

SELECTIVE INHIBITION OF THROMBOXANE SYNTHESIS BY 1-(3-HYDROXY-1-OCTENYL)-IMIDAZOLE AND ITS NICOTINIC ESTER IN HUMAN AND RAT PLATELETS. C. Bonne, B. Martin, D. Sincholle* and F. Regnault. Centre de Recherche sur les Maladies de la Rétine, INSERM FRA N°45, Paris. *Centre de Recherche Chauvin-Blache, Montpellier (France).

Since Thromboxane A₂ (TxA₂) is considered to play an important role in platelet aggregation, attempts to develop selective inhibitors of TxA₂-synthetase are being made. In this report the activity of 1-(3-hydroxy-1-octenyl)-imidazole, chlorhydrate (CBS612) and of its nicotinic ester, dichlorhydrate (CBS634) will be presented.

The inhibitory activity was determined in isolated platelets from human and rat by radiochemical assay and in whole blood by radioimmunoassay of TXB₂ and of PGE₂ as a criteria of specificity. Human enzyme was more sensitive than the rat one's (CBS634 : IC₅₀ human = 0.7µM, IC₅₀ rat = 10µM) and the two compounds were more active than imidazole in human platelets (IC₅₀ : imidazole = 50µM, CBS612 = 2µM ; CBS634 = 0.7µM). Collagen-induced aggregation was inhibited by the drugs at concentrations which suppressed TXB₂ formation.

The results of these experiments showed that CBS612 and CBS634 selectively inhibited thromboxane-synthetase since they increased the formation of PGE₂. Besides, these compounds did not inhibit PGI₂ release from rat aorta ring. The selectivity of the inhibition by the drugs was further shown by the absence of any effect on the first phase of ADP-induced platelet aggregation.

These compounds seemed potentially interesting for the management of thrombotic diseases and for elucidating the role of Thromboxane in physiological and pathological processes.

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INTERNAL Ca²⁺ INTERFERING DRUGS ALTER ARACHIDONIC ACID METABOLISM AND INHIBIT IN VITRO PLATELET FUNCTIONS. H. de la Baume, J. Maclouf, S. Levy-Toledano and J.P. Caen. INSERM U. 150, Hôpital Lariboisière, 6 rue, Guy Patin, Paris, France.

Internal Ca²⁺ fluxes are known to play a critical role in the activation of human blood platelets as well as for the liberation of arachidonic acid from the phospholipids.

When exogenous arachidonic acid is added to platelets, cyclooxygenase inhibitors, aspirin, flurbiprofen and indomethacin inhibit its conversion into prostaglandin G₂ and H₂ and therefore aggregation as well as ¹⁴C-serotonin (5-HT) release but they did not affect bovine thrombin- and ionophore A 23187 induced platelet aggregation and had only little influence on ¹⁴C-5 HT release. On the other hand, the intracellular calcium antagonist TMB8 and chlorpromazine inhibit arachidonic acid-induced platelet aggregation and secretion although they do not affect thromboxane (TX) production. These agents also inhibit platelet aggregation, ¹⁴C-5-HT release as well as TX synthesis when ionophore or thrombin are the stimulators. Using a glass capillary column gas chromatography method developed for this purpose, we studied the quantitative amount of the liberated eicosanoids. At high concentrations, TMB 8 and chlorpromazine inhibited strongly arachidonic release as well as platelet aggregation and ¹⁴C-5HT release but although TX production was abolished, arachidonic acid was still liberated and converted to some extent by the lipoxygenase pathway. These findings suggest that internal Ca²⁺ mobilization is a prerequisite for platelet activation as well as for arachidonic acid liberation. However, at intermediate doses, internal Ca²⁺ - interfering agents may also modulate the oxidative balance that exists between the cyclooxygenase and the lipoxygenase towards the latter pathway.

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THROMBOXANE-SYNTHETASE INHIBITORS COULD BE "LARGE SPECTRUM" ANTI-AGGREGATING AGENTS. C. Bonne and B. Martin. Centre de recherche sur les Maladies de la Rétine, INSERM FRA 45, Paris

"Large spectrum" anti-aggregating activity could be only achieved by agents which increased the c AMP content of platelets. Cyclo-oxygenase inhibitors could only block the Thromboxane (Tx)-dependent pathway of platelet aggregation. Conversely, Tx-synthetase inhibitors could deviate the endoperoxides metabolism to anti-aggregating prostaglandins in particular in the presence of vascular tissues. In this study we have investigated the effect of CBS634 (1-(3-hydroxy-1-octenyl)-imidazole nicotinic ester, dichlorhydrate), a potent inhibitor of TxA₂ synthesis, both on the production of anti-aggregating prostaglandins and on the simultaneous c AMP synthesis in platelets.

Rat aorta fragments pretreated with aspirin were incubated with rat platelet rich plasma in the presence or absence of tested compound. c AMP, TXB₂, PGE₂ and 6-keto-PGF_{1α} were determined by radioimmunoassays.

In the presence of CBS634 (50µM), TXB₂ formation was reduced from 17 ± 2 to 0.2 ng/ml/min. In parallel, PGE₂ production in controls was 1.4 ± 0.6 and 8 ± 1 ng/ml/min in the presence of the drug. On the other hand, 6-keto-PGF_{1α} formation, very low in controls, rose to 4 ± 1.2 ng/ml/min in the presence of CBS634. Radiochemical assays performed with [¹⁴C]-arachidonate confirmed that metabolic deviation. The increased level of c AMP formed in the presence of TxA₂-synthetase inhibitor supports the hypothesis that such a drug could present a "large spectrum" anti-aggregating activity.

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EFFECT OF HEPARIN AND OTHER POLYANIONS ON COLLAGEN INDUCED PLATELET AGGREGATION. M. Lois Tiffany and John A. Penner. Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan, and Department of Internal Medicine, Michigan State University, East Lansing, Michigan, U.S.A.

Despite the fact that relatively high concentrations of polyanions are capable of inducing spontaneous platelet aggregation following a brief incubation period, collagen induced platelet aggregation was actually decreased when a low concentration (1 µg/ml) of the polyanions, Heparin (gut or lung fractionated), Dextran, Sulfate (mol. wt. 500,000) and polyvinyl Sulfate (mol. wt. 100,000) were incubated for 5 minutes with human platelet rich plasma.

An explanation for this apparent anomalous effect of low polyanion concentrations may rest with complement activation. We have proposed that collagen interacts with platelet-bound CIs inducing CIs esterase activity, and have suggested that this interaction results in platelet aggregation and release. Therefore, an explanation of the effect of these polyanions on collagen induced platelet aggregation is found in their known potentiation of the inhibitor, C1-IN_A, on the induction of esterase activity in CIs.

In support of this concept is our findings that the polyanion, polyanethanol sulfonate, which inhibits the activity of C1-IN_A, enhances collagen induced platelet aggregation when used at the same low concentration and under the same experimental conditions as used with the other polyanions discussed.