



EFFECTS OF MACROPHAGE- AND GRANULOCYTE-COLONY STIMULATING FACTORS ON THYMIDYLATE SYNTHASE AND THYMIDINE KINASE ACTIVITY IN RAT HEMATOPOIETIC CELLS

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ABSTRACT

The effects of macrophage (M) and granulocyte (G) colony-stimulating factors (CSFs) on the activity of thymidylate synthase and thymidine kinase, which are involved in *de novo* and salvage pathways for pyrimidine nucleotide synthesis, were investigated in the hematopoietic cells of rats treated with cyclophosphamide. Thymidine kinase activity, but not that of thymidylate synthase, was markedly enhanced in these cells by M- and G-CSF treatment

($p < 0.05$ and $p < 0.01$). G-CSF directly, and M-CSF indirectly stimulate myeloid cells and lead to S-phase predominantly *via* the salvage pathway for pyrimidine nucleotide synthesis. The present study indicates that these CSFs can be effective inducers of complete remission in acute leukemias when employed together with chemotherapy.

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Key words: G-CSF, M-CSF, thymidine kinase, rat bone marrow cells

Granulocyte colony-stimulating factor (G-CSF) is used more frequently than macrophage-CSF (M-CSF) after intensive chemotherapy for patients with cancer.

Thymidylate synthase (TS; EC 2.1.1.45) and thymidine kinase (TK; EC 2.7.1.21) catalyze the formation of deoxythymidine monophosphate (dTMP) by the methylation of deoxyuridine monophosphate (dUMP), with the concomitant conversion of N⁵, N¹⁰-methylene tetrahydrofolic acid to 7,8-dihydrofolic acid *via* the *de novo* pathway and by thymidine phosphorylation *via* the salvage pathway. TS and TK activity is high in rapidly proliferating normal, fetal and neoplastic tissues.¹⁻⁴

In this study, we investigated the effects of M- and G-CSFs on TS and TK activity in the hematopoietic cells of rats treated with cyclophosphamide (Cy).

Materials and Methods

Twelve-week-old male Sprague-Dawley rats were given cyclophosphamide (Cy; 15.0 mg/100 g body weight) by a single intraperitoneal injection with or without a daily subcutaneous injection of M-CSF (0.32×10^6 U/100 g body weight) or G-CSF (10 µg/100 g body weight). The normal control group was given the vehicle for 14 days without Cy injection, and the CSF-control groups were given M- or G-CSF for 7 days without Cy.

Bone marrow cells were flushed from femurs with phosphate-buffered saline (PBS) including 3 mM EDTA, filtered with a cell strainer, washed three times with PBS and stored at -80°C after recording the nucleated cell count (NCC) and the differential count.

TS and TK activity in bone marrow cells was determined by the methods of Dunlap *et al.*¹ and Taylor *et al.*² respectively. Enzyme activity was normalized to the NCC and expressed as pmol/min/femur.

Student's t-test and Wilcoxon's rank test were applied and $P < 0.05$ was considered significant.

Results

Numbers of myeloid series cells in the bone marrow

The myeloid series in bone marrow cells abruptly decreased, reaching a nadir at 3-5 days after Cy injection, and then increased starting from day 7 until a peak was reached at day 14 (data not shown). Daily injections of CSFs markedly raised the number of myeloid series cells at day 7 after Cy ($p < 0.01$). M- and G-CSF enhanced the NCC of the myeloid series to about 1.7- and 2.0-fold, respectively, that of the Cy-control group without CSFs. However, 14 days after Cy administration there was no difference among groups. CSFs given to rats for 7 days in the absence of Cy increased the number of myeloid series cells to over 1.3 times that of the control ($p < 0.05$).

TS and TK activity in bone marrow cells

TS activity in bone marrow cells differed little among the groups given Cy. Daily injections of M-CSF transiently increased TS activity 3 days after Cy administration, though the differences were not statistically significant (Figure 1a).

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Thymidylate synthase activity
(pmol/minute/femur)

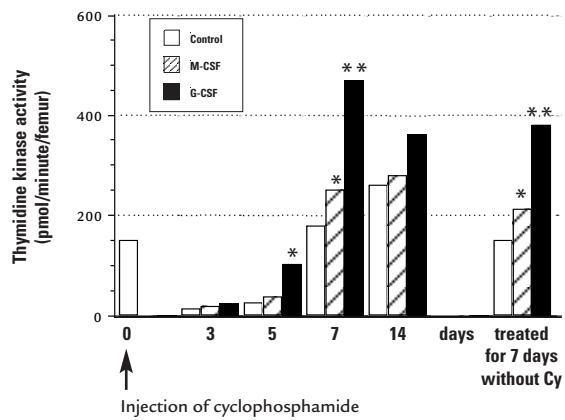


Figure 1. Activity of thymidylate synthase (TS)(upper; 1a) and thymidine kinase (TK)(lower; 1b) in bone marrow cells from rats given daily injections of CSFs after cyclophosphamide (Cy) treatment or not.

M-CSF: macrophage colony-stimulating factor; G-CSF: granulocyte colony-stimulating factor.

* $p < 0.05$ and ** $p < 0.01$: significant difference with respect to the corresponding groups not given CSF.