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Hyperhomocysteinemia in terms of steady-state kinetics

Abstract The plasma level of homocysteine (Hcy) and its oxidized products, i.e., plasma total Hcy (tHcy), is a function of the influx rate of Hcy to plasma and the plasma tHcy clearance. In vitro experiments show that proliferating cells usually export more Hcy than stationary cells and that the Hcy export increases in response to high methionine, low folate or low cobalamin level, and to agents interfering with Hcy remethylation. Comparison between various cell types suggests that hepatocytes have a unique ability to increase the Hcy export in response to extracellular methionine, probably due to its capacity to form adenosylmethionine. Some but not all cell types have an ability to use extracellular Hcy as a methionine source. Clearance studies in healthy subjects indicate that about 1.2 mmol Hcy is supplied from the cells to plasma per 24 h, which is only about 5–10% of total Hcy formed. Comparison of area under the curves after administration of Hcy and methionine shows that about 10% of the methionine administered is released to plasma as Hcy. Notably, only a few percent of Hcy from plasma is excreted unchanged in the urine, and this shows that most tHcy in plasma is metabolized. Folate or cobalamin deficient patients have normal plasma tHcy clearance, which suggests that their elevated tHcy level is due to increased Hcy export from tissues into the plasma compartment. In contrast, the hyperhomocysteinemia in renal failure is accounted for by a marked reduction in tHcy clearance, suggesting an important role of kidney in elimination of Hcy from plasma.

Key words Homocysteine · Kinetics · Kidney · Liver

Abbreviations *Hcy* homocysteine and/or its oxidized products · *k* rate constant for elimination · *tHcy* total homocysteine · $T_{1/2}$ elimination half-life

Introduction

Homocysteine refers to a defined chemical compound: it is a sulfur amino acid with a free thiol (sulfhydryl) group. In this article, the abbreviation Hcy refers to both homocysteine itself (reduced Hcy) and its oxidized products. Plasma total homocysteine (tHcy) denotes the sum of all free and protein-bound Hcy species in plasma determined after quantitative reduction [36]. Elevated tHcy, termed "hyperhomocysteinemia", has been established as an independent risk factor for cardiovascular disease [7] and is caused by both genetic and acquired factors. Severe defects of the Hcy metabolising enzyme cystathionine β-synthase or methylenetetrahydrofolate reductase produce the rare inborn errors termed homocystinuria and are characterized by markedly elevated tHcy, i.e., severe hyperhomocysteinemia [27, 39]. Folate or cobalamin deficiency [1] and renal failure [5] are common causes of moderate to intermediate hyperhomocysteinemia.

Hyperhomocysteinemia is a steady-state condition characterized by enhanced Hcy flux into the plasma compartment or/and impaired elimination of plasma Hcy. Enhanced Hcy delivery into plasma is related to increased Hcy export from tissues owing to imbalance between intracellular Hcy formation and metabolism [37]. The alternative explanation is impaired elimination, but only

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sparse data exist on the site of plasma Hcy clearance and the possibility of re-uptake of plasma Hcy by tissues.

The present work describes the theories of the flux of Hcy through the plasma compartment and reviews the experimental and clinical data on site of formation and elimination of plasma Hcy.

Sites of plasma Hcy formation

The data on the source of Hcy in plasma are sparse and almost solely based on in vitro studies on blood cells [2, 9, 25] and cells in culture [9–11, 15, 30].

Blood cells

When whole blood is left at room temperature, there is a time-dependent release of Hcy from blood cells [40]. Notably, the cellular Hcy is low, suggesting ongoing Hcy production [25]. The process is temperature-dependent, and plasma tHcy usually increases about 5–15%/h at room temperature [40]. Based on studies on Hcy export from blood cells in vitro, it has been suggested that blood cells may be an important source of Hcy in plasma in vivo [2, 25].

We observed that the Hcy increase in the plasma fraction of whole blood is independent of plasma tHcy level [14]. Four hours after collection of blood, tHcy had increased about 2 μM in plasma samples with tHcy level varying from < 5 μM to > 30 μM. These data do not support the statement that the plasma tHcy level in vivo is determined by Hcy export from the blood cells. Moreover, there is no increased Hcy export from blood cells incubated with supraphysiological methionine concentrations [2, 25], probably due to the low K_m methionine adenosyltransferase in these cells [23]. This lack of methionine response contrasts with the marked elevation of plasma tHcy observed in subjects receiving peroral methionine load [39].

Cell culture experiments

Cell lines in culture export Hcy [9, 15, 30, 38] and the export rate is substantially higher in proliferating than in stationary cells [9]. Moreover, high levels of medium methionine [9], low levels of folates [10], nitrous oxide (inactivating methionine synthase) [8, 10, 11, 15] or antifolate methotrexate [30, 38] increases the Hcy export rate two- to three-fold. The maximal Hcy export usually does not exceed 1–2 nmol/10^6 cells/h.

In freshly isolated human lymphocytes, the export rate is 20–30 pmol/10^6 cells/h [9]. This is about 100 times higher than the export rate from (red) blood cells (0.3 pmol/10^6 cells/h at 37° C) [25]. In PHA-stimulated (proliferating) lymphocytes, the export rate approaches that observed in the transformed and malignant cell lines [9]. In comparison, freshly isolated hepatocytes export 1–2 nmol/10^6 cells/h. Moreover, the Hcy export rate increases almost 15-fold when the methionine concentration in the medium is increased from 15 to 1000 μM [9]. Thus, both in low and high methionine medium, the non-proliferating hepatocytes export 5–25 times more than proliferating cells. Compared to stationary lymphocytes [9] and red cells [25], the export rate from the liver cells is 3 to 4 orders of magnitude higher.

Liver

The massive Hcy export from hepatocytes in vitro [9] and the metabolic apparatus of the liver suggest a major role of this organ in the overall Hcy homeostasis and Hcy production. In addition to high levels of the ubiquitous enzymes methionine synthase and cystathionine β-synthase, liver contains the enzyme catalyzing the alternate pathway for Hcy remethylation, betaine-Hcy methyltransferase [12]. Most enzymes involved in Hcy metabolism show a particularly high activity in the liver, which seems to possess about 75% of total body capacity for transmethylation [41]. Liver contains a high level of guanidoacetate methyltransferase involved in the biosynthesis of creatine, which accounts for ~ 90% of the net flow of methionine to homocysteine [22, 26]. Liver has an efficient mechanism to handle excess methionine through a sequence of reaction, the first step of which is catalyzed by the high K_m methionine adenosyltransferase. This enzyme directs methionine via S-adenosylmethionine to the transmethylation-transsulfuration pathway [13]. Such a catabolic sequence requires the consumption of a non-essential methyl acceptor, forming an inert product. The conversion of glycine to sarcosine catalyzed by the enzyme glycine methyltransferase may serve this function [12]. Notably, this multifunctional enzyme is extremely abundant in liver [42].

Hyperproliferative disorders

In children with acute lymphoblastic leukemia, plasma tHcy is related to the number of white blood cells (mostly leukemic cells), and treatment with cytotoxic drugs leads to a simultaneous decline in plasma tHcy and the white cells [24, 31]. Moreover, patients with psoriasis, a hyperproliferative disorder, also have higher plasma tHcy than healthy subjects and other dermatological patients [29]. These data support the premise that a high burden of rapidly proliferating cells may lead to elevated plasma tHcy. Whether this is related to massive Hcy export from such cells or metabolic effects including redistribution of the folate pool is uncertain.

Elimination of plasma Hcy

The elimination of a compound from plasma is usually by metabolism or urinary excretion. For plasma tHcy, urinary excretion is related to the plasma tHcy level, but probably

represents a minor elimination pathway; only 6 μmol/day [28] or less than 0.05% of total cellular Hcy production [26, 35] is excreted unchanged in the urine. The evidence that Hcy can be taken up and metabolized by cells is not new. Nearly 50 years ago, it was demonstrated in laboratory animals that Hcy can function as the sole source of sulfur amino acids as long as vitamin and cofactor supply is adequate [4], and later cell-culture studies have shown that most benign and some transformed cell lines can utilize Hcy for growth [21].

The homocysteine loading test

We have studied the kinetics of plasma tHcy by administering Hcy (65 μmol/kg) both to healthy subjects [16] and patients with hyperhomocysteinemia due to folate or cobalamin deficiency [19] or chronic renal failure [18, 20]. In contrast to the methionine loading test [33], which mainly 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 receiving peroral Hcy loading [16], tHcy in plasma declines at a rate of $k = 0.2$/h ($T_{1/2} = 3.7$ h). In two healthy subject receiving both a peroral and an intravenous administration, we found a bioavailability (AUC_{po}/AUC_{iv}) of 50–65% [16, 20], and clearance was about 0.1 l/min. With Hcy doses varying four-fold, the elimination kinetics were constant and AUC showed a linear increase, suggesting first-order kinetics independent of the Hcy dose [16].

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 [19], in subjects with renal failure [20], and in heterozygous and homozygous homocystinurics (A. B. Guttormsen et al., unpublished results).

In subjects with hyperhomocysteinemia due to cobalamin or folate deficiency, the mean plasma Hcy clearance was not significantly different from that in healthy subjects. Furthermore, vitamin therapy, which normalized the plasma tHcy level, only marginal influenced the elimination half-life. In fact, several subjects with marked hyperhomocysteinemia (> 50 μM) had $T_{1/2}$ for tHcy, which was shorter than observed in healthy subjects [19]. Thus, our data suggest that the high plasma tHcy in cobalamin and folate deficiency is not due to delayed elimination, but is probably related to increased export of Hcy from tissues into the plasma compartment (see below).

Preliminary data from homozygous and heterozygous homocystinuria subjects ($n = 17$) indicate that they usually have normal $T_{1/2}$ and thereby Hcy clearance. However, most of these subjects had relatively normal plasma tHcy (≤ 15 μM). In one homozygote with elevated tHcy level (33 μM), the decline in plasma tHcy initially seemed normal, corresponding to an elimination half-life of less than 3 h, but 4–6 h after the Hcy administration, there was a rebound increase in plasma tHcy possibly due to food intake (A. B. Guttormsen et al., unpublished).

In patients with chronic renal failure and fasting hyperhomocysteinemia, we found a markedly prolonged $T_{1/2}$ (about 13 h) due to a marked reduction in tHcy clearance to about 0.03 l/min [20]. Notably, high-dose folic acid reduces fasting tHcy in renal patients even in the absence of overt folate deficiency [5], but it did not affect tHcy clearance in our patients [20]. This observation is in accordance with normal tHcy clearance in folate and cobalamin-deficient patients before and after vitamin supplementation [19].

Evidence that the kidney is an important metabolic site for removal of plasma Hcy has recently been presented by Bostom et al. [6]. They showed that in rats, there is a substantial renal arteriovenous difference in the plasma tHcy level, which would correspond to a metabolism of about 1 mmol/day in the human kidney [6].

Single dose and steady-state kinetics

Equations and models

The plasma tHcy level is relatively stable over a 24-h period, and in healthy young subjects, the fluctuation corresponds to mean tHcy ±10% [17]. This indicates that the supply of Hcy to and its elimination from plasma are relatively constant. The level of plasma tHcy can therefore be expressed as a steady-state concentration, C_{ss}:

$$C_{ss} = R_0/\mathrm{Cl} \quad (1)$$

where R_0 denotes the delivery of Hcy to plasma per unit time and *Cl* refers to total plasma clearance.

The clearance can be obtained by following the plasma concentration after giving a single dose *(D)* with a bioavailability *F*, using the equations [32]:

$$\mathrm{Cl} = F \cdot D/\mathrm{AUC} \quad (2)$$

$$\mathrm{Cl} = k \cdot V_d = \ln 2 \cdot V_d/T_{1/2} \quad (3)$$

By combining Eqs. 1 and 3, we get:

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

AUC, or area under the plasma concentration curve, is a measure of the systemic exposure [32]. The rate constant for elimination, *k*, denotes the fractional reduction in concentration per unit time, and V_d is the distribution volume.

From the equations above follow that the basal plasma tHcy (i.e., C_{ss}) depends on the amount of Hcy released from the tissues into plasma, the volume in which tHcy is distributed (V_d) and the ability of the system to remove Hcy (Cl).

Notably, the clearance of a compound can take place in different organs, and this is expressed as:

$$\mathrm{Cl}_{total} = \mathrm{Cl}_{hepatic} + \mathrm{Cl}_{renal} + \mathrm{Cl}_{other} \quad (5)$$

Since our experiments with increasing Hcy doses suggest that tHcy clearance is independent of plasma concentration [16], we assume that the clearance is the same both during fasting and after a peroral Hcy load. Studies with

subjects receiving a radioactive trace of Hcy that does not influence plasma tHcy level support this assumption (A. B. Guttormsen et al., unpublished). We can then use Eqs. 1–5 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 μM, a total clearance of 80 ml/min was found after administration of an intravenous dose of Hcy [16]. The estimated delivery rate of Hcy to plasma in this subject according to Eq. 1 is 1.2 mmol/24 h, or about 5–10% of total cellular Hcy production [26, 35].

In methionine loading, the sulfur amino acid dose administered is ten-fold higher than in Hcy loading. Still, the AUC for plasma tHcy after methionine loading is lower than after an intravenous Hcy loading [16, 29]. 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 μM, we found normal elimination half-life, suggesting that his high level is due to increased rate of Hcy influx into plasma. Using a clearance of 0.1 l/min [16, 20] and Eq. 1, the cellular release of Hcy was estimated to be about 20 mmol/24 h, or a major portion of cellular Hcy production [26].

Hcy eliminated from plasma

Our data point to the kidney as an important organ in plasma tHcy homeostasis. In renal failure, the tHcy clearance is 0.03 l/min as compared to 0.1 l/min in healthy subjects [20], i.e., a reduction by 70%. If the reduction in the elimination is due to impaired renal metabolism, this suggests that the normal kidney metabolizes about 70% of tHcy in plasma (Eq. 5). This is in the same range estimated by Bostom et al. studying arteriovenous extraction in rats [6]. Notably, based on the concentration of non-protein-bound Hcy in plasma [28] and a glomerular filtration rate of 125 ml/min [3], the amount of Hcy subjected to glomerular filtration is only about 0.5 mmol/day in healthy subjects. Since the amount of Hcy released to plasma is estimated to be 1.2 mmol/day, this would imply that both reabsorption from the tubular lumen and uptake of Hcy from plasma takes place as described for the sulfur amino acids cysteine and glutathione [34]. Conclusions on the fate of plasma Hcy require additional studies on renal and extrarenal handling of Hcy in healthy subjects and in patients with renal failure.

Conclusion

Based on the available experimental and kinetic data, we propose that the major fraction of Hcy in plasma derives from the liver and is eliminated in the kidneys. Further studies are required to substantiate this hypothesis.

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