Cancer Chemother Pharmacol (1982) 10: 1–6

Cancer Chemotherapy and Pharmacology

Review

Thymidine as an Anticancer Agent, Alone or in Combination

A Biochemical Appraisal

Peter H. Ellims

Monash University Department of Medicine, Alfred Hospital, Prahran, 3181, Victoria, Australia

Summary. *The value of thymidine as a cytotoxic drug alone or in combination with other pyrimidine antimetabolites has received considerable attention in recent years.*

In this paper, the biochemical basis for the cytotoxicity of thymidine and its interaction with other pyrimidine antimetabolites is described. It is indicated that early clinical trials have largely failed to substantiate data from experimental studies that have shown thymidine to be an effective antimetabolite and capable of potentiating the antineoplastic effect of several other agents.

It is suggested that tumours likely to respond to thymidine alone or in combination may be identified by measuring in clinical tumour specimens known biochemical determinants of thymidine efficacy.

Introduction

The growth-inhibitory and cytotoxic effects of the pyrimidine nucleoside thymidine on mammalian cells in vitro have been known for two decades and have been used extensively to synchronize mammalian cells in the S phase (DNA synthesis) of the cell cycle [49, 52, 64]. However, the potential clinical value of these phenomena has only recently received attention. Interest in the use of thymidine as an anticancer agent has developed since the nucleoside has been found to produce tumor regression in human tumor xenograft models [22, 37, 39], and the intracellular biochemical changes produced by exogenous thymidine appear to be capable of modulating the cytotoxic effects of some of the antimetabolites used widely in clinical practice [43]. Thymidine is now undergoing clinical assessment both as a single agent and in combination with other antimetabolites.

In this paper, current concepts of the biochemical basis for thymidine cytotoxicity and the interaction of the nucleoside with other antimetabolites will be reviewed, and their relevance to the available data on the clinical efficacy of thymidine will be critically discussed.

Thymidine Alone

The biochemical basis for these effects of thymidine is believed to be mediated by an increase in the intracellular content of the metabolite thymidine 5′-triphosphate (TTP) (Fig. 1). TTP

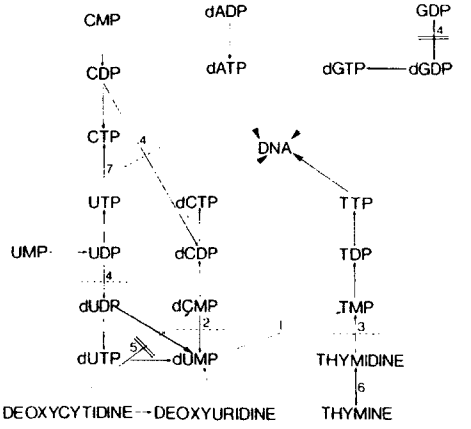

Fig. 1. Deoxyribonucleotide metabolism. Thymidine metabolism and effects of TTP. 1, thymidylate synthetase; 2, deoxycytidylate deaminase; 3, thymidine kinase; 4, ribonucleotide reductase; 5, deoxycytidine kinase; 6, thymidine phosphorylase; 7, CTP synthetase. *Broken lines* indicate inhibition by TTP; *double bars* indicate activation by TTP

exerts a significant regulatory effect over several key pyrimidine enzymes (Fig. 1), inhibiting thymidine kinase and deoxycytidylate deaminase and activating deoxycytidine kinase, while thymidylate synthetase is indirectly controlled by TTP modulation of the intracellular deoxyuridine 5′-monophosphate (dUMP) pool [10–12, 29, 30]. However, the major effect of exogenous thymidine is believed to be inhibition of ribonucleotide reductase. TTP is an allosteric inhibitor of ribonucleotide reductase and inhibits the reduction of cytidine 5′-diphosphate (CDP) to deoxycytidine 5′-diphosphate (dCDP) (Fig. 1), resulting in a decrease in the size of the dCTP pool, which is a critical determinant of cell deoxyribonucleic acid (DNA) synthesis [14, 54]. As a consequence of dCTP pool depletion, DNA synthesis and cell proliferation are suppressed [3, 54]. This concept is supported by the ability of deoxycytidine to reverse the cytotoxic effect of thymidine [17]. A less important effect of TTP is the allosteric activation of the ribonucleotide reductase-catalyzed reduction of guanosine 5′-diphosphate (GDP) to deoxyguanosine 5′-diphosphate (dGDP) (Fig. 1), which leads to expansion of cellular dGTP content [3].

Interest in the possible clinical use of thymidine as anticancer agent developed from the finding that continuous infusions of thymidine produced regression in human tumor xenografts borne by nude mice [22, 37, 39]. Furthermore,

PHE is an Applied Health Fellow of the National Health and Medical Research Council of Australia

0344-5704/82/0010/0001/$ 01.20

other data suggest that some human tumor cell lines are selectively sensitive to thymidine when compared with their non-neoplastic counterparts [38, 55, 61]. The biochemical basis for this phenomenon has been determined in vitro, using cultured human T and null leukemia cell lines, which are extremely sensitive to low concentrations of thymidine compared with B cell lines [15, 16, 18]. In these cell lines, increased sensitivity to thymidine correlates with a sustained elevation in the intracellular TTP pool, which is due to the lack of or low activity in T and null leukemic cells of enzymes capable of catabolizing thymidine and its nucleotides [5, 15, 18].

Current data on the clinical activity of thymidine as an anticancer agent indicate that the nucleoside alone has limited effectiveness [4]. However, partial responses have been observed in acute non-lymphocytic leukemia and in T cell leukemia at thymidine doses in the order of 75 g/m^2/day [4, 25, 27, 36]. These responses occurred in previously treated patients, and correlated with greater activity of the thymidine anabolic than of the thymidine catabolic pathway, the intracellular accumulation of TTP, and arrest in leukemic cell DNA synthesis [25, 27, 36]. These findings are consistent with the experimental concepts, previously described, that TTP is the active metabolite of thymidine and that its cytotoxic effect occurs through inhibition of DNA synthesis. Toxicities encountered included myelosuppression, gastrointestinal side-effects (anorexia, nausea, vomiting, diarrhea), and neurologic sequelae, including headache, somnolence, illusions and memory impairment [4, 36]. A wide range of other tumor types failed to respond [4]. Although further clinical studies are required to establish the place of thymidine as a single anticancer agent, it seems likely that its effectiveness will be limited to tumors composed of cells with the ability to adequately expand intracellular TTP content after exposure to clinically tolerable concentrations of exogenous thymidine.

Thymidine and Cytosine Arabinoside (Ara-C)

Ara-C is a primary agent for the treatment of acute leukemia [9]. The active metabolite is are-C 5′-triphosphate (ara-CTP), which produces cytotoxicity by inhibition of DNA polymerase and the direct incorporation of ara-C into DNA [7, 40, 65]. Both these effects are opposed by the naturally occurring nucleotide dCTP, which also inhibits deoxycytidine kinase, the rate-determining enzyme for incorporation of ara-C into the nucleotide pool [9]. As a consequence, the intracellular level of dCTP is an important determinant of sensitivity to ara-C. Ara-C cytotoxicity is cell-cycle phase-specific, being limited to the S phase of the cell cycle [1]. In view of these data, there is considerable experimental and clinical interest in the combination of thymidine and ara-C.

An early study using cultured Novikoff hepatoma cells showed that thymidine increased the incorporation of ara-C into DNA, but without alteration in ara-C nucleotide content [53]. In another study, the sensitivity of some types of cultured mouse tumor cells to ara-C was found to be markedly enhanced by thymidine concentrations which were below the level required for growth inhibition by thymidine alone [21]. The phenomenon occurred in two lymphoma lines at a thymidine concentration as low as 10 μM. In keeping with present concepts of the biochemical pharmacology of ara-C, the enhancement in the cytotoxicity of ara-C produced by thymidine directly correlated with a fall in tumor cell dCTP content. Conversely, absence of the thymidine effect was associated with lack of significant change in the dCTP pool, despite an appropriate rise in intracellular TTP content. The reason(s) for the absence of a reduction in dCTP levels in response to TTP pool expansion are unclear, but may involve an alteration in ribonucleotide reductase sensitivity to thymidine. However, the phenomenon also draws attention to the potential importance of other enzymes in regulating the dCTP pool. Amongst these enzymes are deoxycytidylate deaminase and cytidine 5′-triphosphate (CTP) synthetase (Fig. 1), which have been found to make an important contribution to the dCTP pool of some mammalian cell lines [10, 30, 56]. Several investigations have found that thymidine enhances the cytotoxicity of ara-C to L1210 mouse leukemia cells and this effect is both schedule- and exposure duration-dependent [19, 33, 41]. Optimum cytotoxicity of the thymidine and ara-C combination required exposure of L1210 cells to in excess of 100 μM thymidine for at least 5 h before ara-C treatment. In keeping with the role given to dCTP pool depletion in the cytotoxicity of this combination, the addition of deoxycytidine or cytidine to the culture medium abolished the thymidine effect. The optimum cytotoxic combination of thymidine and ara-C for L1210 cells correlated with expected biochemical changes, which were a substantial reduction in dCTP content, increased ara-CTP formation, and enhanced incorporation of ara-C into DNA. The results from an in vitro study of the efficacy of the thymidine and ara-C combination against human leukemic blast cells, and a human B cell line [59]), diverged from the findings in the L1210 model. The thymidine and ara-C combination was synergistic against a virus transformed B cell line with the anticipated biochemical phenomena predicted by the L1210 model. However, at clinically relevant concentrations of thymidine (1 mM) and ara-C (0.1 μM) different effects occurred in leukemic blast cells. Thymidine produced a uniform reduction in ara-C nucleotide formation, and only a marginal enhancement in ara-C incorporation into DNA. Although these in vitro results were not correlated with clinical responsiveness to the thymidine and ara-C combination, they do suggest the combination will only be effective in subgroups of acute leukemia. Other in vitro studies have indicated another potentially serious limitation to the clinical use of the thymidine and ara-C combination. A mutant clone of Chinese hamster fibroblasts resistant to ara-C as a result of dCTP pool expansion exhibits simultaneous resistance to thymidine and ara-C, as the expanded dCTP pool is not suppressed by thymidine [56, 57]. The biochemical basis for the dCTP pool expansion is an alteration in the regulation of the CTP synthetase activity of these cells, with loss of the normal CTP feedback inhibition on enzyme activity. These studies indicate that a single-step mutation could produce clinical resistance to both thymidine and ara-C, thereby limiting the clinical efficacy of the combination. Although dCTP pool expansion could be suppressed by the addition of the investigational drug 3-deazauridine [46], it is not known whether the mutant CTP synthetase enzyme is sensitive to inhibition by the active metabolite deaza-uridine-5′-triphosphate (deaza-UTP).

In vivo data on the efficacy of the thymidine and ara-C combination obtained from mice bearing L1210 leukemia cells are consistent with the findings of cell culture studies and emphasize the importance of pretreatment with thymidine to obtain maximum enhancement of ara-C efficacy [33]. Similarly, in another in vivo study, which used a rat-borne colon carcinoma model, enhanced tumor sensitivity to ara-C was achieved with thymidine pretreatment, but increased normal tissue toxicity was also observed [8]. Of some interest was the

observation in this study that pretreatment with thymidine produced a marked decrease in plasma deoxycytidine levels. The mechanism of this is unclear, but it may result from the lower intracellular deoxycytidine nucleotide pools produced by exogenous thymidine [14, 54].

There is a paucity of clinical data on the efficacy of the thymidine and ara-C combination. In a single phase III clinical trial of simultaneous thymidine (8 g/m^2) and ara-C (250 mg/m^2) infusions for 7–12 days in 21 cases of relapsed adult acute leukemia [62], responses were seen in eight patients, all of whom had previously received ara-C. In six patients complete remission was achieved, while two had only partial responses. Responses were seen in acute non-lymphocytic leukemia and the blastic crisis of chronic myeloid leukemia, but not in acute lymphoblastic leukemia. This result is consistent with an in vitro study of human leukemic lymphoblasts, in which thymidine failed to enhance the ara-C effect in lymphoblasts [59]. The duration of response ranged from 28 to in excess of 233 days. These data suggest that a thymidine and ara-C combination may be effective in the treatment of acute leukemia, and the results of further clinical trials are awaited.

Thymidine and 5-Aza-2'Deoxycytidine (5-Aza-dCyd)

5-Aza-dCyd is an investigational anticancer agent that exhibits cytotoxicity against mouse leukemia cells both in vitro and in vivo [44, 45]. The biochemical mechanism of action of 5-aza-dCyd is unclear, but the available data suggest that the incorporation of 5-aza-dCyd into DNA is a major contributor to the cytotoxicity of the antimetabolite, and that this phenomenon is opposed by dCTP [46]. These biochemical data are similar to those for ara-C, and it is probable that thymidine, through its TTP-mediated suppressive effect on cellular dCTP content, would enhance the cytotoxicity of 5-aza-dCyd. A single in vitro study of a thymidine and 5-aza-dCyd combination in L1210 cells and HL-60 human promyelocytic leukemia cells [46] has shown very similar results to those described for the thymidine and ara-C combination. The pretreatment of both cell lines with thymidine produced significant potentiation in 5-aza-dCyd cytotoxicity. For an optimum thymidine effect, the two cell lines differed with respect to the duration of exposure and concentration of thymidine required. For the human leukemic cell line these were clinically relevant, being a 24-h exposure to 1 m*M* exogenous thymidine. The thymidine effect was not dependent on the 5-aza-dCyd concentration over the concentration range 1–100 μ*M*. Thymidine enhancement of 5-aza-dCyd cytotoxicity correlated with substantially increased formation of 5-aza-dCyd nucleotides and the incorporation of 5-aza-dCyd into DNA. The dose- and schedule-dependent thymidine enhancement of 5-aza-dCyd cytotoxicity found in this study could be of considerable value for the design of future clinical trials.

Thymidine and Methotrexate

Since it was first demonstrated that thymidine could reverse methotrexate toxicity in mice bearing L1210 leukemia cells without loss of anticancer activity [60], considerable interest has arisen in the interaction between these antimetabolites. Particular attention has been given to the apparent ability of thymidine to enhance methotrexate selectivity for tumor cells. The cytotoxic action of methotrexate is due to inhibition of dihydrofolate reductase, which leads to depletion of the intracellular pool of tetrahydrofolate cofactors, a biochemical abnormality resulting in impaired TTP synthesis and an antipurine effect [6, 47]. The relative importance of the two effects varies between different cells, including normal and neoplastic types [31, 32, 60]. The phenomenon of thymidine enhancement of the selectivity of methotrexate for tumor cells has been ascribed to the persistence of the antipurine effect of methotrexate in tumor cells [31, 32, 60]. The biochemical basis of this is that tumor cells in vitro are dependent upon de novo synthesis of purines, while normal tissues including bone marrow and intestinal epithelium preferentially use preformed plasma purines and are protected from the block in TTP synthesis by exogenous thymidine [24]. Recent in vitro experimental data indicate that other factors also determine the efficacy of this combination [31, 48]. Of importance is the temporal relationship in the use of the two antimetabolites. Prior treatment with thymidine antagonizes the cytotoxic effects of methotrexate in both normal and neoplastic cell lines, by depleting dUMP levels and therefore limiting the thymidylate synthetase reaction and the consumption of tetrahydrofolate cofactors. In contrast, thymidine given after methotrexate treatment provides a substantial degree of protection for normal cell lines but not for neoplastic cells. This differential effect is most marked in neoplastic cells with a high rate of thymidylate biosynthesis and therefore greater depletion of tetrahydrofolate content. Another biochemical factor of likely importance to the efficacy of selective thymidine 'rescue' from methotrexate cytotoxicity is the ratio between the activities of thymidine anabolic and catabolic pathways in human cells [28], which will determine the ability of exogenous thymidine to bypass the block in TTP synthesis.

Clinical data have not confirmed the experimental concept of thymidine enhancement of methotrexate selectivity for tumor cells. Clinical trials have shown thymidine to be capable of reversing methotrexate toxicity in vivo, but it has yet to be clearly demonstrated that thymidine enhances methotrexate selectivity for tumor cells [13, 23, 24, 26]. In fact, recent data on human marrow and tumor xenograft cells indicate that in view of the wide range of human plasma thymidine levels and high bone marow and tumor hypoxanthine content, differences in the requirements of these cells for thymidine and hypoxanthine for protection against methotrexate toxicity would be insufficient to allow for selectivity [28].

The optimum regimen for thymidine rescue of methotrexate toxicity is not yet established. When high-dose methotrexate (3 g/m^2) is given as a single bolus dose, there is a steep dose-response relationship between serum thymidine and rescue of normal tissues, and adequate rescue is achieved when thymidine is given at a dosage of 1 g/m^2/day, 24 h after the methotrexate [26]. This is considerably lower than the 8 g thymidine per m^2 per day given in other studies using continuous methotrexate infusion [13, 23, 24], and could enhance the antitumor effect of methotrexate on the basis of a differential requirement for thymidine between normal and neoplastic cells [26]. Thymidine rescue of methotrexate toxicity can be associated with significant toxicity [13, 23, 26], and it remains to be documented whether thymidine rescue of methotrexate toxicity is clinically superior to the established folinic acid rescue technique.

Thymidine and 5-Fluorouracil (5-FU)

The pyrimidine analogue 5-FU exerts its cytotoxic effect through two biochemical mechanisms, both of which require

Table 1. Antimetabolites for which modulation of cytotoxicity by thymidine has been demonstrated in vitro

Agent	Biochemical mechanism (mediated by TTP)	Result
Cytosine arabinoside (ara-C)	Inhibition of ribonucleotide reductase	Decreased dCTP pool, enhanced ara-CTP inhibition of DNA polymerase, increased ara-C incorporation into DNA
	Stimulation of deoxycytidine kinase	Increased ara-CTP formation
5-Aza-2′-deoxycytidine (5-aza-dCyd)	Inhibition of ribonucleotide reductase	Decreased dCTP pool, enhanced 5-aza-dCyd incorporation into DNA
5-Fluorouracil (5-FU)	Inhibition of ribonucleotide reductase	Increase 5-FUTP formation, enhanced 5-FU incorporation into RNA
Methotrexate	Bypass of block to TTP synthesis	Antipurine effect retained, which is selective for tumor cells

intracellular activation of the drug. The metabolite 5-fluorodeoxyuridine 5′-monophosphate (5-FdUMP) is a tight-binding inhibitor of thymidylate synthetase, an effect which results in TTP pool depletion and impaired DNA synthesis [2, 42, 50]. The alternative active metabolite is 5-fluorouridine 5′-triphosphate (5-FUTP), which interferes with ribonucleic acid (RNA) metabolism in a number of different ways, but particularly through direct incorporation of 5-FU into RNA [2, 35, 42]. The relative importance of the DNA and RNA effects for the cytotoxicity of 5-FU appears to vary with the cell types examined [2, 43], and this probably in part reflects the relative effectiveness of the metabolic pathways determining either 5-FdUMP or 5-FUTP formation. In vitro experiments have shown that thymidine antagonizes the cytotoxicity of 5-FU for some cultured tumor cells, suggesting that inhibition of thymidylate biosynthesis is the major effect of 5-FU [2, 43]. However, recent data from a variety of experimental mammalian tumor systems have shown that when thymidine is used to bypass the anti-DNA effect of 5-FU there is actual enhancement of 5-FU cytotoxicity [2, 51, 58]. This suggests that interference with RNA metabolism is the primary mode of action of 5-FU in these cell lines. The biochemical basis for this thymidine effect is thought in the main to be mediated by TTP inhibition of ribonucleotide reductase reduction of 5-fluorouridine-5′-diphosphate (5-FUDP) to 5-fluoro-deoxyuridine-5′-diphosphate (5-FUDP) [2], thus promoting the formation of 5-FUTP. In addition, excess thymidine also suppresses the catabolism of 5-FU and its metabolites [34]. Of potential clinical interest is the finding in experimental tumors that while thymidine selectively enhances 5-FU incorporation into the RNA of sensitive tumors, the effect is virtually absent in normal bone marrow and gut cells [58]. This suggests that thymidine could be used to protect normal cells against 5-FU cytotoxicity while potentiating the antitumor effect of 5-FU.

The preliminary clinical data from two studies of patients with advanced solid tumors are at variance with these experimental findings, as there is no in vivo evidence that thymidine enhances the antitumor effects of 5-FU [34, 63]. Furthermore, in vivo thymidine enhances 5-FU bone marrow toxicity [63]. The use of thymidine appears to enhance 5-FU toxicity by competing with 5-FU for catabolic pathway enzymes, which thereby prolongs 5-FU clearance [34]. More clinical data are needed on the combination of thymidine and 5-FU before conclusions on the antitumor efficacy of this combination can be reached.

Conclusion

Although the biochemical basis of the cytotoxic action of thymidine and its interaction with several pyrimidine antimetabolites is well defined in experimental systems (Table 1) its clinical role is speculative, and further studies are needed. An important finding from early clinical trials is the considerable lack of correlation between the results of experimental studies and these trials. The reasons for this are unclear, but must include the greater biochemical heterogeneity of clinical tumors than of experimental models. Future clinical trials should include direct measurement in isolated tumor cells of the biochemical determinants of thymidine efficacy which have been identified from experimental studies, so that tumors likely to benefit from therapy with thymidine either alone or in combination can be identified.

References

1. Aglietta M, Colly L (1979) Relevance of recruitment-synchronization in the scheduling of 1-β-D-arabinofuranosyl-cytosine in a slow-growing acute myeloid leukemia of rat. Cancer Res 39: 2727
2. Ardalan B, Glazer R (1981) An update on the biochemistry of 5-fluorouracil. Cancer Treat Rev 8: 157
3. Bjursell G, Reichard P (1973) Effects of thymidine on deoxyribonucleoside triphosphate pools and deoxyribonucleic acid synthesis in Chinese hamster ovary cells. J Biol Chem 248: 3904
4. Bruno S, Poster DS, Bono VH Jr, MacDonald JS, Kubota TT (1981) "High-dose" thymidine in clinical oncology. Cancer Treat Rep 65: 57
5. Carson DA, Kay J, Matsumoto S, Seegmiller JE, Thompson L (1979) Biochemical basis for the enhanced toxicity of deoxyribonucleosides toward malignant human T cell lines. Proc Natl Acad Sci USA 76: 2430
6. Chabner BA, Johns DG (1977) Folate antagonists. In: Becker FF (ed) Cancer: A comprehensive treatise. Plenum, New York, p 363
7. Cozarelli NR (1979) The mechanism of action of inhibitors of DNA synthesis. Annu Rev Biochem 46: 641
8. Danhauser LL, Rustum YM (1980) Effect of thymidine on the toxicity, antitumor activity, and metabolism of 1-β-D-arabinofuranosylcytosine in rats bearing a chemically induced colon carcinoma. Cancer Res 40: 1274
9. Ellims PH, Donehower RC, Chabner BA (1981a) Modulation of cytosine arabinoside therapy. In: Burchenal JH, Oettgen HF (eds) Cancer. Achievement, challenges and prospects for the 1980s, vol 2. Grune & Stratton, New York, p 123
10. Ellims PH, Kao AY, Chabner BA (1981b) Deoxycytidylate deaminase: Purification and some properties of the enzyme isolated from human spleen. J Biol Chem 256: 6335
11. Ellims PH, Van Der Weyden MB (1981c) Kinetic mechanism and inhibition of human liver thymidine kinase. Biochim Biophys Acta 60: 238

12. Ellims PH, Gan TE, Cosgrove L (1982) Human thymidine kinase: Purification and some properties of the TK1 isozyme from placenta. Mol Cell Biochem 45: 113
13. Ensminger WD, Frei E III (1977) The prevention of methotrexate toxicity by thymidine infusions in humans. Cancer Res 37: 1857
14. Eriksson S, Thelander L, Akerman M (1979) Allosteric regulation of calf thymus ribonucleoside diphosphate reductase. Biochemistry 18: 2948
15. Fox RM, Piddington SK, Tripp EH, Dudman NP, Tattersall MHN (1979) Thymidine sensitivity of cultured leukaemic lymphocytes. Lancet II: 391
16. Fox RM, Tripp EH, Piddington SK, Tattersall MHN (1980a) Sensitivity of leukemic null lymphocytes to deoxynucleosides. Cancer Res 40: 3383
17. Fox RM, Tripp EH, Tattersall MHN (1980b) Mechanism of deoxycytidine rescue of thymidine toxicity in human T-leukemic lymphocytes. Cancer Res 40: 1718
18. Fox RM, Piddington SK, Tripp EH, Tattersall MHN (1981) Ecto-adenosine triphosphatase deficiency in cultured human T and null leukemic lymphocytes. A biochemical basis for thymidine sensitivity. J Clin Invest 68: 544
19. Grant S, Lehman C, Cadman E (1980) Enhancement of 1-β-D-arabinofuranosyl-cytosine accumulation within L1210 cells and increased cytotoxicity following thymidine exposure. Cancer Res 40: 1525
20. Grant S, Rauscher F III, Margolin J, Cadman E (1982) Dose- and schedule-dependent activation and drug synergism between thymidine and 5-aza-2′-deoxycytidine in a human promyelocytic leukemia cell line. Cancer Res 42: 519
21. Harris AW, Reynolds EC, Finch LR (1979) Effect of thymidine on the sensitivity of cultured mouse tumor cells to 1-β-D-arabinofuranosylcytosine. Cancer Res 39: 538
22. Howell SB, Jenkins R (1979) Evaluation of thymidine (TdR) as a chemotherapeutic agent against human tumor xenografts in nude mice. Proc Am Assoc Cancer Res/Am Soc Clin Oncol 20: 945
23. Howell SB, Ensminger WD, Krishan A, Frei E III (1978) Thymidine rescue of high-dose methotrexate in humans. Cancer Res 38: 325
24. Howell SB, Krishan A, Frei E III (1979) Cytokinetic comparison of thymidine and leucovorin rescue of marrow in humans after exposure to high-dose methotrexate. Cancer Res 39: 1315
25. Howell SB, Chu B, Mendelsohn J, Carson DA, Kung FH, Seegmiller JA (1980a) Thymidine as a chemotherapeutic agent. Pharmacologic, cytokinetic, and biochemical studies in a patient with T-cell acute lymphocytic leukemia. J Natl Cancer Inst 65: 177
26. Howell SB, Herbst K, Ross GR, Frei E III (1980b) Thymidine requirements for the rescue of patients treated with high-dose methotrexate. Cancer Res 40: 1824
27. Howell SB, Taetle R, Mendelsohn J (1980c) Thymidine as a chemotherapeutic agent: sensitivity of normal human marrow, peripheral blood T cells, and acute non-lymphocytic leukemia. Blood 55: 505
28. Howell SB, Mansfield SJ, Taetle R (1981) Thymidine and hypoxanthine requirements of normal and malignant human cells for protection against methotrexate cytotoxicity. Cancer Res 41: 945
29. Ives DH, Durham IP (1970) Deoxycytidine kinase. III. Kinetic and allosteric regulation of the calf thymus enzyme. J Biol Chem 245: 2285
30. Jackson RC (1978) The regulation of thymidylate biosynthesis in Novikoff hepatoma cells and the effects of amethoprin, 5-fluorodeoxyuridine and 3-deazauridine. J Biol Chem 253: 7440
31. Jackson RC (1980) Modulation of methotrexate toxicity by thymidine: sequence-dependent biochemical effects. Mol Pharmacol 18: 281
32. Kinahan JJ, Otten M, Grindey GB (1979) Evaluation of ribonucleoside and deoxyribonucleoside triphosphate pools in cultured leukemia cells during exposure to methotrexate or methotrexate plus thymidine. Cancer Res 39: 3531
33. Kinahan JJ, Kowal EP, Grindey GB (1981) Biochemical and antitumor effects of the combination of thymidine and 1-β-D-arabinofuranosylcytosine against leukemia L1210. Cancer Res 41: 445
34. Kirkwood JM, Ensminger W, Rosowsky A, Papathanasopoulos N, Frei E III (1980) Comparison of pharmacokinetics of 5-fluorouracil and 5-fluorouracil with concurrent thymidine infusions in a phase I trial. Cancer Res 40: 107
35. Kufe DW, Major PP (1981) 5-Fluorouracil incorporation into human breast carcinoma RNA correlates with cytotoxicity. J Biol Chem 256: 9802
36. Kufe DW, Beardsley P, Karp P, Parker L, Rosowsky A, Canellos G, Frei E III (1980) High-dose thymidine infusions in patients with leukemia and lymphoma. Blood 55: 580
37. Lee SS, Giovanella BC, Stehlin JS Jr (1977a) Effect of excess thymidine on the growth of human melanoma cells transplanted in thymus deficient nude mice. Cancer Lett 3: 209
38. Lee SS, Giovanella BC, Stehlin JS (1977b) Selective lethal effect of thymidine on human and mouse tumor cells. J Cell Physiol 29: 401
39. Lee SS, Giovanella BC, Stehlin J, Brunn J (1979) Further studies on the long-term effects of high-dose thymidine infusion (TdR) on human tumors heterotransplanted into nude mice. Proc Am Assoc Cancer Res/Am Soc Clin Oncol 20: 945
40. Major PP, Egan EM, Beardsley GP, Minden MD, Kufe DW (1981a) Lethality of human myeloblasts correlates with the incorporation of arabinofuranosylcytosine into DNA. Proc Natl Acad Sci USA 78: 3255
41. Major PP, Sargent L, Egan EM, Kufe DW (1981b) Correlation of thymidine-enhanced incorporation of ara-C in deoxribonucleic acid with increased cell kill. Biochem Pharmacol 30: 2221
42. Mandel HG, Klubes P, Fernandes DJ (1978) Understanding the actions of carcinostatic drugs to improve chemotherapy: 5-fluorouracil. Adv Enzyme Regul 16: 65
43. Martin DS, Stolfi RL, Sawyer RC, Rabindranath N, Spiegelman S, Young CW, Woodcock T (1980) An overview of thymidine. Cancer 45: 1117
44. Momparler RL, Fisher GA (1978) Effect of intravenous infusion of 5-aza-2′-deoxycytidine on survival time of mice with L1210 leukemia. Cancer Res 38: 2673
45. Momparler RL, Goodman J (1977) In vitro cytotoxic and biochemical effects of 5-aza-2′-deoxycytidine. Cancer Res 37: 1636
46. Momparler RL, Vesely J, Momparler LF, Reward GE (1979) Synergistic action of 5-aza-2′-deoxycytidine and 3-deazauridine on L1210 leukemic cells and EMT6 tumor cells. Cancer Res 39: 3822
47. Moran RG, Werkheiser WC, Zakrzewski SF (1976) Folate metabolism in mammalian cells in culture. J Biol Chem 251: 3569
48. Moran RG, Mulkins M, Heidelberger C (1979) Role of thymidylate synthetase activity in development of methotrexate cytotoxicity. Proc Natl Acad Sci USA 76: 5924
49. Morris NR, Fischer GA (1960) Studies concerning the inhibition of the synthesis of deoxycytidine by phosphorylated derivatives of thymidine. Biochim Biophys Acta 42: 183
50. Myers CE, Young RC, Chabner BA (1975) Biochemical determinants of 5-fluorouracil response in vivo. J Clin Invest 56: 1231
51. Nayak R, Martin D, Stolfi R, Furth J, Spiegelman S (1978) Pyrimidine nucleosides enhance the anticancer activity of FU and augment its incorporation into nuclear RNA. Proc Am Assoc Cancer Res/Am Soc Clin Oncol 19: 63
52. Peterson DF, Anderson EC (1964) Quantity production of synchronized mammalian cells in suspension culture. Nature 203: 642
53. Plagemann PGW, Marz R, Wohlhueter RM (1978) Transport and metabolism of deoxycytidine and 1-β-D-arabinofuranosylcytosine into cultured Novikoff hepatoma cells. Cancer Res 38: 978
54. Reichard P (1978) From deoxynucleosides to DNA synthesis. Fed Proc 37: 9

55. Reynolds EC, Harris AW, Finch LR (1979) Deoxyribonucleoside triphosphate pools and differential thymidine sensitivities of cultured mouse lymphoma and myeloma cells. Biochim Biophys Acta 561: 110
56. Robert de Saint Vincent B, Buttin G (1980a) Studies on 1-β-D-arabinofuranosylcytosine-resistant mutants of Chinese hamster fibroblasts. IV. Altered regulation of CTP synthetase generates arabinosylcytosine and thymidine resistance. Biochim Biophys Acta 610: 352
57. Robert de Saint Vincent B, Dechamps M, Buttin G (1980b) The modulation of the thymidine triphosphate pool of Chinese hamster cells by dCMP deaminase and UDP reductase. J Biol Chem 255: 162
58. Spiegelman S, Nayak R, Sawyer R, Stolfi R, Martin D (1980) Potentiation of the antitumor activity of 5-FU by thymidine and its correlation with the formation of (5 FU) RNA. Cancer 45: 1129
59. Streifel JA, Howell SB (1981) Synergistic interaction between 1-β-D-arabinofuranosylcytosine, thymidine and hydroxyurea against human B cells and leukemic blasts in vitro. Proc Natl Acad Sci USA 78: 5132
60. Tattersall MHN, Brown B, Frei E III (1975) The reversal of methotrexate toxicity by thymidine and maintenance of antitumor effects. Nature 253: 198
61. Tsuboi K, Kwong LK (1978) Antiproliferative agents and differential survival between normal and cancer cells. Cancer Res 38: 3745
62. Van Echo DA, Markus S, Wiernik PH (1981) A phase III trial of arabinosylcytosine and thymidine (ara-C + TdR) in adult relapsed acute leukemia (AL). Proc Am Soc Cancer Res/Am Soc Clin Oncol 22: 22: 483
63. Vogel SJ, Presant CA, Ratkin GA, Klahr C (1979) Phase I study of thymidine plus 5-fluorouracil infusions in advanced colorectal carcinoma. Cancer Treat Rep 63: 1
64. Xeros N (1962) Deoxyriboside control and synchronization of mitosis. Nature 194: 682
65. Yoshida S, Yamada M, Masaki S (1977) Inhibition of DNA polymerase and of calf thymus by 1-β-D-arabinofuranosylcytosine 5′-triphosphate. Biochim Biophys Acta 477: 144

Received June 21/Accepted September 27, 1982