Cancer Chemother Pharmacol (1990) 25: 384–386

Cancer Chemotherapy and Pharmacology

Letter to the editors

Methotrexate sensitivity in Down's syndrome: a hypothesis

P. M. Ueland, H. Refsum, and B. Christensen

The Clinical Pharmacology Unit, Department of Pharmacology and Toxicology, University of Bergen, N-5021 Bergen, Norway

Sir,

Patients with Down's syndrome have an increased risk of developing leukemia [15]. The antifolate drug methotrexate (MTX) has proved to be efficient in the management of acute lymphoblastic leukemia. However, patients with Down's syndrome have a decreased tolerance to MTX and often develop severe symptoms of bone marrow and gastrointestinal toxicity [10, 18].

Proposed mechanisms. The mechanism behind the increased toxic effects of MTX in Down's syndrome has not been established; both pharmacokinetic and pharmacodynamic factors have been considered. Higher serum MTX levels and a moderate reduction in clearance have been observed in these patients compared with controls [10], whereas pharmacokinetic differences have been contested by others investigators [18]. These patients have normal serum folate but reduced erythrocyte folate, suggesting decreased body folate stores [11]. This has been explained by increased consumption of reduced folates due to an increased level of folate-dependent enzymes involved in purine synthesis [18]. The increased enzyme activities have been proposed because three such enzymes have recently been assigned to chromosome 21 and have an increased (150%) gene dosage in patients with Down's syndrome [12, 17]. The possibility of increased consumption of reduced folates in purine synthesis is conjectural and not supported by laboratory data.

MTX, folates and homocysteine. MTX is an antifolate drug that inhibits the enzyme dihydrofolate reductase, thereby blocking the regeneration of tetrahydrofolate (THF) from dihydrofolate. Intracellular MTX is a substrate for the folylpoly-γ-glutamate synthase and is converted to MTX polyglutamates [MTX$(Glu)_n$], with up to five glutamyl residues. These anabolites retain MTX within the intracellular compartment but also prevent its degradation and prolong the duration of action. Furthermore, MTX$(Glu)_n$ seem to be more potent inhibitors of dihydrofolate reductase than is the parent drug [14]. Thus, conditions that enhance polyglutamation may increase cytotoxicity and reduce drug elimination.

Homocysteine is linked to folate metabolism through the methionine synthase reaction, in which 5-methyl-THF is converted to THF by donating its methyl group to homocysteine. This is the only way in which 5-methyl-THF may enter the pool of intracellular reduced folates. The methionine synthase reaction also serves as a salvage pathway where homocysteine is converted to methionine [2, 16].

Under normal conditions, 5-methyl-THF represents a major part of the reduced folates and seems to function both as an intracellular reservoir and as a buffer between the fluctuating extracellular folate level and the intracellular pools of reduced folates necessary for DNA synthesis [4]. MTX reduces the intracellular pools of reduced folates, among which 5-methyl-THF is most efficiently depleted [1, 3]. Observations that MTX increases homocysteine egress from cultured cells [24] and plasma homocysteine levels in patients [19, 20] suggest a reduction of 5-methyl-THF below the level required for homocysteine salvage and support the MTX-homocysteine relationship.

Low levels of intracellular reduced folates have been reported to increase MTX cytotoxicity in cultured cells [4]. Inhibition of the methionine synthase reaction may conceivably enhance MTX cytotoxicity by trapping reduced folates such as 5-methyl-THF. The antifolate effect of methionine as an end-product inhibitor of the methionine synthase reaction was recognized in 1975 [8]. Notably, inhibition of this reaction (with nitrous oxide) has recently been shown to enhance the in vivo cytotoxicity of MTX in the rat [13]. Depletion of intracellular homocysteine may have a similar effect. This mechanism is analogous to the methyl-trap hypothesis proposed for cobalamin deficiency [7]. The possibility that altered methionine synthase activity may change the distribution between 5-methyl-THF and other reduced folates, thereby affecting MTX polyglutamation, is strongly supported by the recent observation that excess methionine decreased 5-methyl-THF at the expense of other reduced folates and inhibited MTX polyglutamation in hepatocytes in culture [21].

Hypothesis. A unifying hypothesis of the decreased tolerance to MTX in Down's syndrome is based on increased gene dosage of another enzyme residing on chromosome 21, namely, cystathionine β-synthase. The activity of cystathionine β-synthase is increased (166%) in

Offprint requests to: Per Magne Ueland, The Clinical Pharmacology Unit, Department of Pharmacology and Toxicology, University of Bergen, N-5021 Bergen, Norway

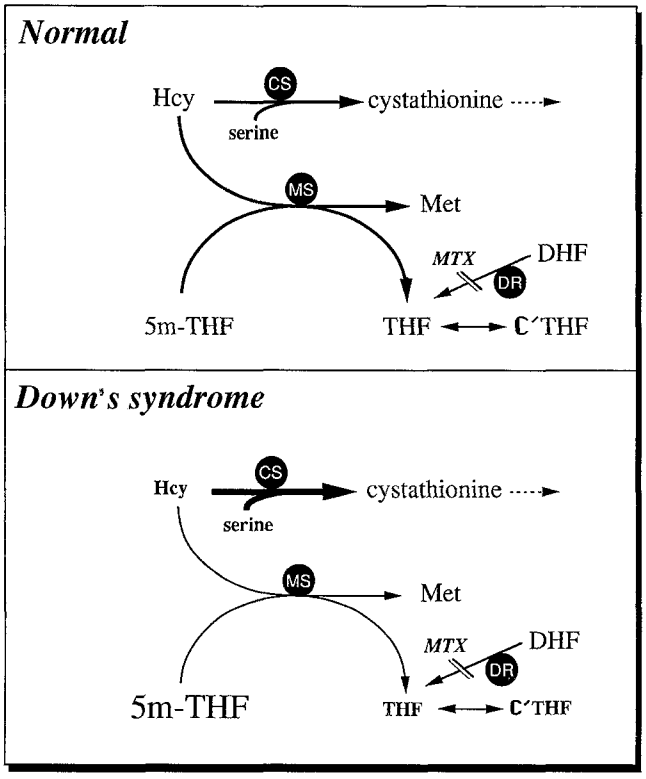

Fig. 1. Alterations in metabolic flux through homocysteine-consuming pathways and resulting changes in folate pool sizes in Down's syndrome. *CS*, cystathionine β-synthase; *DHF*, dihydrofolate; *DR*, dihydrofolate reductase; *Hcy*, homocysteine; *Met*, methionine; *5m-THF*, 5-methyl-THF; *MS*, methionine synthase (5-methyl-THF-homocysteine methyltransferase); *MTX*, methotrexate; *THF*, tetrahydrofolate

these patients [5]. This enzyme catalyzes a rate-limiting step in the catabolism of homocysteine [5]. Evidence suggesting that cystathionine β-synthase is overactive in vivo in Down's syndrome has, in fact, recently been provided by Chadefaux and co-workers [6], showing that plasma homocysteine is significantly reduced in these patients. Lack of homocysteine may retard the methionine synthase reaction that is essential for the conversion of 5-methyl-THF to THF [7]. Decreased flux through this pathway traps reduced folates such as 5-methyl-THF, the predominating species in serum [23]. This is in accordance with normal serum folate and reduced erythrocyte folates in Down's syndrome [11]. 5-Methyl-THF is a poor substrate for polyglutamate synthase, whereas most other reduced folates are efficiently polyglutamated [9, 22] and may, as substrates inhibit MTX polyglutamate formation. Conversely, low levels of these species may enhance MTX polyglutamation, thereby prolonging the duration of action of MTX [14]. Possible alterations in metabolic flux through homocysteine-consuming pathways and the resulting changes in folate pool sizes in Down's syndrome are depicted in Fig. 1.

Conclusion and perspectives. The hypothesis described above encompasses the existing knowledge on folates and MTX in Down's syndrome. The proposed metabolic defect, i. e. homocysteine depletion, would conceivably enhance the folate depletion caused by increased purine and pyrimidine synthesis, as previously suggested by others [18]. Homocysteine depletion has, in fact, recently been verified in vivo by demonstration of reduced plasma homocysteine in these patients [6]. In addition, this model has some potentially important implications that warrant investigation. First, determination of plasma homocysteine values before or during MTX administration may identify those at increased risk of developing severe toxicity; second, homocysteine administration may reduce MTX toxicity.

References

1. Allegra CJ, Fine RL, Drake JC, Chabner BA (1986) The effect of methotrexate on intracellular folate pools in human MCF-7 breast cancer cells. Evidence for direct inhibition of purine synthesis. J Biol Chem 261: 6478 – 6485
2. Barak AJ, Tuma DI, Beckenhauer HC (1984) Methotrexate hepatotoxicity. J Am Coll Nutr 3: 93 – 96
3. Baram J, Allegra CJ, Fine RL, Chabner BA (1987) Effect of methotrexate on intracellular folate pools in purified myeloid precursor cells from normal human bone marrow. J Clin Invest 79: 692 – 697
4. Bunni M, Doig MT, Donato H, Kesavan V, Priest DG (1988) Role of methylenetetrahydrofolate depletion in methotrexate-mediated intracellular thymidylate synthesis inhibition in cultured L1210 cells. J Biol Chem 48: 3398 – 3404
5. Chadefaux B, Rethore MO, Raoul O, Ceballos I, Poissonnier M, Gilgenkrantz S, Allard D (1988) Cystathionine beta synthase: gene dosage effect of trisomy 21. Biochem Biophys Res Commun 128: 40 – 44
6. Chadefaux B, Ceballos I, Hamet M, Coude M, Poissonnier M, Kamoun P, Allard D (1988) Is absence of atheroma in Down's syndrome due to decreased homocysteine levels? Lancet II: 741
7. Chanarin I, Deacon R, Lumb M, Muir M, Perry J (1985) Cobalamin-folate interrelations: a critical review. Blood 66: 479 – 489
8. Cheng FW, Shane B, Stokstad ELRN (1975) The anti-folate effect of methionine on bone marrow of normal and vitamin B 12-deficient rats. Br J Haematol 31: 323 – 336
9. Cichowicz DJ, Shane B (1987) Mammalian folylpoly-γ-glutamate synthetase: 2. Substrate specificity and kinetic properties. Biochemistry 26: 513 – 521
10. Garre ML, Relling MV, Kalwinsky D, Dodge R, Crom WR, Abromowitch M, Pui CH, Evans WE (1987) Pharmacokinetics and toxicity of methotrexate in children with Down's syndrome and acute lymphocytic leukemia. J Pediatr 111: 606 – 612
11. Gericke GS, Hesseling PB, Brink S, Tiedt FC (1977) Leucocyte ultrastructure and folate metabolism in Down's syndrome. S Afr Med J 51: 369 – 373
12. Hards RG, Benkovic SJ, Van Keuren ML, Graw SL., Drabkin HA, Patterson D (1986) Assignment of a third purine biosynthetic gene (glycinamide ribonucleotide transformylase) to human chromosome 21. Am J Hum Genet 39: 179 – 185
13. Kroes ACM, Lindemans J, Schoester M, Abels J (1986) Enhanced therapeutic effect of methotrexate in experimental rat leukemia after inactivation of cobalamin (vitamin B12) by nitrous oxide. Cancer Chemother Pharmacol 17: 114 – 120
14. Matherly LH, Seither RL, Goldman ID (1987) Metabolism of diaminoantifolates: biosynthesis and pharmacology of the 7-hydroxyl and polyglutamyl metabolites of methotrexate and related antifolates. Pharmacol Ther 35: 27 – 56
15. Miller RW (1970) Neoplasia and Down's syndrome. Ann NY Acad Sci 171: 637 – 644
16. Mudd SH, Levy HL (1983) Disorders of transsulfuration. In: Standbury JB (ed) Metabolic basis of inherited diseases. McGraw-Hill, New York, pp 522 – 559

17. Patterson D, Graw S, Jones C (1981) Demonstration by somatic cell genetics of coordinate regulation of genes for two enzymes of purine synthesis assigned to human chromosome 21. Proc Natl Acad Sci USA 78: 405–409
18. Peeters M, Poon A (1987) Down's syndrome and leukemia: unusual clinical aspects and unexpected methotrexate sensitivity. Eur J Pediatr 146: 416–422
19. Refsum H, Ueland PM, Kvinnsland S (1986) Acute and long-term effects of high-dose methotrexate treatment on homocysteine in plasma and urine. Cancer Res 46: 5385–5391
20. Refsum H, Helland S, Ueland PM (1989) Fasting plasma homocysteine as a sensitive parameter of antifolate effect. A study on psoriasis patients receiving low-dose methotrexate treatment. Clin Pharmacol Ther (in press)
21. Rhee MS, Johnson TB, Priest DG, Galivan J (1989) The effect of methionine on methotrexate metabolism in rat hepatocytes in monolayer culture. Biochim Biophys Acta 1011: 122–128
22. Shane B (1986) Folylpoly-γ-glutamate synthetase. In: Cooper BA, Whitehead VM (eds) Chemistry and biology of Pteridines 1986, Walter de Gruyter, Berlin New York, pp 719–728
23. Steinberg SE (1984) Mechanism of folate homeostasis. Am J Physiol 246: G319–G324
24. Ueland PM, Refsum H, Male R, Lillehaug J (1986) Disposition of endogenous homocysteine by mouse fibroblast C3H/10T1/2 CI 8 and the chemically transformed C3H/10T1/2MCA CI 16 cells following methotrexate exposure. J Natl Cancer Inst 77: 283–289

Received 27 April 1989/Accepted 13 August 1989