2020

EFFECT OF DIPYRIDAMOLE ON SPONTANEOUS PLATELET AGGREGATION IN WHOLE BLOOD DECREASES WITH THE TIME AFTER VENEPUNCTURE: EVIDENCE FOR THE ROLE OF ADP. A.R. Saniabadi, G.D.O. Lowe and C.D. Forbes, University Department of Medicine, Royal Infirmary, Glasgow, Scotland, U.K.

Spontaneous platelet aggregation (SPA) was studied in human whole blood at 3, 5, 10, 20, 30, 40 and 60 minutes after venepuncture. Using a whole blood platelet counter (Ultra Flo 100), SPA was quantified by measuring the fall in single platelet count upon rollermixing aliquots of blood at 37°C. The extent of SPA increased with the time after venepuncture, with a correlation coefficient of 0.819. The inhibitory effect of dipyridamole (Dipy) on SPA was studied: (a) 10^{-5}M at each time interval; (b) 0.5-100 x 10^{-6}M at 3 and 30 minutes, and (c) 15 x 10^{-6}M in combination with 2 x 10^{-4}M adenosine (Ad), 8 x 10^{-6}M 2-chloradenosine (2ClAd, a specific ADP receptor blocker) and 5 x 10^{-5}M aspirin. There was a rapid decrease in the inhibitory effect of Dipy with the time after venepuncture; the correlation coefficient was -0.533. At all the concentrations studied, Dipy was more effective at 3 minutes than at 30 minutes after venepuncture. A combination of Dipy with Ad, 2ClAd or aspirin was a more effective inhibitor of SPA than either drug alone. However, when an effective concentration of Dipy and an ineffective concentration of Ad (10^{-4}M) were added together, the inhibitory effect of Dipy was not increased, suggesting that Dipy inhibits platelet aggregation independent of Ad.

The increase in SPA with the time after venepuncture was abolished when blood was taken directly into the anticoagulant containing 2ClAd (5 x 10^{-6}M). We conclude that ADP released from the red blood cells is responsible for the increased platelet aggregability with the time after venepuncture, and makes a serious contribution to the artifacts of in vitro platelet function studies. Furthermore, the decrease in the inhibitory action of Dipy with the time after venepuncture may explain why previously, it has not been possible to observe inhibition of platelet aggregation by Dipy in platelet rich plasma which requires time to prepare.

2021

REVERSIBLE BLOCKADE OF PLATELET ACTIVATION DURING CARDIO-PULMONAR BYPASS IN DOGS AFTER IV ADMINISTRATION OF AJOENE. R. Apitz Castro (1), E. Ledezma (1), A. Jorquera (1) and M.K. Jain (2). Lab. Trombosis Exptl.(IVIC), Caracas-Venezuela and Dept. of Chemistry, U. of Delaware, Newark, DE, U.S.A. (2).

Surgery with extracorporeal circulation (ECC) is associated with platelet activation, which greatly contribute to prolonged postoperative bleeding and increased blood loss after cardiac surgery. Antiplatelet compounds which induce rapid and reversible inhibition of platelet function, without affecting platelet adhesiveness would be potentially useful in the management of the platelet-dependent hemostatic disorder observed in ECC. Ajoene, an organosulfur originally obtained from garlic, inhibits platelet release and aggregation induced ex vivo by all know agonists. It does not affect shape change or adhesion to collagen nor interfere with metabolic pathways relevant to the platelet reaction. Ajoene action is related to its direct interaction with the fibrinogen receptor on the platelet surface which impairs fibrinogen binding to stimulated or chymotrypsin treated platelets. IV administration of ajoene (15 mg/Kg) to mongrel dogs, inhibits platelet aggregation induced ex vivo by collagen (2-5 g/ml) or ADP (10 M). Complete inhibition is attained after 20-35min and recuperation of platelet reactivity is obtained after 2.5-3.5 hours. To study the potential benefit of ajoene for the prevention of platelet activation during cardio-pulmonary bypass, ajoene was administered to anesthesized (heparin-anticoagulated) dogs, as described above, 40min before establishing the ECC. Circulation was mantained at 1.5L/min for a period of 100min Platelet count, and aggregation induced ex vivo by ADP or collagen were meassured inmediately before ajoene administration, 10min after the end of ECC and thereafter, hourly. Platelet count 10min after end of ECC, in non-treated dogs fell to about 57% of pre-pump values, while in ajoene-treated animals circulating platelets represented 80% of pre-ECC values. Recovery of platelet function in ajoene-treated dogs started 2 hr after end of ECC (about 4 hr. after ajoene administration) reaching 70% 4 hr after end of ECC. Surgical bleeding in treated-dogs was not different from controls. Moderate bradicardia and hypotension, which in atropinized dogs returned to normal values within 3 min was observed. Although detailed pharmacological studies are still needed, our results suggest that ajoene is a potentially useful drug for the prevention of platelet activation induced by ECC.

2022

ASPIRIN MODIFIES RED BLOOD CELL BEHAVIOUR (RBC) IN THE PLATELET-RBC INTERACTION. M.T.Santos, J.Aznar ,J.Valles and J.L.Perez-Requejo.Research Center.Hospital "La Fé".Valencia.Spain.

RBC stimulate the initial stages of platelet activation by collagen as evaluated by the BASIC wave (Perez-Requejo et al. Thromb Haemostas 54:799 1985). In order to get some insight into the mechanisms of platelet-RBC interactions, a BASIC wave was induced by 1ug/ml of collagen after mixing "in vitro" platelets and RBC obtained both before and two hours after a single dose of 500 mg of ASA from normal subjects. The TXB2 formed was also evaluated. The results show (Table),

	Exp. Cell Mixture	BASIC	TXB2
1-	Non-ASA-RBC + ASA-PRP	Yes	No
2-	" " " + Non-ASA-PRP	Yes	Yes
3-	ASA-RBC + Non-ASA-PRP	Yes	Yes
4-	" " + ASA-PRP	No	No

that non aspirinized RBC (non-ASA-RBC) increase the BASIC wave intensity of aspirinized platelets (ASA-PRP) by a cyclooxygenase-independent pathway since no increase in TXB2 was observed (Exp 1), while both non-ASA-RBC (Exp 2) and ASA-RBC (Exp 3) activate non-ASA platelets with the participation of the cyclooxygenase system, since an increase in TXA2 was found. A comparison of the effect of non-ASA-RBC (Exp 1) and ASA-RBC (Exp 4) on aspirinized platelets shows that ASA modifies the RBC behaviour associated with estimulation of platelets by a cyclooxygenase-independent pathway. This effect of ASA on RBC is not transient and lasts at least 48 hours after ASA ingestion. In addition, when a small proportion of nonASA platelets (10%) is mixed with aspirinized platelets (90%) and ASA-RBC - a situation that can be encountered "in vivo" in the hours following ASA ingestion - the intensity of the BASIC wave is 89% of that obtained when all the platelets are non aspirinized. This RBC effect on the mixture of ASA and nonASA platelets, may help explain the sometimes contradictory effect of ASA as an antithrombotic agent.

2023

TREATMENT OF PERIPHERAL VASCULAR DISEASE WITH ILOPROST (ZK36374). M.Catalano, S.Belletti, E.Coazzoli, E.Gherardi, F.Lopriore, U.Russo and A.Libretti. Center for Research, Prevention and Treatment of Vascular Diseases, General Medical Clinic, University of Milan, L.Sacco Hospital, Milan, Italy.

The action of a stable analogue of prostacyclin, Iloprost, was studied in 18 male in-patients aged 51-69 yr (mean 61±7) with peripheral vascular disease Fontaine stage IIb and III who gave informed consent. Entry criteria were Fontaine stage III or II with free interval at treadmill test < 100 m. (4 km/h, without slope), ankle/arm arterial pressure index (API)< 0.7 at rest. Patients with arterial hypertension, diabetes, myocardial infarction in the previous 6 mo, heart failure, thrombocytopenia, hemorrhagic diathesis, or kidney or liver failure were excluded. The patients underwent 3 weeks' treatment with 6-h infusion/day of placebo for week 1, Iloprost 1 ng/kg/min for week 2, and Iloprost 2 ng/kg/min for week 3. Before treatment, at the end of each treatment week, and 15, 30 and 60 days after the end of treatment we evaluated: absolute pain-free interval (APFI), relative pain-free interval (RPFI), and API. Platelet aggregation by ADP and collagen and plasma βTG and PF_4 levels were also determined, and complete blood tests and ECG performed. All the patients showed a progressive but variable increase in RPFI and APFI at 15, 30 and 60 days compared with the values at baseline and the end of the week on placebo (basal vs 15, 30 and 60 days, $p < 0.01$ for both parameters, ANOVA BR and Tukey's test). The % variations of APFI compared with baseline were 58.7, 62 and 95.5% at 15, 30 and 60 days. No other significant variations were observed. The results obtained seem to indicate that the drug is effective since there was an increase in the pain-free interval starting from 15 days after treatment suspension which persisted at 60 days. A first hypothesis is that this could be the result of increased flow due to improved collateral circulation by a mechanism not influenced by the platelet parameters considered that merits further study.