Increased expression of procoagulant activity on the surface of human platelets exposed to heavy-metal compounds

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INTRODUCTION
Platelets play two important roles in normal haemostasis. First, by aggregating, they constitute the initial haemostatic plug which immediately curtails bleeding from broken blood vessels. Secondly, the platelet surface can become activated and potentiate blood clotting, a property referred to as platelet procoagulant activity. This is usually observed as an increase in the rate of activation of prothrombin by factor Va in the presence of factor Va and Ca²⁺, referred to as the prothrombinase reaction. The change to the surface of the plateau responsible for procoagulant activity is due, principally, to a reversal of the polarity of the phospholipid membrane: the anionic phospholipids, which are normally maintained by a translocase system at a higher concentration on the inner leaflet, become exposed on the outer membrane surface (reviewed in [1,2,3,4]). The physiological importance of this property of platelets is demonstrated by the moderately severe bleeding disorder, Scott syndrome, in which stimulated platelets reveal abnormally low levels of anionic phospholipid exposure and a correspondingly lower procoagulant activity [5,6].

The generation of platelet procoagulant activity does not occur with all agonists. ‘Weak’ agonists such as ADP, adrenaline and platelet-activating factor hardly affect the procoagulant properties of the platelet surface even though irreversible thromboxane-dependent aggregation can proceed to near completion. In contrast, thrombin, collagen, complement attack complex C5b-9 and calcium ionophore have been demonstrated to enhance the generation of platelet procoagulant activity in the order of potency: ionophore > collagen/thrombin > C5b-9 > collagen > thrombin [4]. Treatment of platelets with local anaesthetics (dibucaine and tetracaine) or with sulphydryl oxidizing agents (diamide or pyryldithiolethanolamine) also cause an increase in procoagulant activity which is dependent upon extracellular calcium [4].

We recently observed an increase in the procoagulant activity of U937 monocyte-like cells upon treatment with mercuric and other heavy-metal compounds [7]. Both the tissue factor activity of the cell and the ability of the surface to support the prothrombinase reaction were rapidly increased, concomitant with a large increase in intracellular calcium concentration ([Ca²⁺]). In the present study we have identified these heavy metals as potent agonists of platelet procoagulant activity. The characteristics of induction of the procoagulant surface are distinct from that promoted by other platelet agonists in that the degree of microvesiculization is low.

EXPERIMENTAL
Materials
Human α-thrombin was obtained as a gift from Dr. J.-M. Freysinnet (Strasbourg, France), human factor X from Enzyme Research Laboratories (Swansea, U.K.) and bovine factor V.

Abbreviations used: PRP, platelet-rich plasma; PKC, protein kinase C; [Ca²⁺], intracellular calcium concentration; [¹⁴C]5-HT, [¹⁴C]5-hydroxytryptamine; MLC, myosin light chain; [Ca²⁺]cyt, cytosolic calcium; Tg, thapsigargin.
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