haematologica

journal of hematology

ISSN 1592-8721
educational edition

volume 87
supplement I to no. 10
october 2002

published by the ferrata-storti foundation, pavia, italy

supplement I to n. 10

Second International Meeting

THE PLATELET ADP RECEPTORS

Biochemistry, Physiology, Pharmacology and Clinical Aspects

Chairmen:

M. Cattaneo (Milan, Italy), C. Gachet (Strasbourg, France)

October 3-5, 2002

S. Margherita di Pula, Italy

samples of anticoagulated human whole blood. The instrument is a controlled microprocessor in which the process of platelet adhesion and aggregation, following a vascular injury, is simulated *in vitro*. This method has been designed to provide an in vitro measure of primary platelet-related hemostasis simply, quickly, quantitatively and accurately in the routine screening of patients with potential hemorrhagic risk due to abnormal platelet plug formation. Herein we report the results obtained by testing chiral analogs of gemfibrozil, in order to evaluate their ability to inhibit human platelet aggregation. All tested compounds revealed a dose-dependent inhibitory activity toward human platelet aggregation. Moreover, a similar inhibitory effect is detectable in their precursor, the well-known gemfibrozil used as the basic reference compound. The inhibitory activity of these compounds is generally detectable at concentrations ranging from 1 to 5 mM. Considering the well-known activity of acetylsalicylic acid against platelet aggregation, we used acetylsalicylic acid as a clearly established reference compound and confirmed that it exerts a good anti-aggregating activity. The findings allow us to surmise that all tested compounds and gemfibrozil act at the platelet level with a mechanism different to that of acetylsalicylic acid, even if with a different potency. In conclusion, the simplicity of the PFA-100® system facilitates preliminary screening tests in order to find new anti-aggregating compounds. The use of this method allows us to demonstrate that the synthesized gemfibrozil analogs inhibit human platelet aggregation. Our study is still continuining as we are setting up an alternative system based on the use of properly collected and anticoagulated bull blood. This new method, used to study the previously described compounds and a new series of gemfibrozil analogs, offers the advantage of predicting the ability of these drugs to modulate the fibrinolytic system using more easily available standardized blood.

THE FREQUENCY OF PLATELET NON-RESPONSIVENESS TO ACETYLSALICYLIC ACID AMONG PATIENTS HANDICAPPED BY VASCULAR THROMBOEMBOLIC DISEASES

Blaskó G, Pál A
Medical Department, Sanofi-Synthelabo Ltd, Hungary

A comparison was made regarding the frequency of non-responsiveness to acetylsalicylic acid (ASA) between 92 cardiology outpatinets of a district hospital and 97 heavily handicapped patients of the Raliway Hospital. The main differences were in the populations of patients: the duration of the treatment with ASA, the numbers of the combined atherothrombotic events, the rate of totally and 67% handicapped patients. There were no significant differences in gender and age. The non-responsiveness to ASA was measured and determined by platelet aggregometry using the Born method. Dose-response curves were plotted with the various concentrations of the following inducers: ADP, epinephrine, arachidonic acid and collagen. Patients were considered as non-responders if the aggregation of the platelets of the ASA-treated patients was not inhibited. Compliance was also taken into consideration. *Results.* The frequency of ASA-non-responders was 28.86% among the non-handicapped cardiology patients of the district hospital, whereas it was 45.8% among the handicapped patients. The latter had been taking the drug for a long period (average 5.33 years) without the pharmacodynamic effect of ASA having been checked, whereas the patients of the other hospital were treated for a shorter period (1.5 years) and the platelet aggregometry was performed in the 1st year of the treatment. The clinical significance of these data and the importance of the pharmacodynamic control of ASA are emphasized along with the appropriate choice of another platelet inhibitor for patients with cardio-, and/or cerebrovascular ischemic events.

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THE FREQUENCY AND CLINICAL SIGNIFICANCE OF NON-RESPONSIVENESS TO ACETYLSALICYLIC ACID AMONG PATIENTS BEING HANDICAPPED FROM VASCULAR THROMBOEMBOLIC DISEASES

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ABSTRACT I.

A comparison was made regarding the frequency of non-responsiveness to acetylsalicylic acid (ASA) between the outpatient's/emergencie' sambulances of cardiology of 92 patients of a district hospital and of 97 heavily handicapped patients of the Railway Hospital. The main differences were found between the patient's population: the duration of treatment with ASA, twhe number of the combined atherothrombotic events, the rate of the totally and of 67% handicapped pts, whereas there were no significant differences in gender, age.
The non-responsiveness to ASA was measured and determined using platelet aggregometry based on the method of Born.Dose-response curves were plotted with the various concentrations of the following inducers being used: ADP., epinephrine, arachidonic acid and collagen.
Pts were considered as non-responders if the aggregation of the platelets of the ASA-treated pts was not inhibited. The compliance was also taken into consideration
Results: The frequeney of ASA-non-responders was 28.86% among the non-handicapped cardiological pts of the district hospital and 45.8% among the handicapped pts. The latter were taking the drug for a long period (average 5.33 years) without controlling the pharmacodynamic effects of ASA, whereas the pts of the other hospital have been treated for a shorter period (1.5 years) with the platelet aggregometry beeing performed in the 1st year of the treatment.
The clinical significance of these data and the importance of the pharmacodynamic control of ASA are emphasized alongwith the appropriate choice of another platelet inhibitor for the pts with cardio-, and/or cerebrovascular ischemic events.

„Until aspirin resistance's true nature and prevalence are better defined, no test of platelet function is recommended to assess the antiplatelet effect of aspirin in the individual patient."

American College of Chest Physicians guideline,
Platelet Active Drugs (Carlo Patrono et al), Chest 2001; 119: 39S.

Aspirin takers with high level of thromboxane B2 urinary excretion face to a 3.5 fold cardiovascular death risk.

Eikelboom Circulation, Apr 2002; 105: 1650

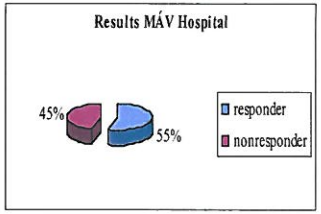
METHOD 1.

The non-responsiveness to ASA was measured and determined using platelet aggregometry based on the method of Born. Dose-response curves were plotted with the various concentrations of the following inducers being used: ADP., epinephrine, arachidonic acid and collagen. The time period between the drug administration and the aggregation examination was av. 25.3 hours
Pts were considered as non-responders if the aggregation of the platelets of the ASA-treated pts was not inhibited. The compliance was also taken into consideration.

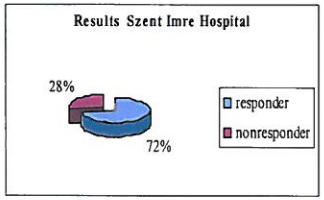
RESULTS I.

MÁV Hosp.:

non-responders: 44, 45,8%

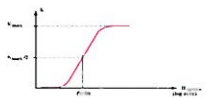


Szent Imre Hosp.:
Incomplete responders: 9
(responds to dose elevation)
non-responders: 26, 28,2%

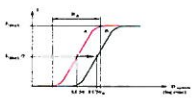


ABSTRACT II.

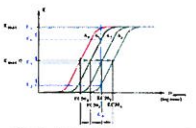
There are a lot of technical concerns to perform a correct aggregometry by the method of Born.
That was the reason why we were looking for a simpler method using fix doses of inductors (ADP, epinephrin, AA, collagen) and an automated measuring system (CARAT- TX4 Aggregometer) in the second part of this study.
Laboratory and/or clinical definition of effective platelet inhibition for the clinician, and methods for routine screening evaluation for the laboratory were studied.
Platelet aggregatory results of 150 untreated and 894 patients treated with antiplatelet agents were evaluated in seven cardiovascular hospital wards. Platelet aggregation measurements were performed under the same conditions in all the centres. Platelet aggregation was measured by using a computerized Carat TX4 platelet aggregometer.
For the interest of mass-screening, fixed doses of different inductors were used (ADP 5 and 10 µM, collagen 2 µg/ml, epinephrine 10 µM, arachidonic acid 0,5 mM).
Maximal platelet aggregatory results of the 150 untreated control cardiovascular patients are as follows:
Results coming from different centres did not differ substantially from each other. **The threshold of the measurable inhibition was postulated as mean of maximal aggregation% minus 2S.D. of the untreated controls**. Treated patients with an antiplatelet agent were considered resistant to that particular drug if results of the maximal aggregation were higher, than this limit. Arachidonic acid was used as a control of drug compliance of aspirin. Epinephrine and collagen were used to evaluate aspirin monotherapy (resistance to any of these two inductors meant aspirin resistance). Epinephrine, collagen and ADP simultaneously were used to evaluate the combination therapy of aspirin and ticlopidine (data not shown).
Aspirin monotherapywas evaluated, and ratios of resistant cases were determined. Cardiovascular patients receiving aspirin (100-125 mg) alone for secondary prevention showed a 25% resistance rate (207 out of 823). Tailored antiplatelet therapy is possible with a relatively easy routine screening method. Patients who are resistant to any oral antiplatelet drug should receive a combination therapy which proved superior in the recent acute coronary syndrome trials.



Traditionally, the EC50 has been used, where platelet aggregation is represented by the 50% of the concentration of a specific inductor causing maximal aggregation.

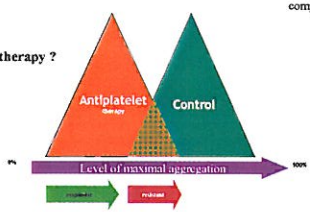


Platelet inhibitor causing a shift to a higher EC50.

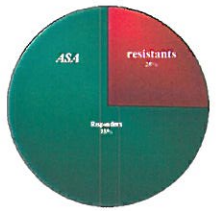


A well defined single inductor concentration can represent the effect of the platelet inhibitor taken. Concentration of inductors used in this study came from previous experiences to reduce the complexity of measurement in case of a single patient.

Who is resistant to antiplatelet therapy ?



RESULT II.



The cutoff value of aggregation inhibition: $Mean_{control} - 2SD$

ASA treated N=823

PATIENTS 1.

Hospital MÁV. 997-1999
N = 97 (handicapped) examined to ASA non responsiveness:
Patients' population in MÁV hospital:
Mean age: 58,18 y, Distribution: female:15, male: 82

Diagnoses

CHD+PAD	5
Stroke+PAD	2
CHD+stroke	2
Hypertension	21
Coronary Heart Disease	47
Cerebrovascular disease	16
PAD	14
Post-thrombotic syndrome	5

Mean time elapsed from the first event and ASA treatment: 5,33 years (1-29 years)

100 % handicapped:	21
67 % handicapped :	82

The grades of handicap

Group III. : 67 % working capacity and incapacity, 100 % diminution of working capacity,
Group II. : 100 % loss of working capacity,
Group I. : 100% loss of working capacity + care and nursing assistance needed.
The time to classification: minimal 6 months - 1 year.

Hosp. Szent Imre 1997-2000
N = 92 cardiovasc. cerebrovasc. examined for ASA non responsiveness:
Patients' population in Szent Imre hospital:
Mean age : 47.3 y, Distribution :female: 37 , male: 55

Diagnoses

Hypertensive disease	39
CHD	41
Cerebrovascular disease	38
PAD	6
amaurosis fugax	7

Mean time elapsed from the first event and ASA treatment: 3,5 weeks (1-5 weeks)

CONCLUSION I.

The ratio of ASA non responsiveness among the non handicapped cardio-vascular patients is in good accordance with the previously obtained epidemiological data (32%): 28,2 %
Among the handicapped patients the ASA non responsiveness ratio is higher: 45,8 %

Patients were treated by ASA for years (mean 5,5 years) without the control of pharmacodynamic effects.

The underlying reason for the higher ASA non-responsiveness ratio among majority of the handicapped patients- when treated without control- may be the progression of the atherothrombotic disease resulting in new vascular events contributing to the handicap.

Data from Szent Imre Hospital mainly came from samples taken after the first event. This fact explains the differences between the centres.
Prospective studies are unacceptable for ethical reasons as, if it is proved that ASA does not inhibit sufficiently platelet aggregation, the continuation of ASA treatment would be unethical.

What are the clinical consequences of ASA non-responsiveness?

1. Control examinations are needed to prove the pharmacodynamic effect
2. ASA should not be prescribed if its non-efficacy is proven!
3. No prospective study!
4. The effect of platelet aggregation inhibitor agents should be controlled by laboratory method (aggregation, PFA-100, etc.)!!!

CONCLUSION II.

According to the recent opinion of Eikelbaum et al. Only 25 % of the cardiological cases are treated properly with ASA or other antiplatelet agents.
The rapid screening scheme described resulted in approximately similar rate of ASA nonresponders, as it was found earlier. The pharmacodynamic control of ASA treatment is essential in order to find the group of patients who need alternative antipletelet therapy. The thienopyridines are better alternative for these patients because of the different underlying mechanism of action, the less number of non-responders and mainly because of the better clinical efficacy