20. HOMOCYSTEINE AND DRUG THERAPY

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Introduction

Several agents other than vitamins involved in homocysteine (Hcy) metabolism affect plasma homocysteine (tHcy) total concentration. The mechanisms behind the hyperhomocysteinemia vary from altered homocysteine production, impaired homocysteine metabolism, and possibly by direct reaction (through thiol-disulphide exchange) with extracellular Hcy. Some drugs change plasma Hcy by mechanisms not known. This review summarizes effects of drug therapy on plasma tHcy, with emphasis on data obtained during the last five years.

Inhibitors of Vitamin Function

ANTIFOLATE

Methotrexate is an inhibitor of dihydrofolate reductase, leading to impaired regeneration and thereby depletion of reduced folate [1], including 5-methyltetrahydrofolate [2,3]. This explains impaired Hcy metabolism in cultured cells exposed to methotrexate [4,5], and hyperhomocysteinemia in patients treated with the drug [6–10].

Plasma tHcy is a sensitive indicator of the antifolate effect of methotrexate, as demonstrated by an increased plasma tHcy concentration, which maximized after about two days, in psoriasis or rheumatoid arthritis patients given only 25 mg [7,9]. In cancer patients receiving intermediate (1–3.6 grams) or high doses (8–33.6 grams/m^2) of methotrexate, there was a rapid increase in plasma tHcy within hours. The elevated tHcy induced by methotrexate was normalized following rescue therapy with folinic acid [6,8,10]. These data show that plasma tHcy is also a responsive parameter following methotrexate exposure.

COBALAMIN INACTIVATION BY NITROUS OXIDE

Prolonged exposure to the anesthetic gas nitrous oxide (N_2O) causes side effects from bone marrow and central nervous system that resemble those observed in cobalamin-deficient patients. The clinical data have recently been reviewed [11,12].

The clinical sequelae stimulated mechanistic studies on the interaction between nitrous oxide and cobalamin. A series of in vitro studies demonstrated that nitrous oxide oxidizes the cob(I)alamin formed as a transient intermediate during the catalytic cycle of methionine synthase (5-methyltetrahydrofolate-homocysteine methyltransferase, EC 2.1.1.13.). Cob(II)alamin and a rogue hydroxyl radical is formed during this reaction:

$$\text{cob(I)alamin} + N_2O + H^+ \rightarrow \text{cob(II)alamin} + N_2 + OH$$

The hydroxyl radical may react at the active site and explain the irreversible inactivation of the enzyme [13,14]. This model predicts that enzyme inactivation is dependent on the catalytic turnover of the enzyme, which was confirmed with isolated rat liver enzyme [15].

Inactivation of methionine synthase has been demonstrated in vivo in experimental animals and in humans exposed to nitrous oxide. Notably, methylmalonyl-CoA mutase is inactivated only after prolonged exposure [11]. Inhibition of methionine synthase explains multiple metabolic effects of nitrous oxide, including trapping of reduced folates as 5-methyltetrahydrofolate, reduction of tissue folate, loss of folate in the urine, and inhibition of thymidylate and purine synthesis [11,16].

The effect of nitrous oxide on methionine synthase and the resulting release of Hcy into the extracellular medium has been studied in cultured cells in the authors' laboratory. These experiments were motivated by the fact the cellular Hcy export is a source of Hcy in extracellular medium, including plasma, and by the possibility of altering the metabolic status of the intact cells by changing the composition of the culture medium. Folate depletion, which decreases the availability of 5-methyltetrahydrofolate [17], enhanced the Hcy export from cultured cells and the

rate of inactivation of methionine synthase was markedly reduced [18]. The rate of inactivation was also drastically reduced in cells depleted with folate following methotrexate exposure (Fiskerstrand et al., unpublished). Furthermore, the rate of inactivation was high at low-medium methionine, while high methionine (>150 μmol/L) partially protected the enzyme [19,20]. These findings fit into the model for inactivation of the enzyme [15], since enzyme kinetic data suggest low catalytic turnover at low folate or high methionine [21]. There was a close relationship between methionine synthase inactivation and Hcy export from cultured cells, which points to Hcy remethylation as a determinant of Hcy export rate and thereby extracellular Hcy concentration [18,19].

PLASMA HOMOCYSTEINE AS AN INDICATOR OF COBALAMIN INACTIVATION

Enhanced Hcy export from cultured cells exposed to nitrous oxide suggests that the export is an important process balancing impaired cobalamin-dependent remethylation. We recently observed that patients with hyperhomocysteinemia and cobalamin deficiency had essentially normal half-life for plasma tHcy [22]. This observation supports the fact that hyperhomocysteinemia is due to increased influx of Hcy into the plasma compartment, and that cobalamin-dependent Hcy remethylation is not responsible for plasma tHcy clearance. The half-life of plasma tHcy (3–4 hours in healthy subjects [23]) also predicts the responsiveness, since it would theoretically [24] take less than 15 hours to reach new steady-state tHcy level in plasma after completion of cobalamin inactivation.

Clinical data on plasma tHcy levels following nitrous oxide exposure are in accordance with the in vitro experiments and data on plasma Hcy kinetics. Plasma tHcy is a responsive indicator of cobalamin inactivation, and is significantly increased after 75 minutes of nitrous oxide exposure [25]. In patients exposed for 1–4 hours, plasma tHcy is increased by 50–100%, but there is a significant residual methionine synthase activity in white blood cells [25,26] (as in cultured cells [18]). Prolonged continuous exposure to nitrous oxide for 24 hours or four days caused a two- to three- and ten- to 15-fold increase in plasma tHcy [27]. This is probably due to further lowering of the methionine synthase activity. Notably, concurrent methionine loading of patients receiving nitrous oxide anesthesia enhanced the restoration of methionine synthase in the white blood cells [26]. Whether this represents a protective effect such as that observed in cultured cells [19], or enhanced synthesis of new enzyme, or a reactivation process remains to be established. Whatever the mechanism, methionine loading may represent useful means to counteract cobalamin inactivation in patients susceptible [11] to nitrous oxide toxicity.

NITRIC OXIDE

There are preliminary reports that nitric oxide inactivates methionine synthase purified from rat liver [28] and brain [29] and human platelets [30]. Nitric oxide was even more potent than nitrous oxide as an inactivator of the platelet enzyme [30]. The versatility of nitric oxide in biologic regulation [31] makes these observations of potentially great importance, and studies on nitric oxide effects on methionine synthase in intact cell and in vivo should be undertaken.

VITAMIN B_6 ANTAGONISTS

Azauridine is an antimetabolite that was initially used for the treatment of refractory psoriasis. It caused hyper homocysteinemia and increased incidence of vascular episodes in patients, and functioned as a vitamin B_6 antagonist, causing hyper homocysteinemia in rabbits. In 1976, FDA abandoned the use of this drug [32].

Several other drugs (isoniazid, cycloserine, hydralazine, penicillamine, phenelzine, and procarbazine) may interfere with functions of vitamin B_6 [32], but impairment of Hcy metabolism has only been demonstrated with isoniazid, which in doses of 300 mg daily for one month is noted to increase urinary homocysteine excretion fivefold [33].

Homocysteine Production

ADENOSINE ANALOGUES

Several adenosine analogues are inactivators or inhibitors of the enzyme S-adenosylhomocysteine hydrolase (EC 3.3.1.1.), which is responsible for the hydrolytic cleavage of S-adenosyl-homocysteine to Hcy [34]. In addition, some adenosine analogues serve as substrate for the enzyme, and are condensed with Hcy to form the corresponding nucleosidylhomocysteine. The resulting accumulation of S-adenosylhomocysteine is the basis of the antiviral properties of these analogues [35].

There is only one ancient report that the treatment of patients with an inhibitor of S-adenosylhomocysteine hydrolase (by 2-deoxycoformycin) reduces plasma Hcy. However, studies on isolated or cultured cells have demonstrated marked inhibition of Hcy production and ex-

port [36,37]. Hcy depletion has been assigned a role in the cytostatic action of some nucleotide analogues against some [38,39] but not all [40–42] cell types. Possible metabolic consequences of inhibition of Hcy formation are methionine depletion [43,44] and trapping of reduced folates as 5-methyltetrahydrofolate [38,45]. These in vitro data should guide future metabolic studies on these drugs in humans.

L-DOPA

L-dopa, used in the treatment of Parkinson's disease, increases the concentration of dopamine in the brain. One major catabolic pathway is O-methylation to 3-O-methyl-Dopa. The reaction is catabolized by the enzyme catechol-O-methyltransferase, which uses S-adenosylmethionine as methyl donor. This explains the low level S-adenosylmethionine and high S-adenosylhomocysteine noted in animals [46,47] and patients [48] given L-Dopa. It has been recently demonstrated that a single dose of L-Dopa elevated total plasma tHcy in rats, and that the hyperhomocysteinemia persisted with chronic L-Dopa administration [49]. Preliminary data from clinical trials suggest that L-Dopa causes hyperhomocysteinemia in humans (Miller and Brattström, personal communication). The hyperhomocysteinemia in explained by enhanced Hcy formation from S-adenosylhomocysteine. This suggests that other drugs or xenobiotics serving as substrates for S-adenosylmethionine-dependent transmethylases [50] may produce hyperhomocysteinemia. The cardiovascular mortality should be investigated in patients taking these drugs.

Sulfhydryl Compounds

Three sulfhydryl-containing drugs investigated have all been shown to suppress plasma Hcy. These are D-β,β-chelating dimethylcysteine (D-penicillamine), a metal changing agent used in the treatment of rheumatoid arthritis; [51] N-acetylcysteine, a mucolytic agent also used for treatment of paracetamol overdose [52]; and 2-mercaptoethane sulfonate (mesna), which is a chemotherapeutic protective agent [53].

These drugs have a free sulfhydryl group and form symmetric and mixed disulfides in plasma. D-Penicillamine and mesna are poorly metabolized and are excreted into the urine as disulfides, whereas N-acetylcysteine is extensively deacetylated and thereby serves as a cysteine precursor [51–53].

D-Penicillamine markedly decreased protein-bound Hcy in stored samples from patients with rheumatoid arthritis and normal Hcy levels [54], and decreased free and protein-bound Hcy (by 50%–90%) and cysteine in homocystinurics [55]. Similar effects were observed in cancer patients given (isosfamide and) mesna, which decreased plasma tHcy and total cysteine by more than 50% within a few days of treatment [56]. Notably, when mesna was given alone as single dose, it first increased free cysteine and then within hours total cysteine declined [57]. A single peroral dose of N-acetylcysteine decreased tHcy (by 20–50%) and total cysteinylglycine. Total cysteine did not change [58], and this is probably related to nearly complete metabolism of N-acetylcysteine to cysteine [52]. The free fraction of all three aminothiols increased after N-acetylcysteine administration [58].

A large portion of Hcy was excreted into the urine as Hcy-penicillamine mixed disulfide in homocystinurics given D-penicillamine [55], and most mesna is excreted as mesna-cysteine mixed disulfide [53]. However, these metabolically stable drugs lower Hcy and other plasma aminothiols to the same extent [54,58] as N-acetylcysteine, which did not influence urinary excretion of Hcy and only moderately increased that of cysteinylglycine, cysteine [58]. Thus, the principal effects of sulfhydryl-containing drugs on plasma aminothiols are most likely alterations in the redox thiol status and protein-binding capacity in plasma [59], i.e., changes resembling those observed after Hcy or methionine loading [60,61]. Administration of sulfhydryl compounds probably increases the total amount of sulfhydryl equivalents in plasma, increases the free reduced fraction of plasma aminothiols through thiol-disulfide exchange reaction, and decreases the protein-bound fraction by displacement. Reduction in total amount may result from distribution of the reduced species into cells and tissues. It is not evident that such redistribution of Hcy and other aminothiols has beneficial effects.

Sex Hormones and Hormone Treatment

The idea that plasma tHcy is related to hormonal status came from consistent observations [62,63] that premenopausal women had lower levels of plasma tHcy than postmenopausal women and men. In addition, plasma tHcy has been reported to decrease in pregnancy [64,65] and in postmenopausal women on hormone replacement therapy [66].

Data on plasma tHcy concentration in subjects taking peroral estrogens are not conclusive. Brattström et al. [76] reported no change in plasma tHcy in women on oral contraceptives, a reduction in plasma tHcy in men with prostatic carcinoma treated

with estrogens, and an increased response to methionine loading was noted in both groups. Steegers–Theunissen et al. [68] made the important observation that young women taking estrogen-containing contraceptives had markedly elevated plasma tHcy in the low hormone phase compared with the high hormonal phase, in which the tHcy level equalled that found in control subjects. Furthermore, plasma tHcy did not show variations during the menstrual cycle in noncontraceptive users.

The mechanism behind the effect of contraceptives on plasma tHcy is uncertain. There are reports that contraceptives containing estrogen decrease serum cobalamin, serum and erythrocyte folate, and may affect folate metabolism or absorption without causing clinically significant vitamin deficiency [69,70]. The young women who showed variations in plasma tHcy related to the phase of contraceptive dosing had normal serum folate and serum cobalamin, and only a slight reduction in whole blood pyridoxal phosphate compared with controls [68]. Thus, estrogens may directly enhance Hcy remethylation or catabolism.

In postmenopausal breast cancer patients the synthetic antiestrogen tamoxifen, which also has some estrogen agonistic effects, decreased mean plasma tHcy by 30% after 6–12 months of treatment. The effect was most pronounced in subjects with high pretreatment values. Several mechanisms were considered, including altered estrogen status, improved folate homeostasis, and antioxidant effects [71]. In contrast, aminoglutethimide, an aromatase inhibitor that blocks the conversion of androgens to estradiol in postmenopausal women, caused a marked increase in plasma tHcy (Anker et al., unpublished). Aminoglutethimide is an efficient inductor of hepatic mixed function oxidase, and may possibly enhance folate turnover. An LH-RH analogue, goserelin, causes medical castration and estradiol reduction to postmenopausal levels. However, this drug did not significantly affect plasma tHcy in six premenopausal breast cancer patients treated for up to eight weeks (Lien et al., unpublished).

In conclusion, the effects of estrogen agonists and antagonists on plasma tHcy are complex, and no unifying hypothesis as to their mechanism has been proposed. However, altered levels of plasma tHcy induced by these agents should be related to their effect on cardiovascular mortality. Notably, the risk for cardiovascular disease is increased in premenopausal women using contraceptives [72]. Among young women using contraceptives, those with documented vascular occlusion had higher plasma tHcy levels than healthy controls [73]. In postmenopausal women, the cardiovascular risk also increased during treatment with aminoglutethimide [74], whereas tamoxifen [75] and estrogen replacement therapy [76] have a protective effect.

Miscellaneous Agents

Preliminary data show that antiepileptic drugs such as phenytoin (Brattström et al., unpublished) and carbamazepine (Refsum et al., unpublished) increase plasma tHcy, and that both drugs have the ability to create a negative folate homeostasis [77]. Notably, chronic users of antiepileptic drugs may have an increased risk of arteriosclerotic disease [78].

Coronary patients receiving bile acid sequestrants such as colestipol and niacin have higher plasma tHcy than patients receiving placebo. These drugs may interfere with folate absorption [79].

Patients with alcoholic liver disease have moderately elevated plasma tHcy and methylmalonic acid, indicating impaired cobalamin transport and function in these patients [80]. The plasma tHcy was markedly elevated (mean 20.5 μmol/L) in 42 alcohol-

TABLE 20-1. Drug effects on plasma total homocysteine and cardiovascular morbidity

Drug	Plasma Homocysteine	Morbidity	Reference
Methotrexate	Increase	Increase	Levine et al., 1988 [84]
Azauridine	Increase	Increase	Shupack et al., 1977 [85]
Peroral contraceptives (premenopausal)	Increase	Increase	Meade et al., 1988 [72]
Estrogen replacement therapy (postmenopausal)	Decrease	Decrease	Grady et al., 1992 [76]
Tamoxifen	Decrease	Decrease	Rutqvist et al., 1993 [75]
Aminoglutethimide	Increase	Increase	Jones et al., 1992 [74]
Antiepileptic drugs	Increase	Increase	Dastur et al., 1988 [78]
Alcohol (heavy intake)	Increase	Increase	Gill et al., 1991 [83]

ics without liver disease hospitalized for detoxification, and almost normal levels were reached within one to two weeks of hospitalization [81]. Thus, alcohol has an acute effect on Hcy metabolism, possibly by interfering with folate distribution [82]. Hyperhomocysteinemia may contribute to the susceptibility to stroke after heavy alcohol intake [83].

Conclusion

Several drugs may produce hyperhomocysteinemia. For some agents, the mechanism is not known. The possible implications of elevated plasma tHcy are threefold:

1. For some drugs, in particular nitrous oxide but also methotrexate, the increased level may serve as a useful indicator of pharmacodynamics.
2. The hyperhomocysteinemia may predict side effects, and the cardiovascular morbidity is increased with many drugs causing hyperhomocysteinemia (table 20-1).
3. Increased plasma tHcy following intake of some drugs should be taken into account when designing protocols for clinical studies of plasma tHcy and human disease.

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