

PHARMACOLOGY OF 12-DEOXYPHORBOL PHENYL ACETATE (12-DOPP) INDUCED HUMAN PLATELET AGGREGATION. J. Westwick, E.M. Williamson, F.J. Evans and V.V. Kakkar. Thrombosis Research Unit, Rayne Institute, King's College Hospital, London, England, and Dept. of Pharmacognosy, School of Pharmacy, (Univ London), 29-39 Brunswick Square, London WC1N 1AX, England.

12-DOPP (0.1 to 3.6  $\mu$ M) induced human platelet aggregation which was dependent upon the presence of divalent cations, intracellular level of C-AMP and an intact microtubular system in common with other aggregating agents. However, the small amount of platelet secretion and thromboxane (Tx) B<sub>2</sub> synthesis did not contribute to 12-DOPP induced platelet aggregation as neither the Tx/endoperoxide antagonists pinane A<sub>2</sub> (0.001-0.004 mM) and trimethoquinone (0.01-0.1 mM), the Tx synthesis inhibitors clotrimazole (0.1 to 0.8 mM) and 9, 11, aza-prosta-5-13 dienoic acid (0.002-0.1) nor the cyclo-oxygenase inhibitor indomethacin (0.03-0.1 mM) inhibited 12-DOPP induced aggregation. Furthermore the free radical scavengers aminopyrine (0.2-2.0 mM), thioanisole (0.2-2.0 mM) and butylated hydroxy toluene (0.07-1.4 mM); the lipoxygenase inhibitor phenidone (0.5 mM) and the leucotriene B and C antagonist FPL55712 (0.005-0.06 mM) failed to modify 12-DOPP induced aggregation.

However compounds which are thought to act as phospholipase inhibitors bromophenacyl bromide (0.3 mM), meprazine (0.2 mM) and propranolol (0.2 mM) were found to be effective inhibitors of 12-DOPP induced aggregation as well as the so-called calmodulin antagonists imipramine (0.12 mM), desmethylinipramine (0.033 mM), promethazine (0.1 mM) and trifluoperazine (0.35 mM).

The aggregation induced by 12-DOPP involves a direct effect upon platelets followed by the release of unknown substances probably phospholipids, which induce further aggregation of platelets.

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IN VITRO AND IN VIVO STUDIES ON CP 1415 S A NEW PLATELET ANTIAGGREGATING COMPOUND. G. Biagi, P. Niebes, J. Roba and G. Lambelin. Continental Pharma, Research Laboratories, Maelen, Belgium.

CP 1415 S is a new phenyloxypropanolamine derivative.

In vitro CP 1415 S inhibits aggregation of human platelets induced by collagen (EC<sub>50</sub> 9  $\mu$ M) or arachidonic acid (EC<sub>50</sub> 12  $\mu$ M). It also inhibits primary and secondary phases of ADP, thrombin and epinephrine induced platelet aggregation. Effective concentrations are in the same range (10  $\mu$ M) as concerns the secondary aggregation and higher as concerns the primary phase. In presence of 50  $\mu$ M CP 1415 S, platelet aggregation is fully prevented. Addition of CP 1415 S to platelets maximally aggregated by ADP causes disaggregation in a dose dependent way (EC<sub>50</sub> 50  $\mu$ M); similar effect is observed only with prostacyclin (PGI<sub>2</sub>) and PGE<sub>1</sub>.

Ex-vivo, the antiaggregating properties of CP 1415 S have been demonstrated after single oral administration to rats and Rhesus monkeys (minimal effective dose 10 and 20 mg/kg respectively). After repeated oral administrations, antiaggregating activity was observed from 3 mg/kg/day in rats.

In vivo, in rats, using the tail transection model, bleeding time was significantly ( $p < 0.01$ ) prolonged by 10 mg/kg p.o. CP 1415 S or ticlopidine. CP 1415 S given orally up to 100 mg/kg had no effect on prothrombin time. CP 1415 S at 1 mg/kg p.o., 2 hours before testing, protected rats ( $p < 0.001$ ) against acute thrombocytopenia induced by i.v. injection of ADP whereas acetylsalicylic acid and dipyridamole were inactive at 100 mg/kg and ticlopidine was unsignificantly active. Prostacyclin-like substance production by rat vascular tissue, measured by platelet aggregation bioassay, was not affected in rats after 5 or 10 mg/kg i.v.

CP 1415 S appears to be a potent inhibitor of platelet aggregation in vitro and ex-vivo. Its ability to disaggregate platelets is currently tested in in vivo models of thrombosis.

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THE ACTIVITY OF PHTHALAZINOL IN BASIC PLATELET FUNCTION TESTS. R.N. Saunders, S.L. Smith and N.S. Nicholson. G. D. Searle & Co., Research & Development Division, Department of Biological Research, P.O. Box 5110, Chicago, Illinois 60680.

Phthalazinol (EG-626, SC-32840) was evaluated in several basic platelet function tests in comparison with known antiplatelet agents. The IC<sub>50</sub>'s for collagen-, ADP-, and thrombin-induced aggregation of human platelets in vitro were 28, 51 and 88  $\mu$ M, respectively. Phthalazinol (100  $\mu$ M) did not alter the cAMP levels of rat platelets in vitro in either the presence or absence of PGE<sub>1</sub>. When administered orally to retired breeder rats, Phthalazinol prevented the formation of platelet aggregates as determined by the Wu and Hoak technique with ED<sub>50</sub>'s of 5.5 and 18.7 mpk, i.g. at 3 and 24 hours, respectively, post injection. Phthalazinol did not alter the platelet serotonin content in rats even when administered at 100 mg/kg orally for three days. In an ex vivo disaggregation assay, arterial blood from anesthetized cats was perfused over rabbit tendon strips and recirculated to the cat. The resulting platelet build-up (200-400 mg) was significantly ( $p < .001$ ) reversed (67% loss) by the i.v. infusion of Phthalazinol at 15 mg/kg. Phthalazinol has demonstrated positive effects in several platelet evaluation models and is a potentially unique antithrombotic agent.

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LU 23051, A NEW POTENT INHIBITOR OF PLATELET AGGREGATION. H.D. Lehmann, J. Gries, D. Lenke. Biologische Forschung und Entwicklung, BASF Aktiengesellschaft, Unternehmensbereich-Pharma, D-6700 Ludwigshafen (FRG).

6-[p-(2-(Chlorpropionylamino)phenyl)-4,5-dihydro-5-methyl-3(2H)-pyridazinone, LU 23051, is primarily characterized by its strong inhibition of platelet aggregation under in vitro and in vivo conditions. In vitro there is a concentration-dependent inhibition of ADP and collagen induced aggregation in platelet rich plasma of man, rat and dog. The inhibitory concentration EC 33 % is 0.0010-0.030 mg/l (man: ADP-0.030, coll.-0.013 mg/l) depending on species and type of aggregation. When administered orally in ex vivo experiments on rats and dogs the substance is found to have a dose-dependent antiaggregatory effect in the range from 0.1-3.16 mg/kg. The ED 33 % is 0.27-0.63 mg/kg.-In addition after oral administration the substance has a good inhibitory effect in models being based on intravascular platelet aggregation. Thus, a dose of 1 mg/kg inhibits laser-induced aggregation in mesenteric venules of rats. Mortality after i.v. injection of collagen in mice is reduced by 50 % after a dose of 0.02 mg/kg. A dose of 0.039 mg/kg prolongs the bleeding time of rats by 50 %. The aggregation-inhibiting action is of long duration (0.1 mg/kg p.o. ~ 24 h). The substance does not interfere with clotting.

Besides its effect on platelet aggregation LU 23051 acts as vasodilator as well. Dilatation of coronary vessels by 100 % is seen in isolated guinea-pig hearts at a concentration of 0.1 mg/l. In spontaneously hypertensive rats the substance has an antihypertensive effect. The ED 20 % is 0.36 mg/kg p.o.

The combination of antiaggregatory and vasodilatory effects opens up interesting aspects with respect to the pharmacotherapeutic use of the new substance.