A denosine—the missing link to understanding homocysteine pathogenicity or more smoke on the horizon?

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See article by Riksen et al. [8] (pages 271–276) in this issue.

Based on epidemiological studies, homocysteine (Hcy) is regarded as an independent risk indicator for occlusive vascular diseases [1,2]. Under experimental conditions, increased Hcy concentrations have been found to result in endothelial dysfunction, leukocyte adhesion, and smooth muscle and collagen proliferation [3–6]. However, the underlying molecular and metabolic links have not been conclusively elucidated [7]. Thus, it still remains to be determined whether Hcy indeed represents a risk factor or rather a risk indicator.

In this issue of the Journal, Riksen et al. [8] present a new hypothesis on the relationship of Hcy and some of its presumed effects. This hypothesis is centered on the metabolism of Hcy with the purine adenosine (Ado) (Fig. 1). Both metabolites originate in the cytosol from the reversible hydrolysis of S-adenosylhomocysteine (AdoHcy) by the action of AdoHcy hydrolase:

$$\text{AdoHcy} + \text{H}_2\text{O} \rightleftharpoons \text{Hcy} + \text{Ado} \quad (1)$$

Under normal physiological conditions, the net flux through this pathway is toward Hcy and Ado production because both compounds are rapidly metabolized, thus, their concentrations are kept low. In addition, AdoHcy is continuously produced from S-adenosylmethionine (AdoMet) via the transmethylation pathway:

$$\text{AdoMet} + \text{R} \rightarrow \text{AdoHcy} + \text{R-CH}_3 \quad (2)$$

a reaction that is nonreversible under physiological conditions. However, the AdoHcy hydrolase-catalysed reaction depends on the concentration of each of the reactants AdoHcy, Hcy, and Ado (reaction (1)). Under conditions when the Hcy concentration increases, the reaction equilibrium will shift toward AdoHcy production and simultaneously tend to lower the free Ado concentration. Riksen and colleagues argue that because Ado has several well known, protective actions in the cardiovascular system mediated by purine surface receptors [9,10], the increased removal of Ado, due to increased Hcy concentrations, would significantly lower the Ado concentration at these receptors and by diminishing the protective actions potentially permit deleterious effects on the cardiovascular system.

The hypothesis by Riksen et al. [8] is not the first proposal that attempts to provide a rationale for understanding the unfortunate effects that are often found to be associated with enhanced Hcy plasma levels. Previous hypotheses that have been considered include (for a metabolic scheme see Fig. 1):

1. **Hcy thiolactone**, which is formed from Hcy by action of methionyl-tRNA synthetase, may lead to homocysteinylation of a variety of proteins, including albumin, fibrinogen, or transferrin [11]. The reaction which takes place at lysine residues of proteins may significantly decrease enzyme activities, as shown for methionyl-tRNA synthetase, trypsin, and lysine oxidase [11]. Hcy thiolactone may be converted to Hcy by the action of thiolactonase/paraoxonase, which is associated with the high-density lipoprotein (HDL) fraction of serum lipoproteins. Recently, this enzyme activity was suggested to contribute to the protective role of the HDL fraction against oxidative damage and Hcy toxicity [12].

2. **Free Hcy** has a thiol group that may interact with the thiol groups of proteins, forming disulfide bonds. Aside from albumin, Hcy binding also has been shown with fibronectin where Hcy seems to bind mainly within or near the fibrin-binding domain, significantly reducing the fibrin binding capacity of the protein [13]. This suggests that Hcy may interfere with normal thrombosis.
or with wound healing. Other actions of free Hcy include such heterogeneous effects as the inhibition of endothelin-1 production by endothelial cells [14] or the inhibition of retinoic acid synthesis [15].

3. Oxygen radicals may be formed during autoxidation of Hcy to homocystine. This has greatly fostered the view of Hcy as a pro-oxidant. Although this view is not uncontested [16], several experimental studies suggest that oxygen radicals formed during Hcy exposure may enhance endothelial dysfunction and T-lymphocyte proliferation and reduce nitric oxide availability [17,18]. Oxygen radicals may be formed during autoxidation of Hcy to homocystine. This has greatly fostered the view of Hcy as a pro-oxidant. Although this view is not uncontested [16], several experimental studies suggest that oxygen radicals formed during Hcy exposure may enhance endothelial dysfunction and T-lymphocyte proliferation and reduce nitric oxide availability [17,18].

4. Sulfane sulfur compounds contain a labile, highly reactive sulfur atom at a reduced oxidation state covalently bound to another sulfur atom. This unstable and reactive compound is formed through enzymatic metabolism of Hcy, homocystine, or its mixed disulfide with cysteine. Sulfane sulfur has effects that are consistent with a role in atherogenesis [19].

5. Nitric oxide (NO) bioavailability may be significantly limited when Hcy concentrations are enhanced. Aside from favoring increased levels of oxygen radicals, which may interfere with NO production (see above), an enhanced concentration of asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, has been shown in the presence of elevated Hcy [5]. This effect might aggravate the effects of oxLDL, which increases endothelial cell ADMA production.

6. Transmethylation reactions may be decreased following an increase of the Hcy concentration. This notion is based on the fact that increased concentrations of Hcy lead to tissue accumulation of AdoHcy [6,20] and that AdoHcy is a potent feedback inhibitor of transmethylation. A reduced methylation rate could result in various unfortunate effects (e.g., impeded DNA and protein methylation). This concept suggests that the increased concentration of ADMA in the presence of elevated Hcy [5] is due to a change in ADMA metabolism (e.g., affected by oxygen radical production) rather than by enhanced precursor methylation.

The hypothesis put forward by Riksen et al. [8] brings additional complexity into the scheme. It inevitably leads to the question of how to deal with the various hypotheses on Hcy-associated pathomechanisms.

The uncertainty surrounding whether and, if so, to which extent Hcy is involved in the pathogenesis of vessel diseases is largely complicated by the following two facts: (1) Hcy exists in various fractions that may have different biological effectiveness, and (2) Hcy may occur in a wide concentration range under pathophysiological conditions.

Re: (1) The largest fraction of Hcy in human plasma is bound to plasma proteins through disulfide bonds with protein cysteines (∼80%). In the protein-unbound fraction, Hcy exists either as free reduced Hcy (physiologically minor subfraction, 1–2%) or as homocystine or mixed disulfides. The respective fractions may differ under conditions of elevated total Hcy concentrations. The sum of the above fractions is referred to as total plasma homocysteine (plasma tHcy) and does not include Hcy thiolactone [21].

Re: (2) Depending on the (patho)physiological condition, the Hcy plasma concentration may vary over a wide range. While the physiological concentration of total Hcy is around 10 μmol/1 [21], mild Hcy elevations (>15 μmol/1) occur in about 1/3 of patients with arterial vessel disease [7,22]. Also, a declining renal function may result in mild to moderate elevations of plasma Hcy. Untreated vitamin deficiencies, e.g., B-12 or folate deficiency, may give rise to moderate (>30 μmol/l) or even severe (>100 μmol/l) increases of plasma Hcy. Finally, inborn errors of Hcy metabolism can result in plasma concentrations of up to 400 μmol/1 [7,21].
With respect to these difficulties, the hypothesis proposed by Riksen et al. [8] is sound and worthy of critical testing in the future. For a critical assessment it seems necessary to test that (1) the production and metabolism of Ado via AdoHcy can be demonstrated for those cells or tissues in which Hcy is known to induce biological actions, (2) inhibitors of Hcy and Ado metabolism and/or transport should evoke effects in accordance with the hypothesis, and (3) quantitative assessments should prove that the concentrations and the kinetics of Ado, Hcy, and AdoHcy are sufficient to explain the observed effects.

References