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ENHANCED PHOSPHOLIPASE A_2 ACTIVITY IN CHOLESTEROL-ENRICHED PLATELETS FROM RABBITS FED A CHOLESTEROL-ENRICHED DIET.
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Phospholipase A_2 (PLA_2) could be the rate-limiting enzyme in the metabolism of arachidonic acid (AA) derived from membrane phospholipid to thromboxane A_2 (TXA_2). Malondialdehyde (MDA) production, which is considered to be an index of TXA_2 synthesis, is increased in platelets which have been enriched in cholesterol by incubation with cholesterol-rich phospholipid dispersions in vitro.

Rabbits were fed a diet supplemented with 0.5% w/w cholesterol for 4 weeks after which serum cholesterol was determined and the platelets examined and compared with rabbits fed a control diet. The cholesterol:phospholipid molar ratio (C/P) in the platelets, MDA production (stimulated by AA (1mM) and basal) and PLA_2 activity were estimated. PLA_2 activity was estimated by measuring the % conversion by resuspended washed platelets of 1-acyl-2-(1-^{14}C)arachidonyl phosphatidylcholine to (1-^{14}C)arachidonic acid on stimulation with collagen (2 µg/ml). Serum cholesterol in the cholesterol-fed group (n=9) was 488 ± 104 mg/100ml compared with the control group (n=3) which was 34 ± 4.7 mg/100ml. Platelets from the cholesterol-fed rabbits showed a 20% increase in C/P ($p < 0.05$); basal and AA stimulated MDA production was increased by 40% and 27% respectively compared with platelets from the control group. PLA_2 activity was 1.26% conversion to products in the cholesterol-enriched platelets compared with 0.10% in the control platelets. This increase in activity was significant ($p < 0.05$).

These results suggest that increased AA metabolism in cholesterol-enriched platelets may in part be due to increased PLA_2 activity. This may reflect a physical effect of cholesterol on the platelet membrane predisposing arachidonyl phosphatidylcholine to PLA_2 catalysed hydrolysis.

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UK-37,248, A NOVEL, SELECTIVE THROMBOXANE SYNTHETASE INHIBITOR WITH ANTI-AGGREGATORY AND ANTI-THROMBOTIC ACTIVITY.
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UK-37,248, 4-(2-(1H-imidazol-1-yl)ethoxy)benzoic acid hydrochloride, potently inhibited human blood platelet microsomal thromboxane (Tx) synthetase, $IC_{50}=3x10^{-9}M$. TxB_2 was quantitated by a specific radioimmunoassay (RIA). In contrast (PG) endoperoxide synthesis by ram seminal vesicle microsomes and prostacyclin (PGI_2) synthesis by pig aortic microsomes were minimally affected by concentrations of UK-37,248 up to $1x10^{-4}M$. In the rabbit isolated perfused lung arachidonic acid (AA) metabolites were quantitated by differential bioassay. Concentrations of UK-37,248 from 10^{-7}-$10^{-6}M$ selectively reduced TxA_2 production from AA but increased the release of PGI_2 and other PGs.

The effect of UK-37,248 in the whole animal was studied by estimating TxB_2 levels in blood samples removed before and after dosing. The samples were allowed to clot and the serum TxB_2 levels were assessed by RIA. In anaesthetised rabbits, 15 minutes after injection of 0.3mg/kg i.v. UK-37,248, serum TxB_2 levels were reduced by 75%. In dogs the compound was similarly effective, 1mg/kg p.o. inhibiting TxB_2 production by 79% two hours after dosing. Aggregation of human platelet-rich plasma in vitro, initiated by threshold collagen, was inhibited by UK-37,248 ($IC_{50}=4.8x10^{-6}M$). In rabbits, UK-37,248 at 2mg/kg i.v. prevented the mortality due to pulmonary embolism and reduced the associated thrombocytopenia and elevation of plasma TxB_2 caused by i.v. AA. In conclusion, UK-37,248 is a selective inhibitor of platelet Tx-synthetase with anti-aggregatory and anti-thrombotic activity.

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IN VITRO AND IN VIVO EFFECTS OF AN INHIBITOR OF THROMBOXANE SYNTHETASE.
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UK-37,248 (4-(2-(1H-imidazol-1-yl)ethoxy)benzoic acid hydrochloride) completely inhibits platelet aggregation in plasma by low concentrations of arachidonic acid at 0.5 uM and thromboxane B_2 (TXB_2) generation in washed platelets at 10 uM. In the latter test system the total amount of cyclooxygenase metabolites is unaltered, the decrease in TXB_2 and hydroxyhepatadecatriënoic acid being compensated by an increase in prostaglandins E_2 and $F_{2\alpha}$. Arachidonic acid challenged platelets pretreated with UK-37,248 do not accumulate cyclic AMP ; they however strongly stimulate the production of prostacyclin by aspirin pretreated cultured endothelial cells.

In a double blind placebo controlled study ingestion of 200 mg of the compound resulted in a complete inhibition of arachidonic acid induced platelet aggregation, whereas the threshold concentration for irreversible platelet aggregation with ADP was unaltered. Serum TXB_2 levels were markedly decreased from the normal pre-values (200-700 pg/ml) to low (60-80 pg/ml). Stimultaneous plasma 6-keto prostaglandin $F_{1\alpha}$ levels from citrate blood increased from 46 ± 23 pg/ml (mean ± SD) to 409 ± 185 pg/ml).

It is concluded that a thromboxane synthetase inhibitor modifies cyclic endoperoxide metabolism in such a way that there is not only a decreased formation of pro-aggregatory thromboxane A_2 but also an increased production of anti-aggregatory prostacyclin. Thromboxane-synthetase inhibitors may be superior to aspirin as antithrombotic agents. The platelet function defect induced by UK-37,248 is only distinct from that in congenital thromboxane synthetase deficiency in that in the latter condition ADP aggregation always is reversible and arachidonic acid challenged platelets accumulate cyclic AMP (Defreyn et al, 1981). Probably UK-37,248, like imidazole itself, activates phosphodiesterase, thus abolishing part of its anti-aggregating effect.

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INFLUENCE OF AN ORALLY ACTIVE THROMBOXANE SYNTHESIS INHIBITOR ON PLATELET FUNCTION IN MAN.
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Several studies have shown that elevated levels of circulating thromboxane and a decreased capacity of the vessel wall to synthesize prostacyclin do occur in patients with cardiovascular diseases and in diabetes. In contrary to the effect of aspirin, selective inhibition of thromboxane synthesis of the platelets would preserve the production of pro-stacyclin. This selectivity is offered by UK-37,248, a new imidazole derivative.

In 11 patients with cardiovascular diseases and enhanced platelet aggregation a single dose of 100 mg UK-37,248 was tested. Before and 1, 2, 4, 6 and 24 h after intake, blood pressure, pulse, bleeding-time were controlled, and blood was taken for in-vitro platelet aggregation and for measurements of plasma as well as serum TXB_2. The plasma TXB_2-level decreased from 130.9±14.2 pg/ml to 85.7±4.6 pg/ml ($p < 0.005$) after 6 h. Serum TXB_2 (319.3±31.4 ng/ml) was lowest 1 h after drug intake (32.9±5.9 ng/ml, $p < 0.001$). 5 h later the level was still significantly decreased (182.1±24.3 ng/ml, $p < 0.005$). The drug had no influence on blood pressure and pulse rate in the lying as well as standing position. Bleeding time was minimally prolonged in 7 patients, the mean prolongation was about 10 %. No effect could be observed on spontaneous, ADP- and collagen-induced platelet aggregation.

The inhibition of thromboxane synthesis in man by UK-37,248 after a single dose of 100 mg could very well be demonstrated. There was no or only a minimal effect on platelet aggregation and bleeding-time. Evaluation of long-term administration is needed.

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