Effect of Bromelain (Ananase®) on Human Platelet Aggregation[1]

Bromelain, a protease mixture prepared from the stems of mature pineapple plants[2], has been used clinically for the reduction of inflammation[3]. In spite of its widespread use, the pharmacodynamics of bromelain are still unknown. In a recent paper[4] we reported that oral ingestion of 100 mg of enteric coated bromelain neither caused any significant change in any of the standard measurements of clinical laboratory tests, nor affected the serum level of antitrypsin, antichymotrypsin or antisulfhydrylprotease. These antienzymatic substances might be expected to reduce inflammation by inhibiting the lysosomal proteases which are released at the site of injury[5].

Previous studies at this laboratory[6,7] showed that the platelet aggregation rate for patients with thromboembolic diseases was significantly higher than it was for a comparable normal age group. The aim of this study was to measure the effect of enteric coated bromelain, orally ingested by volunteers, on the sensitivity of platelets to adenosine diphosphate (ADP) induced aggregation.

Materials and methods. The platelet aggregation test followed the method described by YAMAKIDO et al.[6] and SANO et al.[7]. 8% sodium citrate was used as an anticoagulant at the ratio of one part of citrate solution to nine parts of blood. The mixture was centrifuged at 170 *g* for 10 min to obtain a platelet rich plasma (PRP).

Stock adenosine diphosphate (ADP) solutions were prepared by dissolving 10 mg ADP (Calbiochem, Los Angeles, Calif. 90054) in 10 ml of 0.05 *M tris*hydroxylmethyl aminomethane (*Tris*) buffer. The stock ADP solution was then diluted by a serial two-fold dilution system. One drop of each of the ADP dilutions and 1 drop of PRP were mixed in a plastic tube which was shaken for 1 min on a Vortex mixer.

The platelet aggregation patterns in each of the ADP dilutions were observed under a microscope at 400× magnification. The aggregation patterns were classified into 6 grades as previously described[6,7]. Grade 0: no clumping of platelets; Grade I: small clumps of 3 to 5 platelets scattered among free platelets; Grade II: small masses of the clumps seen in grade I scattered among free platelets: Grade III: similar masses with only a few free platelets; Grade IV: large masses of platelets without a circumscribing marginal line; Grade V: large platelet masses with a marginal line. Grade 0 and I were considered as negative while grade II and above were taken as positive. The absolute value of the exponent of the minimum ADP concentration (2^{-n} mgADP/ml) required to induce aggregation (tube number, *n*, corresponded to this value) was taken as the value of platelet sensitivity. For example, when the endpoint was 2^{-13} mg/ml, the sensitivity was expressed as 13.

Most of the volunteers with high aggregation values were selected from blood bank donors who had been previously tested for platelet sensitivity. In addition the test included several patients who had myocardial infarction or stroke within the past year.

A blood sample was taken from each volunteer before and 2 h after the oral administration of 2 Ananase® '100' tablets. In a few pilot studies, a 3rd blood sample was also taken from the subjects 5 h after the drug administration.

Ananase® '100' tablets were purchased from William H. Rorer Co., Fort Washington, Pa. Each tablet is standardized to contain a specific amount of protease activity.

Results and discussion. According to our previous studies[6,7] the platelet aggregation value for 'normal' individuals ranged from 8 to 13. In our present study, approximately 5% of the random donors had aggregation values higher than 13 (Figure 1). Donors with high aggregation values were chosen for the study of the effect of bromelain on platelet sensitivity. However, during the interval between the original platelet aggregation survey and the bromelain test, which amounted in some instances to 2 months, a drop in aggregation values occurred in some individuals.

Oral administration of 2 bromelain tablets rapidly and significantly reduced the platelet sensitivity to ADP induced aggregation (Figure 2). The figure shows a marked reduction of aggregation values in those individuals who initially had the higher values.

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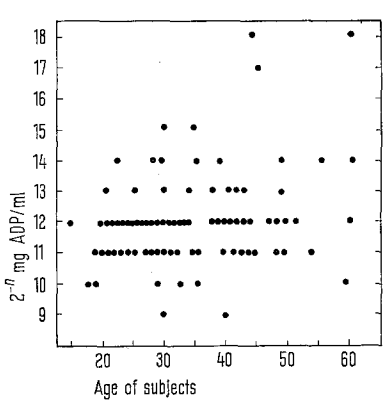

Fig. 1. Distribution of platelet sentitivity rate to ADP in different age groups of the subjects screened for this study. Most of the subjects were volunteer blood donors.

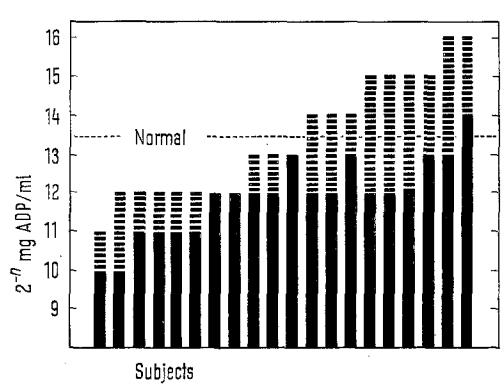

Fig. 2. Change of platelet aggregation rate after oral administration of Ananase® '100' tablets. The solid bar represents the aggregation rate 2 h after the drug ingestion and the discontinuous bar indicates the decrease of the rate from the initial value.

Previous work from our laboratory[6,7] has shown that a high platelet aggregation value is generally associated with certain stress conditions. The rate was high, 15–18, in myocardial infarction, moderately high, 13–18, in cerebrovascular disease, and slightly elevated, 11–14, in cases of diabetes mellitus.

The finding that bromelain significantly reduces high platelet aggregation values of individuals has important clinical implications. Our previous studies[4] indicated that bromelain (administered as Ananase®) did not significantly affect any of the normally measured clinical parameters. Also in its clinical use over a period of more than 10 years, there have been no reports of significant undesirable side effects. This drug would then seem to be worthy of testing as a long term maintenance drug for those individuals who have problems with enhanced platelet aggregation rates.

Zusammenfassung. Die orale Verabreichung von Bromelain in Form von 2 Ananase®-100-Tabletten an freiwillige Versuchspersonen verminderte bei diesen die Empfänglichkeit für die durch ADP induzierte Aggregation der Blutplättchen.

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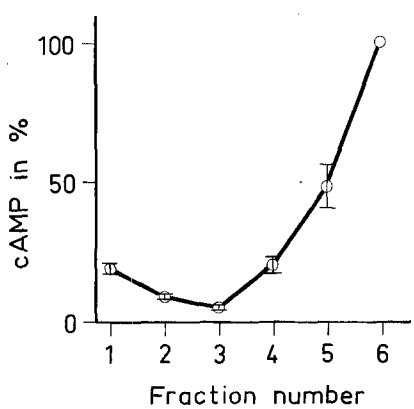
Cyclic AMP of Blood Platelets: Accumulation in Organelles Storing 5-Hydroxytryptamine and ATP

Cyclic 3′,5′-adenosine monophosphate (cAMP) and prostaglandin-activated adenyl cyclase occur in human blood platelets, but their subcellular localization is not known[1–4]. In platelets of rabbits, guinea-pigs, and probably man, non-cyclic phosphonucleotides (e.g. adenosine-5′-triphosphate, ATP) have recently been shown to accumulate in subcellular organelles which also store 5-hydroxytryptamine (5HT)[5–7]. It was therefore of interest to investigate whether cAMP shows a similar subcellular localization as its precursor ATP.

Materials and methods. Blood platelets of rabbits and guinea-pigs were homogenized by ultrasonication, and thereafter the particulate matter (mitochondria, α-granules, 5HT-nucleotide storage organelles, etc.) was subjected to ultracentrifugation in a continuous Urografin® density gradient as previously described[5]. The liquid content of the centrifugation tube was divided into 5 equal parts. Fractions 3 and 4 consisted mainly of mitochondria and α-granules respectively. The fraction containing the organelles storing 5HT and nucleotides was localized as a fine film at the bottom of the tube (fraction 6). It showed virtually no contamination with other subcellular particles as judged by electron microscopy[7]. The level of cAMP in the homogenates of whole platelets, in the isolated storage organelles as well as in the other subcellular fractions (of rabbit platelets only) was determined by a saturation method[8] and by a radioimmuno-assay[9] and related to the amounts of protein[10]. In some experiments, the total nucleotide extract of storage organelles was submitted to chromatography on Dowex AG 50 WX 4, 200–400 mesh. The cAMP of the eluate was assayed[8] before and after incubation at 37°C for 15 min with 3′,5′-cyclic nucleotide phosphodiesterase isolated from rat brain[11].

Results and discussion. The cAMP in the various subcellular fractions of rabbit platelets showed a similar distribution to that previously reported for ATP and 5HT[7,12]. The concentration of cAMP (in *p*moles/mg protein) in the pure 5HT-nucleotide storage organelles markedly exceeded that in the other subcellular fractions (Figure). It was more than 100 times higher than in homogenates of whole platelets (Table). In guinea-pigs,



Subcellular distribution of cyclic AMP (cAMP) in the particulate matter of blood platelets of rabbits determined by the saturation method. The values indicate the cAMP content in *p*moles/mg protein and are expressed in percentages of the values found in fraction 6, i.e. the pure storage organelles (= 100). Averages with S.E. of 8 experiments.

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