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PERTURBATION OF HOMOCYSTEINE METABOLISM BY PHARMACOLOGICAL AGENTS IN EXPERIMENTAL AND CLINICAL USE.

Per M. Ueland, Helga Refsum, Asbjørn M. Svardal, Rune Djurhuus and Svein Helland

Clinical Pharmacological Unit, Department of Pharmacology, University of Bergen, 5000 Bergen, Norway.

INTRODUCTION

Homocysteine, a sulfur amino acid, was discovered over 50 years ago by DuVigneaud as the product of demethylation of methionine This discovery was followed by reports that homocysteine could support growth of animals fed diets deficient in either cysteine, methionine or choline (1).

The possible involvement of homocysteine in human disease has stimulated the interest in this amino acid.

In 1962 homocystine was detected in urine from two mentally retarded children (2). Since then, homocystinuria has been defined as an inherited disorder of homocysteine metabolism. Children afflicted with this disease suffer from premature arteriosclerosis (for review, see ref. 3). This discovery was followed by research into the possible role of homocysteine in the development of arteriosclerotic lesions (4). Recent clinical studies in man strongly indicate a relation between elevated plasma homocysteine and arteriosclerotic disease (5).

During the last 10 years there have been numerous reports that malignant cells cannot use homocysteine instead of methionine to fully support growth. Non-transformed cells thrive under these conditions (6). The biochemical basis of the so-called methionine dependence of cancer cells is not understood, and this concept has been questioned by some workers (7). Nevertheless, the possible role of homocysteine in malignant growth is currently a subject of interest.

The possible centrality of homocysteine in pathological processes like arteriosclerosis and malignant growth, points to the possibility that pharmacological interference with homocysteine metabolism may have important implications. There are examples of pharmacological

agents that either block homocysteine production or perturb the metabolism of this amino acid. This article reviews some recent data on this topic. Some central features of homocysteine metabolism and recent advances in methods for quantitation of homocysteine in biological material, are also reviewed.

METABOLISM AND DISPOSITION OF HOMOCYSTEINE

S-Adenosylmethionine functions as a methyldonor in numerous transmethylation reactions. All these reactions produce stoichiometric amounts of S-adenosylhomocysteine (AdoHcy). This product of transmethylation functions as a negative feed-back inhibitor of this class of reactions. The inhibition is relieved upon metabolic degradation of AdoHcy to homocysteine and adenosine. This reaction is catalyzed by the ubiquitous enzyme, AdoHcy hydrolase (EC 3.3.1.1.) (8).

The structural formulae of homocysteine and related compounds are shown in figure 1.

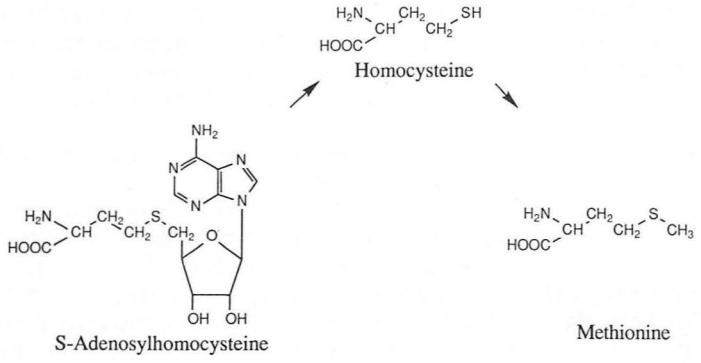

FIG. 1. **Structural formulae of homocysteine and related compounds.**

The AdoHcy hydrolase reaction, the only known source of homocysteine in vertebrates, is reversible and the equilibrium lies in the direction of synthesis of AdoHcy. The metabolic flux *in vivo* is in the hydrolytic direction because both adenosine and homocysteine are continuously removed from the intracellular compartment (8).

Intracellular homocysteine is either methylated to methionine or is condensed with serine to form the thioether, cystathionine. The salvage to methionine is catalyzed by two separate enzymes. One,

betaine-homocysteine methyltransferase (EC 2.1.1.5.), requires betaine as methyl donor and this enzyme is confined to liver and kidney. The other enzyme, 5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13., known as methionine synthase) uses 5-methyltetrahydrofolate, and is widely distributed in tissues of vertebrates. Notably, this enzyme is one of the two enzymes dependent on cobalamin, and homocysteine is thus linked both to the metabolism of reduced folates and vitamin B12 (3)

The methionine synthase reaction is the only known metabolic pathway common to folate, vitamin B12 and methionine. Based on both experimental and clinical evidence, Noronha and Silverman (9) and Herbert and Zalusky (10) advanced the so-called "methyl trap" hypothesis, which states that the consequences of vitamin B12 deficiency stem from reduced activity of the vitamin B12 dependent conversion of 5-methyltetrahydrofolate to tetrahydrofolate. Under these conditions, reduced folates are trapped as 5-methyltetrahydrofolate, thereby causing slowdown in thymidylate and purine biosynthesis. Homocysteine is the methyl acceptor in this reaction, and this implies that the availability of intracellular homocysteine is critical for the quantitative relation between 5-methyltetrahydrofolate and other reduced folates.

Conversion of homocysteine to cystathionine represents the alternative pathway to methylation. This reaction, which is essentially irreversible, is catalyzed by cystathionine ß-synthase (EC 4.2.1.22). The bulk of this enzyme resides in the liver but human brain is also rich in cystathionine ß-synthase. The reaction is an important step in the conversion of methionine to cysteine, i.e. the so-called transsulfuration pathway (3)

The homocysteine content in tissues is low under physiological conditions (1-5 nmol/g,), and a significant amount is associated with proteins (11,12). Similarly, low concentrations can be detected in isolated or cultured cells (12-15). However, cells export large amounts into the extracellular medium (12-17) and the homocysteine egress seems related to the metabolic flux through homocysteine (12).

Homocysteine egress from cultured cells was proportional to the growth rate (16). This should be related to the finding that 5-methyltetrahydrofolate content in human breast cancer cells increased markedly during growth, whereas the levels of other reduced folates remained stable (18).

The efficient export of homocysteine is in accordance with the relatively high concentration of homocysteine in extracellular media like plasma and urine (19). Thus, urinary or plasma homocysteine may be a measure of altered homocysteine metabolism.

In conclusion, homocysteine can be metabolized by different routes (Fig. 2). The quantitative relations between these competing pathways have recently been evaluated by Finkelstein and Martin. (20). Homocysteine is a branch-point metabolite, with relations to the

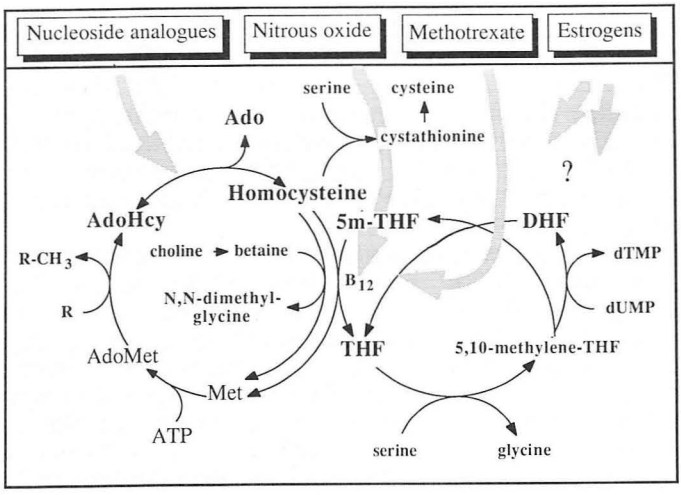

FIG. 2. **Metabolism of homocysteine and related compounds and the metabolic targets of various drugs.** Ado, adenosine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; Met, methionine; THF, tetrahydrofolate; DHF, dihydrofolate.

metabolic fates of several other compounds. This points to homocysteine metabolism as an important target for drug action

METHODS

Investigations into the biological roles of homocysteine have been hampered by lack of sensitive methods for the determination of the small amounts of this compound in biological material.

The homocysteine-cysteine mixed disulfide has been detected in plasma deproteinized by acid using modern amino acid analyzers (21). Homocysteine in plasma has also been determined with HPLC and electrochemical detector (22). For the detection of protein-bound homocysteine in plasma, laborious sample processing is often required (23).

We have recently developed a sensitive radioenzymic assay for the determination of homocysteine in tissues (11,12) and plasma (19). This assay is based on the enzymic conversion of homocysteine to radioactive AdoHcy, and can be carried out in solutions containing native proteins, and therefore allows the determination of both free (acid soluble) and protein-bound homocysteine in biological extracts and plasma.

The radioenzymic assay has been adapted for urine analysis (19). In addition, urinary homocysteine has also been determined with HPLC and electrochemical detector equipped with a mercury electrode (24).

Accurate determination requires rapid processing of tissue or plasma. We have recently observed that rapidly prepared plasma contains somewhat less homocysteine (free plus bound) than serum. This can be explained by a release of homocysteine from blood cells in whole blood left at room temperature. In contrast, when whole blood is kept on ice, there is no change in either free or protein-bound homocysteine within 0.5 hours, and total homocysteine remains stable for 12 hours under these conditions.

There is a redistribution between free and protein-bound homocysteine in plasma at room temperature. Total homocysteine remains constant whereas free homocysteine declines. Repeated freezing and thawing have similar effect.

AGENTS AFFECTING HOMOCYSTEINE METABOLISM AND DISPOSITION

Nucleoside Analogues.

Numerous adenosine analogues function as inhibitors of AdoHcy hydrolase (25). Since this enzyme is the only known source of homocysteine, one might expect that these agents induce depletion of intracellular homocysteine.

This possibility was first investigated in whole mice injected with the drug combination 9-ß-D-arabinofuranosyladenine plus 2'-deoxycoformycin. Although AdoHcy catabolism was profoundly inhibited, depletion of homocysteine in tissues was not observed (11). The effect on homocysteine in extracellular media like plasma and urine was not investigated.

Studies with isolated and cultured cells have enlightened this enigma. Cells in suspension export large amounts of homocysteine, and the homocysteine egress is profoundly inhibited by 3-deazaadenosine or 3-deazaaristeromycin in proportion to their inhibitory effect on AdoHcy catabolism(13,14). Homocysteine associated with the cells is either decreased, as in fibroblasts, or is even increased , as in liver cells, under these conditions. It is conceivable that homocysteine egress may be important as a cellular adjustment to imbalance in homocysteine supply relative to the metabolic need. Such a mechanism may be important for some cell types, but may be of less importance in liver cells where the turnover of AdoMet and related compounds is high (3) .

Cantoni et al. (26) pointed out some metabolic consequences of severe depression of the activity of AdoHcy hydrolase. They suggested that compounds inhibiting this enzyme, like 9-ß-D-arabinofuranosyladenine, may inhibit the regeneration of tetrahydrofolate from 5-methyltetrahydrofolate because of lack of homocysteine. In addition, inhibition of *de novo* methionine synthesis, which supplements dietary methionine, may cause methionine deficiency.

Boss and Pilz (27) have provided some data in favour of these suggestions. They recently demonstrated that the methionine synthesis was inhibited in T-lymphoblasts and to a lesser extent in B-lymphoblasts in the presence of 2'-deoxyadenosine, 9-ß-D-arabinofuranosyladenine or 2'-deoxyguanosine, and the inhibition was reversed by homocysteine (27). The pronounced inhibition of methionine synthesis in T-lymphoblasts correlates with the cytotoxic effects of these nucleosides on these cells. The authors conclude that purine nucleoside toxicity may partly be mediated through inhibition of methionine synthesis, and by trapping reduced folates as 5'-methyltetrahydrofolate. The latter possibility gains support from the observation that some adenosine deaminase and nucleoside phosphorylase deficient patients have megaloblastic anemia (27).

Two reports provide direct evidence that lack of homocysteine plays a role in the cytostatic action of nucleoside analogues inhibiting AdoHcy catabolism. Kim et al. (28) have shown that addition of homocysteine counteracts the cytostatic effect of 3-deazaaristeromycin in mouse macrophages. Likewise, Wolfson et al. (29) have reported that the cytostatic action of neplanocin A was inhibited by homocysteine in rat pituitary cells. These observations contrast to no effect of homocysteine on the cytostatic action of 3-deazaaristeromycin in HL-60 cells (Aarbakke, J., personal communication).

Methotrexate

The primary target for methotrexate is the enzyme dihydrofolate reductase, the enzyme responsible for the regeneration of tetrahydrofolate from dihydrofolate (30). Inhibition of this enzyme is expected to cause cellular depletion of reduced folates, including 5-methyltetrahydrofolate. Since quantitation of intracellular folates has met with technical obstacles, the effect of methotrexate on the cellular folate pools has not been evaluated until recently. In the spring of 1986 two papers were published which describe the differential effect of methotrexate on reduced folates in various cell types in culture, including human breast cancer cells. Notably, both reports independently show that, among the reduced folates, 5-methyltetrahydrofolate is particularly sensitive towards the inhibitory effects of methotrexate. The cellular content of this particular folate was

profoundly reduced within minutes under conditions where the amount of other reduced folates was slightly (18) or not affected (31). Similar results were obtained with cells grown i.p. in mice injected with methotrexate (31). These findings are important in relation to the possible effects of methotrexate on homocysteine metabolism.

We have recently demonstrated that methotrexate increases homocysteine export several-fold in chemically transformed murine fibroblasts. Larger concentrations were required for the enhancement of homocysteine egress from the non-transformed counterpart, which was essentially methotrexate resistant. The increase in homocysteine egress may be explained by lack of 5-methyltetrahydrofolate relative to the metabolic demand (15).

The results with cells in culture prompted us to investigate the effect of high-dose methotrexate on homocysteine in plasma and urine from cancer patients. There was a marked increase in plasma homocysteine and urinary excretion of homocysteine within 12 hours after drug infusion (32). The homocysteine response was extinguished after a few infusions. The acute response is in accordance with the *in vitro* data (15), whereas the mechanism behind the gradual appearance of a refractory state remains an enigma.

In psoriatics receiving low dose (10-25 mg) methotrexate treatment once a week, we observed an acute increase in plasma homocysteine, which subsided within 2-3 days. The homocysteine response regularly appeared, and no refractory state developed in patients receiving low-dose methotrexate (Refsum, H., Helland, S., and Ueland, P.M., unpublished results). Notably, Brattstrøm et al. (33) have recently reported that folic acid therapy resulted in substantial reduction in plasma homocysteine in normal men and women. These data suggest that plasma homocysteine may be a sensitive measure of intracellular folate status

Some cancer patients and most patients with extensive psoriatic lesions have remarkable high plasma homocysteine levels. Plasma homocysteine decreased a few days after high-dose methotrexate treatment, but not in psoriatics receiving low-dose treatment (Refsum, H., Helland, S., and Ueland, P.M., unpublished results). One may speculate whether homocysteine in plasma and urine may, partly at least, originate from rapidly proliferating cells, including cancer cells or basal cells in psoriasis. Exposure to cytostatic concentrations of methotrexate may reduce the number of proliferating cells, which in turn may account for reduction in plasma homocysteine. The possibility that homocysteine egress may be dependent on the proliferation rate is supported by *in vitro* experiments (16), cited in a preceding paragraph.

The clinical consequences of altered homocysteine metabolism during methotrexate therapy have not been settled. Long-term treatment of psoriatics with methotrexate induces liver cirrhosis and

fibrosis in 20-25 % of the patients (34). Based on experimental evidence, Barak et al. (35) have suggested that liver hepatotoxicity is caused by inhibition of the 5-methyltetrahydrofolate dependent synthesis of methionine from homocysteine, which in turn impose lipotrope deficiency. The possible consequences of altered plasma homocysteine, a possible risk factor for arteriosclerotic disease, is an open but intriguing question.

Nitrous Oxide

The anaesthetic agent, nitrous oxide, once considered to be chemically inert, reacts with transition metal complexes in solution, including the cobalt-containing vitamin B12. Oxidation of cobalamin in the enzyme, 5-methyltetrahydrofolate-homocysteine methyltransferase, irreversibly inactivates this enzyme (36). The enzyme is rapidly inactivated in rodents, but biopsi data suggest that the onset of inhibition is slower in man. Plasma methionine is decreased in rat and in man during prolonged (>12 hours) nitrous oxide exposure whereas short exposure does not affect plasma methionine in man (37 and references herein). It is conceivable that plasma homocysteine is a more responsive biochemical parameter during nitrous oxide exposure, but to our knowledge, this has not been evaluated to date.

The data cited above show that methotrexate and nitrous oxide has a common biochemical target, namely the enzymatic conversion of homocysteine to methionine (Fig. 2). In addition, nitrous oxide may affect the cellular content of reduced folates according to the methyl trap mechanism. The clinical data on homocysteine in plasma of patients treated with methotrexate (32) show that this drug in clinical use affects homocysteine metabolism. Therefore, it is conceivable that the drug combination of nitrous oxide plus methotrexate, which is used in some pediatric oncology units, may have some unforeseen clinical effects (38). This possibility is supported by experimental data showing that nitrous oxide enhances the antileukemic effect of methotrexate in rats (39)

Steroids

An experimental study has recently been initiated by Thomson et al. to investigate whether constituents of the oral contraceptives may affect urinary homocysteine excretion (24, 40, 41). Such a study is motivated by the fact that oral contraceptives increase the incidence of thromboembolic disease. In addition, it has been reported that premenopausal women have a more efficient methionine metabolism than men and postmenopausal women (42).

These authors found that injection of the synthetic estrogen, ethynyl estradiol (40) as well as ethynyl estradiol disulfate (41), increased the urinary homocysteine excretion several fold. Progesteron was without effect.

Further experimental and clinical studies of the possible effect of natural and synthetic estrogens and drugs affecting estrogen metabolism on homocysteine metabolism, are warranted.

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