

**INHIBITORY EFFECT OF SIN 1 ON PLATELET FUNCTION : POSSIBLE INTERFERENCE WITH PHOSPHOLIPASE C AND FIBRINOGEN BINDING.** S. Levy-Toledano, D. Weill, J. Maclouf, F. Rendu, and C. Soriat. U-150 INSERM, UA 334 CNRS, Hôpital Lariboisière, Paris. \*Laboratoires Hoeschst, 92080 Paris-La Défense, France.

The mode of action of SIN 1, the main metabolite of an antianginal drug (Molsidomine) was investigated in vitro on human platelet functions. SIN 1 inhibited dose dependently platelet activation as reflected by aggregation and release of serotonin induced by arachidonic acid, prostaglandin endoperoxides analogues (U 46619, U 44069), collagen, and ADP (first and second wave). The maximal inhibition was reached at  $10^{-5}$  M. The thromboxane (TX) synthesis was inconsistently impaired even at high concentrations of SIN 1 ( $10^{-4}$  M) suggesting a discrepancy between the inhibitory effect on platelet activation and TX formation. The main cofactor of ADP-stimulated aggregation is fibrinogen; SIN 1 dose-dependently inhibited the fibrinogen binding to platelets thereby explaining its antiaggregatory properties.

In order to further investigate the mechanism of action of this drug on platelet activation we tested SIN 1 on the thrombin-induced phosphoinositide metabolism and protein phosphorylation on  $^{32}$ P-prelabelled isolated platelets.  $^{32}$ P-phosphatidate (PA) formation was greatly inhibited. Phosphorylations of the myosin light chain (P20) and of 43 kDa protein (P43) were also reduced. These effects were accompanied by an inhibition of serotonin release, TXB<sub>2</sub> synthesis, and platelet aggregation.

SIN 1 would seem to act on early biochemical events and more especially at the level of the membrane phospholipase C.

**THE EFFECT OF PIRACETAM IN ISCHEMIC FLAP VASCULARISATION** Rossillon O., Bayet B., Huvmans M., Englebert P., Stern Ides, Calteux N., Vanwyck R. St. Luc Hospital, Department of Plastic Surgery, Catholic University of Louvain, 1200 Brussels, Belgium.

Piracetam, a drug traditionally used for stimulating the telencephalon has a strong anti-aggregant, antispasmodic and rheological action, due to its antisludge effect and the increase of the red cell deformability capacity. The authors show the interest of this substance in reconstructive surgery: Piracetam significantly increases the circulation in the flaps, reduces the zones prone to necrosis and allows for a faster skin expansion. The first three experiments have been performed on rats - on an epigastric flap with thoracic pedicle-. In the first one, we operated on 150 rats divided into 5 groups: a control series, and 4 series which received the following doses of Piracetam per os: 20, 40, 80 and 100 mg/100 g/day. The necrotic area, as measured daily, represents 13.8% ± 2.6 (± SEM) in the control series, 6% ± 0.8 in the series with 20 mg/100 g, 2.53% ± 0.66 in that with 40 mg/100 g, 1.67% ± 0.4 in that with 80 mg/100 g and 2.77% ± 1.96 in that with 100 mg/100 g. (all these percentages are expressed versus the overall surface). A thorough statistical study including the ANOVA table, and regression tests shows that these differences are significant at 0.0001 for the 40 mg/100 g dosis and 0.0000 for the 80 mg/100 g dosis. A second experiment measured the local blood flow in 4 areas of the flap from its thoracic basis towards its epigastric point thanks to the radio-active microsphere technique described in rats by Mc Devitt and Malik. Measures were carried out on 60 rats divided into 3 groups: control series without flap, series with a flap at the second post-operative day without Piracetam, series with a flap at the second post-operative day with Piracetam. With Piracetam, the increase of the blood flow in the thoracic part proximal to the flap (zone 1) is of 60% ± 13 (p < 0.0000), that of zone 2 of 72% ± 20 (p < 0.0008), that of zone 3 of 92% ± 23 (p < 0.0003), and finally that of the epigastric zone 4 of 117% ± 40 (p < 0.004) (vs the overall surface). A third experiment measured the temperature difference of these 4 zones by means of a thermocouple according to the method described by Jones in 1983 and it compared the results obtained with the flow measured by microspheres. Finally, in a last experiment, the blood flow in expanded tissues with- and without- piracetam 40 mg/100 g was measured with a laser-doppler. Therefore it was concluded that piracetam is a useful drug to prevent necrosis in flaps. It can also be used to expand tissues more quickly.

**TREATMENT OF RAYNAUD SYNDROMES WITH PIRACETAM (NOOTROPIL<sup>R</sup>), A NOOTROPIC AND ANTIPLATELET AGENT.**

M. Moriau, E. Lavenne-Pardone, CH. Col-De Beys. Unité d'Hémostase et Thrombose - Cliniques universitaires St Luc - UCL - Av. Hippocrate 10 - 1200 BRUXELLES - BELGIUM

Three prospective studies have been performed with piracetam (Nootropil<sup>R</sup>) in the treatment of Raynaud disease and syndromes.

The first one, realized in 20 cases of Raynaud disease established that 8 g piracetam daily was the optimal dosage necessary to obtain a significant clinical, ultrasonic and biologic improvement.

In the second one, 58 cases of Raynaud syndromes (47 idiopathic and 11 associated with systemic disease) were treated with 8 g daily piracetam during 6 to 12 months. A clinical and ultrasonic improvement was observed in 75 and 80 % of the cases and a normalisation of the disturbed platelet functions (36/58) in 88 %. Moreover a benefic rheologic effect was noted and related to the membrane deformability, the antiplatelet and the Von Willebrand factor synthese or release inhibiting activity of piracetam

The third one realized in cross over in 30 cases of Raynaud syndromes compare the effects of piracetam alone (dosage 8 g daily) with the combination piracetam (4 g daily) - aspirin (100 mg daily) or with other drugs like buflomedil, calcium antagonists and ketanserine.

A synergic effect was obtained with the combination piracetam - aspirine and piracetam alone seems to be more efficient than the other drugs.

A fourth double blind study is actually performed

**MODE OF ACTION OF A NOVEL ANTI-PLATELET AGENT (E-5510).** T. Saeki (1), K. Harada(1), T. Yoshimura(1), Y. Nakamura(1), T. Fujimori (1), K. Katayama(1), Y. Yamagishi(1), I. Yamatsu(1) and Y. Ikeda (2). Eisai Res. Labs., Tsukuba, Ibaraki, Japan (1) and Blood Center, Keio Univ., Tokyo, Japan (2).

A novel anti-platelet agent, 4-cyano-5,5-bis(4-methoxyphenyl)-4-pentenoic acid (E-5510), has been shown to inhibit platelet aggregation and secretion induced by thrombin as well as by other inducers such as ADP, collagen and PAF. Although E-5510 can act as a cyclooxygenase inhibitor, inhibition of cyclooxygenase may not be the primary mode of action since this compound effectively inhibits thrombin-induced platelet activation. In this paper, effects of E-5510 on arachidonic acid (AA) metabolism, intracellular Ca<sup>++</sup> and cAMP in human platelets are examined.

(1) Effect on AA metabolism. Platelets prelabeled with [<sup>14</sup>C]-AA were stimulated with thrombin. E-5510 inhibited not only thromboxane A<sub>2</sub> and HHT generation but also 12-HETE generation in a dose-dependent fashion. The total AA released was also reduced by E-5510. An almost 50% reduction was obtained by 10 μM of this compound. On the other hand, a cyclooxygenase inhibitor such as U-53059 increased 12-HETE generation in a dose-dependent fashion. In addition to the inhibition of AA metabolism, E-5510 exerted inhibitory effects on phosphatidic acid generation, which suggests the possible inhibition of phospholipase C activity by this compound.

(2) Effect on intracellular Ca<sup>++</sup> and protein phosphorylation. Intracellular Ca<sup>++</sup> mobilization was examined using Fura-2 loaded human platelet suspension. The increase in intracellular Ca<sup>++</sup> induced by thrombin was inhibited by E-5510 and the increase in phosphorylation of 40 K protein was also suppressed by this compound after the stimulation of human platelets by thrombin.

(3) Effect on cAMP. Platelets were incubated with 10-100 μM of E-5510 and the cAMP content in human platelets was measured. E-5510 increased the cAMP content in a dose-dependent fashion. In platelet homogenate, E-5510 inhibited phosphodiesterase activity with an IC50 of 10 μM.

These results suggest that E-5510 may inhibit platelet aggregation and secretion through the multiple modes of action, such as inhibition of phospholipase C, phosphodiesterase and cyclooxygenase, in the process of platelet activation.