3. ON THE FORMATION AND FATE OF TOTAL PLASMA HOMOCYSTEINE

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Introduction

The total concentration of homocysteine (tHcy)* in plasma is a useful marker of impaired function of cobalamin and folate. Moreover, it is an independent risk factor for atherosclerotic disease [1]. These findings have encouraged the search for determinants of plasma tHcy level [1,2].

Most tHcy in plasma probably derives from cellular Hcy. Conceivably, a high plasma tHcy level results from the imbalance between the rate of Hcy entry into and its removal from plasma. The present chapter describes the theory which is relevant for the flux of Hcy through the plasma compartment, and reviews the experimental and clinical data which yield information on site of formation and elimination of plasma Hcy.

Kinetics of Plasma tHcy

The plasma tHcy level is relatively stable during the day. In healthy young subjects, the fluctuation in the level during a 24-hour period corresponded to mean tHcy ±10% [3]. This indicates that the supply of Hcy to and its elimination from plasma do not change substantially during the day. The level of plasma tHcy can therefore be regarded as a steady-state concentration, C_{ss}. The C_{ss} in plasma can be expressed as

$$C_{ss} = R_0/Cl \quad (3\text{-}1)$$

where R_0 denotes the delivery of Hcy to plasma per unit time, and Cl refers to total plasma clearance. The clearance can be calculated in various ways [4]:

$$Cl = Dose/AUC_{i.v.} \quad (3\text{-}2)$$

$$Cl = k \cdot V_d = 1n2 \cdot V_d / T_{1/2} \quad (3\text{-}3)$$

* The abbreviation Hcy indicates that oxidation status of the thiol group of homocysteine is not specified, and includes both the thiol and disulfide forms of homocysteine. The abbreviation tHcy refers to the sum of protein-bound and nonprotein-bound Hcy.

By combining (3-1) and (3-3), we get:

$$C_{ss} = R_0/k \cdot V_d \quad (3\text{-}4)$$

AUC, or area under the plasma concentration curve, is a measure of the systemic exposure [4]. The rate constant for elimination, k, denotes the fraction of concentration removed per unit time. Thus, k = 0.20/h indicates that in 1 hour the concentration will be reduced with 20%, corresponding to an elimination half-life of 3.5 hours. Both $T_{1/2}$ and k depend on clearance and the volume of distribution, V_d [4].

Based on the above equations, it is clear that the basal plasma tHcy (i.e., C_{ss}) depends on the amount of Hcy excreted from cells, the volume in which tHcy is diluted (V_d) and the ability of the system to remove Hcy (Cl).

Notably, the clearance of a compound can be carried out by different organs, and this is usually expressed as:

$$Cl_{total} = Cl_{hepatic} + Cl_{renal} + Cl_{other} \quad (3\text{-}5)$$

By combining (3-3) and (3-4), the rate constant for elimination can be expressed as:

$$k_{total} = k_{hepatic} + k_{renal} + k_{other} \quad (3\text{-}6)$$

These two last equations indicate that if we know how much is eliminated at one site, we can estimate the elimination by other organs.

The Source of Plasma Hcy

The data on the source of Hcy in plasma are sparse, and almost solely based on in vitro studies on blood cells [5–7] and cells in culture [7–11].

BLOOD CELLS

After collection of blood, there is a continuous time- and temperature-dependent release of Hcy from

blood cells [1], and at room temperature, plasma tHcy usually increases about 5%–15% per hour [1]. Notably, the cellular Hcy is low, suggesting ongoing Hcy production [5]. Based on studies on Hcy export from blood cells incubated at 37°C, it has been suggested that blood cells may be an important source of Hcy in plasma in vivo [5,6].

In a study on stability of plasma tHcy in the presence of blood cells (room temperature), we observed that the increase is independent of plasma tHcy level [12]. Thus, 4 and 24 hours after collection of the blood, tHcy had increased approximately 2 μmol/L and 7 μmol/L, respectively (fig. 3-1), in plasma samples with tHcy level varying fivefold. These data may suggest that the plasma tHcy level in vivo is not related to Hcy export from the blood cells. Moreover, blood cells in vitro are unable to increase the Hcy export in response to supraphysiologic methionine concentrations [5,6], probably due to the low K_m methionine adenosyltransferase (<5 μmol/L) in these cells [13]. This lack of response to methionine of the blood cells contrasts to the marked elevation of plasma tHcy observed in subjects receiving peroral methionine load [14].

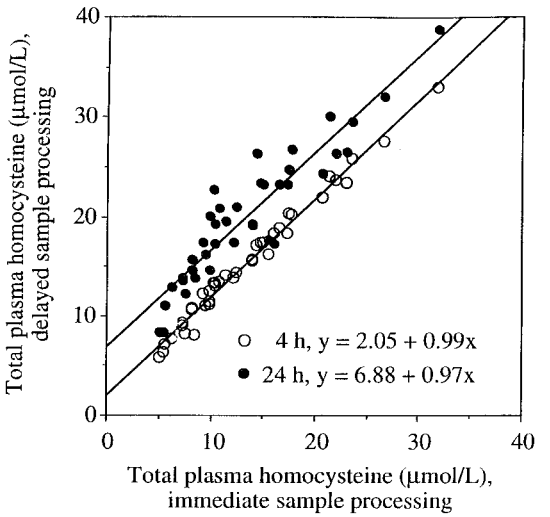

FIGURE 3-1. Release of homocysteine from blood cells. Forty blood samples were collected and either immediately centrifuged to separate the blood cells from plasma (X-axis) or left at room temperature for 4 h or 24 h before centrifugation took place (Y-axis). It can be seen that the increase in total plasma homocysteine in the presence of the blood cells is independent of the initial plasma level. Data modified from [12].

CELL CULTURE EXPERIMENTS

In our laboratory, we have investigated a number of cell lines in culture, and they all export Hcy [7,10,11,15]. Notably, the export rate is substantially higher in proliferating than stationary cells [7]. Moreover, when the cells are exposed to high levels of methionine [7], low levels of folates [8], nitrous oxide (inactivating methionine synthase) [8–10,16], or the antifolate methotrexate [10,11,15], the export rate of Hcy may increase two- to threefold (fig. 3-2), left panel), but usually not exceeding 1 nmol/10^6 cells/hour.

Most of the reported experiments have been with malignant or transformed cells, but we have also investigated Hcy export from freshly isolated lymphocytes and hepatocytes [7]. In lymphocytes, the export rate is only 0.02–0.03 nmol/10^6 cells/hour [7], which is about 100 times higher than the export rate from (red) blood cells (0.3 pmol/10^6 cells/hour) incubated at 37°C [5]. In PHA-stimulated (proliferating) lymphocytes, the export rate approaches that observed in the transformed and malignant cell lines [7]. In comparison, freshly isolated hepatocytes export 1–2 nmol/10^6 cells/hour. Moreover, the Hcy export rate increases almost 15-fold when the methionine concentration in the medium rises from 15 to 1,000 μmol/L [7]. Thus, both in low and high methionine medium, the nonproliferating hepatocytes export 5–25 times more than proliferating cells. Compared to stationary lymphocytes [7] and red cells [5], the export rate from the liver cells is of an order of magnitude three to four times higher.

In humans, the liver is probably the major site of Hcy production, since a high K_m methionine adenosyltransferase affords the liver a unique capacity to drain methionine into the transmethylation pathway [13]. However, enzymatic capacity of the liver to metabolize Hcy by cystathionine β-synthase, methionine synthase, or betaine-Hcy methyltransferase [17] is substantial. Thus, it remains to be shown whether Hcy formed in the liver is exported and functions as the main source of Hcy in plasma.

HYPERPROLIFERATIVE DISORDERS

In vivo data on the source of plasma Hcy are sparse. In children with acute lymphoblastic leukemia, plasma tHcy is related to the number of white cells (mostly leukemic cells), and treatment with cytotoxic drugs leads to a simultaneous decline in plasma tHcy and the white cells [18,19]. Moreover, patients with psoriasis who have a high burden of proliferating cells also have higher plasma tHcy than healthy subjects and other patients with dermatologic conditions [20]. These data suggest that a high burden of

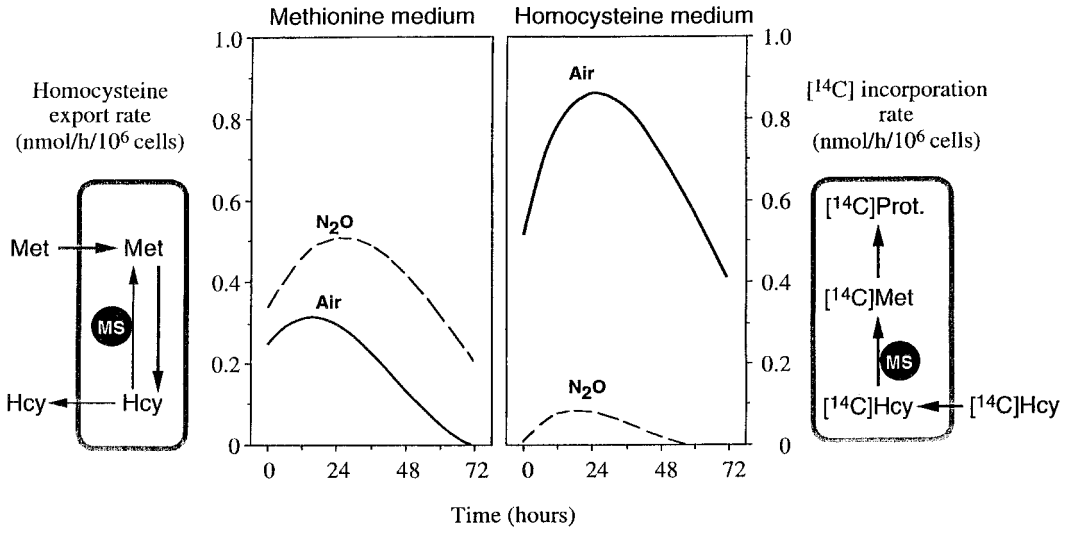

FIGURE 3-2. Methionine synthase and disposition of homocysteine in a glioma cell line. This proliferating glioma cell line has a net export of homocysteine when cultured in a medium containing 50 μmol/L of methionine (left panel). The export rate declines as the cells approaches confluence. By inhibiting the methionine synthase (MS) with N_2O, the homocysteine export rate increases markedly, indicating that substantial remethylation takes place. In a medium without methionine but containing [^{14}C]homocysteine thiolactone (200 μmol/L) (right panel), homocysteine is taken up, remethylated, and then incorporated into protein. Exposure to N_2O reduces incorporation of radioactivity into protein with 90% [23].

rapidly proliferating cells may lead to elevated plasma tHcy.

The Fate of Hcy in Plasma

The elimination of a compound from plasma is usually by metabolism or urinary excretion. For plasma tHcy, urinary excretion probably represents a minor elimination pathway, since only 6 μmol/day [21] or less than 0.05% of total cellular Hcy production [22] is excreted unchanged in the urine. Thus, plasma Hcy must be taken up by cells and then metabolized.

In laboratory animals, Hcy can function as the sole source of sulfur amino acids as long as vitamin and cofactor supply is adequate [24], and most benign and some transformed cell lines in culture can utilize Hcy for growth [25]. This demonstrates that extracellular Hcy can be taken up and metabolized by cells. Notably, the ability of the cells to utilize extracellular Hcy depends on intact function of the Hcy metabolizing enzymes, as shown in fig. 3-2 for a glioma cell line cultivated in the absence and presence of nitrous oxide in a medium supplied with Hcy thiolactone instead of methionine.

THE HOMOCYSTEINE LOADING TEST

We have recently studied the kinetics of plasma tHcy by administering Hcy (65 μmol/kg) both to healthy subjects [26] and patients with hyperhomocysteinemia [27,28]. In contrast to the methionine loading test [29], which primarily reflects Hcy formation and release from cells (probably the liver), the Hcy loading test yields information about the elimination of Hcy from plasma.

In healthy volunteers [26], we found that tHcy in plasma declined at a rate of $k = 0.2$/hour, corresponding to an elimination half-life of 3.7 hours. In a subject receiving both a peroral and an intravenous administration, we found a bioavailability (AUC_{po}/AUC_{iv}) of 0.53, suggesting a substantial presystemic metabolism of Hcy administered perorally. The total plasma clearance was 0.08 L/min. A fourfold increase in Hcy dose did not change elimination half-life, and AUC showed a linear increase with dose, suggesting first-order kinetics in this dose range [26].

We have also investigated the elimination of tHcy from plasma after a peroral Hcy load in subjects with folate and vitamin B_{12} deficiency before and after

therapy with vitamins [27], in subjects with renal failure [28], and in heterozygous and homozygous homocystinurics (AB Guttormsen et al., unpublished results). Moreover, in cancer patients treated with methotrexate, we have administered ^{14}C-labeled Hcy to study the kinetics of tHcy (AB Guttormsen et al., unpublished results). The results of these studies can be summarized as follows: In subjects receiving a radioactive tracer that does not influence plasma tHcy level, the elimination half-life is in the same range as in healthy subjects receiving a peroral Hcy load resulting in markedly elevated plasma tHcy (AB Guttormsen et al., unpublished results).

In subjects with vitamin B_{12} and folate deficiency who had markedly elevated plasma tHcy, the mean elimination half-life was not significantly different from that in healthy subjects. Furthermore, vitamin therapy that reduced plasma tHcy had only marginal influence on elimination half-life. In fact, several subjects with marked hyperhomocysteinemia (>50 μmol/L) had half-lives which were shorter than observed in healthy subjects [27]. Thus, in vitamin B_{12} and folate deficiency, the relative normal $T_{1/2}$ indicates that clearance is not changed (3-3). This strongly suggests that the high plasma tHcy in vitamin B_{12} and folate deficiency is not due to delayed elimination, but probably is related to increased export of Hcy from cells.

The studies with homozygous and heterozygous homocystinurics ($n = 17$) are not completed, but the preliminary data indicate that most of these subjects have normal elimination half-life. However, the heterozygous and most homozygous homocystinurics had relatively normal plasma tHcy (≤15 μmol/L). In one homozygote with elevated tHcy level (33 μmol/L), the decline in tHcy in plasma initially seemed normal, corresponding to an elimination half-life of less than 3 hours. However, 4–6 hours after the Hcy administration, plasma tHcy started to increase once more. Whether this was due to food intake or a response to the administered Hcy is not known (AB Guttormsen et al., unpublished results).

So far, the only condition that we have identified to influence the elimination of tHcy in plasma is renal function. The mean elimination half-life in subjects with chronic renal failure is about 13 hours, corresponding to an elimination rate constant of 0.05/hour, as compared to 0.20/hour in healthy subjects [28].

While the metabolic defects in vitamin deficiency [1] and homocystinuria [30] are well recognized, the role of the kidney in Hcy metabolism is not understood. Notably, evidence that the kidney is an important metabolic site for removal of plasma Hcy has recently been presented by Bostom et al. [31]. They showed that in the renal circulation of rats, there is a substantial arteriovenous difference in the plasma tHcy level, corresponding to a metabolism of about 1 mmol/day in the human kidney. In contrast to these data, Hultberg et al. propose that in renal failure, accumulation of toxic waste leads to disturbed Hcy metabolism (see Chapter 19). Our data on subjects with renal failure do not distinguish between these possibilities, and further investigations are necessary.

Some Kinetic Considerations

Since our experiments with increasing Hcy doses suggest that tHcy clearance is independent of plasma concentration [26], we assume that the clearance is the same both during fasting and after a peroral Hcy load. Studies with subjects receiving a radioactive Hcy that does not influence plasma tHcy level support this assumption (AB Guttormsen et al., unpublished paper). We can then use equations (3-1) through (3-6) to present some tentative kinetic aspects of plasma tHcy.

HCY RELEASED TO PLASMA

In a healthy subject with a fasting tHcy level of 10.8 μmol/L, a total clearance of 0.08 L/min was found after administration of an intravenous dose of Hcy [26]. The estimated delivery rate of Hcy to plasma in this subject according to (3-1) is 1.2 mmol/24 hours, or about 3–10% of total cellular Hcy production [22,23]. Less than 10 μmol/day is excreted unchanged in the urine [21], leaving more than 99% of tHcy in plasma to metabolized.

In methionine loading, the sulfur amino acid dose administered is tenfold higher than in Hcy loading. Still, the AUC for plasma tHcy after methionine loading is lower than after an intravenous Hcy loading [20,26] (fig. 3-3). These data may suggest that less than 10% of the administered methionine is released to plasma as Hcy.

In a cobalamin-deficient subject who had a plasma tHcy level of 140 μmol/L, we found normal elimination half-life, suggesting that his high level is due to increased rate of Hcy influx into plasma [27]. Using a clearance of 0.08 L/min [26] and (3-1), the cellular release of Hcy was estimated to be about 16 mmol/24 h, or a major portion of cellular Hcy production [22].

THE KIDNEY

Our data point to the kidney as an important organ in plasma tHcy homeostasis. In renal failure, the rate constant for plasma tHcy elimination is reduced from

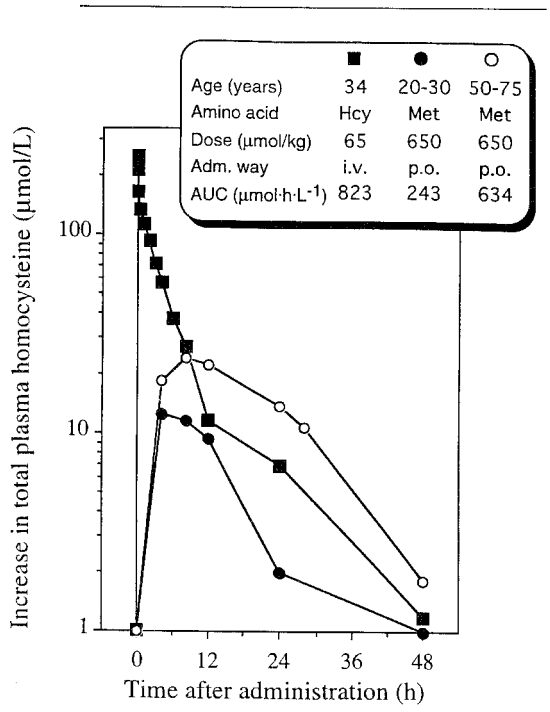

FIGURE 3-3. Increase in total plasma homocysteine after administration of homocysteine or methionine. The increase in total plasma homocysteine in a 34-year-old woman who received homocysteine, and in young (n = 5) and elderly (n = 6) women undergoing a standard methionine loading test, is shown. Intravenous administration of homocysteine ensured 100% systemic availability. The AUC for the increase in total plasma homocysteine was lower in subjects receiving methionine than in the woman receiving homocysteine load. Since the methionine dose is 10 times higher than the homocysteine dose, this suggests that <10% of the administered methionine is released to plasma as homocysteine. Modified data from [14,20,26].

0.20/hour to 0.05/hour, i.e., by 70% [28]. If the reduction in the rate constant for elimination is due to reduction in renal metabolism, this suggests that the normal kidney metabolize about 70% of tHcy in plasma (3-5 and 3-6). This is in the same range estimated by Bostom et al. studying arteriovenous extraction in rats [31]. Notably, based on the concentration of nonprotein bound Hcy in plasma [21] and a GFR of 125 mL/minute [32], the amount of Hcy filtered through the glomeruli is only about 500 μmol/day in healthy subjects. This indicates that both reabsorption from the tubular lumen and uptake of Hcy from plasma (via the peritubular space) may take place. Reabsorption across the brush border membrane and peritubular uptake have been described for other amino acids, including the sulfur amino acids cysteine and glutathione [33].

It is possible that the elevated tHcy in renal failure can be explained by factors other than reduced renal metabolism. Secondary toxic effects on Hcy metabolism in other organs, as suggested by Hultberg et al., may occur (see Chapter 19). A decreased distribution volume, which has been observed for some amino acids in renal failure [34], may increase tHcy level (3-4). However, the elimination half-life would then be expected to be shorter (3-3) and not higher, as we observe. To obtain further information about the influence of renal function, quantitative studies of Hcy metabolism, for instance by the double isotope technique described by Storch et al. [35], may reveal whether remethylation, transsulfuration, or both metabolic pathways are influenced. Finally, experimental studies with animal models on the arteriovenous differences across various organs may yield important information about site of Hcy formation and elimination.

Summary

The site of formation of Hcy in plasma is uncertain, but in vitro experiments point to the liver and proliferating cells as important sources. Hepatocytes seems to be the only cell type with the ability to increase the Hcy export in proportion with extracellular methionine, probably due to its unique capacity to form adenosylmethionine.

Clearance studies in a healthy subject suggest that about 1.2 mmol is supplied to plasma per 24 hours. Notably, only a low percentage of an administered dose of Hcy is excreted unchanged in the urine, suggesting that extensive metabolism takes place.

Clinical studies have not yielded conclusive evidence that either remethylation or transsulfuration determines the clearance of tHcy in plasma. In both folate and B_{12} deficiencies, increased release of Hcy to the plasma compartment probably explains the increased plasma tHcy in these subjects.

Increasing evidence points to the kidney as an important site of clearance of tHcy in plasma, but further studies are needed.

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