

IN VITRO EFFECTS OF 5-FLUOROURACIL ON VASCULAR TISSUE PROSTACYCLIN RELEASE AND PLATELET THROMBOXANE PRODUCTION. L. Caprino, F. Antonetti, M. Lagomarsino and L. Morelli. Chair of Toxicology, Catholic University of Rome and Institute of Pharmacology, University of Perugia, ITALY.

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 ( $\text{PGI}_2$ ) release and platelet thromboxane  $\text{A}_2$  ( $\text{TXA}_2$ ) 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  $\mu\text{l}$  tris buffer containing 5-F.U. (250-500-1000  $\mu\text{g}$ ). The aortic rings were removed and the supernatant was kept 4 hrs at room temperature and the RIA of 6-keto  $\text{PGF}_{1\alpha}$  was thereafter performed.

In 1 ml rabbit PRP containing 5-F.U. (50-100-500  $\mu\text{g}$ ) platelet aggregation was induced by Arachidonic acid (45  $\mu\text{g}$ ). Platelets were then removed by centrifugation and RIA of  $\text{TXB}_2$  was performed on supernatant.

At the dose levels of 250, 500, 1000  $\mu\text{g}$ , 5-F.U. yielded a dose-dependent increase (20, 44 and 68 percent, respectively) in the 6-keto  $\text{PGF}_{1\alpha}$  released by rat aortic rings. Conversely, the  $\text{TXB}_2$  production by platelets during aggregation was reduced of 19, 27, 36 percent at 5-F.U. concentrations of 50, 100, 500  $\mu\text{g}/\text{ml}$ , respectively. 5-F.U. had no effect on platelet aggregation.

Considering the vasodilator and antithrombogenic effects of  $\text{PGI}_2$  and the vasoconstrictor effect of  $\text{TXA}_2$ , 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.

## 1422

CORRELATION BETWEEN PLATELET DISAGGREGATION AND ARACHIDONIC ACID CONVERSION TO PROSTACYCLIN AND THROMBOXANE  $\text{A}_2$  IN THE EX VIVO CAT MODEL. N.S. Nicholson, S.L. Smith and R.N. Saunders. G.D. Searle & Co., Research & Development Division, Department of Biological Research, P. O. Box 5110, Chicago, Illinois 60680.

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 ( $\text{PGI}_2$ ) and thromboxane ( $\text{TXA}_2$ ) were noted 4-8 minutes post initiation of AA infusion (1.0 mpk, i.v.). Plasma levels of  $\text{PGI}_2$  and  $\text{TXA}_2$  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.  $\text{PGI}_2$  infused in the same animal model at 14  $\mu\text{g}/\text{kg}$  was detected in peak levels within 2 minutes after infusion and disappeared rapidly thereafter. Disaggregation of accumulated platelets following  $\text{PGI}_2$  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  $\text{PGI}_2$  and  $\text{TXA}_2$  and that at 1.0 mg/kg AA, sufficient  $\text{PGI}_2$  is produced to result in platelet disaggregation even in the presence of elevated  $\text{TXA}_2$  levels.

## 1421

PLATELET FUNCTION DURING ONE WEEK CONTINUOUS INTRA-ARTERIAL  $\text{PGI}_2$ -INFUSION IN PERIPHERAL VASCULAR DISEASE. K. Silberbauer, H. Sinzinger and Andrea Gall. 2nd Dept. Internal Medicine, University of Vienna, Austria.

In 12 patients with peripheral vascular disease (Fontaine, stage II-IV),  $\text{PGI}_2$  (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 (1 $\mu\text{M}$ ), platelet sensitivity to  $\text{PGI}_2$ , the platelet proteins  $\beta\text{-TG}$  and  $\text{PF}_4$ , thromboxane  $\text{B}_2$  (RIA) and platelet count before, during and up to one week after termination of  $\text{PGI}_2$ -infusion. In some patients platelet life span was performed by autologous 111 In-oxine labelled platelets prior and during  $\text{PGI}_2$ -infusion. Immediately after beginning of  $\text{PGI}_2$ -infusion, in general, a significant decrease in platelet activity can be detected. The response to ADP is diminished, as well as  $\beta\text{TG}$  and  $\text{PF}_4$ . Between 24 and 48 hours after starting the  $\text{PGI}_2$ -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 four-fold), a decrease in platelet sensitivity to  $\text{PGI}_2$  ( $\leq 50\%$ ) and a hyper-reactivity of the platelets to the in vitro ADP-stimulus is found. After termination of  $\text{PGI}_2$ -treatment, the levels are returning back to normal within 48 hours. A trend to a prolonged platelet life span after  $\text{PGI}_2$ -infusion is noted. Beside the clinical data, the prolonged platelet survival suggests a beneficial effect of the intra-arterial  $\text{PGI}_2$ -application. The possible causes and risks of the temporary platelet activation during the  $\text{PGI}_2$ -infusion are discussed.

## 1423

DIFFERENTIAL STABILITY OF PROSTACYCLIN ( $\text{PGI}_2$ ) IN WHOLE BLOOD AND PLASMA. S. Krishnamurthi, J. Westwick, V.V. Kakkar. Thrombosis Res. Unit, King's College Hospital, London, England

The stability of  $\text{PGI}_2$  in human whole blood (WB), platelet-rich plasma (PRP) and platelet-poor plasma (PPP) was studied. Following incubation of  $\text{PGI}_2$  (60nM) in the three media, it was found that the half life ( $t_{1/2}$ ) of  $\text{PGI}_2$  (as measured) by the rate of loss of  $\text{PGI}_2$  equivalents causing platelet inhibition in the supernatant of the incubates) was longer in PPP & PRP ( $t_{1/2}$  49 $\pm$ 4 & 42.5 $\pm$ 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 $\pm$ 1) and WB (pH 7.4 $\pm$ 1). Addition of  $\text{NaHCO}_3$  to raise the pH of WB to 7.8 prolonged the stability of  $\text{PGI}_2$  with a  $t_{1/2}$  of 35 $\pm$ 4 min and addition of HCl to lower the pH of PPP to 7.4 shortened the  $t_{1/2}$  to 18.5 $\pm$ 4 min. However, incubation of  $\text{PGI}_2$  in either Hanks buffer or washed red blood cells (WRBC) at pH 7.8 did not increase  $\text{PGI}_2$  stability.

Since addition of a mixed population of white blood cells ( $7 \times 10^6$  cells/ml in PBS pH 7.8) to PPP (pH 7.8) did not alter the rate of loss of  $\text{PGI}_2$  activity and there was found to be no significant uptake (<15%) of  $^3\text{H}$   $\text{PGI}_2$  in WRBC, the possibility of  $\text{PGI}_2$  conversion to a more stable and platelet-active metabolite such as 6-oxo- $\text{PGE}_1$  in plasma was studied by extraction and TLC of the PPP and WB incubates.  $^3\text{H}$   $\text{PGI}_2$  was found to be converted to  $^3\text{H}$  6-oxo- $\text{PGF}_{1\alpha}$  in both WB (pH 7.4) 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  $\text{NaBH}_4$  (which by reducing free keto groups can distinguish between  $\text{PGI}_2$  and 6-oxo- $\text{PGF}_{1\alpha}$ ) showed that 10-20% of the added  $^3\text{H}$   $\text{PGI}_2$  in PPP (pH 7.8) was unchanged even after 120 mins incubation while virtually all the added  $^3\text{H}$   $\text{PGI}_2$  in WB (pH 7.4) was converted to  $^3\text{H}$  6-oxo- $\text{PGF}_{1\alpha}$  by 50 min with a time course ( $^3\text{H}$   $\text{PGI}_2$  60nM;  $t_{1/2}$  WB-14 min, PPP-35min) similar to the loss of  $\text{PGI}_2$  activity in WB and PPP on bioassay.

We conclude that the prolonged platelet inhibitory activity following incubation of  $\text{PGI}_2$  in plasma compared to that in whole blood is due to unchanged  $\text{PGI}_2$  and not the formation of a 6-oxo- $\text{PGE}_1$  like substance as suggested by Borda and Gimeno in Prostaglandins 19 pp 899 (1979).