Hyperhomocysteinemia in ESRD

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Abstract

In chronic renal failure and in uremia, the role of uremic toxins and in particular of protein-bound molecules, such as AGEs, phenols, and homocysteine, is attracting much attention from scientists interested in understanding the mechanisms leading to the dramatic increase in cardiovascular risk and mortality typical of the condition. Homocysteine is a cardiovascular risk factor in the general population and in uremia. It displays several effects at the genomic level and on protein function, and it represents an interesting candidate in the study of the pathogenesis of the cardiovascular disease specific of uremia. Several aspects of hyperhomocysteinemia, such as clinical epidemiology, therapeutic aspects, and malnutrition, will be discussed.

Key words: homocysteine, folate receptors, uremia, chronic renal failure, hypomethylation, uremic toxins

Homocysteine metabolism

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. Methionine, when it is not employed in protein biosynthesis, is condensed with ATP to form S-adenosylmethionine (AdoMet), a sulfonium compound. AdoMet in turn donates, after decarboxylation, the propyl amino moiety in polyamine synthesis, while its methyl group is utilized 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, which is inhibited by AdoHcy itself (in a competitive product type of inhibition). Homocysteine is then metabolized to cystathionine in the transsulfuration pathway, where CBS is the rate-limiting enzyme. The remethylation pathway leads to methionine formation from homocysteine, which receives a methyl group from methyltetrahydrofolate (MTHF), in a reaction catalyzed by methionine synthase. MTHF reductase is the enzyme which catalyzes the reduction of methylenetetrahydrofolate to MTHF, thereby irreversibly committing one carbon units to MTHF. This represents a "folate trap", because MTHF can be used in this and only this remethylation reaction, while folates in less reduced forms can be used in other reactions and in particular by thymidilate synthase in the synthesis of this DNA precursor.

Hyperhomocysteinemia and the cardiovascular disease in uremia

An increase in plasma homocysteine levels is associated with an elevation of cardiovascular risk (1). The inherited enzymatic defect of CBS represents the most common form of homocystinuria, in which affected patients, who display high homocysteine levels in blood, used to die of premature cardiovascular disease (2, 3). In the general population, even a mild or moderate increase in blood homocysteine levels is associated with an increase in cardiovascular risk (4-7). In uremic patients, both those under conservative treatment and undergoing dialysis, there is an increase in cardiovascular risk, and actually uremia represents a model of generalized atherosclerosis. In addition, traditional risk factors or those typical of uremia can hardly explain this increase. Hyperhomocysteinemia is highly prevalent in this population. Recent studies show that hyperhomocysteinemia predicts mortality and cardiovascular events in uremic patients on hemodialysis (8-12). However, it has been recently proposed that low levels of homocysteine are linked to an increase in cardiovascular risk, so called reverse epidemiology (13-14). A recent study by Ducloux et al (15) clarifies once and for all this controversy. In this work, 459 patients, stratified in relation to the presence or absence of a chronic malnutrition-inflammation state (CISM), were followed prospectically. Homocysteine levels > 30 micromolar were associated with a significantly higher risk of global mortality (Hazard ratio 1.55, CI 1.12-4.72) in patients without CISM, and not in patients with CISM. Also taking into exam cardiovascular mortality, homocysteine levels > 30 micromolar were kinked to an increase in risk (Hazard ratio 1.91, CI 1.23-3.23) in patients without CISM, and not in those with this confounding factor. The conclusion is that hyperhomocysteinemia is a strong risk factor in patients without CISM, while in those with CISM, the association is masked by the combination of malnutrition-inflammation. In this case, in fact, homocysteine levels are lower, an expression of a reduced amino acid pool, and of hypoalbuminemia. Reverse epidemiology is so explained in patients with CISM, while in patients without CISM, the direct relation between homocysteine and mortality is confirmed.

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Causes of hyperhomocysteinemia

As for the cause, this could be theoretically linked to increased production, reduced removal, or reduced excretion. The latter can be ruled out because urinary homocysteine excretion is negligible (16). Considering the possibility of an increase in homocysteine production: homocysteine comes from the hydrolysis of AdoHcy, the demethylated product of AdoMet, the methyl donor in transmethylations. However, the accumulation of homocysteine leads to an increase of its precursor AdoHcy, due to the slowing down of the hydrolysis reaction, and this in turn leads to transmethylation inhibition, and in particular of certain methyltransferases. We have shown, and findings were then confirmed by Loeher et al. and van Guldener et al. (17-19), that this is the case in CRF and uremia. At the high concentrations of AdoHcy present in uremia, and considering the Km and Ki of the various methyltransferases, several methyltransferases are affected, such as DNA methyltransferase, or the protein L-isoaspartate methyltransferase, a repair enzyme of damaged L-isoaspartyl residues, while others are not, such as the quantitatively most important one, guanidinoacetate methyltransferase. This methyltransferase leads to the formation of creatine, and then creatinine forms spontaneously from creatine.

In 1980, Mitch and coworkers administered radiolabeled creatinine intravenously to subjects with severe CRF, and were able to show that the average rate of creatinine production was 150 micromoles/kg/day, similar to the production present in normal subjects (20). Guttormsen et al (21) showed that the average net influx of homocysteine into plasma was the same in controls and renal failure patients. Stam and coworkers (22) using stable isotope techniques have shown that transmethylations are reduced, as well as homocysteine clearance, in the whole body of CRF patients.

All in all, it can be said that in uremia some methyltranferases are inhibited by AdoHcy, while some are relatively affected, and continue to produce AdoHcy and homocysteine, which will lead to AdoHcy accumulation, thus producing a vicious cycle. So, at present current evidence is in favor of the idea that no increased production of homocysteine is present in uremia.

The most likely possibility, at this point of studies, is: decreased metabolic removal, either from the kidney, or from an extrarenal source. Studies from independent groups have shown that there is no difference in homocysteine concentration between the renal artery and the renal vein in humans (23, 24). However, this does not rule out the possibility that the kidney metabolizes homocysteine, because limitations of the measurement, expressing themselves in a relatively high coefficient of variation, could be an issue, that is small differences could still be important, and also we don't know what happens in the fed state. Glomerular filtration of homocysteine is restricted because of protein binding, however, homocysteine could still be removed through peritubular uptake. Peritubular uptake refers to the transport of aminoacids, peptides and proteins which are taken up from the arterial capillaries coming from the efferent arteriole, surrounding the proximal tubules, and go into tubular cells, where they are either secreted into the tubular lumen, or remain into cells and are metabolized. This concept is supported by findings by Garibotto et al (25). In this paper, they show that the fractional extraction of homocysteine is positively linked to renal plasma flow, with a net uptake occurring when renal plasma flow is above 500 ml/min. In addition, they show that homocysteine renal clearance is linked to renal plasma flow, that is, in these subjects, homocysteine clearance goes down to 0 when renal plasma flow is reduced. So at this point, we know that a reduction in homocysteine removal is found in CRF, but little more than that, and the matter is still controversial.

Metabolic consequences of hyperhomocysteinemia

Recently, some unforeseen consequences of hyperhomocysteinemia, coming from other laboratories, and ours, have been explored, which can ultimately affect mortality. We have proposed and explored the "unbalanced methylation" hypothesis in uremia. The accumulation of the homocysteine precursor AdoHcy, occurring when homocysteine levels are high, leads to an inhibition of those methyltransferases which are more sensitive to the inhibitor AdoHcy (high-sensitive methyltransferases, HS-Mtases). The low-sensitive methyltransferases (LS-Mtases) will continue to consume AdoMet and produce AdoHcy to an almost normal extent, thus further maintaining inhibition of the HS-MTases.

For example, we have shown some years ago that methylation-dependent membrane protein repair, a process in which a methylation reaction is involved, is inhibited in erythrocytes of uremic patients (17).

In addition, we have shown that total DNA methylation is reduced in dialysis patients and levels of decrease correlate significantly with plasma homocysteine levels (26).

DNA methylation is viewed as a mechanism for gene silencing and regulation, as, for example, in the case of "imprinted genes". Considering the way through which genes are passed from one generation to another, the allele coming from one of the parents is generally shut off through methylation. Under normal conditions, gene expression is therefore termed monoallelic for these genes (the gene coming from either the mother or the father is expressed, the other is silenced in a non-random manner). SYBL1 (a pseudoautosomal gene, X or Y inactivated) and H19 (an imprinted gene with maternal expression) are regulated in the way we just described. The allelic expression of these genes was used to test the "functional" outcome of DNA hypomethylation in uremic patients. Results show that, for SYBL1, gene expression in patients is biallelic, that is both alleles are expressed. For H19, only in patients with high homocysteine levels (approximately above 60 □M) gene expression is biallelic.

After folate therapy, gene expression returns monoallelic and total DNA methylation improves in parallel with a decrease of homocysteine levels, thus testifying that homocysteine modifies DNA methylation in a reversible fashion. So, we can state that, in patients with higher homocysteine, there is a transcriptional activation of the normally repressed allele, due to DNA hypomethylation. Folate treatment is able to revert the biallelic expression into monoallelic in the patients who had biallelic expression.

Coming to plasma proteins, plasma proteins in hemodialysis patients display a significant increase in the content of L-isoaspartyl residues, so they are significantly altered, or damaged (27).

This alteration under normal conditions can be repaired by a mechanism depending on a specific methyl transfer reaction.

This particular methyltransferase has been shown to be inhibited in uremia and, therefore, this kind of protein damage is increased. This inhibition depends partially on high homocysteine levels, and therefore methylation inhibition, because folate therapy is able to reduce damage levels.

However, the pathogenesis of this alteration, when considering the plasma protein compartment, depends mostly on uremic toxicity. Several uremic toxins, from different chemical groups, can induce protein damage. However, we found that guanidine in particular is able to elicit this protein damage in a dose-dependent manner. Deamidated albumin, that is *in vitro* damaged albumin, was prepared with a standard protocol, and the binding capacity of various substances to this damaged albumin was tested. A reduced binding of homocysteine to serum albumin was found. We can conclude that increased protein damage, due to the uremic milieu and hypomethylation, induces protein damage, with reduced homocysteine binding to proteins, and possible increase in free homocysteine levels.

Among the possible consequences of hyperhomocysteinemia, there is protein homocysteinylation, that is the binding of homocysteine to proteins, which occurs basically as a post-biosynthetic acylation of free amino groups (protein-N-homocysteinylation, mediated by homocysteine thiolactone). This protein modification in *in vitro* experiments leads to functional derangements, such as a loss of enzymatic activity. Another type of protein homocysteinylation is through the formation of a covalent disulfide bond found primarily with cysteine residues (protein-S-homocysteinylation). We have been able to demonstrate the presence of a significant increase of homocysteinylated proteins in uremia (28). We obtained, with a new method combining gel filtration, hydrolysis, and HPLC chromatography, data for both protein-S-Hcy and protein-N-Hcy, which were significantly increased in the plasma of uremic patients on hemodialysis. This type of protein alteration occurs in uremia, because of the high homocysteine levels present in this condition, thus representing another example of a widespread presence of a derangement of the peptide link in uremia. Protein homocysteinylation could be one of the principal mediators of homocysteine toxicity, contributing to determine structural and functional alterations at the molecular and cellular level.

Therefore, it can be stated that in chronic renal failure and end stage renal disease, both altered gene expression and the alterations in protein structure, dependent on hyperhomocysteinemia and acting through an increase of a homocysteine-related metabolite, may play a crucial role in terms of macromolecule functional derangement.

Therapy

In view of the epidemiological data and the high frequency of cardiovascular disease in chronic renal failure patients, numerous attempts have been made to lower plasma total homocysteine concentrations in these patients. In the general population, it is possible to reduce homocysteine levels by means of dietary intervention or with small folate supplementation. In chronic renal failure patients, possible tools conducive to a reduction of homocysteine levels are folate therapy, therapy with betaine, serine, N-acetylcysteine, or B vitamins (vitamin B_6, B_{12}, B_2), and improved dialysis. Betaine, serine, and B vitamins are either not effective, or can add only a modest additive effect. The mainstay of therapy is represented by folic acid, or folic acid in its active, circulating form, MTHF (29-30 for review).

Folic acid therapy in chronic renal failure patients have been shown to reduce, albeit not to normalize, plasma total homocysteine concentrations, particularly in dialysis patients, who express therefore a resistance to folates. Folic acid supplementation of 1 mg daily, in contrast to what is usually observed in the general population, does not have any effect on plasma total homocysteine concentration in chronic failure failure patients, despite the induction of supernormal plasma folate levels. Oral supplementation with high doses of folic acid (up to 15 mg daily), which leads to a 20 to 50-fold increase of plasma folate concentrations, is only partially effective in reducing plasma total homocysteine. This relative resistance to folate action does not appear to be caused by defects in folate absorption or impairment in folic acid conversion in the plasma to the active metabolite MTHF. Moreover, active reduced forms of folic acid did not lead to a greater decrease in plasma total homocysteine levels than those observed with native folic acid supplementation in hemodialysis patients. However, MTHF provides a moiety which does not need to be further metabolized. This is important because a polymorphism, the C677T transition of MTHF reductase, is very common in the population (20 % in the homozygous, 30-40 % in the heterozygous). Providing the active form circumvents the possibility that the specific patient genetic pattern could affect folate utilization.

Other abnormalities in homocysteine metabolism, as for instance a relative resistance to vitamin B12 action, have been observed in chronic renal failure patients. Theses abnormalities may also participate to the genesis of hyperhomocysteinemia in these patients. However, as mentioned previously, the correction of these abnormalities in folate-replete patients has only a partial additional effect on fasting total homocysteine in chronic renal failure patients.

For the sake of completeness, it has to be mentioned that more efficient dialysis procedures could allow an improved removal of uremic toxins and/or homocysteine. The main reason for the genesis of hyperhomocysteinemia, and the reduced efficacy of folate therapy in dialysis chronic renal failure patients, as mentioned previously, is unclear at present. The accumulation of uremic toxins and the decrease in homocysteine clearance and metabolism owing to a decreased functioning renal mass are the two most probable explanations. Standard dialysis procedures using low-flux dialysers or high-flux dialysis are unable to remove sufficient amounts of homocysteine to maintain total homocysteine within the normal range. In contrast, dialysis in super-flux mode significantly lowered total homocysteine concentrations, possibly due to a greater reduction in uremic toxin concentration. This may also be partially due to albumin removal, since the major part of circulating homocysteine is protein-bound. Recently, it has been also demonstrated that total homocysteine levels were significantly lower among patients undergoing daily nocturnal HD.

A displacement of homocysteine from protein-binding sites, allowing increased free homocysteine availability for plasma clearance by dialysis procedures could be an interesting alternative strategy to reduce total homocysteine concentrations. It has been reported by Scholze et al (31) that the acute intravenous administration of N-acetylcysteine (5 g in 5% glucose for 4 hours) during a hemodialysis session, which

presumably can displace homocysteine from protein-binding sites, was able to completely normalize total homocysteine concentrations at the end of the session, with residual efficacy for the next two days. The acute decrease of total homocysteine concentrations induced by N-acetylcysteine supplementation during the dialysis session has been also shown to improve pulse pressure and endothelial function in hemodialysis patients. In a previous study, the same group has also shown that acetylcysteine reduces cardiovascular events, when given 600 mg per os, for two years (32). Friedman et al (33) have shown that long-term oral N-acetylcysteine administration (1.2 g twice a day) total homocysteine levels were reduced by 19 % in hemodialysis patients, compared with an 8 % reduction in patients treated with placebo (p = 0.07). Patients were vitamin-replete. Possibly, this study was underpowered to detect a statistically significant difference, and also acetylcysteine was administered orally.

Although these results are promising, the efficacy and safety of intravenous administration of N-acetylcysteine needs, however, to be evaluated before drawing a definite conclusion. In any case, these data suggest that maneuvers aimed to displace homocysteine from protein-binding sites may represent a valuable strategy to normalize total homocysteine in dialysis patients.

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