

THE EFFECTS OF TICLOPIDINE HYDROCHLORIDE ON BLEEDING TIME AND PLATELET FUNCTION IN MAN. D.J. Ellis, R.L. Roe, J.J. Bruno, B.J. Cranston and M.M. McSpadden. Institutes of Clinical Medicine and Biological Sciences, Syntex Research, Palo Alto, California.

Ticlopidine hydrochloride (T) was given to healthy male and female subjects in doses ranging from 125-500 mg/day for up to 8 days to study the rate of onset and offset of action and to define the optimal dosage for inhibition of platelet function tests.

Template bleeding time (BT) using the Simplate® device and *ex vivo* platelet aggregation were done every day from the start of dosing through the post-dosing period until platelet function had returned to baseline.

Achievement of maximal bleeding time prolongation and maximal inhibition of platelet aggregation induced by ADP, collagen, and epinephrine required 3-5 days of dosing. Rate of onset was higher at higher drug doses. Post-dose return to baseline took 4-8 days.

BT prolongation was dose-dependent with 2.0 x prolongation at 125 mg/d, 2.5 x at 250 mg/d, 3.4 x at 375 mg/d and 4.7 x at 500 mg/d.

At steady state, ADP aggregation (1st and 2nd phase) was inhibited by 50-60%, epinephrine aggregation was inhibited by 40-80%, and collagen aggregation was inhibited by 55-80%. Inhibition was dose-dependent. Arachidonic acid ($1 \times 10^{-3}M$) induced aggregation was not inhibited. ATP release during collagen aggregation was inhibited by 20-50% and malondialdehyde production induced by thrombin was inhibited 20-55%, both in a dose-related fashion.

Ticlopidine hydrochloride is a potent inhibitor of platelet function with a broad spectrum of activity and a delayed onset and offset of action. The drug is neither a prostaglandin synthetase nor a phosphodiesterase inhibitor and appears to act by a novel mechanism.

HEMORHEOLOGICAL EFFECT OF TICLOPIDINE IN RATS.

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The hemorheological effect of ticlopidine was studied in rats *ex vivo*. Ticlopidine (30-300 mg/kg) was orally given to rats. Heparinized blood samples were taken from the carotid artery under pentobarbital anesthesia 3 hr after the drug administration for measurement of whole blood viscosity (ELD type cone-plate viscometer), micropore filtrability of red cells (Nuclepore membrane, 5 μm), erythrocyte sedimentation rate (ESR), hematocrit (Ht) and plasma fibrinogen. Red cell deformability was measured by counting the shear stress-induced *cap-form cells* under a scanning electronmicroscope. Mechanical flexibility of red cells was also studied by measuring hemolysis caused by turbulent flow.

Ticlopidine treatment caused a significant decrease in whole blood viscosity (9.13 ± 0.15 and 6.17 ± 0.08 versus 9.80 ± 0.18 and 6.74 ± 0.09 Cp in control at 19.2 sec^{-1} and 76.8 sec^{-1} , respectively) and a significant increase in micropore filtrability of the red cells (0.54 ± 0.01 versus 0.40 ± 0.02 ml/min in control) without any changes in ESR, Ht and plasma fibrinogen. Ticlopidine also significantly stimulated the shear stress-induced shape change of the red cells to *cap-form cells* (12.08 ± 0.13 versus 8.66 ± 0.23 % in control) and prevented mechanical hemolysis caused by a turbulent flow (16.8 ± 1.6 versus 30.5 ± 2.5 % in control).

In addition to the platelet aggregation inhibitory action the hemorheological action of this agent may be useful for improving microcirculation and protecting red cells from mechanical disruption by turbulent blood flow.

Increase in the adenylate cyclase and Mg^{2+} -activated adenosine triphosphatase activities in red cell membranes may be associated with the effect of ticlopidine to increase red cell deformability.

ANTI-PLATELET EFFECTS OF TICLOPIDINE ARE NOT DIMINISHED BY EFA DEFICIENCY OR INDOMETHACIN ADMINISTRATION. A.L. Willis, J.M. Fisher, D. Donegan, D.L. Smith. Department of Physiology, Institute of Biological Sciences, Syntex Research, Palo Alto, Ca. 94304

It has been suggested that ticlopidine may act to sensitize platelets to the effects of anti-aggregatory prostaglandins (I_2 , E_1 , D_2) or enhance endogenous production of such PG's. Rats (male, Sprague Dawley, 280-575g, Simonsen, Gilroy, CA) or guinea pigs, (male, Hartley strain, 330-410g, Simonsen) were dosed orally with ticlopidine hydrochloride (RS 99847) at 100 mg/kg for 3 days. At 2h following the final dose, platelet function (ADP-induced aggregation, retention by glass beads) was examined within 5 min of blood withdrawal via the abdominal aorta. In rats chronically maintained on a fat-free diet, there is a deficiency in tissue levels of essential fatty acid (EFA) precursors for PG biosynthesis. Consequently, a marked (~90%) reduction in platelet PG production and vascular PGI_2 production was seen. Under such conditions, administration of ticlopidine hydrochloride inhibited platelet function in a manner indistinguishable from that in control animals. Similarly, in both guinea pig and rat, intraperitoneal administration of indomethacin (100 mg/kg) 1h before the final dose of ticlopidine failed to interfere with the anti-platelet effects of ticlopidine, even though vascular PGI_2 production (in thoracic aorta) was shown to be virtually abolished. It is concluded that EFA's and their PG metabolites are not necessary for the platelet inhibitory effects of ticlopidine to be exerted.

ACTION OF TICLOPIDINE ON THE OXYGENATED METABOLISM OF ARACHIDONIC ACID IN THE MOUSE PERITONEAL MACROPHAGES. M. Rigaud, H. Rabinovitch, J. Durand, J.C. Breton, G. Rigaud*. Biochemistry Laboratory and * Anesthesiology Department, Limoges, France.

When ticlopidine is added to macrophages cultures, in the absence of exogenous arachidonic acid, there is a production of both "prostanoids" and "eicosanoids" in the culture medium. These products have been measured using glass capillary gas chromatography prior to multiple ion mass spectrometry. The quantitative determinations are made 5,15,25, 35 and 45 minutes after the drug was added to the macrophages cultures. The three drug concentrations used ($10^{-4}M$, $5.10^{-5}M$ and $2.5 \cdot 10^{-5}M$) induce a liberation of 6-keto- $PGF_{1\alpha}$ in the culture medium. As in our system 6-keto- $PGF_{1\alpha}$ seems to be the major metabolite of PGI_2 , ticlopidine is likely to act by releasing important quantities of PGI_2 in macrophages. These results suggest an increase of liberation of the endogenous arachidonic acid from the membrane phospholipids of the macrophages or a lack in the acyltransferase system of the cell membranes. The lipoxygenase pathway was also studied. When ticlopidine is added to macrophages, two products are liberated: 12-HETE as measured by single ion detection, and 10-hydroxy-11-12-epoxy, -5,8,14-eicosatrienoic acid which comes from an internal rearrangement of the 12-HPETE. In these results, there is a discrepancy between the fact that ticlopidine increases the concentration of 12-HETE and surely its precursor the 12-HPETE and the fact that the synthesis of PGI_2 is not inhibited by these high concentrations of hydroperoxide. To understand this phenomenon we studied the production of hydroperoxide when arachidonic acid is incubated with soybean lipoxygenase. When ticlopidine ($10^{-4}M$) is added to the reaction mixture (AA + soy lipoxygenase) there is an increase of the initial rate and an extent of the reaction as if the enzyme irreversible deactivation was postponed. Ticlopidine could act by suppressing the classical inhibition of PGI_2 synthetase by hydroperoxides, in particular 12-HPETE and 15-HPETE, both produced by mammalian cells.