TIPS REVIEWS

# Clinical significance of pharmacological modulation of homocysteine metabolism

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*The metabolic fate of homocysteine is linked to vitamin $B_{12}$, reduced folates, vitamin $B_6$ and sulfur amino acids. Clinical and experimental data suggest that elevated plasma homocysteine is an independent risk factor for premature vascular disease. This is particularly significant because plasma homocysteine levels are altered in several diseases, including folate and vitamin $B_{12}$ deficiencies, and because many commonly used drugs have now been shown to interfere with homocysteine metabolism. In summarizing the data,* Helga Refsum and Per Ueland *highlight the clinical implications for these metabolic changes.*

Homocysteine is a sulfur amino acid which is not itself incorporated into proteins, but is important as an intermediate in the metabolism of methionine and cysteine, and because its metabolism is linked to the function of some vitamins.

In the late 1960s, inborn errors of homocysteine metabolism (homocystinuria) were demonstrated in patients with mental retardation, skeletal abnormalities, lens dislocation and premature vascular disease[1]. Research into the physiological and pathological roles of homocysteine was subsequently promoted. Since the pioneering work of Wilcken and coworkers in 1976, accumulated epidemiological and experimental evidence has shown that homocysteine may provoke vascular lesions, and that moderate homocysteinemia is an independent risk factor for premature vascular disease[2].

During the last few years, improved analytical techniques have allowed the investigation of plasma homocysteine in healthy subjects and in disease states other than homocystinuria. Folate and cobalamin (vitamin $B_{12}$) deficiencies cause very high plasma levels of homocysteine, and plasma homocysteine has been established as a sensitive and responsive indicator of intracellular folate and cobalamin function[3].

Some drugs influence homocysteine metabolism and plasma levels. This may have some important implications. First, plasma homocysteine may reflect pharmacodynamic effects of some drugs, as most clearly demonstrated with methotrexate and nitrous oxide (see below). Secondly, the increased plasma levels induced by some agents may have implications for their side-effects. Finally, drugs decreasing plasma homocysteine may reduce the risk of vascular disease imposed by homocysteinemia[3].

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## Homocysteine metabolism

Homocysteine holds a unique position in metabolic regulation. Its metabolism is linked to sulfur amino acids, reduced folates and vitamins $B_{12}$ and $B_6$. Its metabolism is summarized in Fig. 1.

The only source of homocysteine in vertebrates is the hydrolysis of *S*-adenosylhomocysteine, an inhibitor and product of *S*-adenosylmethionine-dependent transmethylation[4]. The fate of intracellular homocysteine is either salvage to methionine through remethylation, or conversion to cysteine via the trans-sulfuration pathway. In most tissues, the former reaction is catalysed by the ubiquitous enzyme methionine synthase (Fig. 1). This enzyme requires vitamin $B_{12}$ [methyl(I)cobalamin] as a cofactor and 5-methyltetrahydrofolate as methyl donor; thus 5-methyltetrahydrofolate enters the pool of reduced folates, and homocysteine is remethylated to methionine[1].

There are two cobalamin-dependent enzymes in vertebrates: methionine synthase which utilizes methylcob(I)alamin and is cytosolic, and methylmalonyl-CoA mutase which is a mitochondrial enzyme containing adenosylcobalamin. A major fraction of intracellular cobalamin is associated with these two enzymes[5].

Homocysteine remethylation is also catalysed by an alternative enzyme, betaine–homocysteine methyltransferase, requiring betaine as methyl donor. However, this enzyme is generally confined to the liver.

The metabolism of homocysteine along the trans-sulfuration pathway is catalysed by two vitamin $B_6$-dependent enzymes. The first step is the cystathione β-synthase reaction, where homocysteine is condensed with serine to form cystathionine. Cystathionine is then cleaved to α-ketobutyrate and cysteine, catalysed by cystathionine lyase[1].

## Homocysteine and vascular disease

Patients with homocystinuria suffer from premature vascular disease, localized to the central and peripheral arteries and large veins. This is the major cause of the high mortality (20–75% before the age of 30) in these patients. Thromboembolism may occur at any age and has even been described in children[1]. Both clinical and experimental evidence suggest that high homocysteine levels cause the vascular lesions (see Ref. 1).

Even moderate homocysteinemia may provoke venous thrombosis and premature vascular lesions in the cerebral, peripheral and coronary arteries (L. Brattström, Thesis, University of Lund, 1989). Such a relation was suggested 10 years ago from clinical studies based on measurement of acid-soluble mixed disulfides in plasma from a small number of patients[2]. This has later been confirmed in several investigations, most of

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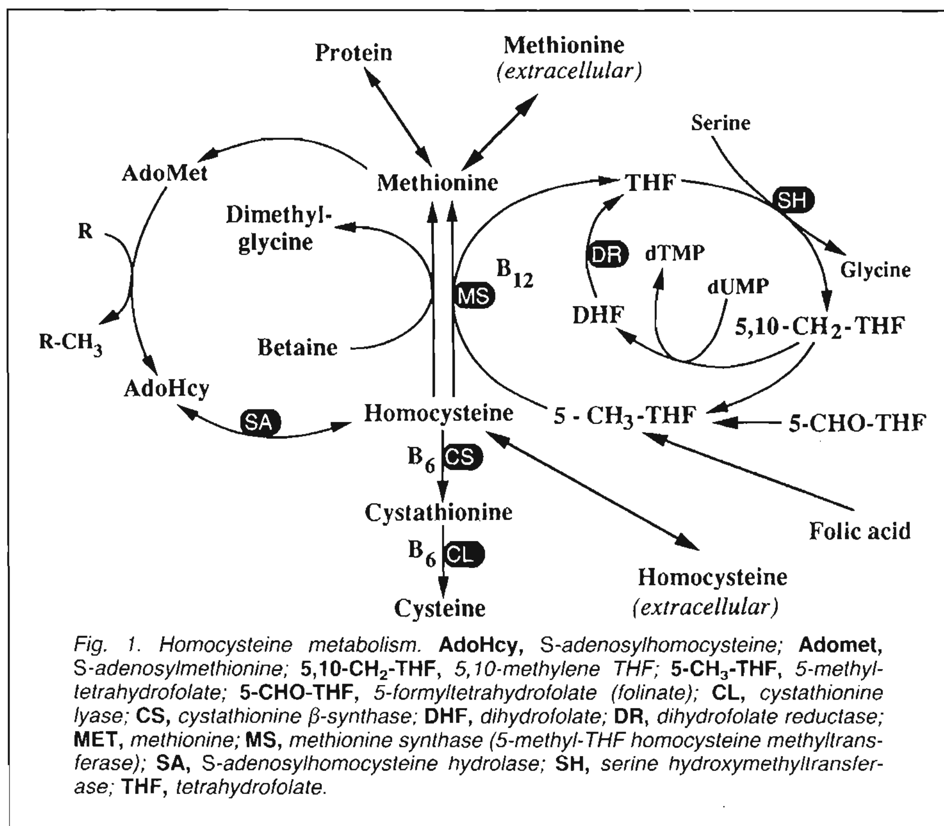


*Fig. 1. Homocysteine metabolism.* **AdoHcy,** *S-adenosylhomocysteine;* **Adomet,** *S-adenosylmethionine;* **5,10-$CH_2$-THF,** *5,10-methylene THF;* **5-$CH_3$-THF,** *5-methyltetrahydrofolate;* **5-CHO-THF,** *5-formyltetrahydrofolate (folinate);* **CL,** *cystathionine lyase;* **CS,** *cystathionine β-synthase;* **DHF,** *dihydrofolate;* **DR,** *dihydrofolate reductase;* **MET,** *methionine;* **MS,** *methionine synthase (5-methyl-THF homocysteine methyltransferase);* **SA,** *S-adenosylhomocysteine hydrolase;* **SH,** *serine hydroxymethyltransferase;* **THF,** *tetrahydrofolate.*

which determined total plasma homocysteine[3,6–10]. Furthermore, an increased incidence of heterozygous homocystinuria has been demonstrated in patients with early onset vascular disease[7].

Other clinical and experimental data support an association between raised homocysteine levels and vascular lesions (reviewed in Ref. 3). Plasma homocysteine levels show age- and sex-dependent variations resembling those described in arteriosclerotic disease. Men and postmenopausal women have higher plasma homocysteine levels during fasting and after methionine loading than young women. Plasma homocysteine is significantly increased in chronic renal failure, severe psoriasis, and in some patients with cancer. These are conditions associated with increased risk of vascular disease not adequately explained by risk factors like smoking, lipid abnormalities, hypertension or other known predisposing conditions[3]. Moderate homocysteinemia should be considered as a possible cause of vascular disease in those cases (15–30%) when other risk factors cannot be identified. Down syndrome, on the other hand, is an abnormality characterized by low plasma homocysteine levels, probably due to increased gene dosage for the enzyme cystathionine β-synthase residing on chromosome 21 (Ref. 11). In 1977 Murdoch and co-workers suggested this state as an atheroma-free model because of the remarkable absence of arteriosclerotic lesions observed in five patients aged 44–66 years[12].

There is also more direct evidence that high levels of homocysteine mediate the thrombogenesis and accelerated atherogenesis observed in homocystinuria, and that homocysteine may also be responsible for the vascular disease associated with moderate homocysteinemia. Homocysteine damages human endothelial cells in culture, possibly by producing hydrogen peroxide in an oxygen-dependent reaction. Moreover, endothelial cells from patients heterozygous for cystathionine β-synthase gene deficiency may have an increased susceptibility to injury by homocysteine. Mechanisms linking mild homocysteinemia and vascular effects could also involve production of free radicals and oxidation of low-density lipoprotein. Conflicting data exist on possible roles of platelet sequestration in the development of atherosclerotic lesions under conditions of elevated plasma homocysteine levels[2].

## Folate and cobalamin deficiency

Cobalamin deficiency may increase plasma homocysteine to levels observed in homocystinurics (high micromolar range); there is a negative correlation between serum cobalamin and total plasma homocysteine. Homocysteine levels may also be elevated in cobalamin-deficient patients devoid of typical signs like anemia, macrocytosis and reduced serum cobalamin. Levels are normalized following cobalamin therapy[3,13].

Similarly, folate deficiency is a common cause of elevated plasma homocysteine levels, and a close negative correlation with serum folate has been demonstrated; again, folate therapy normalizes levels. Moderate elevation of plasma homocysteine is also observed in subjects with low but normal serum folate levels, suggesting that increased homocysteine levels in these subjects may reflect an intracellular folate content insufficient for optimal folate-dependent remethylation of homocysteine[3].

Measurement of plasma homocysteine is therefore a promising laboratory test for evaluating cobalamin or folate deficiency states. It may be particularly useful when used in conjunction with serum methylmalonic acid, which is a specific measure of disturbances of cobalamin metabolism[13].

## Agents decreasing homocysteine concentrations

Compounds serving as cofactors in homocysteine catabolism or remethylation may enhance homocysteine metabolism and thereby reduce plasma homocysteine levels in inherited enzymic defects. Thus, vitamin $B_6$ reduces plasma homocysteine in homocystinurics with residual cystathionine β-synthase activity, and vitamin $B_{12}$ acts similarly in some mutations of cobalamin metabolism. Betaine and folic acid have been shown to efficiently reduce plasma homocysteine in patients with cystathionine β-syn-

thase deficiency who are unresponsive to vitamin $B_6$ (Ref. 1).

### *Folic acid*

Folic acid (5 mg daily) efficiently decreases plasma homocysteine levels. It reduces elevated plasma homocysteine in renal transplant recipients and even in those without overt folate deficiency. Treatment of healthy subjects with folic acid for 14 days significantly reduced plasma homocysteine, especially in persons with high pretreatment levels[14].

The marked effect of high doses of folic acid on the concentration of homocysteine in plasma is important. Since moderate homocysteinemia may provoke vascular lesions, folic acid may prevent atherosclerotic disease in selected subjects. This intervention is particularly attractive because folic acid intake has essentially no side-effects[14]. Interestingly, the effect of folic acid suggests that the intracellular folate content is insufficient for an optimal remethylation of homocysteine. This may be a more common state than hitherto recognized, as it may not be detected by established laboratory procedures, including determination of folate in serum or erythrocytes[3].

Folic acid probably decreases plasma homocysteine levels by increasing the availability of intracellular 5-methyltetrahydrofolate, thereby enhancing homocysteine remethylation (Fig. 2). This reaction forms tetrahydrofolate, which enters the pools of reduced folates carrying one-carbon units via the serine hydroxymethyl transferase reaction (Figs 1 and 2). In this reaction, serine is consumed and glycine formed, as would be expected from the moderate reduction in plasma serine levels and increase in plasma glycine levels that follow folic acid administration[14].

### *D-Penicillamine*

D-Penicillamine (D-β,β-dimethylcysteine) is currently used for the treatment of heavy metal poisoning, rheumatoid arthritis, hepatolenticular degeneration, cystinuria and scleroderma. It is metabolically stable, chelates heavy metals, produces disulfides and forms a thiazolidine ring with aldehydes and ketones. In plasma, penicillamine forms symmetrical penicillamine disulfides and mixed disulfides with cysteine, homocysteine and plasma proteins. The low molecular weight disulfides undergo rapid renal excretion, which explains the short plasma half-life and the therapeutic effect in cystinuria[15].

Penicillamine efficiently reduces both free and protein-bound plasma homocysteine in homocystinurics[16] and total plasma homocysteine in patients with rheumatoid arthritis, a condition with normal homocysteine pretreatment levels[17]. This reduction in plasma levels may be associated with an intracellular homocysteine depletion sufficiently pronounced to decrease homocysteine remethylation and thereby induce methionine deficiency and secondary effects on folate metabolism. If such effects occur, penicillamine may act as an antifolate agent and may therefore interact adversely with methotrexate used in low doses in the management of rheumatoid arthritis (see below)[18].

A further clinical implication of this work is that penicillamine may be a useful means to reduce plasma homocysteine.

### *Adenosine (nucleoside) analogs*

The cleavage of S-adenosylhomocysteine to adenosine and homocysteine, catalysed by S-adenosylhomocysteine hydrolase (Figs 1 and 2), is the only known source of homocysteine in vertebrates. Several nucleoside analogs block this reaction by serving either as an inactivator or inhibitor of the enzyme. In addition, some analogs act as substrate and are thus converted to the corresponding S-adenosylhomocysteine analogue[4]. Inhibition of S-adenosylhomocysteine hydrolase leads to massive accumulation of S-adenosylhomocysteine in isolated cells, whole animals, and in patients. This is important for the antiviral effects of this class of compound[4,19].

The other immediate consequence of S-adenosylhomocysteine hydrolase inhibition, reduction of homocysteine formation (Fig. 2), has been studied only recently. Homocysteine depletion and inhibition of homocysteine export have been demonstrated in isolated cells exposed to nucleoside analogs[20,21]. A reduction in plasma homocysteine was found in patients with acute leukemia treated with 2-deoxycoformycin, which indirectly inactivates S-adenosylhomocysteine hydrolase[22,23]. Inhibition of homocysteine formation plays an important role in the cytostatic action of some nucleoside analogs against some[24,25] but not all[26–28] cell types, and probably mediates the differentiation of HL-60 cells induced by adenosine dialdehyde[29]. The consequences of cellular homocysteine deficiency are twofold. First, nucleoside analogs may induce severe methionine deficiency, since homocysteine salvage is a significant source of methionine in humans[30,31]. Secondly, lack of homocysteine may trap reduced folate as 5-methyltetrahydrofolate because homocysteine is the methyl acceptor in the methionine synthase reaction catalysing the conversion of 5-methyltetrahydrofolate to tetrahydrofolate. In this way, lack of tetrahydrofolate may ensue, and thereby inhibit folate-dependent purine and thymidylate synthesis. Both mechanisms have been demonstrated in cultured cells[24,32].

Induced deficiencies of methionine and folates might be avoided clinically by appropriate supplementation which would be expected to reduce associated side-effects. Moreover, adverse interactions of adenosine analogues with drugs that also interfere with folate or methionine metabolism such as nitrous oxide or methotrexate should be considered. This has been suggested for the antiviral agent vidarabine (9-β-D-arabinofuranosyladenine) and high-dose methotrexate with folinic acid rescue[30]. Reduction in plasma homocysteine levels might however have beneficial vascular effects.

## Inhibitors of homocysteine remethylation and degradation

Several important drugs interfere with homocysteine metabolism.

### *Nitrous oxide*

The anesthetic agent nitrous oxide was used for a century before it was discovered that long-term exposure caused megaloblastic and aplastic bone marrow changes, anemia and myelopathy.

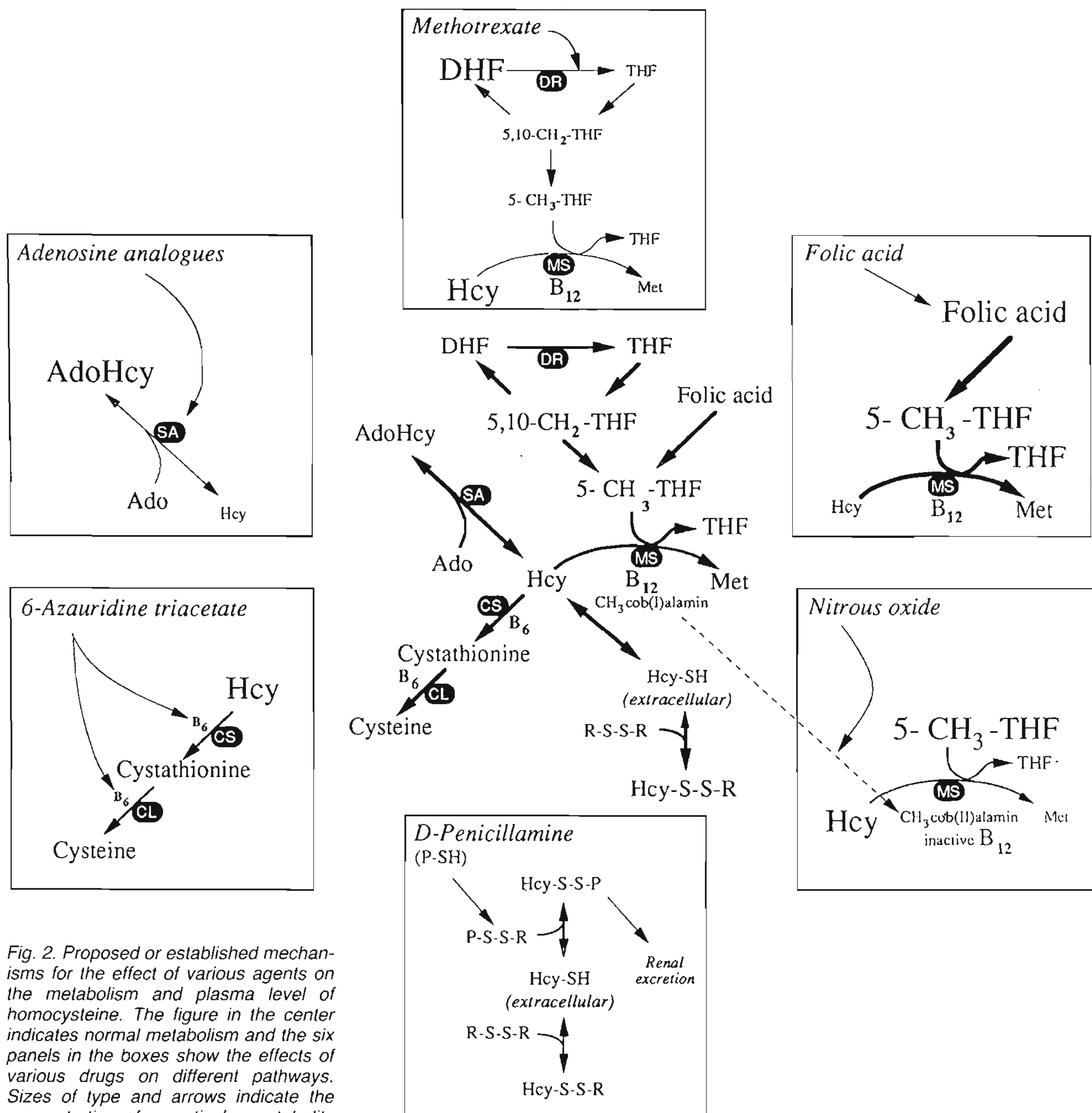


*Fig. 2. Proposed or established mechanisms for the effect of various agents on the metabolism and plasma level of homocysteine. The figure in the center indicates normal metabolism and the six panels in the boxes show the effects of various drugs on different pathways. Sizes of type and arrows indicate the concentration of a particular metabolite and the flux through the pathway, respectively. Folic acid probably increases the cellular content of 5-methyltetrahydrofolate* **($5$-$CH_3$-THF)**, *which increases the homocysteine* **(Hey)** *remethylation catalysed by methionine synthase* **(MS)**. *D-Penicillamine* **(P-SH)** *forms a mixed disulfide with homocysteine* **(Hcy-S-S-P)**; *this disulfide has a high renal clearance. Adenosine analogs are inhibitors of S-adenosylhomocysteine hydrolase* **(SA)**. *Nitrous oxide oxidizes methylcob(I)alamin to methylcob(II)alamin and thereby irreversibly inhibits methionine synthase* **(MS)**. *Methotrexate inhibits dihydrofolate reductase* **(DR)**, *thereby inhibiting regeneration of tetrahydrofolate* **(THF)** *from dihydrofolate* **(DHF)**. *Tetrahydrofolate supplies 5-methyltetrahydrofolate via 5,10-methylenetetrahydrofolate* **(5,10-$CH_2$-THF)**. *Methotrexate may therefore induce depletion of 5-methyltetrahydrofolate, and inhibit homocysteine remethylation catalysed by methionine synthase. Azauridine is a vitamin $B_6$ antagonist, and may inhibit some vitamin $B_6$-dependent enzymes, including cystathionine β-synthase* **(CS)**.
**Hcy**, *homocysteine;* **Met**, *methionine;* **Ado**, *adenosine;* **P-SH**, *D-penicillamine reduced form;* **Hcy-S-S-R**, *homocysteine mixed disulfide;* **Hcy-S-S-P**, *a mixed disulfide between homocysteine and D-penicillamine;* **P-S-S-R**, *D-penicillamine mixed disulfide.*
*For other abbreviations, see Fig. 1.*

These side-effects are similar to the symptoms of vitamin $B_{12}$ deficiency[33].

Nitrous oxide oxidizes the cobalamine species methylcob(I)-alamine, inactivating specifically the enzyme methionine synthase, without affecting the adenosylcobalamine cofactor of methylmalonyl-CoA mutase[33]. Inactivation of methionine synthase causes a cascade effect on folate metabolism including trapping of reduced folates such as 5-methyltetrahydrofolate, loss of folate in the urine, and reduction in tissue folate levels. There is a subsequent decrease of folate-dependent purine and thymidylate synthesis, as demonstrated by the deoxyuridine suppression (dU)

test, which reveals changes in human bone marrow within 5–6 hours. In humans, 50% of methionine synthase is inactivated in about two hours, and there is a reduction in plasma methionine after 8–24 hours of exposure. Megaloblastic bone marrow changes can be detected after 12–24 hours of exposure[33].

Data on the effect of nitrous oxide on homocysteine metabolism are sparse, but this drug has been reported to increase the urinary excretion of homocysteine in sheep, and increase plasma homocysteine levels in fruit bats[3]. We have recently demonstrated that nitrous oxide induces a marked increase in plasma homocysteine within 90 minutes, with a concurrent increase in urinary homocysteine excretion. In patients receiving nitrous oxide for 3–6 hours, plasma homocysteine remained above normal for at least 7 days (Ermens, A. A. M. *et al.*, unpublished data). The homocysteine response evolved before other early signs of nitrous oxide-induced cobalamine inactivation[33]. Thus, significant cobalamine oxidation and methionine synthase inactivation occurs even after short-term exposure, which previously has not been regarded as harmful. The resultant increase in plasma homocysteine observed in some patients may in itself be detrimental. Furthermore, the homocysteine response may reflect loss of functional cobalamine and folate, and may enhance sensitivity towards antifolate drugs such as methotrexate[34] (Ermens, A. A. M., PhD Thesis, University of Rotterdam, 1990). Since an increase in plasma homocysteine levels is both an early and sensitive measure of cobalamine oxidation, plasma homocysteine monitoring could be useful in the detection of such effects in the clinic.

### *Methotrexate*

Methotrexate is an antifolate drug which has been used extensively in intermediate and high doses in the treatment of leukemia and some solid tumors. Low-dose methotrexate is used in the management of some non-malignant diseases such as rheumatoid arthritis and psoriasis.

Methotrexate acts by inhibiting dihydrofolate reductase, thereby blocking the regeneration of tetrahydrofolate from dihydrofolate (see Ref. 35). This leads to depletion of reduced folates, including 5-methyltetrahydrofolate[36,37]. Thus, methotrexate may also inhibit the folate-dependent remethylation of homocysteine catalysed by methionine synthase. This would explain the increased homocysteine export from cultured cells exposed to methotrexate, and the methotrexate-induced homocysteinemia and urinary homocysteine excretion in patients[38].

Low-dose methotrexate (25 mg daily) given to psoriatics induced increased plasma homocysteine levels, which maximized after about two days, and normalized within one week[39]. This shows that plasma homocysteine is a sensitive measure of the antifolate effect.

Intermediate doses (1–13.6 g) given to patients with solid tumors, induced a rapid increase in plasma homocysteine within hours, which was reversed on administration of folinic acid 24 hours after start of infusion. This response was observed following several methotrexate doses in a single patient[40]. High doses of methotrexate (8–33.6 $m^{-2}$) given to children gave a similar response, i.e. a rapid increase a few hours after start of administration and a decline following 'rescue' therapy[41]. This is analogous to the results obtained with cultured cells[38].

Basal homocysteine levels in patients with acute lymphoblastic leukemia were often above normal before treatment, and declined markedly following treatment with cytotoxic agents including methotrexate (Refsum, H. *et al.*, unpublished). This may be due to eradication of proliferating cells exporting large amounts of homocysteine.

The high-dose methotrexate regimen also induced a transient but marked reduction in plasma methionine[41] which may contribute to the killing of tumor cells or toxicity of methotrexate. Plasma homocysteine response and methionine depletion may correlate with the therapeutic as well as the side-effects of methotrexate, including liver toxicity[42] and an increased incidence of thromboembolism[43]; plasma homocysteine measurements could provide a useful adjunct to serum methotrexate determination in the management of methotrexate therapy.

### *Vitamin $B_6$ antagonists*

Azauridine is an antimetabolite interfering with *de novo* synthesis of uridine-5′-monophosphate. It was initially used for the treatment of refractory psoriasis, but was withdrawn by the FDA in 1976 because its use was associated with an increased incidence of vascular episodes[44]. This may be due to effects on homocysteine metabolism. Azauridine causes homocysteinemia, abnormal homocysteine excretion and a significant increase in serum methionine levels in patients. Studies in rabbits suggest that it functions as a pyridoxal 5′-phosphate antagonist and causes homocysteinemia by inhibiting vitamin $B_6$-dependent cystathionine synthesis[44]. This suggests that supplementing vitamin $B_6$ would prevent the inhibition of homocysteine catabolism; determination of plasma homocysteine may identify patients at risk of vascular episodes.

Several other drugs also interfere with the function of vitamin $B_6$: isoniazid, cycloserine, hydralazine, penicillamine, phenelzine and procarbazine[44]. Perturbation of homocysteine metabolism in patients has been demonstrated with isoniazid[45]. In one out of six patients given 300 mg isoniazid daily for one month, urinary homocysteine excretion was fivefold higher than normal. Inhibition of cystathionine metabolism in these patients is supported by increased excretion of this compound after methionine loading[45].

### *Other agents*

Premenopausal women have lower plasma homocysteine than men and postmenopausal women[46], and plasma levels are low during pregnancy. However there is no conclusive evidence that homocysteine metabolism and plasma homocysteine levels are under the influence of estrogens. Preliminary data in women given contraceptive steroids or the antiestrogen tamoxifen suggest a polymorphic response. In some women, altered estrogen status may cause a decrease, and in

others, an increase in plasma homocysteine levels. Because of the widespread use of contraceptives, and the suggested use of tamoxifen as prophylactic intervention in healthy women at high risk of developing breast cancer, the effect on plasma homocysteine from altered estrogen status is a question of major concern to public health[3].

Various antiepileptic drugs, particularly phenytoin but also phenobarbital, primidone, carbamazepine, and valproic acid, may induce folate deficiency[47]. The activity of methylenetetrahydrofolate reductase, the enzyme producing 5-methyltetrahydrofolate, is altered in mouse liver following exposure to these drugs. Preliminary data suggest that phenytoin and possibly carbamazepine may increase plasma homocysteine, but its relation to overt folate deficiency has not been established[3].

Several other drugs are known to interfere with folate metabolism or function. These include some phenothiazines and tricyclic antidepressants, oral contraceptives, possibly some tuberculostatic drugs, and antifolate drugs such as trimethoprim[47]. Thiols or disulfide forming drugs, such as cysteamine and *N*-acetylcysteine are actual candidates as modifiers of plasma homocysteine levels, due to a possibly unique disposition of the mixed disulfides with homocysteine, as demonstrated with penicillamine in humans[16].

□ □ □

Various pharmacological agents have been shown to enhance homocysteine remethylation and urinary excretion, or inhibit homocysteine production, remethylation or catabolism thereby affecting plasma homocysteine levels. Modulation of homocysteine metabolism and plasma concentrations may be an important component of drug action. Agents that reduce plasma homocysteine (e.g. folic acid, penicillamine) may prevent vascular disease, while agents increasing plasma homocysteine (e.g. nitrous oxide, methotrexate, azauridine) may provoke vascular episodes. Also for some drugs (e.g. nitrous oxide and methotrexate), elevation of plasma homocysteine is an early and sensitive measure of drug action and plasma homocysteine has been shown to be useful in the diagnosis and follow-up of some diseases, in particular homocystinuria, folate deficiency and cobalamine deficiency.

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