/mL). Release of ATP-ADP induced by collagen was decreased in CHS platelets, compared with control platelets. Release of ADP in CHS platelets that was induced by collagen at 30 µg/mL was approximately a tenth of that in control platelets. The ratio of ATP-to-ADP release was higher in control platelets than in CHS platelets. For comparison, thrombin-induced ATP release was measured (ADP was not measured in this case). Thrombin (0.1 U/mL) released ATP to a degree comparable to the amount of ATP re-
leased by collagen at 10 or 30 µg/mL in control or CHS platelets, respectively. Release of ATP induced by this concentration of thrombin (0.1 U/mL) was maximal because ATP release induced by thrombin at 10 U/mL was similar to that induced by thrombin at 0.1 U/mL (data not shown).

The effect of ADP receptor antagonists or aspirin was tested on ATP release induced by collagen (1 and 10 µg/mL or 10 and 30 µg/mL for control or CHS platelets, respectively) to establish whether released ADP or TXA₂ secondarily induced release of ATP (Figure 3). Release of ATP was not significantly affected by ADP receptor antagonists or aspirin in either control or CHS platelets. A combination of ADP receptor antagonists and aspirin did not affect the release.

Collagen-induced production of TXB₂, in control and CHS platelets—In this series of experiments, TXB₂ was measured as an index of TXA₂ production induced by collagen. Collagen induced TXB₂ production in control and CHS platelets in a concentration-dependent manner (Figure 4). The production of TXB₂ in CHS platelets in response to collagen at 1 µg/mL was decreased, and that induced by collagen at 10 µg/mL was slightly less than in control platelets. For collagen at 30 µg/mL, however, TXB₂ production was not different between control and CHS platelets.

Synergism between ADP and U46619 or collagen—to evaluate the interaction between TXA₂ and ADP, the combined effect of ADP and U46619 (a TXA₂ analogue) was compared with platelet aggregation induced by the sole use of ADP or U46619 (Figure 5). Adenosine diphosphate at a low concentration (1µM) induced no or slight aggregation (5.0 ± 1.5% in control platelets [n = 3]; 3.2 ± 1.3% in CHS platelets [5]), whereas ADP at a high concentration (10µM) caused maximum aggregation (79.0 ± 2.3% in control platelets [3]; 77.5 ± 2.1% in CHS platelets [4]). By comparison, U46619 (0.1 to 10µM) did not induce aggregation, although it did cause a shape change in both types of platelets. A combination of ADP (1µM) and U46619 (0.1µM) induced aggregation (37.4 ± 5.1% in control platelets [n = 6]; 32.2 ± 4.2% in CHS platelets [7]). Furthermore, when 0.3µM ADP, which did not induce aggregation, and 1µM U46619 were combined, these agonists caused aggregation of 40.9 ± 2.9% and 31.2 ± 1.8% in control (n = 3) and CHS (3) platelets, respectively.

Whether synergism existed between ADP and collagen was tested for control platelets. When subthreshold amounts of collagen (0.2 µg/mL) and ADP (0.3µM) were simultaneously applied to control platelets in the presence of ADP receptor antagonists, no potentiation of aggregation was observed.

Discussion

Bell et al⁵ and Kim et al¹⁸ determined that ATP or ADP content in platelets from cattle with CHS is low, compared with that in platelets from healthy cattle. However, to our knowledge, no reports exist on how the release of ATP or ADP upon stimulation of a platelet receptor is altered in platelets from animals with CHS, except for a study¹² in which the results indicate that collagen-induced release of ATP is inhibited in platelets from cats with CHS. In the study reported here, release
of ATP or ADP induced by collagen was markedly inhibited in platelets from cattle with CHS. The ratio of ATP-to-ADP release was higher in control platelets than in CHS platelets. In other studies\textsuperscript{5,10,11} in which ATP and ADP contents in platelets were measured, the ratio of ATP-to-ADP content was higher in platelets from animals with CHS than in platelets from healthy animals. Thus, the ratio of ATP-to-ADP release did not match the ratio of ATP-to-ADP content. The discrepancy between the ratio of ATP-to-ADP release and the ratio of ATP-to-ADP content suggests that several factors are involved in the release of ATP or ADP, and the ratio of ATP-to-ADP release does not necessarily reflect the ratio of ATP-to-ADP content in dense granules. In any case, the amount of released ADP in CHS platelets was likely higher than that expected on the basis of the ratio of ATP-to-ADP content.

Results of studies on human platelets indicate that a TXA\textsubscript{2} analogue, U46619, increases ATP release\textsuperscript{19} and that indomethacin inhibits collagen-induced ATP release,\textsuperscript{11} indicating that TXA\textsubscript{2} stimulates ADP secretion. In the study reported here, collagen (30 \(\mu\)g/mL)-induced release of ATP from control platelets was slightly inhibited by aspirin, but the inhibition was not significant. These data are inconsistent with the results of studies on human platelets and suggest that the action of TXA\textsubscript{2}, which is generated by collagen, to induce secondary ADP release was weak in bovine platelets, whereas other studies\textsuperscript{13-15} have found that the aggregatory action of TXA\textsubscript{2} is weak in bovine platelets. Therefore, it is reasonable to suggest that TXA\textsubscript{2} could not release ADP from platelets in the study reported here. Although ADP receptor antagonists inhibited ATP release induced by collagen at 10 \(\mu\)g/mL, the inhibition was not significant. This suggests that ADP release induced by collagen did not cause detectable secondary release.

Collagen at the highest concentration (10 \(\mu\)g/mL) induced release of approximately 80 pmol of ADP from 6 \(\times\) 10\textsuperscript{7} control platelets. In the aggregation experiment, 3 \(\times\) 10\textsuperscript{8} platelets/mL were used. From these, it was calculated that ADP in the external medium increased to approximately 400 pmol/mL (400nM) following stimulation of control platelets with the highest concentration of collagen (10 \(\mu\)g/mL), provided released ADP was uniformly diffused in the medium. Adenosine diphosphate at this concentration (400nM) was below the threshold for inducing aggregation when ADP was added exogenously. However, assuming that ADP concentration in the vicinity of platelet membranes was higher than the mean concentration in the external medium, ADP may have activated platelets by itself when the highest concentration of collagen was applied to control platelets. Release of ADP by collagen at 1 \(\mu\)g/mL was approximately 40% of that induced by collagen at 10 \(\mu\)g/mL. Therefore, it is more probable that ADP released by collagen at 1 \(\mu\)g/mL, which was used to evaluate the involvement of ADP and TXA\textsubscript{2}, was not enough to cause platelet aggregation by its own action.

Collagen at 1 and 10 \(\mu\)g/mL caused aggregation to a similar extent (approx 70%) in control and CHS platelets. Either aspirin or ADP receptor antagonists (A3P5PS and AR-C66096) greatly inhibited control platelet aggregation, which was induced by collagen at...
By contrast, in CHS platelets, each treatment halved aggregation, which was induced by collagen at 10 µg/mL. These results indicated that the aggregation of control platelets mostly depended on a cyclooxygenase product (TXA₂) and ADP released by collagen, whereas the aggregation of CHS platelets involved a TXA₂- and ADP-dependent component and an independent component. The greater inhibition by aspirin or ADP receptor antagonists in control platelets than in CHS platelets indicated that the involvement of endogenous agonists in collagen-induced aggregation was less in CHS platelets than in control platelets.

Despite the fact that control platelet aggregation induced by collagen at 1 µg/mL was dependent on ADP and TXA₂, it was almost abolished by either aspirin or ADP receptor antagonists. Similarly, the inhibitory effect of a single application of aspirin or ADP receptor antagonists on CHS platelet aggregation was similar to the effect induced by a combination of all inhibitors. Namely, when either of the effects produced by the 2 endogenous agonists, TXA₂ and ADP, was blocked, the effect dependent on the other agonist was not evident in response to an intermediate concentration of collagen. Abolishment of the ADP-dependent component of platelet aggregation in response to aspirin was not a consequence of inhibition of ADP release because it was unlikely that TXA₂ stimulated ADP release. More probably, ADP did not cause platelet aggregation by itself when TXA₂ was absent.

The action of ADP can be potentiated by other agonists such as epinephrine, serotonin, or thrombin. In the study reported here, an alternative possibility for abolishment of the ADP-dependent component of platelet aggregation by aspirin was that TXA₂ and ADP release in response to an intermediate concentration of collagen was too low to induce aggregation, but they did cause platelet aggregation when both agonists were present. Certainly, a combination of subthreshold U46619 (1 µM) and ADP (0.3 µM), which were applied exogenously, caused platelet aggregation of approximately 40%. Synergism exists between the actions of ADP and TXA₂ in human or mouse platelets. An important finding in the study reported here was that ADP or TXA₂, released in response to a low or intermediate concentration of collagen was insufficient to cause platelet aggregation on their own, but they were able to act together synergistically to cause platelet aggregation. Besides the synergism between ADP and TXA₂ to cause platelet aggregation, ADP and collagen also worked synergistically in the study reported here.

In the study reported here, production of TXB₂ in response to collagen at 1 and 10 µg/mL was modestly decreased in CHS platelets, whereas it was not significantly different between control and CHS platelets when challenged with collagen at 30 µg/mL. Jin et al. reported that ADP stimulates the arachidonic acid cascade, producing TXA₂ in human platelets, although an argument against this finding also exists. Decreased production of TXA₂ in CHS platelets in response to collagen at 1 or 10 µg/mL could be explained by the decreased amount of ADP released from CHS platelets in response to a low or intermediate concentration of collagen.

Figure 5—Effect of the combination of U46619 and ADP on aggregation of control and CHS platelets. A—Typical tracings of platelet aggregation induced by ADP (1 µM), U46619 (0.1 µM), and a combination of ADP (1 µM) and U46619 (0.1 µM). B—Summarized data for platelet aggregation induced by ADP alone, U46619 alone, and a combination of ADP and U46619. Data are mean ± SEM of 5 or 6 experiments. *Significantly (P < 0.05) different from ADP or U46619 alone. See Figure 1 for remainder of key.

Figure 6—Effects of a combination of collagen and ADP and of collagen and U46619 on control platelet aggregation. A—Collagen (0.2 µg/mL) was combined with subthreshold ADP (0.3 µg/mL) in the presence of aspirin (1 mM) to exclude involvement of TXA₂. B—Collagen (1 µg/mL) was combined with U46619 (1 µM) in the presence of ADP receptor antagonists A3P5PS (100 µM) and AR-C66096 (100 nM) to exclude involvement of endogenous ADP. Data are mean ± SEM of 5 experiments. *Significantly (P < 0.05) different than collagen alone.
response to collagen. Alternatively, a smaller increase in cytosolic Ca²⁺ concentration following stimulation with collagen⁹,¹⁰ may be responsible for smaller TXB₂ production in CHS platelets than in control platelets. Considering the fact that CHS platelets were much less sensitive to collagen than were control platelets, the collagen-induced TXB₂ production in CHS platelets was unexpectedly high. This was emphasized by the result that collagen significantly increased TXB₂ production in CHS platelets at 1 µg/mL, a concentration at which collagen causes neither aggregation nor an increase in cytosolic Ca²⁺ concentration.⁶

Likewise, the maximum ATP release induced by collagen (30 µg/mL) in CHS platelets was similar to that induced by thrombin (0.1 U/mL). Therefore, the reduction of collagen-induced ATP and ADP release may not be attributable to a decrease in the signal from collagen receptors to stimulate dense granule release, but rather to a direct result of a decrease in nucleotide content in CHS platelets.³,⁴,¹³,¹⁶ Data of the study reported here indicated that the collagen signal to produce TXA₂, or to release ADP from dense granules in CHS platelets was not impaired. On the contrary, the direct action of collagen is inhibited in platelets from cattle with CHS because collagen-induced cytosolic increases in Ca²⁺ concentration¹³ and platelet aggregation in the presence of ADP receptor antagonists and aspirin are weak, compared with those in platelets from healthy cattle. Therefore, divergent pathways may exist downstream of collagen receptors, one of which links to the release or generation of secondary agonists and the other of which is related to direct Ca²⁺ mobilization, with impairment of the latter in platelets from cattle with CHS.

Bovine platelets are insensitive to TXA₂,¹⁴,¹⁵ In fact, U46619 up to 10µM caused a slight increase in cytosolic Ca²⁺, but not platelet aggregation.⁹ As discussed, however, TXA₂, is likely to play a role in aggregation of bovine platelets if ADP is present. Synergism between ADP and TXA₂ is important in bovine platelets because they are insensitive to TXA₂ alone. Unlike the interaction with ADP, TXA₂ does not seem to have a cooperative relationship with collagen because U46619 and collagen at low concentrations did not result in potentiation of platelet aggregation in the study reported here.

Taken together, results of the study reported here indicated that ADP release induced by an intermediate concentration of collagen did not cause platelet aggregation by itself, but may play multiple roles with other agonists, including synergism with collagen and TXA₂. Platelet aggregation was induced by ADP alone when large amounts were released in response to high concentrations of collagen. In platelet aggregation, an interrelationship was found between collagen, ADP, and TXA₂. Furthermore, results of the study reported here indicated that TXA₂ was important for aggregation of bovine platelets.

References


