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INHIBITION OF GUINEA-PIG PLATELET FUNCTION IN VIVO AND EX VIVO USING THE THROMBOXANE A2 ANTAGONISTS, AH23848 AND GR32191.
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The thromboxane $A_2$ antagonist, GR32191 (Lumley et al., this meeting) was tested as an inhibitor of platelet aggregation in the guinea-pig and compared with another Tx-antagonist, AH23848 (Brittain et al, 1985). Guinea-pigs were dosed with AH23848 or GR32191 at 0.01-1.0mg/kg. At intervals, blood was taken and PRP was prepared for ex vivo aggregation studies. Collagen concentrations causing half maximal aggregation ($IC_{50}$) were calculated for test and vehicle-dosed groups. Inhibition was expressed as a concentration ratio ($IC_{50}$ test/$IC_{50}$ vehicle). For in vivo studies, $^{111}$In-labelled platelets (12µCi, 200µl) were injected into anaesthetised guinea-pigs and 24 hrs later oral doses of AH23848 or GR32191 (0.01-1.0mg/kg) or indomethacin (5mg/kg) were given. After one hour, blood was taken for platelet and radioactivity counting. The carotid artery was exposed under anaesthesia and a current of 2mA was applied for 60 sec. After 90 min, 1cm of the damaged and contralateral carotid vessels were removed for gamma-counting. Inhibition of accumulation of platelets on the injured artery was measured by comparison with the undamaged contralateral artery. Numbers of platelets deposited were calculated from the radioactivity of each section of artery and the radioactivity and platelet count in the blood. Oral doses of AH23848 or GR32191 inhibited ex vivo platelet aggregation induced by collagen. Maximum inhibition occurred one hour after dosing, and was still present at 6 hours for AH23848 (1.0mg/kg) and GR32191 (0.3mg/kg). GR32191 and AH23848 were active in vivo causing inhibition of platelet deposition at doses of 0.01-1mg/kg. The maximum inhibition of deposition was 58% for AH23848 (0.1mg/kg) and 63% for GR32191 (0.1mg/kg), with 50% inhibition at 0.02mg/kg for both. Indomethacin (5mg/kg p.o.) caused maximum inhibition of 58% at 5mg/kg p.o. suggesting that this represents the total thromboxane involvement in platelet deposition. GR32191 and AH23848 are thromboxane $A_2$ antagonists with antithrombotic activity after oral dosing to guinea-pigs.
Brittain R.T. et al Circulation, 72, 1208-1218, 1985.

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INHIBITION OF PLATELET ACTIVATION BY THE NOVEL THROMBOXANE RECEPTOR ANTAGONIST BM 13.505.
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The effects of BM 13.505 (4-[2-(4-Chlorobenzenesulfonylamino)ethyl]-benzene acetic acid = BM) on human washed platelets and platelet-rich plasma (PRP) were studied in vitro and after oral application in 10 male volunteers ex vivo/in vitro. BM inhibited the shape change, aggregation and [$^3$H]serotonin release when the platelets were activated by agents that stimulate via the thromboxane $A_2$/prostaglandin $H_2$ ($TXA_2/PGH_2$) receptor. Such agonists were collagen, methyl mercury chloride (methyl-Hg), arachidonic acid and the $PGH_2$ analogue U 46,619. BM was 9 times more potent an inhibitor than sulotroban (= BM 13.177). The slope of the Schild plot for U 46,619-induced shape change was close to unity, which is consistent with competitive antagonism. The $pA_2$-value in PRP was 6.5. BM did not inhibit the primary platelet activation induced by ADP, PAF or serotonin in aspirin-treated platelets, indicating that the inhibition by BM was specific for the platelet $TXA_2/PGH_2$ receptor. BM also suppressed platelet activation by $PGH_2$ which accumulated when the platelets were stimulated by collagen, methyl-Hg or arachidonic acid in the presence of the thromboxane synthase inhibitor dazoxiben. At concentrations beyond about 600 times the apparent $K_D$ (200 µM in PRP and 10 µM in washed platelets), BM induced a transient shape change. This effect was inhibited by sulotroban and might indicate a slight intrinsic activity of BM. BM 10 µM exerted no effect on the formation of $^{14}$C-labelled $TXB_2$, $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, HHT and 12-HETE from $^{14}$C-labelled arachidonic in washed platelet suspensions, irrespective of whether exogenous or endogenous (by methyl-Hg mobilized) arachidonic acid was metabolized. Volunteers who received 7 oral doses of 400 mg in 12 hour intervals reached peak plasma concentrations between 9.5 and 45 µM 1 hour after dosage. The bleeding time was prolonged by 90 %. Platelet activation by collagen, methyl-Hg and U 46,619 was inhibited for at least 9 hours after a single dose. No objective and subjective side effects were observed in any of the subjects. Thus, BM 13.505 is a specific, well-tolerated and long-acting $TXA_2/PGH_2$ receptor antagonist. (Supported by the DFG, Grant Pa-263).

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**R 68 070, A COMBINED $TXA_2$-SYNTHETASE/$TXA_2$-PROSTAGLANDIN ENDOPEROXIDE RECEPTOR INHIBITOR, REDUCES CEREBRAL INFARCT SIZE AFTER PHOTOCHEMICALLY INITIATED THROMBOSIS IN SPONTANEOUSLY HYPERTENSIVE RATS.**
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The effects of R 68 070, an oxime-alkane carboxylic acid derivative combining specific thromboxane $A_2$ ($TXA_2$) synthetase inhibition with $TXA_2$/prostaglandin endoperoxide receptor blockade in one molecule, were investigated in a model of photochemically induced stroke in spontaneously hypertensive rats.
Each experimental group was compared with an untreated control group. All animals were anesthetized with halothane in $N_2O/O_2$ and artificially ventilated. After incision of the scalp and stereotaxic positioning of a fibre optic light source, halothane was discontinued. When physiological variables reached normal values, a focal cortical infarction was produced by injection of 10 mg.kg$^{-1}$ rose bengal and 20 min irradiation of the brain through the intact skull. Four hours later the brains were perfusion fixed and damaged areas measured on consecutive histologic sections. Infarct size was calculated by numerical integration.
R 68 070 (40 mg.kg$^{-1}$ p.o., -3 h) significantly reduced the cerebral infarct size to 2.32 mm$^3$ compared with 5.78 mm$^3$ in controls (median values; n = 5; $p < 0.05$). At 2.5 mg.kg$^{-1}$ the lesion was reduced from 11.75 mm$^3$ in the control group to 7.82 mm$^3$ in the treated group (n = 5; p = 0.095). Serum $TXB_2$ levels were reduced by > 80 %.
Production of damage in this model is based upon photodynamic generation of singlet molecular oxygen, resulting in peroxidative endothelial cell injury and subsequent platelet thrombus formation. Protection with R 68 070 can be explained by the anti-thrombotic effect of the compound. The relative contribution to this protective effect of synthetase and receptor blockade by R 68 070 are being investigated.

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TREATMENT OF ADVANCED STAGES OF PERIPHERAL OBLITERATIVE DISEASE WITH THE THROMBOXANE RECEPTOR ANTAGONIST BM 13.177: A PLACEBO-CONTROLLED DOUBLE BLIND STUDY.
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Twenty patients (67.2 ± 12.3 yrs; aged 39-86 yrs, 10 males, 10 females) with stage IV of peripheral obliterative arterial disease received at random either a) BM 13.177, a thromboxane receptor antagonist, at a dose of 6 g/24 hrs for 7 days i.v., followed by oral treatment of 6.4 g/day in four doses for 2 weeks and a subsequent placebo week, or b) placebo alone with the same protocol. The clinical course was followed by measurement of blood pressure (by Riva-Rocci on the left brachial artery and by Doppler of the ankle artries), blood flow at restand after 3 min of tourniquet ischemia (by venous occlusion plethysmography), $TcpO_2$ at the wrist of the affected limb, and by subjective estimation of pain with visual analog scale before, at the end of the infusion period, as well as on day 7 and 14 of oral treatment, and 7 days after treatment while patients of both groups were on placebo. In addition spontaneous platelet aggregation by PAT III, induced platelet aggregation in whole blood by collagen and in PRP by ADP at different doses on the same days were measured on the same days as described above.
Results: BM 13.177 completely inhibited aggregation in whole blood induced by collagen 0.3 µg/ml, however one week after treatment a rebound phenomenon was observed with 16.0 ± 3.8 OHM compared to pretreatment value of 11.0 ± 3.8 OHM ($p < 0.01$). At a higher dose of 1.2 µg/ml the same inhibiting effect on platelet aggregation was observed. Spontaneous platelet aggregation as measured by PAT III was evident in only 2/10 pat. pre-treatment, was abolished in all patients on i.v. BM 13.177, returned in 1/10 pat. during oral treatment, but in 4/10 pat. on day 7 after treatment, while being on placebo, again indicating a rebound phenomenon ($p < 0.05$) ADP induced platelet aggregation was not significantly affected by BM 13.177. In the placebo group, too, no significant differences were observable between the different treatment regimens. Clinical data did not show any significant alteration in either verum or placebo group during the six week period, indicating no benificial effect of thromboxane receptor antagonists in advanced stages of peripheral obliterative arterial disease, although platelet inhibiting effects were clearly demonstable.