Pharmacological determinants and biochemical correlates of nitrate-induced vasodilatation and tolerance development

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by

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Summary

The experiments described in this thesis addressed some of the determinants of acute and chronic responsiveness to the anti-ischaemic vasodilator agent glyceryl trinitrate (GTN; nitroglycerin). In particular, it sought to examine the mechanisms of induction of nitrate tolerance, a phenomenon of decreasing nitrate efficacy during long-term therapy. Experiments were performed largely utilizing an in vitro model (isolated bovine coronary artery rings) and an in vivo haemodynamic model (systemic haemodynamics in patients undergoing cardiac catheterization).

In vitro studies

(1) Interaction between S-nitrosothiols and S-nitrosoproteins and tolerance induction

S-nitrosothiols including S-nitrosoalbumin are present in biological system and may be formed during nitrate administration. In experiments designed to test whether the S-nitrosocompounds influenced the action of GTN, it was shown that low concentrations of this class of compounds were capable of limiting the extent of GTN tolerance.

(2) GTN–endothelin interactions; influence of contractile agents

Endothelin-1 (Et-1) is a vasoconstrictor agent released from endothelial cells and may act as a physiological antagonist to nitric oxide (NO), the active product of GTN metabolism. GTN proved to be a more effective vasodilator in vessels contracted with Et-1 than with the thromboxane mimic U46619. Furthermore, the extent of tolerance to GTN were much less in the vessels contracted with Et-1 than with U46619. In the course of this study, it was shown that vessels contracted with Et-1 failed to maintain steady-state tone and that this was probably due to Et-1-stimulated release of prostanoid and NO.
(3) **Nitrate withdrawal and the phenomenon of “rebound” vasoconstriction**

Abrupt discontinuation of organic nitrate therapy may aggravate ischaemia. The possibility that the bovine coronary artery may serve as an in vitro model of this “rebound” ischaemia was examined by testing responsiveness of the vessel to constrictor agents following induction of GTN tolerance. The results indicated that tolerance induced in vitro was not associated with increased vascular responsiveness to a variety of constrictor agents such as endothelin-1, serotonin, thromboxane analogue U46619 and potassium. The results argue against the possibility that rebound seen in vivo is due to increased reactivity of the vascular muscle.

(4) **Interactions of glyceryl dinitrate with GTN tolerance induction**

Metabolism of GTN is followed by formation and accumulation of high concentrations of 1,2- and 1,3-glyceryl dinitrates (GDNs) and there is evidence that these metabolites may inhibit further metabolism of GTN. The possible effects of these dinitrate metabolites on GTN vasodilator effect and tolerance induction were investigated in this in vitro model. It was found that addition of 1,2- and 1,3-GDN affect neither the vasodilator response to GTN nor the extent of GTN tolerance. These findings suggest that accumulation of GDNs does not inhibit the vasodilator action of and the tolerance induction to GTN.

**B. In vivo haemodynamic studies**

These studies were performed in an attempt to develop a convenient marker of biochemical events during chronic nitrate therapy. Since soluble guanylate cyclase is the key enzyme system mediating nitric oxide-mediated vasodilator response, cGMP was selected as the potential marker.

In patients undergoing cardiac catheterization for investigation of chest pain, it was demonstrated that 10 min of GTN infusion (10 µg/min) induced significant increase in plasma transfemoral cGMP production which was associated with significant decreases in both systolic and pulmonary capillary wedge pressures. The results imply that transfemoral plasma cGMP concentration gradient may be a sensitive marker of the acute effects of GTN.
It was also demonstrated that during chronic nitrate therapy, there was increased plasma ANP levels which may also contribute to the increased plasma cGMP gradient. Thus, under this conditions, plasma cGMP gradient was no longer a specific marker of nitrate effects, instead, it can only be used as a marker for net vasodilator effects.
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