A Study To Determine The Effect Of Oral N-Acetyl-L-Cysteine On Platelet-Leukocyte Interaction In Patients With Type-2 Diabetes

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Patients with type-2 diabetes are at increased risk of thrombotic events on account of oxidative stress, endothelial dysfunction, platelet hyperaggregability, a sustained hypercoagulable state, and alterations in the fibrinolytic pathway. The thrombotic risk is compounded by the lack of benefit of aspirin in primary prevention in this group. Oxidative stress is known to depress intra-platelet glutathione (GSH) levels and is associated with platelet hyperaggregability in people with diabetes. Consequently, replenishing GSH levels represents a possible therapeutic option to reduce platelet activity. However, the clinical effectiveness of GSH is limited due to its lack of oral availability and cell penetrance. N-acetyl-L-cysteine (NAC) is an alternative means of increasing intracellular GSH through delivery of L-cysteine, a crucial substrate for GSH synthesis. On account of our previous positive in vitro findings with NAC at orally available concentrations on platelet aggregation, we set out to test the hypothesis that oral dosing with NAC would depress markers of platelet activation ex vivo.

The study was a double-blind, placebo-controlled randomized crossover design (n=15). Patients with well-controlled type-2 diabetes not on aspirin (age range 43-79 years, HbA1C 7.0% +/- 0.2) were recruited through local general practices; those that met the study criteria at an initial screening visit attended the Highland Clinical Research Facility for 4 further visits: day 0 and day 7 for each of the placebo and NAC arms of the study, with at least 1 week washout between study arms. At each visit, blood was sampled at baseline prior to administration of NAC (oral, 1200 mg) and at t=2 h. Patients were provided with sufficient placebo or NAC capsules for daily dosing (1200mg) for 6 days. The primary outcome measure for the study was platelet-leukocyte conjugation (events positive for both CD41a and CD14) by flow cytometry—a measure that has been suggested to be associated with risk of myocardial infarction and injury. Secondary measures included flow cytometric assessment of plasma microparticles and platelet P-selectin exposure, ex vivo platelet aggregation in response to collagen (2.5 µg.ml⁻¹), thrombin (500 mU.ml⁻¹), ADP (2-8 µM) and the thromboxane analogue, U46619 (8 µM), as well as measurement of plasma levels of plasminogen activator inhibitor (PAI-1) antigen and activity.

The proportion of monocytes conjugated to platelets was significantly reduced (~12%) at 2 h after NAC treatment compared to baseline (P=0.006); there was no significant effect of placebo at 2h (P>0.05). The inhibitory effect of NAC was maintained after oral dosing for 7 days (P=0.037 compared to placebo).

Despite evidence for plasma NAC levels of ~3 µM at 2 h after oral dosing, there was no impact on any of the secondary flow cytometry, platelet aggregometry or fibrinolytic markers measured in this study (P>0.05 for all).

We conclude that oral NAC has a modest yet significant effect on platelet-leukocyte interaction that is both fast in onset and maintained after a week of dosing. The clinical significance of the effect has yet to be determined, but given the association of platelet-leukocyte binding with myocardial infarction, the concept of a protective effect of NAC remains a realistic proposition in patients with type-2 diabetes.
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