Cellular resistance to homocysteine: a key for longevity?

Recent works have identified moderately elevated plasma levels of homocysteine as a risk factor for atherosclerosis [1]. Indeed, the mechanism by which homocysteine induces atherosclerosis is still not completely understood. Several hypotheses have been suggested for explaining the negative impact of homocysteine on endothelium; (i) homocysteine might lead to an increase in oxidative stress but future studies in humans are needed to confirm such a possibility; (ii) studies in hyperhomocysteinemic vascular patients have shown that endothelial antithrombotic properties appear to be more severely impaired than in patients with normohomocysteinemia; (iii) a negative impact of homocysteine on NO-production also cannot be ruled out [2,3]. We have recently found in vitro an inhibitory action of homocysteine on platelet NO production both in healthy and — to a larger extent — in diabetic subjects [4] and we hypothesise that one of the mechanisms causing the homocysteine-linked vascular damage might be the platelet activation caused by a reduction in NO release [4].

Healthy centenarians (HC) have recently gained the attention of researchers looking for the biological mechanisms at the basis of longevity and successful ageing. HC have been protected during the course of their life from the development of atherosclerotic damage, but the defending mechanisms have not been clarified. Indeed, it has been recently demonstrated that HC have a less degree of oxidative stress [5] and a preserved response to NO production at vascular level [6] compared with healthy aged subjects. Whether cellular production of NO in response to a proxidant factor is also different between HC and healthy aged subjects might be a further piece of the puzzle which could help us to understand the reason for extreme longevity. Thus, the aim of the present work was to study in vitro the effect of the incubation with homocysteine on the platelet production of NO in HC.

The study was performed on ten healthy centenarians (age > 100 years, three men, seven women) and 20 middle aged healthy subjects (15 men, five women, age = 48 ± 14 years). Both the centenarians and control subjects were submitted to the following inclusion crite-ria; liver, kidney, and thyroid function tests within the normal range; absence of history of diabetes, hypertension or coronary heart disease; no drug or vitamin supplement in the 4 weeks before the study; absence of Alzheimer’s disease or secondary dementia. Blood was drawn in the fasting state for the isolation of platelet rich plasma (PRP), which was divided into three aliquots, used, respectively, for an immediate determination of platelet NO production and for a 3-h incubation with or without 100-μM homocysteine and subsequent measurement of NO production. NO released by the platelets was directly measured in the PRP using an isolated NO meter and its associated probe (IsoNO Mk-11, World Precision Instruments, Sarasota, FL) equipped with the Duo. 18 Data Acquisition System, as recently described by Chakravarthy et al. [7].

All results were mean ± S.D. Changes (%) with the basal value equal to 100%. Difference between values at baseline and those found after adding homocysteine in the medium were evaluated by paired Student’s t-test.

No significant differences in basal NO-formation in HC versus middle aged control group (HC, 2.89 ± 0.53 nmol NO/min per 10⁶ cells; controls, 3.05 ± 0.52 nmol NO/min per 10⁶ cells P = NS) were found. Incubation of freshly isolated platelets with 100 μM HCys for 3 h, significantly decreased the rate of NO-formation (~ 27%) in the control subjects (2.13 ± 0.31 nmol NO/min per 10⁶ cells, P < 0.01 vs. basal levels), but only slightly (~ 12%) in HC (2.61 ± 0.28 nmol NO/min per 10⁶ cells, P = NS vs. basal levels).

The data obtained in vitro in middle-aged healthy subjects confirm the hypothesis that high homocysteine plasma levels cause an increase platelet aggregation by inhibiting the NO production. Several possible mechanisms by which HCys can inhibit NO-production in platelets have been hypothesised; among them the inhibition of L-Arg transport across the plasma membrane or the direct blockade of nitric oxide synthase (NOS) [3] are the most likely ones. Interestingly enough, platelets from HC displayed resistance to the homocysteine-induced inhibition of NO production, so that the NO-formation in platelets from HC after incubation with homocysteine was not significantly different from basal NO production and strongly different from the result...
found in the middle aged group. To the best of our knowledge, this the first report showing a cellular (platelet) resistance to a NO-inhibition due to homocysteine. Such data is in agreement with the earlier findings showing HC to have a lower degree of oxidative stress [5] and more preserved endothelium function [6] than the aged subjects. It should also be pointed out that due to the low prevalence of atherosclerosis in HC [8], one might speculate that a cellular resistance to homocysteine might be viewed, as one of the mechanisms at the basis of successful ageing in HC. Nevertheless, it should underlined that the molecular mechanisms at the basis of the resistance need further investigations.

References


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