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S_2-SEROTONERGIC RECEPTOR INHIBITION (KETANSERIN), COMBINED WITH THROMBOXANE A_2/PROSTAGLANDIN ENDOPEROXIDE RECEPTOR BLOCKADE (BM 13177) : ENHANCED ANTI-PLATELET EFFECT.

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Subsequent to an initial activation, several products are released from the platelets, and -potentially- contribute to the recruitment of new cells to build up the eventual platelet haemostatic plug or thrombus. Among the various accussates, serotonin as well as the arachidonic acid derivatives thromboxane A_2 (TXA_2) and prostaglandin endoperoxides (PGEND) seem to be involved as causative mediators, in particular when acting in concert on the same target cells. Indeed, in vitro at concentrations achievable after oral administration to man, either ketanserin (K), an S_2-serotonergic receptor antagonist or BM 13177 (BM), an antagonist at TXA_2/PGEND receptors, pre-incubated separately for 5 min at 37°C with human citrated platelet-rich plasma, reduces to some extent the rate of the second-wave, irreversible platelet aggregation elicited by critical concentrations (1 to 2 x 10^{-6} M) of ADP. However, the combindation of both agonists results in a significantly more pronounced inhibition than achieved with the single products (K 1 x 10^{-7} M : 26.1 ± S.E.M. 9.9 %, n = 8, $p < 0.05$; BM 1 x 10^{-7} M : 25.1 ± S.E.M. 8.7 %, n = 8, $p < 0.05$; K + BM at 1 x 10^{-7} M each : 65.5 ± S.E.M. 10.3 %, n = 8; $p < 0.05$ versus K or BM separately). In vivo in rats, a modest prolongation of the bleeding time occurs with K (1.25 mg/kg orally -2 h : 410 sec, median values n = 9, $p < 0.05$) but not with BM (40 mg/kg orally, -2 h : 250 sec, n = 9) in comparison with controls (190 sec, n = 9); the amount of initial blood loss as a parameter for anti-vasoconstriction is significantly increased by K (140.4 µl/30 sec) but not by BM (77.2 µl/30 sec) in comparison with controls (35 µl/30 sec). Again, the combined treatment with both agonists results in a marked prolongation of the bleeding times (> 2065 sec, n = 9, $p < 0.05$) without further significant increase of the initial blood loss, indicating the interplay between serotonin and TXA_2/PGEND to be of primary importance for the aggregation of platelets in damaged vessels.

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PLATELET TXA_2 SYNTHETASE INHIBITION AND TXA_2/PROSTAGLANDIN ENDOPEROXIDE RECEPTOR BLOCKADE COMBINED IN ONE MOLECULE (R 68070).

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F 68070, an oxime-alkane carboxylic acid derivative (Janssen Pharmaceutica), is a potent inhibitor of thromboxane A_2 (TXA_2) synthetase activity (IC50 in vitro against thrombin-stimulated human platelets in plasma : R 68070 : 2.9 x 10^{-8} M; CGS 13080 : 6 x 10^{-8} M; OKY-1581 : 8.2 x 10^{-8} M; dazmegrel : 2.6 x 10^{-6} M; dazoxiben : 2.3 x 10^{-6} M).
The compound specifically inhibits platelet TXA_2 synthetase activity (^{14}C-arachidonic acid metabolism by washed human platelets) without effect on the cyclo-oxygenase, lipoxygenase (platelets, RBL cells) or prostacyclin synthetase activities (rat aortic rings).
The inhibitory effect of R 68070 against human platelet TXA_2 synthetase activity increases upon prolongation of the contact time (IC_{50} at 0.5 min of contact : 5.2 x 10^{-7} M; at 5 min : 8.3 x 10^{-8} M; at 30 min : 2.5 x 10^{-8} M) and is reversed by washing of the platelets.
In vivo, the compound has a comparatively strong inhibitory effect on platelet TXA_2 synthetase activity after oral administration to rats (ED_{50} - 2 h : R 68070 0.013 mg/kg; CGS-13080 : 0.8 mg/kg; OKY-1581 : 0.61 mg/kg; dazmegrel : 1 mg/kg; dazoxiben : 4.1 mg/kg) and a protracted duration of action in rats and dogs (inhibition 8 h after 1.25 mg/kg orally > 80 %).
In vitro, R 68070 inhibits the aggregation of human platelets in plasma stimulated with collagen (IC_{50} : 4 x 10^{-6} M), but also with U 46619 (IC_{50} : 3.8 x 10^{-6} M) without affecting the primary aggregation reaction elicited by ADP, 5-HT or adrenaline. The compound thus also produces platelet TXA_2/prostaglandin endoperoxide receptor blockade.
In rats and in dogs R 68070 (1.25 mg/kg I.V.) potently prevents thrombus formation in carotid and coronary arteries damaged by electrical stimulation.
The combination of platelet TXA_2 synthetase inhibition with TXA_2/prostaglandin endoperoxide blockade in one molecule thus might offer an improved anti-thrombotic effectiveness.

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RESTING MYOCARDIAL ISCHEMIA AFTER INTRAVENOUS INFUSION OF BM 13.177, A THROMBOXANE RECEPTOR ANTAGONIST

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We conducted a double blind placebo controlled trial of BM 13.177, a thromboxane receptor antagonist, given intravenously in patients (PTS) with stenoses ≥70 % of the left anterior descending coronary artery and stable exertional angina pectoris (AP). The study had to be stopped after enrollment of 8 PTS, because 2 had developed resting AP after initiation of the study medication. Both proved to belong to the 4 PTS who had received BM 13.177 (12.5 mg/min). While in one PT, AP was mild, transient and associated with only slight decreases in coronary sinus blood flow (CSBF) and myocardial lactate extraction (MLE), in the other, AP was severe and persisted for 30 minutes in spite of antianginal therapy. Severe clinical symptoms in this PT were associated with a marked fall in MLE from +24 to -121 %. Two PTS under BM 13.177 and 4 on placebo underwent supraventricular stimulation. For both groups, no change in clinical symptoms, CSBF or MLE occured in comparison to a former control stimulation without medication. BM 13.177 led to an inhibition of ex vivo platelet aggregation induced by collagen 1 µg/ml (mean reduction in rate of aggregation by 41 %, $p < 0.05$), while aggregation was not influenced with collagen 5 µg/ml or ADP. This effect of BM on platelets is explained by its thromboxane receptor blocking properties. The induction of resting myocardial ischemia, however, in 2 of 4 PTS with formerly stable exertional AP may have been the result of either a coronary steal mechanism or an intrinsic stimulation of vascular thromboxane receptors, followed by coronary vasoconstriction.

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INITIAL STUDIES IN MAN WITH A NOVEL THROMBOXANE RECEPTOR BLOCKING DRUG GR32191.

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In-vitro GR32191 is a potent and specific thromboxane receptor blocking drug on platelets, and vascular and airways smooth muscle (Lumley et al this meeting). We have undertaken studies in healthy male subjects (n) to examine the effects of oral GR32191 upon platelet aggregation ex-vivo and template bleeding time. Platelet aggregation was monitored in whole blood by counting platelets electronically. Concentration-effect curves to U-46619 and ADP were constructed prior to and following drug or placebo. The degree of rightward displacement of a curve due to treatment was expressed as a concentration-ratio (CR) which was calculated at the 50% aggregation level (ECso post-treatment ÷ ECso pre-treatment). Plasma concentrations of GR32191 were determined by h.p.l.c. After single doses of GR32191 mean peak CR's of 8 and 80 were achieved with 0.125 and 0.25mg/kg (n=4) and values of 74 and 234 with 0.5 and 1mg/kg (n=4). Peak effects were seen within 2 hours of dosing while activity was still present between 8 and 24 hours. ADP-induced aggregation was unaffected by drug (CR<2) and placebo was without significant effect upon the sensitivity to either aggregating agent (CR<2). GR32191 was rapidly absorbed and the plasma elimination half-life was about 2 hours. GR32191 17.5mg 12-hourly for 10 days (n=6) produced a progressive antagonism of U-46619 induced aggregation which resulted in a large continuous blockade in all subjects (range of 12h trough CR's 85 to 287). However, plasma concentrations of GR32191 did not accumulate on repeated administration. In a double-blind, placebo-controlled, cross-over study (n=16), a statistically significant (p= 0.002) increase in bleeding time was seen following treatment with GR32191 40mg twice daily for 7 days (pre-treatment mean 3.79 min, post-placebo mean 3.47 min, post-GR32191 mean 5.42 min). Rectal bleeding (n=1) has occurred with GR32191 but otherwise tolerability has been good. No drug related changes have been seen in routine laboratory safety screens. Clinical studies are in progress.