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Hyperhomocysteinemia and Cardiovascular Disease in Uremia: The Newest Evidence in Epidemiology and Mechanisms of Action

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In the general population, hyperhomocysteinemia is an independent risk factor for cardiovascular disease (ischemic disease, such as stroke and myocardial infarction, and arterial and venous thrombosis). We can presume that the association is causal, based on the example of homocystinuria, and on the evidence put forward by several basic science and epidemiologic studies. However, the results of large intervention trials, which may grant further support to this hypothesis, are not yet available. In chronic renal failure and in uremia, the evidence that is offered by carefully performed prospective studies also indicate the presence of an association, although some studies suggest reverse epidemiology. The mechanisms underlying the association, and able to explain the several toxic effects of homocysteine, related or not to cardiovascular disease, are unclear. Oxidation, nitrosylation, and hypomethylation are among the postulated mechanisms. In uremia, protein hypomethylation interferes with protein repair; DNA hypomethylation impairs regulation of gene expression, whereas folate treatment reverts such alterations. Acylation, another structural modification able to impair protein function, is a possible mediator of homocysteine toxicity.

KEYWORDS homocysteine, folate therapy, uremia, chronic renal failure, hypomethylation

Several observational studies, both retrospective and prospective, have shown (with some exceptions) that hyperhomocysteinemia is an independent risk factor for cardiovascular disease in the general population.[1] In a recent meta-analysis, including 30 studies and more than 6,000 events, a 25% lower homocysteine level (a 3 μmol/L decrease) was associated with an 11% lower ischemic heart disease risk and a 19% lower stroke risk. Data were adjusted for regression dilution bias and known cardiovascular risk factors. These authors concluded that homocysteine is a modest cardiovascular risk factor in healthy people. Nevertheless, the implications of decreasing homocysteine levels still could be substantial in a general strategy to lower cardiovascular risk.[2]

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In normal individuals, plasma total homocysteine concentrations span from 5 to 12 μmol/L, and over 97% is in its oxidized form. Hyperhomocysteinemia is defined as mild degree if less than 16 μmol/L, as moderate degree between 16 and 30 μmol/L, as intermediate between 31 and 100 μmol/L, and as severe if greater than 100 μmol/L.[3] In nontreated homocystinuria levels are in the severe range, and in chronic renal failure levels are in the moderate-intermediate range, whereas in the general population mild increases (12–16 μmol/L) still can provide for an increase in cardiovascular risk.[4]

Homocystinuria is the first described human model of hyperhomocysteinemia. In its most common form it is caused by the inherited defect of cystathionine-β-synthase, with accompanying hypermethioninemia, but other forms are characterized by hyperhomocysteinemia alone. In all forms, affected patients, who display a variety of clinically relevant derangements attributable to homocysteine accumulation (marfanoid features, ectopia lentis, and so forth), used to die of premature cardiovascular disease, in particular arterial and venous thrombosis, myocardial infarction, and stroke.[5] It has

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been concluded that in this monogenic disease hyperhomocysteinemia causally induces high mortality levels caused by cardiovascular disease, and in fact therapy leads to a significant increase in survival rates.[6]

Homozygosity for the methylenetetrahydrofolate reductase 677C→T transition, leading to the unstable thermolabile form of this enzyme, is a particularly frequent polymorphism, responsible for mild hyperhomocysteinemia. Studies on genetic variants are useful in assessing the nature of associations, such as that between homocysteine and cardiovascular disease, that is whether homocysteine causes the disease or not. A recent meta-analysis has shown that this polymorphism is associated with increased cardiovascular risk, and the risk becomes patent when folate levels are low.[7]

Other causes of hyperhomocysteinemia are reduced B vitamin intake or defective intestinal absorption and administration of drugs (eg, those that consume methyl groups, similar to L-DOPA, or that interfere with folate metabolism such as methotrexate). Another cause of hyperhomocysteinemia is chronic renal failure, with a high prevalence of mild-moderate hyperhomocysteinemia (85% to 90%) in uremic patients, in whom high cardiovascular mortality rates also are observed.

Very briefly, homocysteine is a sulfur amino acid whose metabolism is related to methionine, an essential amino acid, contained either in the normal diet or originating from protein breakdown.[8] Methionine, when it is not used in protein biosynthesis, is condensed with adenosine triphosphate to form *S*-adenosylmethionine (AdoMet), a sulfonium compound. AdoMet in turn donates, after decarboxylation, the propyl amino moiety in polyamine synthesis, whereas its methyl group is used in the transmethylation pathway to methylate various methyl acceptors (proteins, DNA, and small molecules, such as guanidino acetate, in creatine biosynthesis). AdoMet demethylated product is *S*-adenosylhomocysteine (AdoHcy). AdoHcy is hydrolyzed to adenosine and homocysteine in a reversible reaction that is inhibited by AdoHcy itself (in a competitive product type of inhibition). Homocysteine then is metabolized to cystathionine in the transsulfuration pathway, where cystathionine-β-synthase is the rate-limiting enzyme. The remethylation pathway leads to methionine formation from homocysteine, which receives a methyl group from methyltetranydrofolate (MTHF), in a reaction catalyzed by methionine synthase. Methylenetetrahydrofolate reductase is the enzyme that catalyzes the reduction of methylenetetrahydrofolate to MTHF, thereby irreversibly committing one carbon unit to MTHF. This represents a folate trap because MTHF can be used in this and only this remethylation reaction, whereas folates in less reduced forms can be used in other reactions and in particular by thymidilate synthase in this DNA precursor synthesis.

Hyperhomocysteinemia in Renal Failure: Causes

As for the cause of hyperhomocysteinemia in renal failure, it previously was thought that impaired renal excretion could be responsible, but it has been ascertained that homocysteine excretion is negligible.[9] Also, no increased production of homocysteine is reported to be present in uremia.[10] This is also confirmed by the observation of Mitch et al,[11] who showed that creatinine production is not increased in renal failure (creatinine derives from creatine, which is the methylated product of guanidinoacetate, a major AdoMet methyl-acceptor). Regarding homocysteine metabolism by the kidney, results obtained in the rat model show decreased renal metabolism in uremia.[12] However, in this experimental animal model homocysteine is mostly in the free, non–protein-bound, form. On the contrary, in human plasma only a small fraction of homocysteine circulates in a free form, and is therefore available for glomerular filtration. Homocysteine levels were measured in the renal vein and renal artery of patients with normal renal function undergoing a coronarography, in the fasting state, and there was no significant difference.[13] Guttormsen et al[10] have shown that, after peroral and intravenous homocysteine loading in chronic renal failure patients, total body homocysteine clearance from plasma is impaired (70% reduction). After folate treatment homocysteine levels are reduced, but homocysteine half-life is not. It therefore has been concluded that folic acid enhances homocysteine remethylation in tissues, thereby decreasing homocysteine efflux into the plasma compartment, but does not affect the reduced total body clearance of homocysteine.

Recent findings support the view that high homocysteine levels in uremia are not caused by defective folate absorption or folate interconversion to the active forms.[14,15]

A study by Garibotto et al[16] investigated the balance of total homocysteine, cysteine-glycine, and cysteine across the kidney, splanchnic bed, and the lower limbs in humans in the postabsorptive state, a condition in which the steady state of arterial levels is maintained by the constancy of the uptake and tissue release rates.

The average kidney removal of homocysteine was not statistically significant, thereby confirming the findings of van Guldener et al.[13] These findings may reflect the low sensitivity in the methods currently available to detect small arteriovenous differences of homocysteine, as argued by Blom and De Vriese[17] and Brosnan.[18] However, the important finding is that the fractional extraction (eg, the arteriovenous difference in percent of the arterial concentration) of homocysteine across the kidney varies directly with renal plasma flow. However, a big interindividual variability in homocysteine metabolism across the kidney was found. The fractional extraction of homocysteine across the kidney was related positively with renal plasma flow with a net uptake taking place when renal plasma flow was greater than 500 mL/min. By examining the relationship between renal homocysteine clearance and renal plasma flow, it appears that homocysteine renal clearance decreases rapidly from 70 mL/min to values close to 0 when plasma flow decreases from 650 mL/min to values around 400 to 500 mL/min. This finding is in agreement with data by Guttormsen et al,[10] indicating that whole-body homocysteine clearance is reduced from 100 to 30 mL/min when renal function is impaired. Taken together, these data suggest the dependence of homocysteine uptake

by the human kidney on a nonreduced blood supply. Besides glomerular filtration, which is restricted because of protein binding, homocysteine may be taken up by the peritubular basolateral surface,[19,20] and conditions decreasing renal plasma flow, such as diuretic therapy, are characterized by hyperhomocysteinemia.

Hyperhomocysteinemia in Renal Failure: Effects

Consequences of hyperhomocysteinemia can be viewed directly in relation to cardiovascular risk. Interventional trials can help to answer the issue of causality, that is, whether or not hyperhomocysteinemia is linked causally to cardiovascular risk. Several intervention trials currently are under way that will test the hypothesis that decreasing homocysteine levels in the general population will reduce cardiovascular events. In patients undergoing coronary angioplasty, the prevalence of restenosis decreases with folic acid, vitamin B_6, and B_{12} therapy.[21] Because of the mandatory folic acid flour fortification program in the United States and other countries for prevention of neural tube defects, the idea has been put forward that these intervention trials will lose their statistical power because blood homocysteine levels will be lower than expected at the start of trials.[22] Therefore, the difference between pre- and posttherapy levels will be much lower. It has been proposed that these trials should enroll more patients and be longer in duration, and that only a meta-analysis of these trials, once available, will assess the relevance for public health of decreasing homocysteine levels for prevention of cardiovascular disease.[23]

In uremic patients, a recent study, in which a population of 175 chronic hemodialysis patients was followed-up for 29 months, showed an increase in mortality rates that was higher with higher plasma homocysteine levels at baseline.[24] Statistical adjustment for confounders and traditional and nontraditional risk factors was performed. However, it also has been shown that lower homocysteine levels (as expression of general malnutrition) are associated with increased mortality.[25] It is important in testing the reverse epidemiology hypothesis[26] that data should be adjusted carefully for confounders, and in this particular case for plasma albumin levels. Low albumin levels are a strong predictor of mortality, and low homocysteine concentration, if not adjusted, could therefore be just a surrogate for malnutrition.

A trial in kidney transplant patients funded by the National Institutes of Health in the United States is currently (July 2004) in its initial stages. Transplant patients display unique characteristics because they are at high risk for cardiovascular disease, their homocysteine levels are high (in the mild range), and they are susceptible to be normalized on folate treatment. This trial most probably will keep its statistical power even in the era of folate fortification because homocysteine levels are high to start with. The results of this trial are eagerly awaited.[22]

Consequences of hyperhomocysteinemia can be viewed also in terms of a general toxicity, more or less directly related to cardiovascular risk. For example, it has been shown that homocysteine induced the expression of T-cell death–associated gene 51 (TDAG51) in human umbilical vein endothelial cells (HUVECs), and ApoE −/− mice fed hyperhomocysteinemic diets showed an increase in TDAG51 expression that was correlated with apoptosis in atherosclerotic lesions. Apoptosis increases the risk for rupture of the atherosclerotic lesion by decreasing its stability. Therefore, TDAG51 promotes detachment-mediated apoptosis in vivo and in vitro, and contributes to the development of atherosclerosis in hyperhomocysteinemia.[27]

It recently has been shown that folate treatment reverts impairment in DNA methylation and alterations of gene expression in hemodialysis patients.[28] In these patients, global DNA methylation is impaired owing to the inhibition of methyltransferases, exerted by the accumulation of the homocysteine precursor AdoHcy. In earlier studies it has been shown that membrane protein repair, a process in which a methylation reaction is involved, is inhibited in uremia.[29–31] Given the levels of AdoHcy in uremia, and the Km and Ki of the individual enzyme, the extent of inhibition for each enzyme has been calculated, and DNA methyl transferases were among the enzymes likely to be affected.[32] In fact, DNA methylation is impaired and its levels correlate significantly with plasma homocysteine levels.

DNA methylation is viewed as a mechanism that can silence (and more generally regulate) gene expression. For example, this is the case of imprinted genes. Considering the way through which genes are passed from one generation to another for the imprinted genes the allele coming from one of the parents is generally shut off through methylation. Under normal conditions, gene expression therefore is termed *monoallelic* for these genes (the gene coming from either the mother or the father is expressed, the other is silenced in a nonrandom manner, ie, not casually).

SYBL1 (a pseudoautosomal gene, X or Y inactivated) and H19 (an imprinted gene with maternal expression), genes regulated in the way described earlier, were chosen for this study. The allelic expression of these genes was therefore used to test the functional outcome of DNA hypomethylation in these patients (ie, if this hypomethylation could result in significant changes of gene expression). Allelic expression of SYBL1 and H19 was checked by using a suitable restriction fragment length polymorphism as a marker. This means that only heterozygous patients for a given restriction fragment length polymorphism could be monitored for allelic expression, once the complementary DNA obtained from RNA through reverse-transcriptase polymerase chain reaction was run, whereas in the others it was not possible to identify by this technique if one or both alleles (paternal and maternal) actually were activated. Results show that for SYBL1 the gene expression in patients is biallelic, that is, both alleles are expressed, and for H19, only in patients with high homocysteine levels (roughly >60 μmol/L) is the gene expression biallelic. After therapy with MTHF for 2 months the homocysteine levels decreased in the H19 heterozygous patients, with a parallel decrease of total DNA methylation, and gene expression returned to monoallelic, thus confirming the

working hypothesis that homocysteine modifies DNA methylation in a reversible fashion. The interpretation of results is that, in patients with higher homocysteine levels (>60 μmol/L), there is a transcriptional activation of the normally repressed allele caused by DNA hypomethylation. The subsequent folate treatment was able to revert the biallelic expression into monoallelic expression in the 3 patients who had biallelic expression; whereas in the other 4 patients (who acted as controls) H19 expression already was monoallelic (ie, normal) before folate and remained monoallelic afterward.

In conclusion, SYBL1 and H19 were used as marker genes for testing alterations of gene expression induced by hyperhomocysteinemia because SYBL1 and H19 are well-known methylation-regulated genes. Therefore, folates were used to test the hypothesis that high homocysteine levels induced DNA hypomethylation, and this resulted in alterations of gene expression.

Another possible mechanism of homocysteine toxicity is protein homocysteinylation, occurring after the formation of excess homocysteine thiolactone. Normally, it is not possible to measure homocysteine thiolactone levels in blood, probably because of a low concentration owing to the high molecule reactivity. However, when there is an increase in blood homocysteine level, an increase in homocysteinylated proteins can be observed.[33] Taking as a model plasma proteins incubated in the presence of homocysteine thiolactone, the formation of homocysteinylated proteins occurs spontaneously through a non enzymatic mechanism, and rapidly, with complete disappearance of thiolactone from the medium after 3 hours. Consequences of protein homocysteinylation are: protein damage with an altered electrophoretic mobility, and loss of enzymatic activity (with protein denaturation), in several model systems such as plasma proteins, methionyl tRNA synthetase, tripsyn, lysin oxidase, and so forth.

Therapy

In the general population, it is possible to reduce homocysteine levels by means of dietary intervention or with folate supplementation. In chronic renal failure patients, a resistance to folate therapy is present in the sense that it is possible to normalize homocysteine levels in only a subset of patients, whereas the majority show varying degrees of reduction. It is possible that the whole issue of therapy will be addressed in a much more specific manner when the exact cause of hyperhomocysteinemia is clearer. Resistance is present even with folates at a high dosage, as shown in the Vienna Multicenter Study.[34] It appears from this randomized controlled trial comparing 15, 30, and 60 mg folic acid that to treat with high-dose folate is not necessary because there was no difference between the groups. Folates are present in several chemical forms, from the less (folic acid, folinic acid) to the most reduced forms, represented by MTHF. There is no significant difference between these forms in terms of homocysteine reduction.[35] As for betaine, serine, and vitamin B_6, these compounds are not effective in uremic patients.

In general, it must be noted that until the assessment of the benefits in terms of cardiovascular risk is available, there is no general recommendation to screen or to treat hyperhomocysteinemia in renal failure. However, some scientists deem it necessary to screen and to treat individuals with especially high cardiovascular risk.

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