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MODULATION OF THROMBIN-MEDIATED REACTIONS IN HAEMOSTASIS BY TREQUINSIN AND ITS ANALOGUES.

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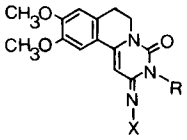
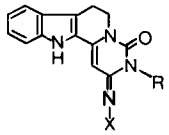
A new pyrimido/6,1-a/-isoquinoline derivative,/Ref.1/ developed by Hoechst and named Trequinsine has been reported to be a powerful inhibitor of cAMP phosphodiesterase and platelet aggregation having a potency nearly similar to prostacyclin. We synthesized Trequinsin (1), its indole derivative (4), as well as their precursors (2 3) and subjected them to a study in order to evaluate their effects on haemostasis. Surprisingly they exhibited a modulatory effect only on reactions mediated mainly by thrombin. They inhibited platelet aggregation (IC_{50} for Trequinsin: 13 ± 2.5 ng/ml, for indole derivatives: 1-3 μg/ml); 1, 2, 4 enhanced the amidolytic activity of α-thrombin towards S-2238 substrate within a narrow range of submicromolar concentrations but they did not exert any effect on the activity or the inactivation of Factor Xa and plasmin.



Compound 2 proved to be an inhibitor of prostacyclin production of rat aortic tissue (Trequinsin has much less effect) but in contrary to this 3 and 4 exerted an enhancing effect on the total prostacyclin production.



1 : R = CH_3 2 : R = H

3 : R = H 4 : R = CH_3

X = 2,4,6-trimethylphenyl-

Reference: 1. Lal,B., Dohadwalla,N., Dadkar,N.K.,D'Sa, A., De Souza N.J.: J.Med.Chem.28, 1470, 1984.

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CORRELATION BETWEEN INHIBITION OF PLATELET PHOSPHODIESTERASES (PDEs) AND PLATELET FUNCTION TESTS.

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A systematic investigation of over 100 PDE inhibiting pyrimidopyrimidines was performed regarding a possible correlation between influence on platelet function tests and inhibition of platelet PDEs. Basically, the compounds tested were congeners of Dipyridamole but lack substitution in position 6. The concentration necessary for a 50% inhibition of PDE activity in platelet homogenates ranged between 0.000045 and 35 μmoles/L.
The inhibition of platelet PDEs was measured in freeze-thaw homogenates of human platelets using 3H-cAMP as substrate. Intraplatelet cAMP changes were measured by prelabelling the ATP pool with 3H-adenine and isolation of 3H-cAMP. Inhibition of platelet retention was determined using Morris' method (rotating citrated whole blood with glass beads) and the aggregation tests were performed according to Born and Cross in citrated platelet rich plasma.
The rather large number of compounds allowed the application of correlation analysis. The results of linear regression tests (IC_{50} and EC_{200} values) are shown in the table.

PDE	vs	Morris	r = 0.566	N = 61
PDE	vs	ADP	0.560	30
PDE	vs	Coll	0.763	53
cAMP	vs	Morris	0.778	54
cAMP	vs	ADP	0.819	27
cAMP	vs	Coll	0.851	45

It appears that increased intracellular cAMP caused by PDE inhibition shows a strong correlation to the inhibition of the above mentioned platelet function tests. A weaker relation exists between inhibition of isolated PDEs and platelet function. Thus, determination of intrinsic PDE inhibitory potency is not sufficient to predict the influence of a given compound on platelet function tests.

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PHARMACOLOGICAL STUDIES OF PLATELET ANTIAGGREGANTS USING AN IN VITRO MODEL OF PRIMARY HEMOSTASIS.

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We used a new device simulating in vitro primary haemostasis : more precisely the reactivity of blood to collagen and ADP. Thus an artificial vessel was created consisting of two main parts : a glass capillary (ID 140 um, length 16 mm, siliconized) simulating the haemodynamic resistance of an arteriole and an aperture (ID 150 um) reflecting the injured part of a cut arteriole. This aperture was performed in a cellulose acetate filter covered with collagen type I (3 mg/ml) to provide a defined surface for the adhesion of platelets and soaked with ADP in a concentration similar to that of injured endothelial cells (2×10^{-2} M). The mean ± sd control values were 110 ± 24 s, 156 ± 40 ul (n = 25) and correlated well with in vivo bleeding time values ($p < 0.01$). We studied the effect on this test of classical antiaggregant drugs which act on primary hemostasis by different mechanisms of action. Acetylsalycilic acid (Egic laboratories) prolonged this test for concentrations above 10^{-5} M, ticlopidine (Millot-Solac laboratories) above 3×10^{-4} M, prostacyclin (Wellcome laboratories) above 5×10^{-9} M, the synthetic octapeptide LYS-PRO-GLY-GLU-PRO-GLY-PRO-LYS derived from type III collagen (gift from Y. Legrand) above 5×10^{-4} M. We evidenced a synergistic action between collagen octapeptide and ticlopidine. Thus this device permits the screening of new drugs for their effects on primary hemostasis and the study of ex vivo repeated measurements for the monitoring of antiaggregant therapy.

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MECHANISMS FOR THE IN VIVO ANTIPLATELET EFFECTS OF ORGANIC NITRATES.

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Organic nitrates (nitroglycerin, isosorbide dinitrate) are inhibitors of platelet function more effective in vivo than in vitro (Am J Cardiol 1984; 53:1683), the in vivo effect requiring concentrations 10-100 times lower than in vitro. We have previously excluded that such difference is due to elicitation by nitrates of prostacyclin synthesis in human endothelial cells or vascular fragments (Circulation 1985; 71:176). In the present study we evaluated alternative explanations: that the difference is due (1) to generation of more active drug metabolites; (2) to synergism between nitrates and prostacyclin in the inhibition of platelet function. Isosorbide dinitrate (ISDN) and its two main in vivo metabolites, isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN), were compared in their ability to inhibit platelet aggregation and thromboxane (TX) B2 formation (RIA) in respone to threshold doses of ADP, adrenaline, collagen, arachidonic acid and thrombin in citrated platelet-rich plasma. The same tests were performed in 10 healthy volunteers before, during (at 5, 15 and 30 min) and after infusion of the three drugs at 8 mg/h for 30 min in 3 different days. Finally, the concentration of prostacyclin (and its stable analogue Iloprost) added in vitro to platelets, and required to inhibit platelet aggregation by 50% (IC50) after 5 min pre-incubation of platelets with nitrates was determined. In vitro incubation of platelets with IS-2-MN resulted in greater inhibition of both aggregation and TX formation (by ADP and adrenaline) than with ISDN and IS-5-MN. At 10^{-7}M, only IS-2-MN significantly inhibited aggregation (-12%, $P<0.05$) and TX formation (from 9.2±1.8 to 5.9±0.6 ng/ml) by ADP, while minimum effective concentrations were 10^{-6}M for ISDN and 10^{-4}M for IS-5-MN. These in vitro differences are unlikely to be the explanation of in vivo findings, since IS-2-MN, ISDN and IS-5-MN were equipotent when administered in vivo (complete abolition of secondary wave after ADP and adrenaline at 30 min of infusion). At supra-threshold doses of all the aggregating agents, all three drugs, at 10^{-7}M, decreased IC50 for prostacyclin from 2.9±1.3 to 0.32±0.18 nM ($P<0.01$). Synergim with prostacyclin is most likely to account, at least partially, for in vivo antiplatelet effects by organic nitrates.