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EFFECTS OF HOMOCYSTEINE-LOWERING THERAPY ON ENDOTHELIAL FUNCTION, CAROTID WALL THICKNESS AND MYOCARDIAL PERFUSION

SUMMARY

Homocysteine is a physiological product of the human metabolism. It has got primary atherogenic and prothrombotic properties. Moderate hyperhomocysteinemia is widely accepted as a risk factor for venous thromboembolism; its role in atherosclerotic vascular disease is still not well-defined. The objective of this small pilot study was to relate a homocysteine-lowering therapy with folic acid in coronary artery disease patients with mild hyperhomocysteinemia to benefits concerning endothelial function, carotid wall thickness and myocardial perfusion.

A total of 15 patients with stable coronary artery disease and hyperhomocysteinemia was randomized, of whom 10 were treated with folic acid 5 mg/daily per os over a period of 6 months. Five patients received (single-blinded) placebo. Measurement of endothelial function by determination of the forearm blood flow, measurement of wall thickness of arteria carotis communis by MRI and of myocardial ischemia by adenosin-stress MRI was performed initially and after the treatment period of 6 months.

Homocysteine levels fell significantly by 34% under therapy with folic acid as expected. Endothelium function improved by 27 % under the treatment, while under placebo, a minor improvement by 3 % was seen. This trend of a positive treatment effect reached no significance however because of a small number of patients. Carotid structure and myocardial perfusion did not show any significant improvement, also.

In this pilot study, treatment of mild hyperhomocysteinemia in patients with stable coronary artery disease did not improve the vascular status despite lowering homocysteine levels. Maybe only the treatment of severe forms of hyperhomocysteinemia shows a noticeable benefit.

Key words: **hyperhomocysteinemia, endothelial dysfunction, coronary artery disease**

INTRODUCTION

The role of hyperhomocysteinemia (HHCy) in atherosclerotic diseases has not been well-defined. Up to 40 % of patients with premature coronary heart disease (CAD), peripheral vascular disease or recurrent venous thrombosis present with HHCy (1). Although a relationship to a higher risk of CAD, stroke and peripheral vascular disease has been shown in several large trials, it is not called a major risk factor. The American Heart Association does not recommend the use of folic acid and B vitamins to reduce the risk of heart disease and stroke (2). The „D.A.CH.-Liga Homocystein e. V. “ (I. E. homocysteine-league of Germany, Austria and Switzerland) recommends lowering the plasma homocyste-

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ine (Hcy) levels to below 10 µmol/l in patients at high risk of vascular disease (3). During the annual meeting of the European Society of Cardiology in 2005, a controversy on this topic took place. Based on the data of a topical trial called „NORVIT“ (not yet published), opponents of a vitamin therapy rated treatment of HHCy in secondary prophylaxis after myocardial infarction worthless to dangerous. Supporters indicated to positive effects of treating HHCy in primary prophylaxis and also in patients with CAD. In regard to a directed intervention in CAD-patients undergoing successful coronary stenting, the results of two larger studies are contrary also: lowering of plasma HCy levels lead to a decreased rate of coronary restenosis in one trial with 205 patients (4). The treatment caused an increased risk of in-stent-restenosis and need for revascularization in another trial with 636 patients (5). The list of contrary data could be continued.

Our approach to treatment of HHCy was to explore whether it is possible to influence endothelial dysfunction as an early stage of atherosclerosis. We chose subjects with angiographically confirmed CAD-manifestation to influence both endothelial dysfunction and manifest atherosclerosis (carotid wall thickening and myocardial ischemia). The vision withstanding is to prevent manifestations of atherosclerosis in subjects being at high risk in sense of primary prophylaxis.

MATERIAL AND METHODS

Two hundred and thirty-eight patients with angiographically confirmed CAD (> 50% luminal stenosis) or a history of myocardial infarction were screened on HHCy within a period of two years. HCy values above 12 µmol/l were taken as elevated. Fourty-three patients were found to have HHCy after this definition. Most of them could not be included because of fulfilling exclusion criteria (almost carrying an implantable cardioverter / defibrillator which makes peformance of MRI impossible; atrial fibrillation) or lack of interest in taking part. At last, 15 patients were randomised successively in a single-blinded fashion. To study a possible positive therapy effect, 10 subjects were enclosed as verum-group and treated with 5 mg folic acid/day. Five patients served as control and took in one pill of placebo per day. Any of the following procedures was done two times: at baseline and after the treatment period of six months (follow - up).

HCy-levels were measured in patient's plasma by fluorescence-polarisation immunoassay (Ax SYM, ABBOTT, Wiesbaden, Germany).

Main effect of endothelial dysfunction is an impaired intravasal production of nitric oxide (NO) by endothelial NO-synthetase (eNOS). NO-production of endothelium cells as a response to administration of acetylcholine is impaired in endothelial dysfunction, followed by a minor vasodilatation. This is expressed by a diminished forearm blood flow. Measurement of endothelial function was performed using endothelium-dependent vasodilatation as a main parameter. We used venous occlusion pletysmography with Periquant 4000 (Gutmann Medizinelektronik, Eurasburg, Germany) for measurement of forearm blood flow. After puncture of the A. brachialis of the patient's nondominant arm (mostly left) with a 19 ga needle and bringing in a 18 ga x 6´´ arterial catheter (Argon medical devices), a rest period of 30 minutes was obtained. Then, forearm blood flow was determined natively (normal range 2 – 3,5 ml/100 ml tissue/min.) and after intra-arterial administration of acetylcholine 20 µg/min and 40 µg/min. (Miochol, Novartis), followed by 15 minutes rest. Afterwards, endothelium independent vasodilatation was determined during intra-arterial infusion of nitroprusside 4 µg/min. and 8 µg/min (Nipruss, Schwarz Pharma).

This second measurement after nitroprusside-infusion is seen as independent from an existing vascular damage. The difference between the effects of acetylcholine and nitroprusside can therefore be taken to compare the amount of vascular damage in a single individual.

Determination of the wall thickness of arteria carotis communis: MR-images were acquired by T1-classified TSE-sequences (Siemens Sonata, 1,5 Tesla scanner). The total wall thickness of distal common carotid artery, taken as cross sectional area including adventitia to intima was measured on both sides. The respective sides were compared before and after the treatment.

For examination of myocardial ischemia, a stress-MRI of the heart was performed, as it is used for frequent clinical questions. Myocardial perfusion and wall motion in rest and under exercise under adenosine (140 µg/kg bw/min over 6 minutes) was measured. For this, a contrast medium (gadolinium-DPTA, 0,2 ml/kg bw, injection velocity 8 ml/s) was used. Furthermore, an evaluation of the myocardial vitality was performed. Images were acquired by FLASH-3D-inversion-pulse-sequences (ECG-triggered, TI frequency dependent 150-320 ms, SLT 5 mm). Image acquisition was to be started five minutes after infusion of the contrast medium and was to be continued for approximately 15 minutes. The existence of a “late enhancement“allows to distinguish between vital and avital myocardium.

Statistical analysis was done with the aim of software WinSTAT using U-test (Mann-Whitney).

RESULTS

Of 15 patients included, 10 were treated with folic acid 5 mg/day (group I). The mean plasma HCy level in this group was 19,2 ± 5,38 µmol/l and thus markedly higher than in the placebo-treated group II showing a level of 13,3 ± 2,28 µmol/l. In group I, the mean HCy level fell to 12,6 ± 1,6 µmol/l (- 34 %), while in group II a spontaneous decline to 11,6 ± 3,0 µmol/l (-13 %) was observed *(Figure 1)*.

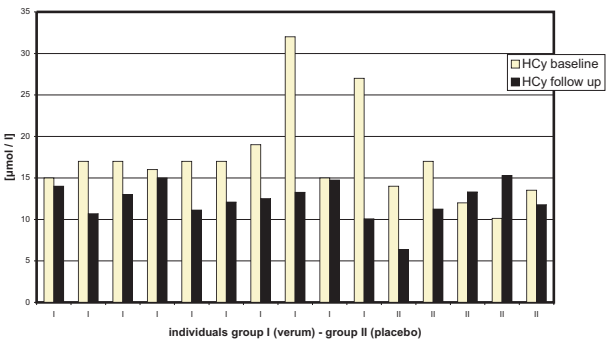

Figure 1. Homocysteine plasma levels [µmol/l]: individual values group I (treatment) and group II (placebo)

The differences in HCy levels between the groups were statistically insignificant because of different initial values.

The endothelium-dependent vasodilatation measured as maximum increase in forearm blood flow (FABF) [ml/100 ml tissue/min] under intra-arterial infusion of acetylcholine was highest under infusion of 40 µg/min acetylcholine. For analysis, the percentual growth of mean maximum increase in FABF was compared in both groups. In group I mean maximum increase in FABF was 5,83 % at baseline and 7,43 % at follow up (+ 27 %) *(Figure 2 A)*.

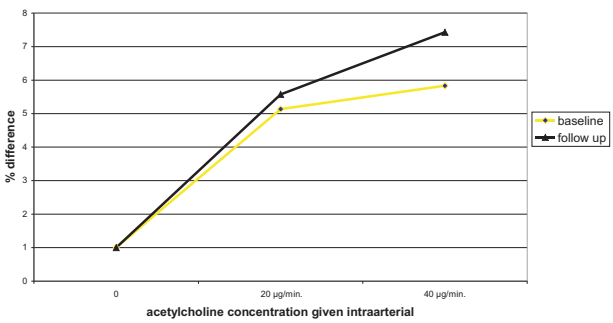

Figure 2 A. Forearm blood flow [ml/100 ml tissue/min] under acetylcholine infusion in two different dosages; group I (treatment; folic acid 5mg daily for 6 months)

Group II showed a mean maximum increase of FABF of 8,30 % at baseline and 8,58 % at the follow-up (+ 3 %) *(Figure 2 B)*.

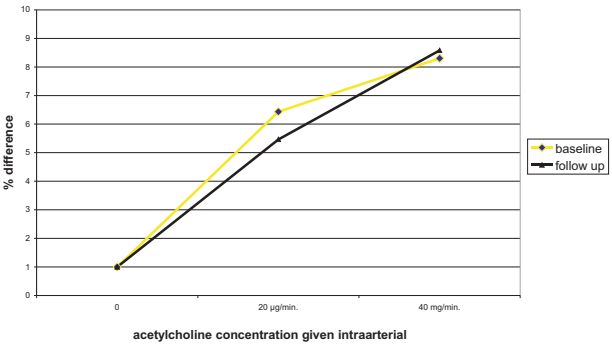

Figure 2 B: forearm blood flow under acetylcholine - group II (placebo)

The difference in maximum FABF increase was higher in group I, and statistical analysis did not show any significance (p = 0,3 in group I, p = 0,6 in group II). Measurements of endothelium independent vasodilatation by detecting maximum increase in FABF under intra-arterial infusion of nitric oxide was found highest under 8 µg/min. Group I showed an increase from 5,37 to 6,00 % (+ 12 %) (figure 3 A), group II from 7,33 to 10,03 % (+ 37 %) (figure 3 B).

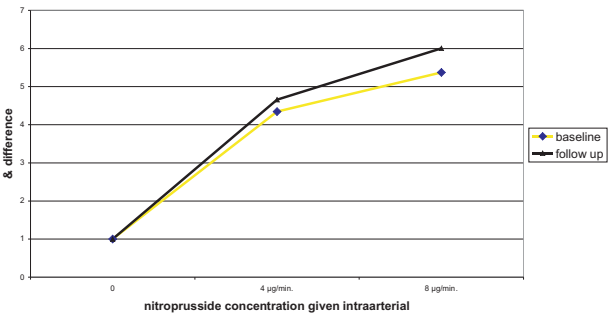

Figure 3 A. Forearm blood flow [ml/100 ml tissue/min] under nitroprusside infusion in two different dosages; group I (treatment; folic acid 5mg daily for 6 months)

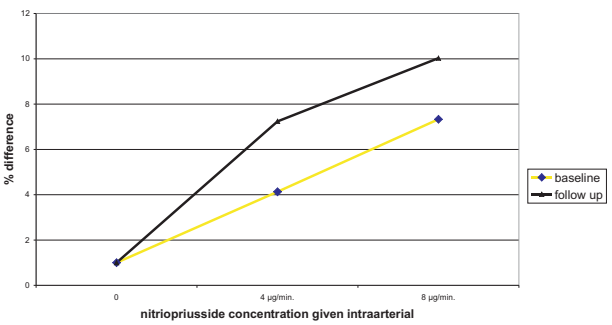

Figure 3 B: forearm blood flow under nitroprusside - group II (placebo)

MRI-measurement of the wall thickness of arteria carotis communis resulted as follows: in group I mean thickness was 0,64 cm² mean (right side) and 0,58 cm² mean (left side) at baseline. At follow up, the right side was measured with 0,60 cm² mean and the left side with 0,52 cm² mean *(Table 1)*.

Thus, a regression in wall thickness was seen, but reached no significance in this group of patients. In group II, the wall thickness decreased from 0,53 cm² mean to 0,45 cm² on the right and from 0,57 cm² mean to 0,48 cm² on the left side which was statistically not significant *(Table 2)*.

Myocardial perfusion finally showed the following results: MRI under adenosine administration was unaffected in the follow up versus baseline in 9 patients of group I and 4 patients of group II. The findings in these patients were not indicating on myocardial ischemia beyond infarction scarves, neither at baseline nor in the follow-up. In each group, there was one patient with a finding of a myocardial perfusion defect beyond existing scarves in the baseline examination. Because of being free of symptoms, no invasive diagnostics was performed. In the follow-up, both patients showed no more evidence of perfusion defects.

DISCUSSION

Several data indicate positive correlation of hyperhomocysteinemia and vascular disease, i.e. ischemic heart disease, deep vein thromboembolism and stroke, for example a meta-analysis of 72 studies from 2002 (6). Some trials are indicating even a relationship between HCy-levels and cardiovascular mortality in patients with acute coronary syndromes (7) respectively and overall mortality in patients with CAD (8) .

Measurements of the forearm blood flow and common carotid wall thickness are seen as showing an early manifestation of atherosclerosis and predicting manifestations as CAD/ myocardial infarction. The positive correlation of HHCy on carotid artery intima-media thickness is well-established (9-11) .

The mechanisms of vascular damage by HCy are widespread. It has got primary atherogenic and prothrombotic properties. Both in vivo and in vitro, a direct toxic endothelium damage by HCy is described. This toxic effect can be caused by free oxygene radicals (oxidative stress), by stimulation of smooth-muscle-proliferation, stimulation of LDL-oxidation and by diminishing the function of endothelial NO-synthase via higher levels of ADMA (asymmetric dimethylarginine). This can lead through diminished production of endothelial nitric oxide to impaired endothelium dependent vasodilatation. Furthermore, HCy leads to formation of aggregates together with LDL-cholesterol, that become foam cells after being taken up by vascular macrophages in the intima. The prothrombotic effects include altered platelet reactivity, production of proinflammatory cytokines, induction of apoptotic cell death, for example in endothelial cells, attenuation of endothelial cell tissue plasminogen activator binding sites, activation of factor VIIa and V, inhibition of protein C and heparin sulphate and many more (12-13).

Despite this bold pathophysiological influence of HHCy, positive effects of HCy lowering therapy on endothelial dysfunction are rarely evidenced. A study from 2000 in 75 CAD-patients with HCy-values > 9 µmol/l resulted in a significant improvement of endothelial dysfunction, correlating with the reduction of HCy-levels by the intake of folic acid (14). The positive study in CAD-patients after coronary intervention was mentioned above (14). Other studies found no correlation between lowering plasma homocysteine and improvement in flow-mediated artery dilatation (15).

In our investigation, no significant improvement of parameters standing for endothelial function and vascular integrity was seen, while the HCy level was nearly normalized in the treatment group. Comparison of HCy level with placebo group was not significant because of the distinct lower initial levels in group II. All parameters ranged within accidental variation. It seems remarkable that FABF was initially higher in group II correlating to lower HCy levels. Lowering the levels in group I under the initial levels of group II does not lead to an adjustment in FABF. Although the number of study patients is too small to come to a strong implication, the question is why neither endothelial function nor carotid wall thickness improved significantly under the therapy. The one point is that the levels of HCy we found were not very high. Probably more severe HHCy shows more benefit by treating it. Very high HCy levels (up to over 100 mmol/l) are found in subjects with genetic defects of the HCy metabolism (polymorphism of the methylentetrahydrofolatreductase, MTHFR). Moderate elevations of the HCy level (< 30 mmol/l) are mostly caused by lack of B-vitamins, cigarette smoking or impaired renal elimination (16).

On the other side, there are considerations that the atherogenic effects of HCy are seen mainly in combination with hyperlipidemia, for example in leading to the generation of foam cells (12). This would lead to the conclusion that under sufficient therapy by inhibitors of cholesterol synthesis (CSE-inhibitors), a positive effect of HCy-lowering treatment might not be seen. All patients in our investigation were treated with high dose CSE-inhibitors.

In conclusion, treatment of mild hyperhomocysteinemia to prevent manifestation of atherosclerosis does not seem to be effective and CAD-patients with mild hyperhomocysteinemia should not be categorically treated with folic acid.

Table 1: group I (treatment)
Common carotid wall thickness (cross sectional area) [cm²]

pat.-No.	baseline	baseline	follow- up	follow -up
	ACC r.	ACC l.	ACC r.	ACC l.
1	0.52	0.74	0.78	0.66
2	0.73	0.56	0.73	0.63
3	0.67	0.7	0.72	0.48
4	0.85	0.65	0.76	0.47
5	0.35	0.26	0.28	0.3
6	0.82	0.69	0.71	0.64
7	0.73	0.67		
8	0.62	0.42	0.49	0.48
9	0.56	0.53		
10	0.57	0.6	0.29	0.48
mean	0.642	0.582	0.595	0.5175

Table 2: group II (placebo)
Common carotid wall thickness (cross sectional area) [cm²]

pat.-No.	baseline	baseline	follow-up	follow-up
	ACC r.	ACC l.	ACC r.	ACC l.
11	0.49	0.51	0.44	0.57
12	0.52	0.61		
13	0.73	0.87		
14	0.5	0.59	0.47	0.62
15	0.4	0.29	0.44	0.24
mean	0.53	0.57	0.45	0.48

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EFEKTI TERAPIJE SMANJENJA HOMOCISTEINA NA ENDOTELNU FUNKCIJU, DEBLJINU KAROTIDNOG ZIDA I MIOKARDNU PERFUZIJU

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SAŽETAK

Homocistein je fiziološki produkt ljudskog metabolizma. Karakteriše se primarnim aterogenskim i protrombotičkim osobinama. Umerena hiperhomocisteinemija je opšte prihvaćeni faktor rizika za nastanak venske tromboembolije. Njena uloga kod aterosklerotične vaskularne bolesti još uvek nije jasno definisana. Cilj ove male pilot studije bio je da prikaže odnos terapije smanjenja homocisteina folnom kiselinom u slučaju koronarne arterijske bolesti praćene blagom hiperhomocisteinemijom i povoljnog uticaja, koji se odnosi na endotelnu funkciju, debljinu karotidnog zida i miokardijalnu perfuziju.

Izabrana je grupa od 15 pacijenata sa stabilnom koronarnom arterijskom bolešću. U okviru ove grupe, 10 pacijenata je bilo lečeno folnom kiselinom, 5 mg/dnevno per os, u toku šest meseci. Pet pacijenata je primalo placebo. Na početku i nakon šest meseci merena je endotelna funkcija određivanjem protoka krvi podlaktice. Izmerena je i debljina zida arterije carotis communis pomoću MRI.

Nivoi homocisteina su značajno pali za 34% pod terapijom folne kiseline, kao što je i bilo očekivano. Endotelna funkcija se poboljšala za 27% u toku tretmana, dok je u grupi koja je uzimala placebo poboljšanje nastalo u 3% slučajeva. Ovaj trend pozitivnog uticaja tretmana nije dostigao nivo značajnosti zbog malog broja pacijenata. Takođe, ni karotidna struktura ni miokardna perfuzija nisu pokazale značajno poboljšanje.

Ova pilot studija je pokazala da lečenje blage hiperhomocisteinemije kod pacijenata sa stabilnom arterijskom bolešću nije dovelo do poboljšanja vaskularnog statusa uprkos sniženim nivoima homocisteina. Možda će lečenje samo ozbiljnijih oblika hiperhomocisteinemije pokazati značajnije poboljšanje.

Ključne reči: **hiperhomocisteinemija, endotelna disfunkcija, koronarna arterijska bolest**