



FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Increased number of thromboxane A2-prostaglandin H2 platelet receptors in active unstable angina and causative role of enhanced

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Increased number of thromboxane A2-prostaglandin H2 platelet receptors in active unstable angina and causative role of enhanced thrombin formation / P.A.MODESTI; COLELLA A; CECIONI I; COSTOLI A; BIAGINI D; MIGLIORINI A; NERI SERNERI GG. - In: AMERICAN HEART JOURNAL. - ISSN 0002-8703. - STAMPA. - 129:(1995), pp. 873-879. [10.1016/0002-8703(95)90106-X]

Availability:

The webpage https://hdl.handle.net/2158/213789 of the repository was last updated on

Published version: DOI: 10.1016/0002-8703(95)90106-X

Terms of use: Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf)

Publisher copyright claim:

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The abovementioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

Increased number of thromboxane A₂-prostaglandin H₂ platelet receptors in active unstable angina and causative role of enhanced thrombin formation

The current study was designed to investigate the number and affinity of platelet thromboxane A₂/prostaglandin H₂ (TxA₂/PGH₂) receptors in patients with unstable angina and, if any, the role played by the increased thrombin formation that is a common finding in these patients. Measurements taken during active unstable angina but not those taken during inactive angina showed an increased number (p < 0.001), without changes in affinity, of platelet TxA₂/PGH₂ receptors, evaluated as the binding capacity of iodine 125–PTA-OH, a stable TxA₂ analogue. Moreover patients with active angina had higher plasma concentrations of fibrinopeptide A (FPA) (p < 0.0001), which were significantly related to the number of platelet TxA₂/PGH₂ receptors (r = 0.76; p < 0.01). Heparin infusion but not aspirin treatment promptly normalized the number of TxA₂/PGH₂ receptors and significantly reduced plasma FPA concentrations. In an in-vitro study thrombin in a concentration similar to that found in vivo significantly increased the number of platelet TxA₂/PGH₂ receptors. These results have important therapeutic implications and indicate the preferential use of heparin rather than aspirin during the acute phase of unstable angina. (AM HEART J 1995;129:873-9.)

Pietro Amedeo Modesti, MD, PhD, Andrea Colella, MD, Ilaria Cecioni, MD, Alessandro Costoli, MD, Debora Biagini, MD, Angela Migliorini, MD, and Gian Gastone Neri Serneri, MD *Florence*, *Italy*

Several studies have shown that in patients with unstable angina platelet activation is increased,^{1, 2} frequently resulting in enhanced thromboxane A₂ (TxA₂) formation and increased platelet aggregation.^{3, 4} Moreover, a decreased number of platelet receptors for prostacyclin I₂⁵ (PGI₂) and prostaglandin E₁⁶ has been found in patients with unstable angina and an increased density of platelet TxA₂/prostaglandin H₂ (PGH₂) receptors in patients with acute myocardial infarction.⁷ The increased TxA₂ formation by platelets in patients with unstable angina has been found to be associated with changes in the fatty acid content of platelet membranes⁸ that might result in functional changes in platelet binding sites.

From the Clinica Medica I, Center for Heart and Thrombosis Research, University of Florence.

Received for publication Feb. 16, 1994; accepted Aug. 2, 1994.

Reprint requests: Pietro Amedeo Modesti, MD, PhD, Clinica Medica I, Center for Heart and Thrombosis Research, University of Florence, Viale Morgagni 85, 50134 Florence, Italy.

Copyright © 1995 by Mosby-Year Book, Inc.

0002-8703/95/\$3.00 + 0 **4/1/61645**

In addition to these functional changes in platelet activity, patients with unstable angina frequently show enhanced thrombin formation, as indicated by increased plasma concentrations of fibrinopeptide A (FPA).⁹⁻¹¹ Thus in patients with unstable angina links might exist among the altered fatty acid composition of platelets, membrane changes in platelet functional activities, and increased thrombin generation. The current study was designed to investigate in vivo and in vitro whether the number or affinity of platelet receptors for TxA_2/PGH_2 is changed in patients with unstable angina and whether the increased thrombin formation may be responsible for any changes.

METHODS

Study population. Participating in the investigation were 21 patients with primary unstable angina with the most recent painful episode having occurred <24 hours before admission to the hospital (active phase), 18 patients who had had unstable angina but who had been free of symptomatic or asymptomatic (by Holter monitoring) ischemic episodes for ≥ 8 weeks (inactive phase), and 11 pa-

	Control subjects (n = 33)	Patients with unstable angina		
		Active (n = 21)	Unactive (n = 18)	Patients with effort angina (n = 11)
Age (yr)	58 ± 9	62 ± 9	59 ± 10	61 ± 10
Weight (kg)	65 ± 10	69 ± 12	67 ± 9	69 ± 12
Fasting blood glucose (mg/dl)	103 ± 6	108 ± 5	$105~\pm~4$	109 ± 8
Triglycerides (mg/dl)	126 ± 19	$141~\pm~33$	132 ± 15	145 ± 23
Total cholesterol (mg/dl)	186 ± 32	$191~\pm~26$	196 ± 38	189 ± 36
High-density lipoprotein cholesterol (mg/dl)	49 ± 9	45 ± 11	$44~\pm~12$	39 ± 11
Platelets $(\times 10^{6}/\text{ml})$	$240~\pm~36$	$233~\pm~36$	229 ± 39	231 ± 33
Megathrombocytes (%)	6.8 ± 1.8	10.1 ± 2.2	7.1 ± 1.8	6.9 ± 1.9
Protein $(mg/10^9 \text{ platelets})$	$3.7~\pm~0.3$	3.9 ± 0.4	3.8 ± 0.2	3.7 ± 0.3

Table I. Patient characteristics

tients with stable angina (effort angina). As control subjects 33 apparently healthy persons of similar ages were studied. None of the control subjects had taken any drugs for ≥ 2 weeks. Patients with angina were excluded from the study if they had enzymatic or electrocardiographic (ECG) evidence of myocardial infarction; were older than 75 years; or had diabetes, immunologic disorders, or neoplastic disease. Subjects also were excluded if they had undergone surgical or invasive procedures in the month preceding the study. Characteristics of the subjects are reported in Table I.

Unstable angina was defined as chest pain occurring at rest or on minimal effort (eg, washing, speaking, or combing) without any increase in creatine kinase MB fraction, with ECG evidence of myocardial ischemia (transient STsegment displacement >0.1 mV during chest pain), and with angiographic evidence of coronary artery disease. Platelet TxA₂/PGH₂ receptors were investigated when patients with active angina were taking only nitrates (isosorbide dinitrate, six 10 mg tablets daily) and before administration of aspirin or heparin or other drugs. In eight patients platelet TxA₂/PGH₂ receptors also were investigated before and after 48-hour continuous intravenous infusion of heparin, priming dose 5000 IU followed by 800 to 1000 IU/hr. In another eight patients TxA₂/PGH₂ receptors were investigated before and after aspirin, 325 mg/day for 3 days.

In all patients coronary angiography was performed after the assessment of platelet receptors. Ten of the 21 patients with active-phase unstable angina were studied again 8 to 12 weeks later during the inactive phase. Eight additional patients with unstable angina in the inactive phase also were investigated at this time. All patients in the inactive phase were taking nitrates (isosorbide dinitrate, 10 mg tablets three or four times per day), and aspirin had been stopped ≥ 2 weeks before the study.

Stable effort angina was defined according to the following criteria: (1) typical angina on effort and no angina at rest during at least the previous 3 months; (2) no asymptomatic ischemic episodes at rest during 3-day Holter monitoring; (3) stable ischemic threshold during at least three stress exercises in the week preceding the study; and (4) angiographic evidence (at least one stenosis >70% of vessel diameter) of coronary artery disease. Patients with effort angina had been taking nitrates for ≥ 1 week before the study. All other drugs were stopped ≥ 2 weeks previously. Nine patients with stable effort angina and 9 control subjects were studied again 8 to 12 weeks later.

All patients gave informed consent to use of part of their blood samples for an experimental study.

Blood sampling and platelet isolation. Blood samples (50 to 60 ml) were collected with a 19-gauge siliconized needle in a syringe containing indomethacin (10 μ mol/L) and acid citrate dextrose (National Institutes of Health formula A) (15% volume/volume). Washed platelets were prepared by sequential centrifugation and resuspended in assay buffer (138 mmol/L NaCl, 5 mmol/L MgCl₂, 1 mmol/L EGTA and 25 mmol/L Tris/HCl, pH 7.5) to 10⁹ platelets/ml as previously described.⁵

Platelet TxA₂/PGH₂ receptor assay. TxA_2/PGH_2 receptors were investigated with 9,11-dimethyl- methano-11,12methano-16(3[¹²⁵I]-4-hydroxyphenyl)-13,14-dihydro-13aza-15-tetranor-TxA₂ (¹²⁵I-PTA-OH) (Amersham, Buckinghamshire, Great Britain) (2000 Ci/mmol), a stable labeled analogue of TxA₂, and with ONO11120, the unlabeled dehydroxylated form of ¹²⁵I-PTA-OH (a gift from S. Narumiya, MD, Kyoto, Japan) as a cold displacer. In brief, 5×10^7 washed platelets were incubated in a final volume of 0.2 ml for 10 minutes at room temperature with increasing concentrations of ONO11120 (0 to 4×10^{-6} mol/L) and a fixed concentration of ¹²⁵I-PTA-OH, 0.05 nmol/L (final concentrations [fc]). Bound radioactivity was separated by rapid filtration under reduced pressure through glass microfiber filters (GF/C, Whatman, Maidstone, England). Previous time course experiments showed that in these conditions binding equilibrium had been reached.¹² The entire washing procedure was completed within about 15 seconds. Nonspecific binding was defined as the residual binding after the addition of ONO11120 2×10^{-5} mol/L (fc). Nonspecific binding of 0.05 nmol/L ¹²⁵I-PTA-OH was 30% to 40% of total binding. The experiments were carried out in triplicate.

The binding affinity (dissociation constant $[K_d]$) and maximum binding capacity (B_{max}) were obtained from a



Fig. 1. Saturation curves and Scatchard plots (inset) corrected for nonspecific binding in control subjects (open diamonds), patients with unstable angina in active phase (open squares) and in inactive (solid diamonds) phase, and patients with effort stable angina (solid squares). plt, Platelets.

nonlinear regression analysis of equilibrium binding performed by a computerized iterative, least-squares algorithmic analysis on a microcomputer according to Scatchard.¹³

Effects of thrombin on platelet ¹²⁵I-PTA-OH binding. To determine the effects of thrombin (from human plasma, SIGMA, St. Louis, Mo.) on platelet TxA₂/PGH₂ receptors, 0.1 ml platelet suspension was incubated in a final volume of 0.2 ml with ¹²⁵I-PTA-OH 0.05 nmol/L plus ONO11120 in increasing selected concentrations (0 to 4×10^{-6} mol/L) in the presence and in the absence of thrombin in increasing concentrations (0, 0.005, 0.01, and 0.05 U/ml). After a 10-minute incubation at room temperature samples were processed as previously described. Similarly the effect of increasing concentrations of unfractionated sodium heparin (Liquemin, Roche, Milan, Italy) (0 to 2 IU/ml) on ¹²⁵I-PTA-OH binding to platelets was assessed.

FPA assay. FPA was evaluated by enzyme-linked immunosorbent assay according to Gaffney et al.¹⁴ and Soria et al.¹⁵ with commercial kits (Boehringer Mannheim, Milan, Italy). The intraassay and interassay variation coefficients were 5.9% and 7.8%, respectively.

Statistics. If not otherwise indicated, all data are given means ± 1 SD of *n* experiments. The receptor numbers and the dissociation constants at each time were compared by analysis of variance and Student's t test for paired or unpaired data. Plasma FPA concentrations in the various groups studied were compared by the Kruskal-Wallis test. Correlation between FPA concentrations and platelet receptor number in the whole population was estimated by Kendall and Spearman rank correlation. All statistical analyses were performed with BMDP (Los Angeles, Calif.) statistical software.

RESULTS

¹²⁵I-PTA-OH binding to platelets. ¹²⁵I-PTA-OH bound to platelets from control subjects with a B_{max} of 303 ± 83 fmol/10⁸ platelets, corresponding to

7000 6000 TxA2/PGH2 receptors/platelet 5000 4000 3000 2000 1000 0 UA EΑ Controls UA n=11 n=33 (active (inactive phase) phase)

Modesti et al.

875

Fig. 2. Number of TxA_2/PGH_2 receptors per platelet in control subjects and in patients with unstable angina (UA) or stable effort angina (EA).

n = 21

n=18

1830 \pm 498 sites/platelet, and with a K_d of 30 \pm 7 nmol/L (Fig. 1). In comparison, in patients with active unstable angina the number of binding sites was significantly greater ($B_{max} = 539 \pm 188$ with 3247 ± 1132 sites/platelet, p < 0.001; K_d 30.5 ± 6 nmol/L, difference not significant [NS] (Figs. 1 and 2) and was greater than that in patients with stable effort angina ($B_{max} = 364 \pm 58 \text{ fmol}/10^8$ platelets with 2194 \pm 349 sites/platelet, p < 0.001; K_d = 30 \pm 9 nmol/L, NS), whose results did not significantly differ from those of control subjects. The increased number of platelet binding sites for TxA₂/PGH₂ found in patients with active unstable angina was not associated with changes of affinity, as indicated by the lack of differences in K_d.

In patients with inactive unstable angina the number of binding sites ($B_{max} = 313 \pm 96 \text{ fmol}/10^8$ platelets with 1889 \pm 577 sites/platelet) was lower than that of patients with active angina (p < 0.001)and was similar to that of control subjects (Figs. 1 and 2). In particular, in all 10 patients initially studied in the active phase and studied again in the inactive phase the number of binding sites was significantly reduced (from 2895 ± 735 sites/platelet, $B_{max} =$ 480 ± 122 fmol/10⁸ platelets to 1688 ± 471 sites/ platelet, $B_{max} = 280 \pm 78$ fmol/10⁸ platelets; p < 0.002) and was not different from that of control subjects (Fig. 3). On the contrary no significant changes were observed when patients with effort angina and control subjects were studied again 8 to 12 weeks later (Fig. 3).



Fig. 3. Number of TxA_2/PGH_2 receptors per platelet in patients with unstable angina studied in active phase (A) and 8 to 12 weeks later in inactive phase (B). Patients with effort stable angina and control subjects also were studied at baseline (A) and 8 to 12 weeks later (B).

All patients with active unstable angina showed increased thrombin generation, as indicated by increased plasma FPA concentrations. Plasma FPA levels in patients with active unstable angina $(8.34 \pm 3.96, \text{ median } 8.39, \text{ range } 2.1 \text{ to } 14.9 \text{ ng/ml})$ were significantly higher than those in control subjects $(1.79 \pm 0.69, \text{ median } 1.79, \text{ range } 0.8 \text{ to } 3.4$ ng/ml; z = 6.04, p < 0.05) or those in patients with effort angina (1.97 \pm 0.99, median 1.97, range 0.6 to 4.3 ng/ml; z = 4.36, p < 0.05). In patients with inactive unstable angina the plasma FPA concentrations were lower than those in patients in the active phase (z = 4.72, p < 0.05) and were similar to those in control subjects (2.17 \pm 1.21, median 2.17, range 1 to 5 ng/ml). In particular all 10 patients with active angina studied again 8 to 12 weeks later in the inactive phase showed a significant reduction of plasma FPA concentrations (from median 7.92, range 2.2 to 12.3 ng/ml to median 1.96, range 0.6 to 4.2 ng/ml; p = 0.003), at this time not differing from control subjects. A significant correlation was observed between the plasma FPA concentrations and the $TxA_2/$ PGH_2 receptor number (n = 83, Kendall rank correlation coefficient = 0.32, p < 0.01; Spearman rank correlation coefficient = 0.44, p < 0.001) (Fig. 4).

No differences in the platelet count was observed among the different groups (Table I). Patients with active unstable angina had an higher percentage of megathrombocytes when compared with patients with effort angina and to controls (Table I).

Effect of heparin infusion on ¹²⁵I-PTA-OH binding in unstable angina. Heparin infusion significantly decreased the plasma FPA concentrations in all patients (from 8.61 ± 1.96 to 2.07 ± 1.21 ng/ml,



Fig. 4. Spearman rank correlation (r_s) between plasma FPA concentrations and number of TxA₂/PGH₂ receptors per platelet in all subjects (n = 83).

p < 0.0001), with a parallel reduction in platelet TxA₂/PGH₂ binding sites (from 3407 ± 901 sites/ platelet to 1819 ± 266 sites/platelet, p < 0.001). The affinity of the binding sites was not significantly changed (K_d from 29.6 ± 8.6 to 26.9 ± 5.6 nmol/L, NS). On the contrary in none of the patients treated with aspirin did the number of platelet TxA₂/PGH₂ receptors decrease (from 3500 ± 527 to 3498 ± 577 sites/platelet, NS). In five of eight patients the ¹²⁵I-PTA-OH binding capacity was even increased (Fig. 5).

Effects of thrombin and heparin on ¹²⁵I-PTA-OH binding in vitro. To investigate whether thrombin may be involved in the increased exposition of TxA_2/PGH_2 platelet binding sites, normal platelets were incubated with increasing concentrations of thrombin. After a 10-minute incubation thrombin induced a significant increase in platelet ¹²⁵I-PTA-OH binding. The number of receptors increased from 1889 \pm 798 sites/platelet in a control sample to 3534 ± 1033 sites/platelet (p < 0.01) in platelets incubated with thrombin 0.05 U/ml (Fig. 6). On the contrary heparin in concentrations ≤ 2 IU/ml affected neither the number nor the affinity of platelet TxA_2/PGH_2 receptors. In the presence of heparin 2 IU/ml the receptor number passed from 2160 \pm 414 to 2223 \pm 452 sites/platelet (NS).

DISCUSSION

The current results indicate that the number of platelet TxA_2/PGH_2 receptors was significantly increased in patients with active unstable angina but not in patients with inactive angina in comparison with control subjects and patients with effort angina,

FPA (ng/ml)



Fig. 5. Effect of heparin, continuous intravenous infusion 800 to 1000 IU/hr, or aspirin per os, 325 mg/day (ASA), on number of TxA₂/PGH₂ receptors per platelet and on plasma FPA concentrations in eight patients with active unstable angina. *Bas.*, Baseline.



Fig. 6. Effects of increasing concentrations of thrombin (0 to 0.05 U/ml) or heparin (0 to 2 IU/ml) on number of TxA_2/PGH_2 receptors per platelet. *p < 0.05, **p < 0.001.

and that enhanced thrombin formation seems to be responsible for the increased number of platelet TxA_2/PGH_2 receptors. The increased TxA_2/PGH_2 binding density in patients with active unstable angina was confined to the active phase of disease because the number of binding sites was similar to that in control subjects when the same patients were studied 8 to 12 weeks later, in the inactive phase of the disease.

The increase in platelet binding sites might only be apparent in relation to the increased number of megathrombocytes found in current and previous studies.^{4, 5, 16} In patients with active unstable angina, however, the average increase (3.3%) in megathrombocytes in patients with active unstable angina is not enough to justify the increased number of TxA_2/PGH_2 binding sites/platelet (from 6.8% in control subjects to 10.1% in patients with angina). In fact TxA_2/PGH_2 receptors were found on average at 1830 sites/platelet in control subjects; even if the mega-thrombocytes from patients with unstable angina had a number of receptors 10 times higher than that of platelets, the average number of binding sites per platelet could increase no more than 25% instead of the 75% found (from 1830 ± 498 in control subjects to 3247 ± 1132 sites/platelet in patients with angina). Moreover, heparin infusion did not significantly change the number of megathrombocytes but quickly induced a significant decrease in platelet binding capacity. It is stressed that this finding cannot be the

result of a direct effect of heparin on platelet binding sites because heparin in vitro did not affect TxA_2/PGH_2 receptors. Therefore the increased number of TxA_2/PGH_2 receptors in patients with active unstable angina actually results from enhanced exposition of platelet binding sites.

Increased thrombin generation seems to be responsible for the enhanced number of TxA_2/PGH_2 platelet receptors. This hypothesis is supported by (1) the normality of TxA₂/PGH₂ receptor number in patients with normal plasma FPA concentrations; (2) the close relation between FPA concentration in plasma and TxA_2 receptor number; (3) the decrease in TxA₂/PGH₂ receptor number after heparin administration simultaneously with the decrease in thrombin formation, as indicated by the reduction in the plasma FPA concentrations; and (4) the capability of low or very low thrombin concentrations to induce in vitro an increase in the number of $TxA_2/$ PGH₂ receptors in platelets from controls, whereas heparin did not affect TxA₂/PGH₂ binding sites. These arguments strongly indicate a causative role for thrombin.

Thrombin exerts various effects on the cell membrane, modifying the phospholipid composition by direct activation of phopholipases $(A_2, C, and D)^{17}$ and modifying the physical array of the lipidic leaflet as a consequence of the enhanced aminophospholipid translocase activity.^{18, 19} Moreover thrombin induces release of lysophosphatidylcholine from plasma membrane at the same concentration found to enhance in vitro exposition of TxA₂/PGH₂ platelet receptors.²⁰ This phospholipid fraction contains polyunsaturated fatty acid, especially docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3), that play a relevant role in the modulation of the TxA₂/PGH₂ receptor exposition and function.^{21, 22} In thrombin-stimulated platelets 20:5n-3 and 22:6n-3 are released from most of the phosphatidylcholine fractions.²³ A previous study by our group⁹ demonstrated that 20:5n-3 and 22:6n-3 were significantly reduced in membrane phospholipid fractions, especially in the phosphatidylcholine of platelets from patients with active unstable angina. Thus thrombin can enhance exposition of TxA₂/PGH₂ receptors on the platelet membrane by reducing the membrane's content of polyunsaturated fatty acid.

Another mechanism underlying the increased number of the platelet TxA_2/PGH_2 receptors may be the oxidation of platelet membrane with formation of disulphide bonds during the passage of the platelet through myocardial ischemic areas. The formation of disulfide bonds has been found to increase rapidly the number of TxA_2/PGH_2 receptors.²⁴

The current results have important pathophysiologic and therapeutic implications. From the pathophysiologic point of view the increased number of $T_{x}A_{2}/PGH_{2}$ platelet receptors can further favor platelet aggregation and thrombus formation in patients with active unstable angina by means of enhanced TxA_2 formation by platelets²⁵⁻²⁷ and activated monocytes.²⁸ The therapeutic implications seem to be more relevant because the causative role of thrombin in the enhancement of platelet binding sites can be better controlled by anticoagulant drugs rather than by antiaggregating drugs. Aspirin inhibits platelet aggregation by blocking TxA₂ synthesis but has little effect on thrombin-induced platelet aggregation and in the current study was unable to reduce the increased ¹²⁵I-PTA-OH binding capacity. Heparin in contrast is able to inhibit thrombin formation and thrombin-induced platelet activation. This assessment is supported by both the demonstrated efficacy of heparin in controlling myocardial ischemia in the active unstable angina^{29, 30} and by the observation that heparin prevents myocardial infarction better than does aspirin during the acute phase of unstable angina.³¹ Thus heparin or specific TxA₂/ PGH₂-receptor-blocking agents seem to be preferable to aspirin for the treatment of patients with acute unstable angina.

REFERENCES

- Sobel BM, Salzman EW, Davies GC, Handin RI, Sweeney J, Ploetz J, Kurland G. Circulating platelet products in unstable angina pectoris. Circulation 1981;63:300-6.
- Hamm CW, Lorenz RL, Bleifeld W, Kupper W, Wober W, Weber PC. Biochemical evidence of platelet activation in patients with persistent angina. J Am Coll Cardiol 1987;10:998-1004.
- FitzGerald DJ, Roy L, Catella F, FitzGerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986;315:983-9.
- 4. Neri Serneri GG, Gensini GF, Abbate R, Mugnaini C, Favilla S, Brunelli C, Chierca S, Parodi O. Increased FPA formation and thromboxane A₂ production in patients with ischemic heart disease: relationships to coronary pathoanatomy, risk factors and clinical manifestations. AM HEART J 1981;101:185-94.
- Neri Serneri GG, Modesti PA, Fortini A, Abbate R, Lombardi A, Gensini GF. Reduction in prostacyclin platelet receptors in active spontaneous angina. Lancet 1984;2:838-41.
- Jaschonek K, Karsch KR, Weisenberger H, Tidow S, Faul C, Renn W. Platelet prostacyclin binding in coronary artery disease. J Am Coll Cardiol 1986;8:259-66.
- Dorn GW II, Liel N, Trask JL, Mais D, Halushka PV. Increased platelet thromboxane A₂/prostaglandin H₂ receptors in patients with acute myocardial infarction. Circulation 1990;81:212-8.
- Prisco D, Rogasi PG, Matucci M, Abbate R, Gensini GF, Neri Serneri GG. Increased thromboxane A2 generation and altered membrane fatty acid composition in platelets from patients with active angina pectoris. Thromb Res 1986;44:101-12.
- Neri Serneri GG, Gensini GF, Abbate R, Laureano R, Parodi O. Is raised plasma fibrinopeptide A a marker of acute coronary insufficiency? Lancet 1980:1:982-3.
- Gallino A, Haeberli A, Baur HR, Straub PW. Fibrin formation and platelet aggregation in patients with severe coronary artery disease: relationship with the degree of myocardial ischemia. Circulation 1985;72:27-30.

- Theroux P, Latour JG, Leger-Gauthier C, De Lara J. Fibrinopeptide A and platelet factor levels in unstable angina pectoris. Circulation 1987;75:156-62.
- Modesti PA, Abbate R, Gensini GF, Colella A, Neri Serneri GG. Kinetic analysis of a TxA2 receptor antagonist: evidence of the existence of an uptake system. Eicosanoids 1990;3:115-20.
- Scatchard G. The attraction of protein for small molecules and ions. Ann N Y Acad Sci 1949;51:660-4.
- Gaffney PJ, Mahmoud JF, Fossari CA, Spitz M. A novel radioimmunometric approach to the assay of components of human haemostasis I: assay of plasma fibrinopeptide A levels. Thromb Res 1980;19:815-22.
- Soria J, Soria C, Ryckewaert JJ. A solid phase immuno enzymological assay for the measurement of human fibrinopeptide A. Thromb Res 1980;20:425-35.
- Neri Serneri GG, Abbate R, Panetta A, Pinto S, Favilla S, Prisco D, Gensini GF. Altered intraplatelet arachidonic acid metabolism during the acute state of unstable angina. Thromb Res 1987;46:303-16.
- Rubin R. Phosphatidylethanol formation in human platelets: evidence for thrombin-induced activation of phospholipase D. Biochem Biophys Res Commun 1988;156:1090-6.
- Bevers EM, Tilly RHJ, Senden JMG, Comfurius P, Zwaal RFA. Exposure of endogenous phosphatidylserine at the outer surface of stimulated platelets is reversed by restoration of aminophospholipid translocase activity. Biochemistry 1989;28:2382-7.
- Bassé F, Gaffet P, Rendu F, Bienvenue A. Translocation of spin-labeled phospholipids through plasma membrane during thrombin- and ionophore A23187-induced platelet activation. Biochemistry 1993;32:2337-44.
- McHowat J, Corr PB. Thrombin-induced release of lysophosphatidylcholine from endothelial cells. J Biol Chem 1993;268:15606-10.
- Swann PG, Parent CA, Croset M, Fonlupt P, Lagarde M, Venton DL, Le Breton GC. Enrichment of platelet phospholipids with eicosapentaenoic acid and docosahexaenoic acid inhibits thromboxane A2/prostaglandin H2 receptor binding and function. J Biol Chem 1990; 265:21692-7.

- 22. Parent CA, Lagarde M, Venton DL, LeBreton G. Selective modulation of the human platelet thromboxane A2/prostaglandin H2 receptor by eicosapentaenoic and docosahexaenoic acids in intact platelets and solubilized platelet membranes. J Biol Chem 1992;267:6541-7.
- Croset M, Bayon Y, Lagarde M. Incorporation and turnover of eicosapentaenoic and docosahexaenoic acids in human blood platelets in vitro. Biochem J 1992;281:309-16.
- Dorn GW. Cyclic oxidation-reduction reactions regulate thromboxane A2/prostaglandin H2 receptor number and affinity in human platelet membrane. J Biol Chem 1990;265:4240-6.
- FitzGerald DJ, Roy L, Catella F, FitzGerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986;315:983-9.
- Vejar M, Fragasso G, Hackett D. Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. Thromb Haemost 1990;63:163-8.
- 27. Koike T, Hattori A, Igarashi Y. Elevation of 11-dehydro-thromboxane B2 levels in unstable angina. J Cardiol 1991;21:899-904.
- 28. Neri Serneri GG, Gensini GF, Poggesi L, Modesti PA, Rostagno C, Boddi M, Gori AM, Martini F, Ieri A, Margheri M, Abbate R. The role of extraplatelet thromboxane A₂ in unstable angina investigated through a dual antithromboxane inhibitor: importance of activated monocytes. Coron Artery Dis 1994;5:137-45.
- Theroux P, Ouimet H, McCans J, Latour JG, Joly P, Levy G, Pelletier E, Juneau M, Stasiak J, DeGuise P, Pelletier GB, Rinzler D, Waters DD. Aspirin, heparin or both to treat acute unstable angina. N Engl J Med 1988;319:1105-11.
- 30. Neri Serneri GG, Gensini GF, Poggesi L, Trotta F, Modesti PA, Boddi M, Ieri A, Margheri M, Casolo GC, Bini M, Rostagno C, Carnovali M, Abbate R. Effect of heparin, aspirin or alteplase in reduction of myocardial ischaemia in refractory unstable angina. Lancet 1990;335: 615-8.
- Theroux P, Waters D, Shiquiang Q, McCans J, de Guise P, Juneau M. Aspirin versus heparin to prevent myocardial infarction during the acute phase of unstable angina. Circulation 1993;88(part 1):2045-8.

AVAILABILITY OF JOURNAL BACK ISSUES

As a service to our subscribers, copies of back issues of the AMERICAN HEART JOURNAL for the preceding 5 years are maintained and are available for purchase from the publisher, Mosby, at a cost of \$10.00 per issue. The following quantity discounts are available: 25% off on quantities of 12 to 23, and one-third off on quantities of 24 or more. Please write to Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call (800)453-4351 or (314)453-4351 for information on availability of particular issues. If unavailable from the publisher, photocopies of complete issues are available from University Microfilms International, 300 N. Zeeb Rd., Ann Arbor, MI 48106, (313)761-4700.