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PRELIMINARY COMMUNICATIONS

ENHANCEMENT OF ARTERIAL THROMBOFORMATION BY URIC ACID, A FREE RADICAL SCAVENGER

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A technique was developed, permitting the induction of repetitive thrombogenesis in a branch of the mesenteric artery of the Wistar rat (1). The microscopic image of the white platelet thrombus is continuously registered and monitored by optical and electronic devices.

The thromboformation is induced by superfusion of the exposed arterial segment with adenosine-diphosphate (ADP), following a local desendothelialization of a small area of approximately 300 μm in diameter, by means of a weak DC current of 30 μA. When the superfusion with ADP is discontinued, the thrombus embolizes. The endothelial cells surrounding the cell-free area have a normal appearance at the ultra-structural level (2); in these experimental conditions, few if any platelets adhere to the intimal structures, when ADP superfusion is resumed.

A computerized photometrical analysis of the image of the thrombus permits the registration of the phenomenon and the measurement of its amplitude.

The hypothesis of a definite role played by the prostaglandin biochemical pathway in the platelet/vessel interactions, resulting in local arterial thrombogenesis, was clearly sustained by different findings : non steroidal antiinflammatory substances such as aspirin, indomethacin and flubiprofen (3,4) significantly inhibited thromboformation, while tranylcypromine (5) and 15-hydroperoxyarachidonic acid (6), two inhibitors of PGI_2 synthetase, markedly enhanced the ADP-induced thrombogenesis. The addition of exogenous arachidonic acid further enhanced this phenomenon.

The hypothesis of a balance between the cyclic endoperoxides and thromboxane A_2 (pro-aggregating agents) and PGI_2 (anti-aggregating), controlling the platelet/vessel wall interactions, was then verified. The marked enhancement of thrombus sizes after a superfusion of an inhibitor of the PGI_2 synthetase (tranylcypromine or 15-hydroperoxyarachidonic acid) suggests firmly (6) that, the pathway PGG_2 , $PGH_2 \longrightarrow PGI_2$ being blocked, the endoperoxides remain in excess in the endothelial cells and are released in the vessel lumen; they can therefore act directly on platelets as aggregating agents, or they can be metabolized into thromboxane, in these corpuscules.

However, another phenomenon remained unclear. When tranylcypromine was superfused continuously, it was shown that after an initial increase in thrombogenesis, a gradual reduction in the ADP-induced thromboformation was observed, and after a time interval of approximately 30 minutes, the enhancing effect was lost altogether.

The biochemical explanation of this "tachyphylactic" phenomenon is here proposed. A cyclo-oxygenase-dependent production of activated oxygen species was proposed by Marnett et al.(7). Egan et al.(8)

hypothesized that cyclooxygenase deactivation occurs during its activity, by formation of oxygen species generated by conversion of PGG_2 to PGH_2. In support of this theory is the observation that radical scavengers enhance the cyclooxygenase activity, probably by inhibiting its self-deactivation.

Uric acid was found to be a potent stimulator of prostaglandin synthesis (9); it is a potent radical scavenger, as evidenced by Deby et al.(10), and more recently confirmed by Ames et al.(11). The question then arose if uric acid could suppress the "tachyphylactic" effect occuring under tranylcypromine.

Table 1 : Effects of uric acid (UA : 10^{-3}M) on the enhancement of the ADP-induced thrombosis by tranylcypromine (TR : $1,5.10^{-3}$M).

Parameter	TR + UA superfusion				TR alone superfusion			
Time (min)	1	13	25	37	1	13	25	37
t_1	76,6	107,7	97,3	88,9	77,9	106,7	101,0	92,1
m_T	305,5	246,2	242,9	266,9	204,3	152,1	128,9	98,2
m_o	132,9	124,9	135,2	135,2	130,0	106,1	109,7	96,4
TTV	561,5	329,4	318,5	389,5	300,7	183,3	153,3	83,9

The data (means of 5 experiments) are expressed as percentages of the controls.

In table 1 are represented the results of five experiments. Different thrombotic parameters are evaluated, such as the lag period (t_1), the maximal instantaneous thrombus value (m_T), the thrombus surface (m_o), and the total thrombus value integrated (TTV). All values are expressed as percentages of the control values. In fig.1 is represented the effect of the local superfusion of uric acid on thrombogenesis induced by ADP in the presence of tranylcypromine, as measured by the changes in the maximal thickness of the thrombus (m_D). In C, ADP is superfused alone and produces a thrombus taken as control. After C, tranylcypromine is continuously superfused, alone or together with uric acid (10^{-3}M). In these conditions, the first assay with ADP produces thrombi of greater sizes than controls, particularly in the presence of uric acid. But during the following assays with superfusion of tranylcypromine alone, the increment afforded by this drug falls significantly; the thrombus size returns to the control value at the fourth experiment (time 37). On the contrary, the enhancing effect of tranylcypromine is maintained by the administration of uric acid. Uric acid alone is of slight effect on the thromboformation.

Inspection of these observations clearly demonstrates : firstly, that uric acid further increases the thrombus enhancement by tranylcypromine, and secondly, that the "tachyphylactic" phenomenon is markedly inhibited. Mannitol, another oxygen species scavenger (12),exhibits a comparable but less marked effect (13); formate, which is also a hydroxyl scavenger (14) was found to be active (15).

The possible clinical implications of these findings are, from a first viewpoint, very limited; indeed UA was used at high concentration (10^{-3}M). But concentrations up to 5.10^{-4}M of UA can be found in plasma in cases of severe hyperuricemia; it is evident that, in cells presenting xanthine-oxidase, higher levels of UA can be present as sometimes cristal depots of UA can be observed.

These findings suggest that the cyclooxygenase self-deactivation could be responsible for the decrease of platelet/vessel wall interactions, as ultimately less pro-aggregating endoperoxides are synthesized.

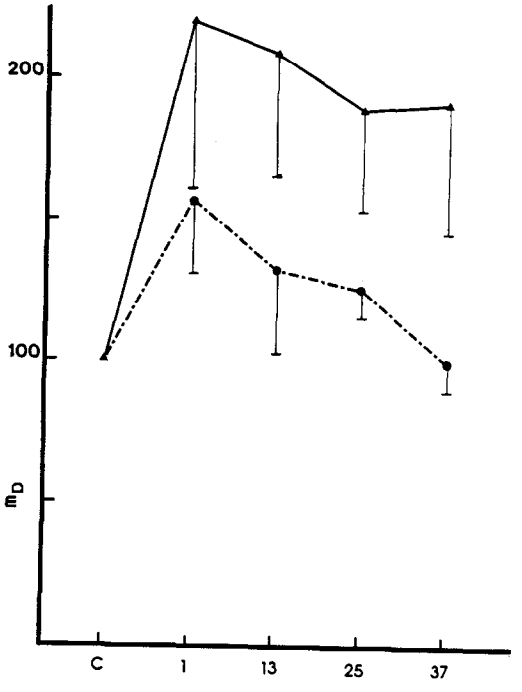

Fig. 1 : Effect of uric acid (UA : 10^{-3}M) on the maximal thickness of the thrombus (m_D) induced by ADP (4.10^{-4}M) and amplified by tranylcypromine (TR : $1,5.10^{-3}$M). Ordinate : values of m_D expressed in percentages of controls. Abscissae : time in minutes. C : effect of ADP alone. Continuous line : superfusion with TR + UA . Dotted line : superfusion with TR alone.

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