

621

TICLOPIDINE AND PREVENTION OF ARTERIAL REOCCLUSION AFTER DIRECT PERIPHERAL VASCULAR SURGERY. P. Castelli, A. Basellini, G.B. Agus, E. Martelli, R. Zucca. Istituto di Chirurgia Vascolare - Università degli Studi di Milano, Milano, Italy.

Late thrombosis after arterial revascularization surgery is a relatively frequent complication especially when the more peripheral districts are involved. Among the causes of these failures, in addition to progression of the atherosclerotic disease, the behavior of platelet aggregation is of particular importance. With the aim of preventing this pathology we performed two controlled clinical 6-month studies on patients undergoing thrombo-endoarterectomy (TEA) of the femoropopliteal district and on patients undergoing aortobifemoral bypass.

In the first study 47 patients were randomly allocated to receive ticlopidine, 500 mg/day, (23 patients) or placebo (24 patients), and in the second study 125 patients were evaluated (25 treated with ticlopidine, 500 mg/day, and 100 controls).

Physical examination, doppler sonography, angiography, and blood tests were performed on all patients at baseline, during and at the end of treatment.

In the patients who underwent TEA of the femoropopliteal district doppler sonography revealed 3 reocclusions in the ticlopidine-treated patients versus 6 reocclusions and 6 significant stenoses in the placebo group ($p = 0.007$).

Furthermore, clinical symptomatology persisted or reappeared in 5 patients in the ticlopidine group versus 15 in the placebo group ($p = 0.003$).

The active treatment significantly reduced the incidence of acute ischemia. In the patients who underwent a bypass 5 reocclusions were observed in the control group, whereas the bypass remained patent in all the patients who received the active treatment. Ticlopidine was well tolerated in all the patients and no alterations of blood composition or other side effects were observed.

623

DIPYRIDAMOLE INDUCES CHANGES IN THE THROMBOGENIC PROPERTIES OF ENDOTHELIAL CELL EXTRACELLULAR MATRICES. J. Aznar-Salatti, G. Escobar, L. Almirall, A. Ordinas, and E. Bastida. Hospital Clinic i Provincial, Facultat de Medicina, Universidad de Barcelona, Barcelona, Spain.

Dipyridamole (DIP) is a pyrido-pyrimidine drug widely used as an antiplatelet agent. DIP has also been demonstrated to have effects on various cultured cells. We studied platelet reactivity of extracellular matrices (ECMs) produced by untreated (ECM-c) and DIP-treated (ECM-DIP) endothelial cells (ECs). Confluent monolayers of EC were incubated in buffer containing 0 or 10 μ M DIP for 48 h. The ECMs were prepared by removing the EC with 2% EGTA (1 h at 37°C). Platelet deposition onto the ECMs was measured under flow conditions using a flat chamber perfusion model. ECM-c and ECM-DIP were exposed for 5 min to whole blood at shear rates of 300 or 1300 sec^{-1} . Then, the perfused ECMs were processed for cross sectional morphometric evaluation using a computer system. The platelet deposition is expressed as total surface covered with platelets (mean \pm SEM) and shown in the table.

	% Coverage Surface	
	300 sec^{-1}	1300 sec^{-1}
ECM-c	22.3 \pm 2.7	48.8 \pm 3.1
ECM-DIP	19.5 \pm 2.7	39.9 \pm 2.8 *

* $p < 0.05$

Platelet aggregate formation on ECM-DIP at 1300 sec^{-1} was also reduced (by 25%) as measured morphometrically. We conclude that DIP-treatment of EC results in ECM modifications, which in turn decrease ECM-platelet interactions. These data also suggest that pharmacological treatment of the endothelium influences basement membrane reactivity.

622

ANTIPLATELET ACTIVITY OF DYPYRIDAMOLE IN HUMAN WHOLE BLOOD WITH PHYSIOLOGICAL LEVELS OF IONIZED CALCIUM. J. L. Pérez-Requejo*, J. Aznar**, T. Santos**, J. Vallés**. *Universidad de Carabobo, Valencia - Venezuela. **Hospital La Fé, Valencia - España.

In spite of the widespread use of dipyridamole (DP) as an anti-thrombotic drug, its usefulness has not been generally proved. The clinical trials have been somewhat inconclusive or challenged, and most of the studies were not able to show a clear inhibitory action of DP on platelet aggregation. Using the impedance aggregometer it has been shown that the antiplatelet action of DP can be demonstrated in whole blood (WB) *ex vivo* as it was suggested that this was due to the inhibition of adenosine reuptake by the red cells. We have recently described (Thrombosis & Haemostas 54: 799, 1985) a method, that detects the early platelet-collagen interaction in whole blood, which has been called BASIC wave. Using slight modifications of the BASIC wave method, we were able to study the platelet-collagen interactions in native WB with physiological levels of calcium and its inhibition by DP *ex vivo*. We performed the BASIC wave in 25 human healthy volunteers in WB without sodium citrate, WB with citrate and citrated platelet rich plasma (PRP) before and 2 hours after the oral administration of DP 3mg/kg. We observed that in WB with citrate, DP produced a 66% inhibition of the BASIC wave ($p < 0.0005$) in PRP the inhibition was 40.7% ($p < 0.005$) but it was a non-significant 21.7% in WB without citrate. Using hirudin 400U/ml as anticoagulant, the inhibition was the same as in WB without citrate. There were no correlation between the oral dose and the DP plasma levels or with the observed inhibition of the BASIC wave. We postulate that the antiplatelet action of DP is only demonstrable in citrated samples, been negligible in native blood with physiological levels of calcium. The present report could explain the lack of firm data supporting the antithrombotic action of DP in clinical trials. Furthermore, the method here presented allows the study of anti-thrombotic drugs *ex vivo* in whole blood in its normal calcium environment.

624

EFFECTS OF SIN 1 ON PLATELET ACTIVATION INDUCED BY THROMBIN IN HUMAN PLATELETS. M.F. SIMON, H. CHAP, L. DOUSTE-BLAZY. INSERM 101, Biochimie des Lipides, Hôpital Purpan, 31059 TOULOUSE

The mechanism of platelet activation is well known. The interaction of agonist such as thrombin, on specific membrane receptor induces phosphatidylinositol-specific phospholipase C activation, with a concomitant formation of two second messengers (from PIP_2): inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 is able to induce a rapid discharge of Ca^{2+} from internal stores and Ca^{2+} influx through plasma membrane by unidentified Ca^{2+} channels linked to receptor activation. The increase of cytoplasmic free calcium concentration leads to the activation of the calcium calmodulin dependent myosine light chain kinase which phosphorylates 20 kD proteins (myosine light chain). DAG is a potent activator of protein kinase C, which phosphorylates 40 kD proteins. These different pathways act in synergism.

Sin 1 is a platelet aggregating inhibitor. This compound is an active metabolite of molsidomine, which activates platelet guanylate cyclase, inducing a rapid rise in cyclic GMP level. The precise role of cyclic GMP in platelet activation is not yet known. In order to study the mechanism of action of this drug, we tried to determine the effect of Sin 1 on the different steps described above. We measured Ca^{2+} fluxes and phospholipase C activation in thrombin (0.5 U/ml) stimulated platelets in the presence of different doses of Sin 1 (10^{-7} M - 10^{-3} M). Serotonin secretion was inhibited by 30 % with Sin 1 (10^{-4} M - 10^{-5} M). A parallel inhibition of phospholipase C was detected by measurement of (^{32}P)-PA level. Platelets loaded with Quin 2 and stimulated by thrombin showed a 70 % inhibition of external Ca^{2+} influx as soon as a concentration of 10^{-7} M of Sin 1 was added. A study on platelet loaded with ($^{45}\text{Ca}^{2+}$) and Quin 2 confirmed these results. On the contrary, discharge of internal Ca^{2+} store seemed to be unaffected.

In conclusion, the major effect of Sin 1 on platelet phospholipase C pathway is an inhibition of Ca^{2+} influx through plasma membrane. Some further experiments are necessary to show whether this inhibition is correlated with cyclic GMP formation (the major effect of Sin 1) and try to establish a relation between this inhibition and that exerted on phospholipase C. Sin 1 was a generous gift of Hoechst.