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CAN ELEVATED PLASMA HOMOCYSTEINE LEVELS RESULT IN THE INHIBITION OF INTRACELLULAR METHYLTRANSFERASES?

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We have been interested in the biochemical mechanisms by which elevated plasma total homocysteine concentrations are associated with premature cardiovascular disease and neurological impairment in humans (1). In this paper, we would like to consider the possibility that a part of the pathology may result from the inhibition of one or more members of a class of *S*-adenosylmethionine (AdoMet)-dependent methyltransferases. These intracellular enzymes are often subject to potent inhibition by their *S*-adenosylhomocysteine (AdoHcy) product (2). This product is normally metabolized by AdoHcy hydrolase, which gives rise to adenosine and homocysteine. However, the equilibrium of this reaction strongly favors AdoHcy formation (3), thus linking increases in intracellular homocysteine to increases in AdoHcy and resulting in the inhibition of methyltransferases.

MAMMALIAN METHYLTRANSFERASES CATALYZE NUMEROUS CRUCIAL CELLULAR REACTIONS

In Table 1, we list the functions of mammalian AdoMet-dependent methyltransferases for which kinetic data are available. The list encompasses two DNA methyltransferases, ten RNA methyltransferases, three lipid methyltransferases, eleven protein methyltransferases, and thirteen small molecule methyltransferases (4). These enzymes catalyze reactions involved in the biosynthesis and degradation of small molecules, in the posttranslational modification of proteins, and in the posttranscriptional modifications of DNA, mRNA, RNA, and tRNA. Many of these reactions are involved in metabolic control. The total number of mammalian methyltransferases is unknown, but may be 100 or more based on the pace of discovery of new types of enzymes (4).

Table 1 - Functional roles of methyltransferases in mammalian cells (4)

1) Small molecule biosynthesis: Phosphatidyl choline, coenzyme Q, creatine phosphate, melatonin, epinephrine
2) Inactivation and elimination of small molecules/xenobiotics: neurotransmitters, pyridine compounds, sulfur compounds, arsenite, neurotoxins
3) Modulating RNA function in mRNAs, tRNAs, and ribosomes
4) Stabilization of DNA and proteins: Genomic stability, protein repair, protein "capping"
5) Cellular signaling: PP2A modulation, protein arginine methylation
6) Metabolic control: glycine methylation

ELEVATED PLASMA TOTAL HOMOCYSTEINE LEVELS ARE CORRELATED WITH ELEVATED INTRACELLULAR ADOHCY LEVELS AND METHYLTRANSFERASE INHIBITION

The possible connection between elevated plasma homocysteine levels and methyltransferase inhibition was pointed out in the early studies on homocystinuria (5). However, only recently has experimental evidence been produced that demonstrate that mild increases in plasma total homocysteine can be correlated with increases in intracellular AdoHcy, and that these raised levels of AdoHcy are sufficient to significantly inhibit known methyltransferases.

Analysis of patients with chronic renal failure, where plasma total homocysteine is mildly elevated and may be associated with the premature occlusive arterial disease, has revealed a clear connection between plasma homocysteine accumulation, AdoHcy accumulation, and the inhibition of one specific methyltransferase (6, 7). It has been shown in this uremia model that erythrocyte AdoHcy levels are four- to eight-fold higher in patients with little change in AdoMet levels (Ref. 6, Table 2). This change is accompanied by an up to 50% inhibition in the activity of the erythrocyte protein repair L-isoaspartate (D-aspartate) O-methyltransferase (6).

In a recent human study, mild increases in plasma total homocysteine (range of 5 - 16 μM) have been correlated with a five-fold increase in lymphocyte AdoHcy levels and an increase in DNA hypomethylation, reflecting reduced activity of DNA methyltransferases (Ref. 8, Table 2).

INHIBITION OF SPECIFIC METHYLTRANSFERASES BY ELEVATED ADOHCY LEVELS

We are now interested in asking how the increased levels of AdoHcy found in the uremic and mild homocysteinemia models described above may affect other types of methyltransferases. The activity of methyltransferases can be given as:

Fraction of maximal velocity =
[AdoMet]/(Km+Km[AdoHcy]/Ki+[AdoMet])

Here, Ki is the inhibition constant for AdoHcy and Km is the Michaelis constant for AdoMet of each enzyme. One important factor in methyltransferase activity is the ratio Km/Ki; when this ratio is high, increased AdoHcy will have a proportionally larger effect on the reaction rate.

***Table 2** - Uremia and mild homocysteinemia models for homocysteine-induced methyltransferase inhibition*

	Intracellular [AdoMet]	Intracellular [AdoHcy]
"Uremic" Erythrocyte Model (Ref. 6)		
11.8 µM plasma Hcy (control)	2.7 µM	0.8 µM
40.6 µM plasma Hcy (uremic)	2.7 µM	6.7 µM
"Mild homocysteinemia" Lymphocyte Model (Ref. 8)		
6 µM plasma Hcy	5.7 µM	0.6 µM
16 µM plasma Hcy	5.7 µM	2.6 µM

We compiled literature values for the kinetic constants of mammalian methyltransferases (4) and then calculated their expected activity in either the uremia or the mild homocysteinemia model (Table 3). These results show a range of kinetic values such that some methyltransferases will be much more susceptible to the inhibitory effect of AdoHcy than others. In fact, for many of these enzymes, little or no inhibition would be expected under either the uremic or the mild homocysteinemia model. However, several methyltransferases appear to be particularly sensitive to AdoHcy inhibition. At the elevated homocysteine levels seen in uremic patients, only the guanine-*N*-1 tRNA methyltransferase and the protein repair L-isoaspartate (D-aspartate) methyltransferase are inhibited more than 70%, with 15% and 14% residual activity, respectively (Table 3). (In the mild homocysteinemia model, these enzymes would be expected to each have 31% activity (Table 3)).

We can now ask if the inhibition of either the guanine-*N*-1 tRNA methyltransferase or the protein repair L-isoaspartate (D-aspartate) methyltransferase might be connected with the pathologies seen in humans

with elevated plasma homocysteine. The function of the guanine-*N*-1 methyltransferase is to prevent translational frameshifting by

Table 3 - Potential inhibition of mammalian methyltransferases (MTs) when plasma total homocysteine levels are raised in either an uremic model or a mild homocysteinemia model (Table 2). Kinetic values are taken from Ref. 4.

	Km AdoMet µM	Ki AdoHcy µM	Km/Ki	Calculated MT Activity Uremics vs. controls	Mild homocysteinemia vs. controls
Small molecule methyltransferases					
Phosphatidyl ethanolamine MT	18.2	3.8	4.8	47%	74%
Glycine MT	100	35	2.9	86%	95%
Guanidinoacetate MT	49	16	3.0	75%	90%
Histamine MT	1.7	11.8	0.1	84%	96%
Phenylethanolamine MT	10	1.4	7.1	30%	58%
Beta-carboline-2 MT	81	14.8	5.5	73%	89%
Indolethylamine MT	54.3	8.65	6.3	63%	84%
Hydroxyindole MT	14	2.1	6.7	36%	64%
Catechol MT	3.1	1	3.1	31%	63%
Thiopurine MT	3	5.8	0.5	67%	90%
Thioether MT	1	40	0.03	96%	99%
Nucleic acid methyltransferases					
DNA 5-cytosine MT	1.4	1.4	1.0	45%	79%
tRNA 5-cytosine MT	0.5	0.9	0.6	53%	85%
tRNA N-1-guanine MT	3	0.11	27	15%	31%
tRNA N-2-guanine MT	2	23	0.1	90%	98%
tRNA N-1-adenine MT	0.3	0.85	0.4	61%	90%
rRNA O'-2-ribose MT	0.24	0.17	1.4	33%	71%
Protein methyltransferases					
L-Isoaspartyl MT	2	0.08	25	14%	31%
Isoprenylcysteine MT	2.1	9.2	0.2	79%	95%
Calmodulin lysine MT	2	15.2	0.1	86%	97%
Histone lysine MT	12.5	5.9	2.1	57%	82%
MBP arginine MT	4.4	1.8	2.4	39%	70%
PRMT1 arginine MT	8	2.3	3.5	40%	69%

modifying a base adjacent to the anticodon of several tRNAs (9). Defects in this enzyme have been linked to mitochondrial myopathies (10). Such myopathy may be linked to the cardiovascular effects seen in humans with elevated homocysteine levels in plasma. The protein repair L-isoaspartate (D-aspartate) methyltransferase, which has already been shown to be inhibited in uremic patients (6), has a fundamental role in cells of limiting spontaneous aging damage to proteins; mice lacking it accumulate altered proteins and die of seizures at young ages (11). This enzyme is also trapped in the extracellular space when blood vessels are injured and may play a role in the repair of damaged collagen molecules (12). It is thus possible that the accumulation of damaged proteins in humans with high

plasma homocysteine levels could be correlated with both the cardiovascular and neurological pathologies seen.

CONCLUSIONS

It is now clear that mild hyperhomocysteinemia can be linked to the inhibition of intracellular methyltransferases. Based on their relative affinity for AdoMet and AdoHcy, some methyltransferases may be significantly affected; others may not be. Further work is clearly warranted both in studying the function and kinetics of individual methyltransferases as well as in identifying new methyltransferases.

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