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Hyperhomocysteinemia and venous thrombosis : studies into risk and therapy

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Chapter 5

Oral anticoagulant treatment with coumarin derivatives does not influence plasma homocysteine concentration

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Abstract

Introduction

High circulating levels of homocysteine are a risk factor for arterial and venous thrombosis. This association has been established in numerous case-control studies. In some of these studies patients were treated with anticoagulants at the time of venapuncture. It is not clear whether homocysteine concentrations are influenced by anticoagulants. If there is an effect of anticoagulation on homocysteine levels this might under- or over-score the possible association of homocysteine levels and vascular disease.

Methods

In this study we used two different groups to investigate the association of coumarin derivatives on homocysteine concentrations. Homocysteine levels were measured in patients (N=40) who were on the waiting list for orthopedic surgery and were expected to receive prophylactic anticoagulant therapy after the operation. We measured homocysteine concentrations before the operation, and during and after coumarin therapy. In a second study group, we measured homocysteine concentrations in 12 healthy volunteers who were treated with oral anticoagulants.

Results

Mean homocysteine concentrations increased 6% (95% CI 2% to 10%) during the treatment with coumarin derivatives. This corresponds with 1 µmol/l increase in homocysteine concentration. After the anticoagulant treatment period the concentrations decreased again. We calculated that this slight increase does not influence the interpretation of epidemiological studies. No influence on homocysteine concentrations was observed: decrease 3.6% (~0.6 µmol/l) (95% CI –17.5% to 8.5%) after 13 weeks of treatment with anticoagulants in healthy volunteers.

Conclusion

We conclude that there is no important effect of anticoagulation on homocysteine concentrations.

Introduction

Homocysteine is an aminoacid, formed after demethylation of methionine. It is either transsulphurated to cysteine or remethylated to methionine. Increased homocysteine concentrations are associated with arterial and venous thrombosis[1-4]. The association between homocysteine levels and arterial and venous thrombosis has been established in prospective studies, but mainly in retrospective case-control studies. In several of these studies on venous thrombosis, the patients were on treatment with anticoagulants at the time of venapuncture. In studies on arterial vascular disease many patients are also using coumarin derivatives, even in a prospective design. If anticoagulants influence the homocysteine concentrations, relative risk estimates will have been overestimated or underestimated. An influence of anticoagulants on homocysteine values could interfere with the outcome of these studies, especially since the odds ratios in the presented studies are usually small. To our knowledge there is no theoretical reason to believe that anticoagulants do influence homocysteine values. The aim of our study was to assess whether coumarin derivatives affect plasma homocysteine concentration *in vivo*. For this we measured plasma homocysteine concentrations before during and after anticoagulant treatment in two study groups. The first group underwent surgery and was treated with anticoagulants as prophylaxis; the second group were healthy volunteers who participated in a pharmacologic study.

Methods

Patient selection

We selected two study groups to assess the influence of coumarins on homocysteine. The first group were patients selected from the department of Orthopedics of the Leyenburg Hospital. Patients were all waiting for a hip- or knee-replacement operation and were asked to participate in this study. They were scheduled to take coumarin derivatives (acenocoumarol or phenprocoumon) until 3 months after the operation. Patients were excluded if they were using drugs that influence homocysteine- or folate concentrations (vitamin B, folic acid, methotrexate, phenytoin). 64 Consecutive patients were enrolled. Of these 64 patients, 24 patients were excluded for the following reasons: missing blood samples due to not showing up at follow-up visits, continued use of oral anticoagulant therapy, use of vitamin B after the start of the study, previously unmentioned use of oral anticoagulant therapy before the start of the study. Homocysteine concentrations of the remaining 40 patients (9 male, 31 female; age 47-88 years, median 71) were used for the analysis.

A second study was carried out in 12 healthy volunteers (6 men, 6 women, age 25-31 years) who took part in a study to evaluate the effect of low-dose vitamin K on INR levels. All volunteers were treated with coumarins (acenocoumarol) and adjusted to an INR of 2.0. The subjects were first adjusted to a stable INR (week 1-4) and subsequently supplemented with increasing doses of synthetic vitamin K_1 (in tablet form) over a 7-week period (weeks 5-11). Each K_1 dose was taken daily for a one-week period (Monday to Sunday), and in successive weeks the dosage was increased in increments of 50 μg K_1 over the range 50 μg to 300 μg, increasing to 500 μg K_1 for the final week. After the vitamin K supplementation period was a two week wash-out period (week 12 and 13).

Blood sampling and homocysteine measurements

Non-fasting blood (acidic citrate, Biopool, Stabilyte®) was taken three times from each patient in the first group of patients: before the operation, two months after the operation (on coumarin derivatives) and two months after cessation of the coumarin therapy. Blood was centrifuged within 30 minutes after collection at 2000*g* for 10 minutes. Plasma was separated and stored at -30ºC until serial determinations. In a subset of the patients blood was collected in EDTA (Vacutainer®, Beckton&Dickinson) for determination of folate and vitamin B 12 concentrations, according to standard techniques.

In the second group we determined homocysteine concentrations in standardized non-fasting morning samples (EDTA plasma). Blood was centrifuged within 30 minutes and stored at –20ºC. Samples were taken at the start of the study (before coumarin therapy was started), after week 4 (before the start of the low dose vitamin K), after week 10 (after combined vitamin K and coumarin treatment), and after week 13 (after the wash-out period of the vitamin K). The inclusion of this study group gives us the possibility to study both the effect of the drug medications as well as the anticoagulant effect on homocysteine levels. Details of the second study group have been published by Schurgers *et al.*[5].

Homocysteine concentrations of both study groups were determined using an automated high-performance liquid chromatography with reverse phase separation and fluorescence detection (Gilson 232-401 sample processor (Gilson Medical Electronics Inc., Middleton,WI), Spectra-Physics 8800 solvent delivery system and Spectra-Physics LC 304 fluorometer (San Jose,CA)), according to the method described by Fiskerstrand *et al.*[6] with some modifications[7].

Folate and vitamin B12 concentrations are determinants of the homocysteine concentration[8]. We measured folate and B12 with IMx Automated Immunoassay Analyzer (Abbott®) in a subset of patients of the surgery group.

Statistics

Paired-samples T-tests were used to compare geometric means of homocysteine, vitamin B12 and folate concentrations. Results are presented in percentages with 95% confidence intervals.

The study protocol was approved by the Leyenburg Ethics Committee for the surgery group. For the second study group, approval was granted by the Medical Ethics Committee of the University of Maastricht. All persons gave their informed consent according to the revised Helsinki declaration (1975).

Results

Figure 5.1 shows the individual values of the mean homocysteine concentration before during and after coumarin therapy in 40 patients. Geometric mean homocysteine concentration during coumarin therapy was 6% higher than before the start of coumarin therapy (95% confidence interval (CI) 2% to 10%; p=0.01). Absolute values increased from 16.4 µmol/l to 17.4 µmol/l. After cessation of the coumarins homocysteine decreased 4% (95% CI –1% to 10%; p=0.1) (absolute values 17.4 µmol/l to 16.7 µmol/l). The geometric mean difference between the concentrations before and after coumarin therapy was 1% (95% CI –4% to 7%; p=0.6).

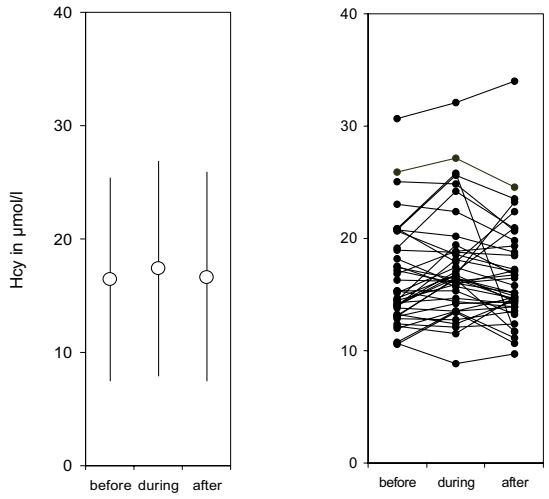

Figure 5.1 Mean homocysteine concentrations (Hcy) ± 2SD and individual homocysteine concentrations before the start of coumarin therapy, during coumarin therapy and after coumarin therapy in a group of surgery patients. See text for values.

Although the possible effect of anticoagulans on homocysteine values is very low, we analyzed if the 1 µmol/l increase in homocysteine concentration found in the surgery group was indeed due to treatment with anticoagulant drugs, we measured homocysteine concentrations in 12 volunteers who were treated with oral anticoagulants for 13 weeks. We found that geometric mean homocysteine concentrations decreased with 1.6% (95% CI –3.9% to 7.0%; p=0.5) after 4 weeks of treatment with oral anticoagulants, subsequently increased after vitamin K (2.9% (95% CI –5.2% to –0.4%); p=0.02), and decreased again after the wash-out of vitamin K (4.7% (95% CI –2% to 11%; p>0.05) The absolute values were 10.7, 10.1, 10.8, and 10.0 µmol/l, respectively. The individual concentrations and the mean INR levels in the study are shown in Figure 5.2. We conclude that there is no influence of anticoagulants on homocysteine values in healthy volunteers.

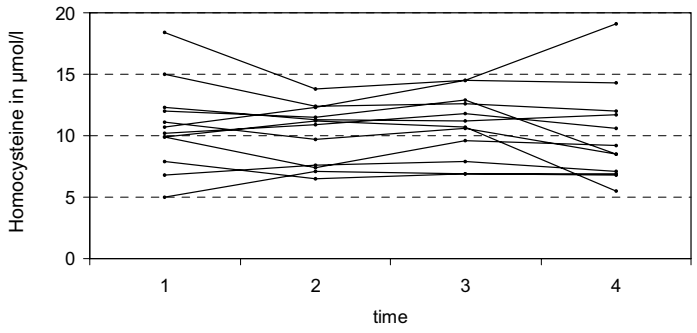

Figure 5.2 Homocysteine concentrations in 12 healthy volunteers treated with oral anticogulants and low-dose vitamin K.
1 = week 0. Before coumarins, mean INR=1.0
2 = week 4. After 4 weeks of coumarin therapy, mean INR=2.1
3 = week 10. After 11 weeks of coumarin therapy in combination with increasing doses of vitamin K, mean INR=1.4
4 = week 13. After the wash-out period of vitamin K, mean INR=1.9

We determined plasma vitamin B12 and folate concentrations in a subset of 27 surgery patients. No influence on vitamin B12 was observed (data not shown). However, folate level declined slightly (mean value from 6.7 nmol/l to 5.7 nmol/l), but not significantly (13%, 95% CI -3 to 27%; p=0.1). This was, however not associated with anticoagulant drugs since we saw no recovery after anticoagulant withdrawal but even a further decline from 5.7 nmol/l to 5.2 nmol/l (10 %, 95% CI –3% to 24%; p=0.1).

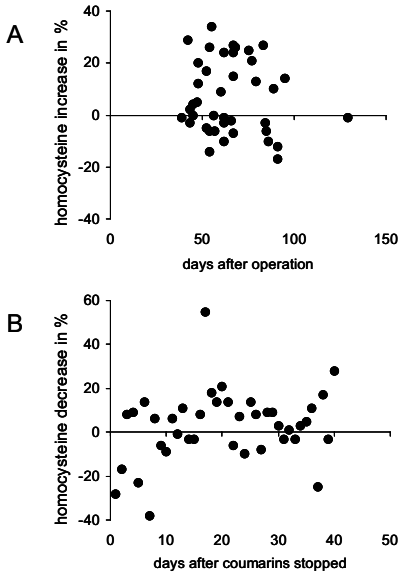

Figure 5.3 Percentile increase of homocysteine levels while on coumarin therapy in relation to the time interval passed since the operation(A) and percentile decrease of homocysteine levels after cessation of coumarin therapy(B)

Discussion

In this study we found a slight increase in homocysteine concentration in patients on prophylactic anticoagulant therapy for surgery. However, this effect was very small with a difference of 6% only, which corresponds to 1 µmol/l. In a group of healthy volunteers we found no influence of anticoagulants on homocysteine values at all.

The question is whether the slight increase in the surgery group was found due to coumarin use, or as an effect of the operation. We hypothesized that if there is an effect of the operation, it will diminish in the course of time. We compared the percentile increase in homocysteine concentration between the first (before coumarins) and the second (on coumarins) blood sample with the time elapsed after the operation. As shown in Figure 5.3A there was no relation between times elapsed after the operation and the percentile increase in homocysteine concentration (r=-0.1). In addition, we also determined the decrease in homocysteine concentration after the cessation of coumarins in relation to the time elapsed. Also, if the rise would be an effect of the coumarin use, the

percentile decrease after cessation of the coumarins would be greater when more time has passed. However, we found no relation between the time elapsed after the cessation of coumarins and the percentile decrease (Figure 5.3B) (r=0.07). A control group having similar surgery without anticoagulants is not possible for ethical reasons. A control group having a different form of thrombosis prophylaxis - like heparins – could have given information on the possible effect of different forms of anticoagulant treatment on homocysteine values versus the surgery procedure per se, but was not available.

The effect we observed in the surgery group was much smaller than the effect of coumarin use on homocysteine levels as found by Murua *et al.*[9]. They found a median difference of 9 µmol/l between a group of chronically anticoagulated patients with mitral valve replacement, atrial fibrillation and dilated cardiomyopathy in comparison to a group of patients with atherosclerosis who were not treated with coumarins. The authors concluded that this increase in homocysteine concentration was due to the lower folate levels in the patients on coumarins. The difference between the observation of Murua *et al.* and this study might be explained by the duration of coumarin treatment. Our study was aimed at analysing anticoagulants per se interfering with homocysteine values. Following patients for a longer period of time could coincide with a change in other variables such as folate levels observed by Murua.
The slight increase in homocysteine levels we found in the surgery group can be influenced by several factors. First, all our patients had a knee or hip replacement the day after the first blood sample was drawn. The use of nitrous oxide during operations can cause increments in homocysteine by cobalamin dependent blockage of methionine synthase[10-12], an enzyme necessary for the re-methylation of homocysteine. It has been shown to affect homocysteine concentrations up to one week postoperatively[12], but it is not known whether this effect lasts longer. When nitrous oxide is not used as an anesthetic, homocysteine is shown to remain stable[11] or even decrease[13]. Long-term effects of operations on homocysteine concentrations have not been reported. We reasoned that the effect would diminish in the course of time. The data shown in Figure 5.3A do not support the hypothesis that homocysteine concentrations increase due to a late effect of the operation: there is no relation between the time elapsed since the surgery and the increase in homocysteine, making an effect of aneshtesia on homocysteine concentrations unlikely.
Despite the fact that the influence on homocysteine concentration was very low in the surgery group, people who underwent this kind of major surgery, may have alterations in dietary habits, e.g. reduced folic acid and vitamin B12 intake. We did not observe an influence on vitamin B12 levels but a slight decline in plasma folate levels. The folate concentrations decreased even further after withdrawal of anticoagulants. Possibly, folate levels are an inpact

determinant of homocysteine values after surgery. This mechanism would fit to the analysis of Murua *et al*.[9].

Since we observed a minor effect in the surgery group, we subsequently studied the effect of coumarin treatment on homocysteine levels in a group of healthy volunteers. This way we were able to separate the effect of coumarins and surgery. In this group we found no effect in homocysteine concentrations during anticoagulant treatment. Since we found no effect of anticoagulant use in the healthy controls it is most plausible that the small effect in the surgery group was caused by clinical circumstances and not to the anticoagulant per se.

Many interactions of drugs and homocysteine concentrations have been described. Examples of these drugs are: B-vitamins, anti-folates like methotrexate, anti-epileptic drugs like phenytoin, the nitrous oxide and estrogens[8]. Most of these interactions are a result of direct influence of the drug on the metabolic pathways involved in homocysteine metabolism. As far as we know, the metabolic pathways of homocysteine and coumarins are not interchanged, and our results support the conclusion that there is no interaction.

Although an influence of coumarins on homocysteine levels is unlikely, we calculated wether a slight change in homocysteine levels as observed in the surgery group would affect the risk estimates in case-control studies in which only the cases used anticoagulant therapy. When homocysteine levels increase during anticoagulant therapy, more cases will have hyperhomocysteinemia and fewer cases will have a normal homocysteine concentration, resulting in a falsely elevated odds ratio. We calculated the impact of the 6% increase we found in our surgery group and we obtained correction factors of 0.89 for the 75th, 0.88 for the 90th and 0.88 for the 95th percentile (using reference values of a case control study we performed earlier[2]). Thus, if an effect of coumarins on homocysteine concentration would indeed exist - at a level 1 µmol/l increase - this would have had only a very small effect on the odds ratios from comparing studies in which coumarins were used in patients and not controls.

Usually, the increase in homocysteine concentration in patients at risk for vascular disease is expressed as odds ratio per 5 µmol/l. For venous thrombosis this odds ratio is approximately 1.6[1], for stroke and ischemic heart disease 1.4 and 1.2 respectively[3]. A change of 1 µmol/l in homocysteine concentration, as we found in the surgery group, corresponds with a risk ratio of approximately 1.01. Therefore, when homocysteine concentrations are measured in a screening program for cardiovascular risk factors in individual patients, the use of anticoagulant therapy during this screening has no significant impact on the risk estimate. Moreover, because results from adequate intervention trials in atherosclerosis are conflicting[14-16], and the

results of intervention trials in venous thrombosis are still limited[17] the benefit of determination of individual homocysteine concentrations remains unclear.
In conclusion, we found a slight but not relevant increase in homocysteine values in patients who were on anticoagulant therapy because of orthopedic surgery, and no influence in healthy volunteers taking anticoagulants for scientific reasons. We conclude that anticoagulants per se do not influence homocysteine values at a level to consider relevant in epidemiological studies nor in individual patient care.

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