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Computer modelling of antifolate inhibition of folate metabolism using hybrid functional petri nets

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Abstract

Antifolates are used in the treatment of various human malignancies and exert their cytotoxic activity by inhibiting folate-dependent enzymes resulting in disruption of DNA synthesis and cell death. Here we devised a computerized hybrid functional petri nets (HFPN) modelling of folate metabolism under physiological and antifolate inhibitory conditions. This HFPN modelling proved valid as a good agreement was found between the simulated steady-state concentrations of various reduced folates and those published for cell extracts; consistently, the simulation derived total folate pool size (11.3 μM) was identical to that published for cell extracts. In silico experiments were conducted to characterize the inhibitory profile of four distinct antifolates including methotrexate (MTX), tomudex, and LY309887, which inhibit dihydrofolate reductase (DHFR), thymidylate synthase (TS) and glycineamide ribonucleotide transformylase (GARTFase), respectively, as well as pemetrexed which has the capacity to inhibit all three enzymes. In order to assess the inhibitory activity of antifolates on purines and pyrimidines, the biosynthesis rates of IMP (20.53 μM/min) and dTMP (23.8 μM/min) were first simulated. Whereas the biochemical inhibitory profile of MTX was characterized by increased dihydrofolate and decreased tetrahydrofolate (THF) concentrations, the remaining antifolates did not decrease THF levels. Furthermore, MTX was 766- and 10-fold more potent in decreasing the production rates of IMP and dTMP, respectively, than pemetrexed. LY309887 indirectly decreased the rate of dTMP production by reducing the levels of 5-CH_2-THF, a folate cofactor for TS. Surprisingly, pemetrexed failed to inhibit DHFR even at high concentrations. This HFPN-based simulation offers an inexpensive, user-friendly, rapid and reliable means of pre-clinical evaluation of the inhibitory profiles of antifolates.

Keywords: Folate metabolism; Purine; Pyrimidine; Enzyme inhibition; Antifolates

1. Introduction

Reduced folate cofactors serve as one-carbon transfer donors in several biosynthetic reactions of purines and pyrimidines essential for DNA replication as well as for the methionine cycle necessary for methylation reactions (Stockstad, 1990; Bailey, 1995; Choi and Mason, 2000). As such, folate-dependent enzymes catalysing this biosynthetic pathway have long been recognized as targets for antifolates used in the treatment of various human malignancies. Methotrexate (MTX), a potent inhibitor of the purine and pyrimidine biosynthesis key enzyme dihydrofolate reductase (DHFR, Fig. 1A), was first introduced as a folic acid analogue and has shown efficacy in the treatment of acute lymphoblastic leukemia, osteosarcoma, breast cancer and choriocarcinoma (Bertino, 1993).

The involvement of reduced folates in various reactions that are essential for the biosynthesis of AMP, GMP and thymidylate has led to the development of novel antifolates that specifically and potently target (i.e. inhibit) key folate-dependent enzymes (Jackman, 1999). Among

Abbreviations: HFPN, hybrid functional petri nets; GON, genomic object net; DHF, dihydrofolate; THF, tetrahydrofolate; 5-CHO-THF, 5-formyl-THF; 5, 10-CH_2-THF, 5, 10-methylene-THF; MTX, methotrexate; IMP, inosine monophosphate; DHFR, dihydrofolate reductase; TS, thymidylate synthase; GARTF, glycineamide ribonucleotide transformylase; CH, methenyl cyclohydrolase; SHT, serine hydroxymethyl transferase

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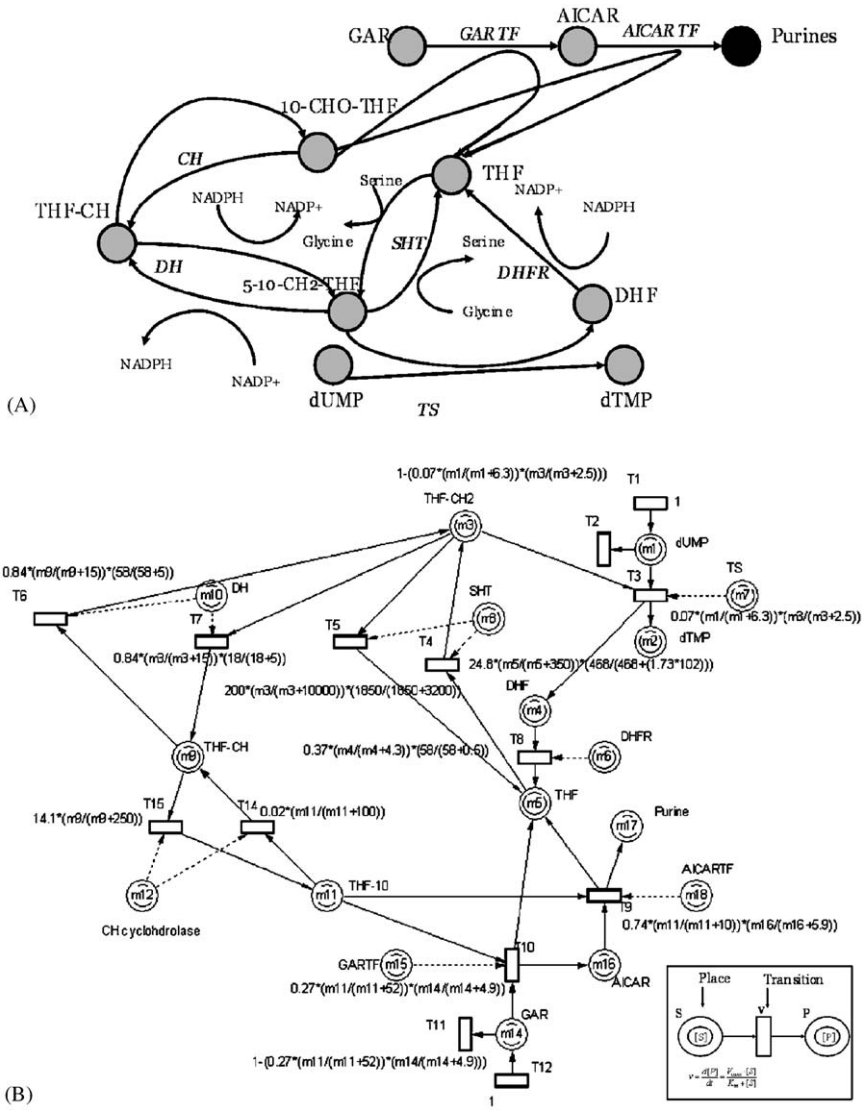

Fig. 1. The pathway of folate metabolism (A) and its HFPN implementation (B).

these are Tomudex (Raltitrexed), a TS inhibitor (Jackman et al., 1991), LY309887, an inhibitor of glycinamide ribonuleotide transformylase (GARTF) (Mendelsohn et al., 1996) and Pemetrexed, a multitargeted antifolate which has the cell-free capacity to simultaneously inhibit TS, GARTF and DHFR (Shih et al., 1997). Tomudex was approved for the treatment of advanced colorectal cancer, whereas Pemetrexed showed activity against a variety of tumors and has been recently registered for the treatment of malignant mesothelioma (Hazarika et al., 2005).

The central cellular role that folate metabolism plays has led to extensive experimental research aimed at delineating the characteristics of this pathway under antifolate inhibitory conditions. However, an experimental understanding of the dynamics of this intracellular pathway as well as other metabolic pathways requires significant resources and is clearly time and labour-intensive. In contrast, powerful computer simulations can be highly informative and yet minimize the time and cost. Appropriate simulation tools and methods empower life scientists with the opportunity to perform virtual experiments that entail both quantitative as well as qualitative analysis (Levasseur et al., 1998). Several bio-simulation methods attempt to model various aspects of a different biological pathways, such as metabolism, signal transduction, and gene regulation pathways (Matsuno et al., 2003a, b; Doi et al., 2004; Nijhout et al., 2004). Indeed, the pathway of folate metabolism was first simulated by Seither et al. (1989, 1990, 1991) under physiological and antifolate inhibitory conditions using the old version of the electrical circuits simulation tool SPICE2 (see SPICE); folate metabolism was subsequently modeled with emphasis on folate homeostasis (Nijhout et al., 2004). However, SPICE2 was never intended for biological purposes and using it demands a high level of skill in electrical engineering. Moreover, the development of many rationally designed novel antifolates and the increasing availability of many kinetic constants along the folate