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IN VITRO EFFECTS OF 5-FLUOROURACIL ON VASCULAR TISSUE PROSTACYCLIN RELEASE AND PLATELET THROMBOXANE PRODUCTION.
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Severe chest pain (angina attacks) and myocardial infarction has been recorded during 5-Fluorouracil (5-F.U.) treatment. The present study was undertaken to evaluate the "in vitro" activity of 5-F.U. on vascular prostacyclin (PGI_2) release and platelet thromboxane A_2 (TXA_2) formation, which play a role in the onset of cardiovascular disorders.

Rat aortic rings (about 20 mg wet/weight) were incubated at 30°C for 15 mins in 300 µl tris buffer containing 5-F.U. (250-500-1000 µg). The aortic rings were removed and the supernatant was kept 4 hrs at room temperature and the RIA of 6-keto $PGF_{1\alpha}$ was thereafter performed.

In 1 ml rabbit PRP containing 5-F.U. (50-100-500 µg) platelet aggregation was induced by Arachidonic acid (45 µg). Platelets were then removed by centrifugation and RIA of TXB_2 was performed on supernatant.

At the dose levels of 250, 500, 1000 µg, 5-F.U. yielded a dose-dependent increase (20, 44 and 68 percent, respectively) in the 6-keto $PGF_{1\alpha}$ released by rat aortic rings. Conversely, the TXB_2 production by platelets during aggregation was reduced of 19, 27, 36 percent at 5-F.U. concentrations of 50, 100, 500 µg/ml, respectively. 5-F.U. had no effect on platelet aggregation.

Considering the vasodilator and antithrombogenic effects of PGI_2 and the vasoconstrictor effect of TXA_2, the present results are not in agreement with the already described cardiotoxicity of 5-F.U.. The "in vitro" results, however, if confirmed "in vivo", show a new aspect of the mechanism of 5-F.U. cardiotoxicity.

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PLATELET FUNCTION DURING ONE WEEK CONTINUOUS INTRA-ARTERIAL PGI_2-INFUSION IN PERIPHERAL VASCULAR DISEASE.
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In 12 patients with peripheral vascular disease (Fontaine, stage II-IV), PGI_2 (3-5ng/kg/min) was given intra-arterially continuously during 7 days. Besides angiological and hormonal control, the platelet behaviour was monitored by ADP-induced platelet aggregation (1µM), platelet sensitivity to PGI_2, the platelet proteins β-TG and PF4, thromboxane B_2 (RIA) and platelet count before, during and up to one week after termination of PGI_2-infusion. In some patients platelet life span was performed by autologous 111 In-oxine labelled platelets prior and during PGI_2-infusion. Immediately after beginning of PGI_2-infusion, in general, a significant decrease in platelet activity can be detected. The response to ADP is diminished, as well as βTG and PF4. Between 24 and 48 hours after starting the PGI_2-infusion, the levels reach again the starting level, followed thereafter by a significant increase in most of the patients. An increase in platelet count (in one patient to the fourfold), a decrease in platelet sensitivity to PGI_2 (≤50%) and a hyper-reactivity of the platelets to the in vitro ADP-stimulus is found. After termination of PGI_2-treatment, the levels are returning back to normal within 48 hours. A trend to a prolonged platelet life span after PGI_2-infusion is noted. Beside the clinical data, the prolonged platelet survival suggests a beneficial effect of the intra-arterial PGI_2-application. The possible causes and risks of the temporary platelet activation during the PGI_2-infusion are discussed.

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CORRELATION BETWEEN PLATELET DISAGGREGATION AND ARACHIDONIC ACID CONVERSION TO PROSTACYCLIN AND THROMBOXANE A_2 IN THE EX VIVO CAT MODEL.
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Arachidonic Acid (AA) infusion in the ex vivo cat model was performed to determine the effects of the products produced from the metabolism of AA on the disaggregation of platelets accumulated on a rabbit Achilles tendon. Apparent peak blood levels of both prostacyclin (PGI_2) and thromboxane (TXA_2) were noted 4-8 minutes post initiation of AA infusion (1.0 mpk, i.v.). Plasma levels of PGI_2 and TXA_2 were determined by radioimmunoassay. Significant platelet disaggregation was noted 6-8 minutes post AA infusion with peak effects attained at 20-24 minutes. In other experiments no disaggregation occurred when AA was infused at 0.5 mg/kg. PGI_2 infused in the same animal model at 14 µg/kg was detected in peak levels within 2 minutes after infusion and disappeared rapidly thereafter. Disaggregation of accumulated platelets following PGI_2 infusion occurred immediately after the completion of infusion and continued over the 20 minute observation period. This data shows that AA infused into the cat can be converted to both PGI_2 and TXA_2 and that at 1.0 mg/kg AA, sufficient PGI_2 is produced to result in platelet disaggregation even in the presence of elevated TXA_2 levels.

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DIFFERENTIAL STABILITY OF PROSTACYCLIN (PGI_2) IN WHOLE BLOOD AND PLASMA.
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The stability of PGI_2 in human whole blood (WB), platelet-rich plasma (PRP) and platelet-poor plasma (PPP) was studied. Following incubation of PGI_2 (60nM) in the three media, it was found that the half life ($t_{½}$) of PGI_2 (as measured by the rate of loss of PGI_2 equivalents causing platelet inhibition in the supernatant of the incubates) was longer in PPP & PRP ($t_{½}$ 49±4 & 42.5±5 min respectively, n=10). On investigation this was found to be largely due to the pH difference observed between PPP&PRP (both pH 7.8±1) and WB (pH 7.4±1). Addition of $NaHCO_3$ to raise the pH of WB to 7.8 prolonged the stability of PGI_2 with a $t_{½}$ of 35±4 min and addition of HCl to lower the pH of PPP to 7.4 shortened the $t_{½}$ to 18.5±4 min. However, incubation of PGI_2 in either Hanks buffer or washed red blood cells (WRBC) at pH 7.8 did not increase PGI_2 stability.

Since addition of a mixed population of white blood cells ($7x10^6$cells/ml in PBS pH 7.8) to PPP (pH7.8) did not alter the rate of loss of PGI_2 activity and there was found to be no significant uptake (<15%) of ^3H PGI_2 in WRBC, the possibility of PGI_2 conversion to a more stable and platelet-active metabolite such as 6-oxo-PGE_1 in plasma was studied by extraction and TLC of the PPP and WB incubates. ^3H PGI_2 was found to be converted to ^3H 6-oxo-$PGF_{1\alpha}$ in both WB (pH7.4) and PPP (pH 7.8) with no other detectable metabolites in three different solvent systems. Treatment of the incubates prior to extraction and TLC with $NaBH_4$ (which by reducing free keto groups can distinguish between PGI_2 and 6-oxo-$PGF_{1\alpha}$) showed that 10-20% of the added ^3H PGI_2 in PPP (pH 7.8) was unchanged even after 120 mins incubation while virtually all the added ^3H PGI_2 in WB (pH7.4) was converted to ^3H 6-oxo-$PGF_{1\alpha}$ by 50 min with a time course (^3H PGI_2 60nM; $t_{½}$ WB-14 min, PPP-35min) similar to the loss of PGI_2 activity in WB and PPP on bioassay.

We conclude that the prolonged platelet inhibitory activity following incubation of PGI_2 in plasma compared to that in whole blood is due to unchanged PGI_2 and not the formation of a 6-oxo-PGE_1, like substance as suggested by Borda and Gimeno in Prostaglandins 19 pp 899 (1979).