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THE NEWLY SYNTHESIZED COMPOUND E-5510 IS A HIGHLY POTENT ANTI-PLATELET AGENT. K. Harada (1), T. Fujimori (1), M. Kogushi (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).

Our newly synthesized compound, 4-cyano-5,5-bis(methoxyphenyl)-4-pentenoic acid (E-5510) has highly potent antiplatelet activity. In this paper, the effects of E-5510 on platelet functions in vitro and ex vivo in human and in various experimental animals are examined.

E-5510 inhibited human platelet aggregation induced by collagen, arachidonate, ADP, PAF and epinephrine (IC50: 1.5, 0.7, 2.0, 1.6 and 1.1 uM, respectively). Thrombin-induced platelet aggregation, which was not inhibited by aspirin and U-53059 (IC50s: 100 uM), was also inhibited by this compound (IC50: 21 uM). The IC50 of E-5510 in thrombin-induced ATP secretion from human platelets was only 2 uM. Platelet adhesion to a collagen coated disk, which was measured by the method of Buchanan et al (Prost. Leuko. Med., 21, 157, 1986) was inhibited by E-5510 (IC50: 19.3 uM) but not by aspirin and U-53059. In the PRP of the guinea pig, the beagle and the monkey, E-5510 inhibited collagen-induced platelet aggregation in vitro to the same degree as in human PRP(IC50: 1.2, 0.6 and 1.5 uM, respectively). After being administered orally to guinea pigs, E-5510 exhibited extremely potent ex vivo inhibitory effect in collagen-induced platelet aggregation with a very low ED50 of 0.05 mg/kg. In contrast, the ED50's of ticlopidine, aspirin and U-53059 were 300 , 27.2 and 1.0 mg/kg, respectively. In beagles and monkeys E-5510 also showed ex vivo antiplatelet effects at 0.01 and 0.003 mg/kg, respectively. This effect continued for more than 8 hrs. and disappeared within 24 hrs. The antiplatelet effect in human PRP was highly correlated with that in PRP of experimental animals in which the ex vivo effects were confirmed at a very low dose. Thus, E-5510 will ensure to exert the antiplatelet effect after oral administration to human subjects.

In summary, E-5510 is unique among the known antiplatelet agents since it has potent inhibitory effects on thrombin-induced platelet activation and platelet adhesion to collagen. It was also shown that this compound had an ex vivo antiplatelet effect at an extremely low ED50. Our results suggest that E-5510 will be a beneficial agent for antiplatelet therapy in humans.

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ANTI-THROMBOTIC EFFECTS OF E-5510 IN EXPERIMENTAL THROMBOSIS MODELS. T. Fujimori (1), T. Saeki (1), K. Harada (1), M. Sato (2) and N. Ohshima (2). Eisai Res. Labs., Tsukuba, Ibaraki, Japan (1) and Inst. Basic Medical Sciences, Univ. Tsukuba, Ibaraki, Japan (2).

A new agent developed in our laboratory, 4-cyano-5,5-bis(4-methoxyphenyl)-4-pentenoic acid (E-5510), suppressed various human platelet functions in vitro. The compound also showed quite potent ex vivo anti-platelet effects in many experimental animals. It is well known that anti-platelet effects are not always parallel to anti-thrombotic effects. Thus, in order to predict the efficacy of E-5510 in thrombotic disorders, the anti-thrombotic effects were examined in 3 different animal models of thrombosis.

(1) Anti-thrombotic effect in an extracorporeal shunt model

Two hrs after oral administration of the drug to guinea pigs, an extracorporeal shunt between the right carotid artery and the left jugular vein was performed. The thrombus formation on a silk thread inserted in the shunt tube was quantitated by measuring the time from the onset of circulation to the stenosis of blood flow. E-5510 dose-dependently inhibited thrombus formation, the minimum effective dose being 0.03 mg/kg.

(2) Effect on microthrombus formation in mesenteric arteriole

In order to evaluate the effect on intravascular platelet thrombus formation, thrombosis was induced in vivo in mesenteric arteriole in guinea pigs with filtered light from a mercury lamp and an intravenous fluorescent dye in an intravital microscope system (M. Sato and N. Ohshima, Thromb. Res.,35, 319, 1984). The thrombus formation was quantitated by measuring the time taken for circulating platelets to begin to adhere to vessel wall and the time taken for blood flow to stop completely due to fully developed thrombus. Both the time required for platelet adhesion to vessel wall and for platelet thrombus formation were significantly prolonged after oral administration of E-5510.

(3) Effect on pulmonary thromboembolism

Acute pulmonary thromboembolism was induced in mice by a bolus intravenous injection of arachidonic acid, and mortality was determined 3 min later. E-5510 dose-dependently reduced pulmonary thromboembolic mortality, and its ED50 was 0.11 mg/kg.

The results described above indicate that E-5510 may have beneficial effects in clinical treatments of thrombotic disease.

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PROTECTIVE EFFECT OF A NEW SYNTHETIC COMPOUND: PCA-4230, ON SEVERAL *in vivo* THROMBOSIS MODELS. M.P. Ortega, C. Sunkel & J.G. Priego. Dpto. Investigación, ALTER, S.A., Madrid - SPAIN.

PCA-4230 is a new anti-thrombotic compound which inhibits platelet aggregation *in vitro* and *ex vivo* in several species, including man, prolongs the bleeding time and has potent protective activity in several thrombosis models. Phase I trials with different dosage schedules have recently been initiated.

In the present study, the effects of PCA-4230 on bleeding time and on several *in vivo* thrombosis models were studied in mice.

Mice were treated with one single oral dose of PCA-4230 (1-10 mg/kg). One hour after treatment, mice were injected intravenously with four thrombotic challengers {arachidonic acid (AA), thromboxane agonist (U46619), Platelet Activating Factor (PAF) or collagen/epinephrine combination (C/E)} at a dose which induced 80-90% of mortality. The thrombotic agents were prepared in saline. The appropiate doses were dissolved in a volume of 100 µl/mouse.

Bleeding time was measured in non-anesthetized mice by the tail transection technique.

Effects of compound were recorded from 1 to 4 hours after dosage.

Acute pre-treatment with PCA-4230 showed a significant dose-dependent protective effect.

Results of each series of experiments are given in the next table.

Agonist injected	DOSE OF PCA-4230 (mg/kg) 0	1	3	5	10
U46619 (% protection)	0	n.t.	n.t.	20	54*
PAF (% protection)	0	n.t.	22	38*	45*
C/E (% protection)	0	30*	60***	70***	80***
AA (duration of distress, sg)	930±283	n.t.	n.t.	845±190	750±120#
Bleeding time (AUC)	234	224	254*	330*	432**

n.t.: not tested; #P(t) < 0.05; *P(χ^2) < 0.05; **P(χ^2) < 0.01; ***P(χ^2) < 0.001; AUC: area under curve (% effect versus time).

The compound inhibited thrombotic sudden death induced by U46619, PAF or C/E combination, reduced the duration of respiratory distress induced by AA and prolonged bleeding time. Thus, PCA-4230 is protective against a variety of thrombotic stimuli.

These results suggest that PCA-4230 may be a promising anti-thrombotic drug.

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INHIBITION OF HUMAN PLATELET FUNCTION BY PCA-4230. M.P. Ortega, C. Sunkel & J.G. Priego. Dpto. Investigación. ALTER, S.A. Madrid - SPAIN.

PCA-4230 (3-{2-(N-1,2-benzisothiazolyl-3(2H)one-1,1-dioxide) ethoxycarbonyl}-2,6-dimethyl-5-ethoxycarbonyl-4-methyl-1,4-dihydropyridine) is a new synthetic compound which has been selected after evaluation of several series of molecules included in an extensive program of synthesis and biological screening.

The purpose of this study was to investigate the *in vitro* effects of PCA-4230 on human platelet function.

Platelet aggregation (PA) was measured, in platelet rich plasma (PRP) or washed platelets, according to Born's technique. Release reaction (RR) was measured by the luminiscence method as adenosine triphosphate (ATP) release in response to stimulation.

Aggregating agents were adenosine diphosphate (ADP), epinephrine (Epi), collagen (Col), thrombin (Thr), calcium ionophore (A23187) arachidonic acid (AA), thromboxane agonist (U46619) or platelet activating factor (PAF). Incubation with PCA-4230 (1 to 10 µM) were carried out at 37°C for 15 minutes. PCA-4230 potentiation of Prostacyclin (PGI_2) anti-aggregatory activity was also studied by addition to PRP of PGI_2, PCA-4230 or both, and PA by ADP.

PCA-4230 inhibited PA and RR in PRP in a concentration-dependent fashion when Col, Epi, U46619 or PAF were used as agonists. AA- and Thr-induced aggregation were only slightly impaired and no inhibition was shown on ADP or A23187-triggered activation.

A23187-induced aggregation and RR were inhibited only in the absence of extracellular Ca^{++} in washed platelets. This effect was overcome by addition of Ca^{++} 1 mM. Additionally, the inhibitory effect of PGI_2 on ADP-induced PA, was synergistically potentiated by PCA-4230, suggesting inhibitory activity of the compound on cyclic nucleotide phosphodiesterase.

Since PCA-4230 inhibited PA and RR induced by Epi, U46619 and PAF, mediated via receptor-agonist binding, and Col-induced activation, it is reasonable to suspect that common process(es) may be involved. Recently, it has been suggested that intraplatelet Ca^{++} levels, acting as a second messenger, are directly linked to the degree of platelet activation. Therefore, the ability of PCA-4230 to modulate platelet function appears, at least in part, to be due to regulation of cytosolic Ca^{++} levels. This hypothesis is confirmed by the results with A23187-induced aggregation in absence of extracellular Ca^{++}.