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Is there a role for thymidine in cancer chemotherapy?

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Introduction

In human plasma, thymidine (TdR)§ is present at low but detectable levels. It is incorporated in the triphosphate form into DNA (24). High concentrations of TdR have long been used in tissue culture to study cell synchronization (6, 17, 30, 84, 114), and short-term exposure to 10^{-4} or 10^{-3} M TdR results in accumulation of cells in early S phase both *in vitro* and *in vivo* (17, 30, 64). However, when exposure is maintained for long periods, TdR is also cytotoxic (30).

TdR has been used for a number of years in the tritiated form for cytokinetic and pharmacokinetic studies *in vitro* as well as in human subjects without noticeable toxicity (91). In the early fifties TdR was introduced as a therapeutic agent for the treatment of megaloblastic anemias, based on the assumption that in vitamin B_{12} deficiency the conversion of dUMP to dTMP was impaired (59, 99). The results obtained with low doses of TdR administered intramuscularly were contradictory, but in 1964 Killman described the reversal of megaloblastic changes in pernicious anemia following 48–72-h infusions of TdR in doses up to 14 g (59). This kind of therapy became obsolete when purified vitamin B_{12} preparations were made available.

Recently interest in TdR has grown due to preclinical and clinical evidence that TdR can modulate the toxicity and therapeutic efficacy of antimetabolites such as methotrexate, 5-fluorouracil and 1-β-D-arabinofuranosylcytosine (ara-C) (21, 24, 44, 85, 112, 115). The use of TdR as an anticancer agent has also generated a great deal of interest (15, 68). In this review article the relevant experimental and clinical data of TdR as an anticancer

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§ List of abbreviations: ALL, acute lymphocytic leukemia; AML, ANLL, acute myeloid (non-lymphoctic) leukemia; ara-C, 1-β-D-arabinofuranosylcytosine; CFU-C, colony forming cells in culture, myeloid precursor cells; CML, chronic myeloid leukemia; CSF, cerebrospinal fluid; dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dCyd, deoxycytidine; dGTP, deoxyguanosine triphosphate; dNTP, deoxyribonucleoside triphosphate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FdUMP, 5-fluorodeoxyuridine monophosphate; FH_4, tetrahydrofolate; 5FU, 5-fluorouracil; FUdR, 5-fluorodeoxyuridine; FUMP, 5-fluorouridine monophosphate; FUR, 5-fluorouridine; FUTP, 5-fluorouridine triphosphate; HD-TdR, high-dose thymidine; Hyp, hypoxanthine; MTX, methotrexate; NTP, ribonucleoside triphosphate; TdR, thymidine; dTTP, thymidine triphosphate.

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drug, the pharmacokinetics of TdR and the role of TdR in the metabolic modulation of other antimetabolites will be reviewed together with our own experience.

Thymidine as an antineoplastic agent

It has been shown in several *in vitro* studies that a high concentration of TdR causes a reversible suppression of cell growth (69, 77, 78, 85). This phenomenon has been attributed to the inhibition of ribonucleoside diphosphate reductase by increased intracellular pools of the triphosphate metabolite of TdR, dTTP. High dTTP pools lead in turn to depletion of dCTP pools (69, 77, 78, 114). Recently, it was shown that high concentrations of TdR are more lethal *in vitro* to some classes of tumour cells than to normal cells (12, 66, 105). Increased sensitivity of leukemic T lymphocytes for instance has been correlated with diminished activity of catabolic enzymes such as thymidine phosphorylase and 5′-nucleotidase, favouring the elevation of dTTP pools following exposure to TdR (12, 22, 27, 62, 87, 113). Another determinant in sensitivity to TdR appears to be the level of dCTP. Less sensitive cells contain more dCTP than do highly sensitive cells (90, 93). This fits with the observations of Morris and Fischer (77) and Xeros (114) that cell growth inhibition by TdR *in vitro*.could be reversed by concomitant addition of dCTP. Resistance to TdR has also been attributed to a lack of thymidine kinase, the enzyme responsible for the conversion of TdR to dTTP, and a decrease in TdR uptake (118). Howell *et al.* found that the sensitivity of normal human T cells was dependent on their proliferative state prior to exposure to TdR (49). T cells in G0 at the start of exposure remained viable *in vitro* in the presence of 10^{-3} M TdR, whereas T cells already proliferating in response to phytohemagglutinin at the start of TdR exposure were killed. Grindey *et al.* recently suggested another mechanism by which dTTP might block DNA synthesis (34). They found that high concentrations of deoxyribonucleoside triphosphates could inhibit calf-thymus DNA-polymerase α, which means that the high intracellular TTP levels induced with high-dose TdR therapy could directly inhibit DNA synthesis. In contrast, Fox *et al.* reported that the growth inhibition of cultured human leukemic T-lymphocytes by TdR could be completely reversed by exogenous deoxycytidine (dCyd) with only minimal reduction of the markedly elevated dTTP and dGTP pools, suggesting that an excess of dTTP and dGTP was not directly inhibitory to DNA replication (28). Most recently Akman *et al.* reported interesting data on growth inhibition of leukemic cells and normal human myeloid progenitor cells by TdR (1). They found that normal myeloid colony formation was 10 times more sensitive to TdR than was colony formation of HL-60, a human acute progranulocytic leukemia cell line. HL-60 growth in liquid suspension culture could be totally inhibited by 5·0 mM TdR, and rescued completely by concomitant addition of dCyd. However, the reduction in cloning potential of HL-60 caused by TdR could not be completely reversed by dCyd, and normal myeloid cloning could not be rescued at all from TdR inhibition (1). Although *in vitro* reversal of TdR toxicity by dCyd is most consistent with dCTP-depletion as the primary cause of inhibition of DNA synthesis, recent data (1, 34) suggest that TdR affects more than just deoxyribonucleotide synthesis.

In 1977 Lee *et al.* published their observations on the effect of high-dose TdR (HD-TdR) on the growth of human melanoma xenografts transplanted in athymic nude mice (67). They found that high doses of TdR could suppress the growth of melanoma cells and prolong the survival of treated mice compared to non-treated controls. However, growth inhibition only lasted for the duration of the treatment. Recently, they reported

comparable results with several other human xenografts in nude mice (68). In 19 out of 40 treated animals, total tumor regression was obtained with intermittent subcutaneous TdR infusion after 150 days, and 3 of these lived more than 300 days. Complete regression occurred only in very rapidly growing tumors. Reiter also reported an increased life span of EL 4 tumor-bearing mice treated with HD-TdR compared to non-treated controls (88). Although EL 4 tumor cells are among the most sensitive to TdR *in vitro* only 3 of 10 treated mice survived more than 60 days. Howell *et al.*, examining the activity of HD-TdR against 4 human tumor xenografts in nude mice, found a significant growth-inhibiting effect, which was cytostatic rather than cytotoxic only in melanoma (46). Even when the melanomas were very small, there were no complete regressions and tumor growth at the same rate as controls resumed when TdR treatment was stopped. Toxicity in the form of myelosuppression was significant. They concluded that the therapeutic ratio in humans would probably not be high due to myelosuppression (46).

Kufe *et al.* investigated the cytokinetic effects of TdR on rapidly proliferating cell populations *in vivo* (63). TdR was given as a continuous infusion to normal Wistar/Furth rats to maintain serum levels of 10^{-4}, 10^{-3} or 10^{-2} M over 24, 48 and 72 h. At serum TdR levels of 10^{-4} M the growth of bone marrow and intestinal mucosa cells was arrested in S phase of the cell cycle, as shown by flow-cytometry and ^{32}P-labeling studies. On release after TdR exposure a partial synchronization of these cell populations was observed. TdR exposure to 10^{-3} M for more than 24 h was associated with bone marrow hypocellularity, while at levels of 10^{-2} M the nucleoside killed all animals after 48 h. The growth kinetics of subcutaneously transplanted myeloblastomas in similar rats was not altered by 10^{-4} M TdR. However, at 10^{-3} M a transient arrest of cellular growth was observed similar to that in normal cell populations.

Clinical studies with high-dose thymidine

Prompted by the data of Lee *et al.*, clinical Phase I and II trials were initiated in several institutes. In this section the available clinical and toxicological data, including our own (unpublished) experience with HD-TdR will be discussed.

At the National Cancer Institute in Baltimore, 12 patients were treated by Chiuten *et al.* with continuous i.v. infusions for at least 5 days, using a daily dose of 75 g/m^2 (15). A minor tumor response, consisting of minimal shrinkage of s.c. nodules, was observed in 1 patient with metastatic melanoma. In 3 patients with acute leukemia (2 ALL, 1 ANLL) peripheral white blood counts fell and 1 ALL patient showed marked marrow hypocellularity. Upon marrow recovery, however, there was no improvement over pretreatment marrow status. Myelosuppression appeared to be the dose-limiting toxicity and non-hematological side-effects consisted of anorexia, nausea, vomiting, diarrhoea, alopecia, somnolence, headache, visual hallucinations and memory impairment. Comparable clinical results were reported by Kufe *et al.* (62). Eleven patients with acute leukemia (3 ALL, 4 ANLL) and malignant lymphoma were treated with TdR as a continuous infusion at 75 g/m^2/day. Courses varied in duration from 2 to 14 days, with the majority of courses being 5 days or longer. In 5 of the 7 patients (3 T cell leukemia, 2 ANLL) there was suggestive evidence of antitumor activity, in the form of a sharp decline in peripheral blast counts during TdR infusions; 1 T-cell leukemia patient also showed resolution of hepatosplenomegaly. Another patient with T-cell leukemia experienced partial clearing of the blasts in the bone marrow, but in this and all the other cases the blasts returned to pretreatment numbers or beyond the end of the

TdR infusion. With flow-cytometric analysis of bone marrow and peripheral blood TdR infusion resulted in an increase in the proportion of cells in S phase in 9 of 10 courses (62). Kufe and coworkers also measured the levels of thymidine kinase and thymidine phosphorylase in an attempt to correlate the intracellular metabolism of TdR with cellular sensitivity to HD-TdR. They found that AML and T-cell leukemia preparations had up to 10-fold higher levels of thymidine kinase and up to 30 times lower levels of thymidine phosphorylase compared to leukemic B cells. Thus, when comparing the ratio of thymidine kinase to thymidine phosphorylase, which provides an indirect measure for predicting the conversion of TdR to dTTP, there appears to be a more than 100-fold differential between these two leukemia types. Three patients (1 AML, 2 T-cell ALL) had high intracellular ratios of thymidine kinase to phosphorylase, and these patients all responded with sharp declines in blast count, in contrast with 2 patients with B-cell ALL who had low kinase to phosphorylase ratios and who failed to demonstrate any cytoreduction after HD-TdR.

Recently, Kufe *et al.* (64) reported that HD-TdR elicited an antitumor effect in patients with mycosis fungoides, another T-cell disorder. Three patients with mycosis fungoides, who were resistant to conventional treatment, responded to HD-TdR (75 g/m^2/day for 4–7 days) with partial clearing of skin lesions in 2 patients and some regression of nodulo-ulcerative lesions and lymphadenopathy in the third patient. The responses however, were short term and the skin lesions recurred within several weeks following therapy. The toxicity observed in these two groups of patients was similar to that reported by Chiuten *et al.* (15). In these studies millimolar concentrations of TdR were maintained in plasma during the entire period of infusion.

At the Memorial Sloan–Kettering Cancer Center a Phase II study of HD-TdR was performed with 10 acute leukemia patients (2 ALL, 8 ANLL). Seventeen continuous i.v. infusions of TdR were given with a duration ranging from 5 to 24 days at dose levels of 70–90 g/m^2/day range. Although a 2–60-fold reduction of peripheral blasts occurred in 16 of the 17 courses (median plasma TdR concentration was 1.14 mM), no patient achieved marrow remission (5). Like Kufe *et al.* (62) this group observed an accumulation of blasts in S phase following HD-TdR infusion and both groups suggested that HD-TdR followed by S phase specific agents such as ara-C might improve therapeutic results (5, 62, 63). Howell *et al.* reported cytokinetic and biochemical studies in a 5-year old T-cell ALL patient, who received one 5-day course of TdR g/m^2/day followed by an 8-day infusion 8 days later at the same dose (43). Both courses were associated with a rapid fall in peripheral lymphoblast count during infusion, followed by an extremely rapid recovery of blasts 2–4 days after the end of TdR exposure. This indicates that TdR caused little lasting damage to the surviving cells. The number of marrow blasts was not reduced. Enzymatic analysis of the T lymphoblasts revealed low levels of dTTP catabolic enzymes but also low thymidine kinase activity. TdR caused an initial accumulation of peripheral blood cells in S phase and a decrease of cells in G1 followed by a rapid reversal of this pattern, indicating a block in late G1 and/or early S phase, which is in agreement with the *in vivo* experimental data of Kufe *et al.* (63). The pharmacokinetic data from this study (43) will be discussed in the next section.

McCormick *et al.* studied immunological parameters in 16 patients with solid tumors, who were treated with HD-TdR (75). TdR at a daily dose of 90 g/m^2 was administered by continuous i.v. infusion for 4 days; the interval between the cycles was 5 days. Differential T- and B-cell counts in peripheral blood were performed before and after treatment and whole blood cultures were used to assess lymphocyte response to phytohemagglutinin, concanavalin A, pokeweed mitogen and streptokinase–streptodornase. They found no difference in the percentage distribution of T- and B-lymphocytes compared with pre-

treatment values, and also no inhibition of functional activity of the patients' lymphocytes following HD-TdR infusion. In their report they made no mention of tumor response (75).

Our own experience (unpublished data) with HD-TdR in 10 patients is in accordance with the above data. The characteristics of the patients are shown in Table 1. Doses ranged from 30–75 g/m^2/day and i.v. infusions were administered for 3 of 5 days. One patient showed some regression of her recurrent melanoma of the soft palate following the first course of TdR, but when due for the second course she appeared to have rapidly progressive pulmonary metastases. There was no suggestion of tumor response in any of the other patients. Toxicity was similar to that described by others (15, 62). One patient with extensive liver metastases from melanoma, however, developed severe hyponatremia on the third day of the TdR infusion, which was followed by fatal acute renal and cardiac failure. She had disturbed renal function tests before treatment. At autopsy the cause of death could not be established. The liver metastases did not seem to have been affected by the TdR infusion.

In summary, in 53 patients reported in the literature with a variety of cancers, who have been treated with HD-TdR as well as in our own group of 10 patients, no complete or partial remission has been achieved with daily infusions of 30–90 g/m^2 over 3–24 days. A further increase in dose levels for the treatment of solid tumors or lymphomas does not seem appropriate because of the increased severity of myelosuppression. Moreover, further dose increments would require larger volumes of administration due to the poor solubility of TdR. The future clinical role of TdR will probably be in combination chemotherapy based on the potential of TdR to modulate the biochemical activity of antimetabolites such as 5-fluorouracil, ara-C and methotrexate (21, 24, 44, 85, 112, 115).

Pharmacokinetics of thymidine

TdR is endogenously present in human plasma in concentrations of 0.19–0.35 μM (24, 96). After rapid injection of tracer doses of ^{3}H-labeled TdR to human subjects, a very rapid plasma clearance has been reported with a $T_{\frac{1}{2}\alpha}$ of 1–2 min and $T_{\frac{1}{2}\beta}$ of approximately 25 min (91). Most of the injected TdR appeared to be metabolized to thymine, which was further broken down in the liver to β-aminoisobutyric acid and CO_2 (26). Between 30 and 80% of the radioactivity was recovered from the urine as ^{3}H-water with only a small amount of ^{3}H-β-aminoisobutyric acid (91). Within one min of injection, ^{3}H-TdR appeared to be incorporated into newly formed DNA of proliferating cells (91).

Ensminger and Frei reported that the serum clearance of TdR after an i.v. 2 g/m^2 pulse dose was 8–20 min (24). TdR concentrations were measured by radioimmunoassay. When TdR was administered as a continuous infusion over 3 days at a daily dose of 8 g/m^2, an 8-fold rise in TdR serum levels was obtained. After cessation of the infusion the TdR levels dropped with a $T_{\frac{1}{2}\alpha}$ of 8–10 min returning to pretreatment levels by 45 min. Less than 2% of the administered dose of TdR was recovered as such in the urine. CSF TdR levels appeared to equal the serum levels throughout the infusion period (24).

During infusion of 24 g/m^2/day, TdR plasma levels of 200 μM were found, a 100-fold higher than plasma levels obtained with infusion of 8 g/m^2/day, suggesting that the elimination of TdR reaches a saturation point (96). Furthermore, hepatic extraction of TdR was reported to be high with an extraction ratio (hepatic artery level : hepatic vein level) of 0.64–0.95 at dose rates up to 16 g/m^2/day of TdR (25). The ratio decreased

Table 1. Patient characteristics, Phase I study of HD-TdR

Patient	Age	Sex	Diagnosis	ECOG[a]	Prior RT or surgery	Prior chemotherapy[b]	Dose of TdR ($g/m^2/d$)	Duration of infusion (days)	Antitumor effect	Toxicity
1	74	M	Squamous cancer of the larynx	3	S, RT	MTX+TdR	34	3	–	–
2	59	F	Squamous cancer of the oropharynx	3	RT	MTX+TdR	50	3	–	–
3	45	F	Neurolemmasarcoma	2	S, RT	Cy, Vcr, Adm D, Ctz, MTX	70	3	–	+
4	59	M	Adenocarcinoma of the colon	2	S	FU, Me-CCNU	70	3	–	±
5	70	M	Squamous cancer of the nasopharynx	3	RT	MTX, Cy, Blm Vcr, HU	75	3	–	+
6	50	F	Adenocarcinoma of the colon	3	S, RT	5FU, Me-CCNU	75	5	–	+
7	61	F	Melanoma of the vulva, liver metastases	2	S (of primary)	–	75	$3\frac{1}{2}$[c]	–	+
8	75	F	Melanoma, soft palate	2	S	–	75	5	±	+
9	62	F	Melanoma, oral cavity	0	–	–	75	5	–	+
10	57	F	Melanoma of the eye, liver metastases	3	S (of primary)	–	75	5	–	+

[a] ECOG: ECOG performance scale.

[b] The abbreviations used are Adm = adriamycin, Blm = bleomycin, Ctz = chlorozotocin, Cy = cyclophosphamide, D = DTIC, 5FU = 5-fluorouracil, HU = hydroxyurea, Me-CCNU = methyl-CCNU, MTX = methotrexate, Vcr = vincristine.

[c] The infusion was terminated after 84 h because acute renal failure.

progressively when doses of 16–128 g/m^2/day were administered i.v. or directly into the hepatic artery, suggesting that the capacity of the liver to remove and catabolize the drug was saturated somewhere around this dose level. The excess TdR was then eliminated from the plasma by extrahepatic processes, such as renal clearance and uptake by proliferating tissues, accounting for the removal of 20–80% of the administered dose (17, 25).

Pharmacokinetic studies of HD-TdR infusions have shown that in man TdR plasma levels, which are 10,000 times higher than those endogenously present, can be achieved and maintained for more than 5 days without major toxicity (15, 116, 117). At dose levels of 75 g/m^2/day, TdR is eliminated mainly by the kidneys, as a result of saturation of both hepatic and extrahepatic metabolic elimination (116, 117). In contrast to the 2% of TdR which is excreted by the kidneys at micromolar concentrations (24) renal clearance of TdR ranged from 41 to 67% of total body clearance when millimolar plasma concentrations were achieved (62, 116). This indicates that the body can metabolize 40% of these high doses, which is supported by the observation that thymine plasma concentrations were maintained for some time after TdR infusion was stopped, suggesting that this major catabolite of TdR was still being produced as TdR levels were rapidly declining (116). After cessation of HD-TdR infusions a $T_{\frac{1}{2}\beta}$ of 80–100 min was found in contrast to a $T_{\frac{1}{2}\beta}$ of 10 min at low plasma levels (116, 117). Howell *et al.* reported a somewhat shorter TdR $T_{\frac{1}{2}\beta}$ of 48 min in their 5-year-old patient (43). This difference is attributed to the higher proportion of TdR being metabolized in the child compared to adults. In all studies the half-life of thymine appeared to be much longer than that of TdR, but could not be accurately determined because it did not follow first-order kinetics (43, 62, 116).

In the monkey, the concentration of TdR in the CSF during HD-TdR infusion was found to be 10 times lower than plasma concentrations in the millimolar range (117). At steady state millimolar plasma levels in humans, CSF concentrations of TdR were approximately one-third of the plasma concentrations (15, 43, 117), in contrast with equal TdR levels in CSF and plasma at micromolar concentrations (24). This suggests that transport of TdR from plasma to CSF is also saturable. During HD-TdR infusion CSF concentrations of thymine, which is less polar than TdR, appeared to be consistently higher than those of TdR (43, 116).

Thymidine and 5-fluorouracil

5-Fluorouracil (5FU) is an effective antineoplastic agent in the palliative treatment of carcinomas of the breast, ovary, gastrointestinal tract and skin (13). 5FU is rapidly converted in the cell to the nucleotide derivates FdUMP and FUMP (Figure 1).

FdUMP is a potent inhibitor of the enzyme thymidylate synthetase, which is involved in the conversion of dUMP to dTMP, which is further phosphorylated to dTTP and incorporated into DNA. Thus, FdUMP indirectly inhibits DNA synthesis by blocking the supply of dTTP (40). FUMP can be either incorporated as FUTP into RNA instead of the natural pyrimidine uracil or converted to FdUMP by the enzyme ribonucleotide reductase. The effect on DNA synthesis via inhibition of the thymidylate synthetase step has generally been considered as the primary mechanism of cytostatic action (40). Recently, however, the importance of the incorporation of FUTP into RNA, altering RNA synthesis and function as well as ribosomal stability, has been stressed (18, 73, 98).

In the early sixties, Burchenal *et al.* observed that the toxicity of 5-fluorouridine (FUR) to L1210 leukemia *in vitro* and *in vivo* could be prevented by uridine but not by TdR (10), in

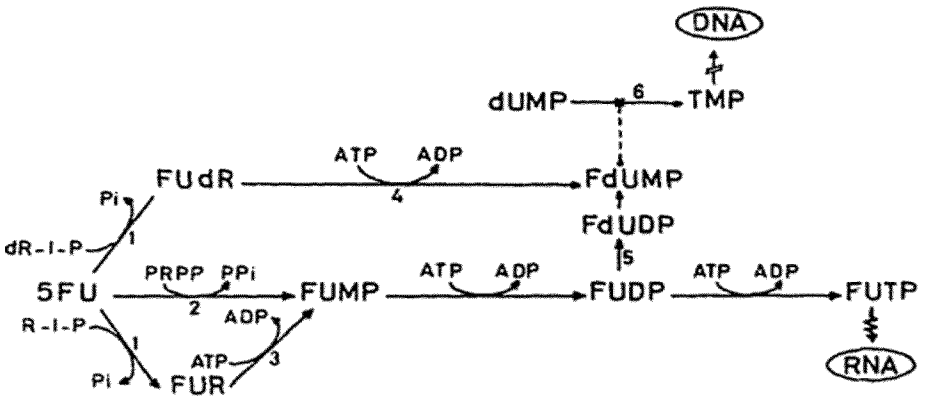

Figure 1 Metabolic pathways for activation of 5-fluorouracil. 5FU = 5-fluorouracil; FUdR, FdUMP and FdUDP are fluorodeoxyuridine and its mono- and diphosphate; FUR, FUMP, FUDP and FUTP are fluorouridine and its mono-, di- and triphosphate; dR-1-P = deoxyribose-1-phosphate; R-1-P = ribose-1-phosphate; PRPP = phosphoribosylpyrophosphate. 1 = thymidine phosphorylase; 2 = phosphoribosyl transferase; 3 = uridine kinase; 4 = thymidine kinase; 5 = ribonucleotide reductase; 6 = thymidylate synthetase.

contrast to 5-fluorodeoxyuridine (FUdR), the *in vitro* toxicity of which was blocked by TdR but not by uridine. The toxicity of 5FU and FUdR to L1210 *in vivo* was affected by neither TdR nor uridine (11). Ten years later it was reported that mitotic inhibition induced by FUdR *in vitro* could be reversed by TdR (42) and that *in vivo* the response to FUdR correlated inversely with the level of thymidine kinase (58). From this observation it was suggested that co-administration of TdR would overcome 5FU cytotoxicity (58). Recently, potentiation of 5FU cytotoxicity by TdR against leukemia cells and murine breast carcinoma *in vivo* was described (71, 94).

Santelli and Valeriote investigated whether large doses of TdR could reverse 5FU cytotoxicity in a mouse model (94, 95). On the contrary, they observed enhanced host toxicity, along with increased cytotoxicity of the TdR–5FU combination to AKR leukemia, No change was seen when L1210 leukemia was used (95). The observed difference in sensitivity to TdR–5FU between AKR and L1210 leukemia was interpreted as a difference in 5FU metabolism; AKR may be more sensitive to an isolated RNA effect of 5FU, as suggested by Martin and Stolfi (71). Martin *et al.* attributed the improvement of the antitumor activity of the combination to modulation of 5FU metabolism by TdR. Thus, increased incorporation of 5FU into RNA and reversal of the inhibition of DNA synthesis may follow TdR exposure (71, 73, 98). An additional substance which is involved in the potentiation of 5FU by TdR is thymine. This base, which is the major metabolite of TdR, competes with 5FU for the same degradation enzymes, hence it reduces the rate of 5FU catabolism (95, 98). This finding has been supported by the observations of other investigators (29, 79), who did not see enhanced antitumor activity of the TdR–5FU combination *in vitro*, if not accompanied by catabolism of 5FU.

By applying continuous infusion of drugs via the tail vein of unrestrained Balb/C mice bearing a chemically induced colon tumor, Danhauser and Rustum examined the antitumor effect of 5FU alone and in combination with TdR (20, 92). They observed a higher therapeutic index for the combination of the two drugs compared to the single agent 5FU (92). The same results were achieved in C57BL/6 mice and Fischer (CDF) rats bearing colon tumors. The TdR–5FU combination resulted in an increased overall host toxicity including several toxic deaths (92).

The first experience with the TdR–5FU combination in man was reported by Martin *et al.* (70). These investigators administered TdR (15 g) as a rapid infusion 60 min prior to a 7.5 mg/kg i.v. bolus of 5FU. This schedule, which was repeated every 4 weeks, invoked an antitumor effect in patients with colon cancer resistant to 5FU alone. Myelosuppression was the major toxicity in this Phase I study (70). Since then, three other Phase I clinical trials with TdR–5FU have been reported. Vogel *et al.* treated 12 patients with advanced colorectal cancer (108). TdR (8 g/m^2/day) was given as a continuous i.v. infusion for $5\frac{1}{2}$ days, simultaneously with a 5-day infusion of 5FU (5–20 mg/kg/day). Myelosuppression was the dose-limiting toxicity, being mild at 5FU doses of 5.0–7.5 mg/kg/day. Severe myelosuppression occurred at doses of 10–20 mg/kg/day resulting in 4 drug-related deaths. Gastrointestinal toxicity was only rarely observed. Due to the small number of patients, no conclusions could be made regarding the antitumor effect (108).

At the Sidney Farber Cancer Institute 7 patients with a variety of neoplastic diseases were treated with a TdR–5FU combination and 5FU pharmacokinetics, the effect of 5FU on bone marrow cytokinetics and clinical toxicology of the combination were reported (61). Patients were initially treated with 5FU (460–525 mg/m^2/day) for 5 days as an i.v. bolus. TdR was administered by continuous infusions for 120 h, commencing 2 h before the initial dose of 5FU. The authors observed that continuous infusion of TdR increased the toxicity of 5FU in all respects. TdR infusion did not reverse the inhibition of DNA synthesis in bone marrow cells, as determined by flow-cytometry and nucleoside incorporation studies, nor did it increase the inhibition of RNA synthesis by 5FU. Pharmacokinetic studies revealed a significant prolongation of the 5FU half-life in both components of the decay curve. Dose-limiting mucositis, myelosuppression and gastrointestinal toxicity were observed with 1 case of fatal toxicity in a patient with poor bone marrow reserve due to previous irradiation (61).

A large Phase I study, including pharmacology, on 53 patients with a variety of cancers was reported by Woodcock *et al.* (112). Six different schedules of the TdR–5FU combination were employed; TdR doses ranged from 3 to 45 g and were infused in either 15 or 90 min. 5FU (7.5 or 10 mg/kg) was given as an i.v. bolus injection 60 min after the start of the TdR infusion. With the combination of 45 g TdR and 7.5 mg/kg 5FU, a 35% reduction of plasma 5FU levels was observed after 10 min. This reduction persisted for more than 2 h and was believed to be due to an equilibrium reaction between TdR and FUdR, with thymine, 5FU and deoxyribose-1-phosphate as the shared intermediates. FUdR was not detected in the plasma or urine of patients who received 5FU alone or in combination with 3 g TdR. Clearance of 5FU from plasma was very fast with a $T_{\frac{1}{2}}$ of 6 min; α or β phases could not be distinguished. With prior TdR treatment, however, plasma $T_{\frac{1}{2}\beta}$ values of 5FU were 33, 135, 188 and 190 min respectively, due to blocked oxidative degradation by excess thymine. Increased hematological toxicity was observed, and was ascribed to an increased concentration $\times$ time factor ($C \times t$) for 5FU. Antitumor effects were insufficient to establish superiority of the combination over 5FU alone. In 5 of 53 patients a neurologic syndrome occurred, characterized by lethargy, confusion, disorientation including nystagmus and dysmetria in 2 cases. The origin of the syndrome has not been clarified (112).

In a Phase II study of TdR plus 5FU reported by Ohnuma *et al.*, 10 patients with advanced solid tumors were given 24 courses of TdR–5FU (83). The major pharmacological effect of TdR was protracted plasma elimination of 5FU, which was more pronounced when TdR (8 g/m^2) was given as a rapid $2\frac{1}{2}$-h infusion than as a slow 24-h infusion. Myelosuppression was the major toxicity. None of the patients responded with more than 50% regression of measurable tumor (83).

In summary, the combination of TdR and 5FU appears to increase the toxicity of 5FU to the host, and does not seem to be superior to 5FU alone in terms of net antitumor action. An enhanced antitumor effect of the combination, however, may be masked by the conversion of 5FU to FUdR, due to an excess of deoxyribose-1-phosphate released from TdR under the influence of thymidine phosphorylase. FUdR would not be growth inhibitory *in vitro* in the presence of TdR (10, 42). The conversion of 5FU to FUdR in the presence of TdR has been observed *in vitro* by Murgo *et al.* (79), and *in vivo* by Woodcock *et al.* (112).

There is insufficient evidence that the increased toxicity of the combination is caused by channelling of 5FU selectively into RNA. It is more likely, that the modulation of 5FU metabolism by TdR and the resultant increased $C \times t$ factor for 5FU is responsible for the aggravated toxicity. An additional contributing factor may be the enhanced inhibition of thymidylate synthetase by 5FU when TdR is co-administered (109).

One preliminary report on combination chemotherapy of TdR with ftorafur, a 5FU analog which was developed in the Soviet Union, suggests an increased antitumor effect of ftorafur against P388 leukemia without significant increase in host toxicity (35).

Further studies on differential toxicity to host and tumor tissues are needed before the combination of TdR with one of the fluorinated pyrimidines enters Phase II trials.

Thymidine and reversal of methotrexate toxicity

The antifolate methotrexate (MTX) is widely used in the treatment of human cancer (4). MTX binds tightly but not irreversibly to dihydrofolate reductase, thus inactivating the enzyme. This leads to a depletion of tetrahydrofolates (FH_4), essential cofactors in the conversion of dUMP to dTMP and in the *de novo* synthesis of purines and some amino acids (Figure 2). MTX also inhibits thymidylate synthetase and this is regarded as the most important aspect of MTX cytotoxicity in man (41, 74).

Folinic acid (5-formyltetrahydrofolate) bypasses the metabolic block caused by MTX and supplies the reduced folates needed for synthesis of dTMP, purines and amino acids; its

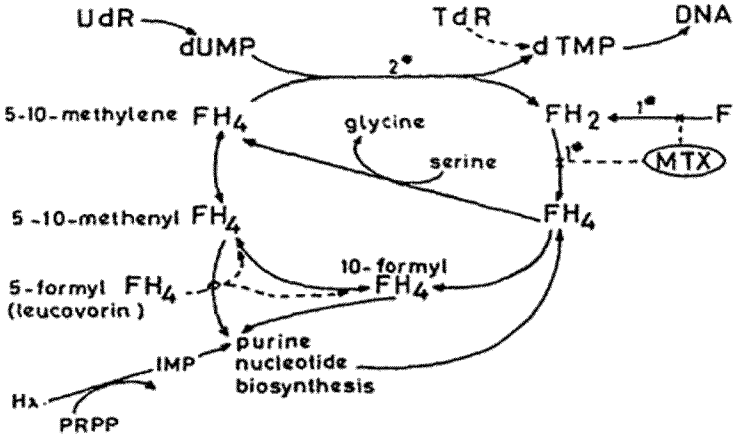

Figure 2. The mechanism of action of methotrexate. F = folic acid; FH_2 and FH_4 are dihydrofolic acid and tetrahydrofolic acid respectively; Hx = hypoxanthine; IMP = inosine monophosphate; PRPP = phosphoribosylpyrophosphate; TdR = thymidine and dTMP = thymidine monophosphate; UdR and dUMP are deoxyuridine and its monophosphate respectively. 1* = dihydrofolate reductase; 2* = thymidylate synthetase.

use thus allows the safe administration of high doses of MTX (3, 31). The reversal of MTX toxicity with folinic acid was extensively investigated in the early fifties by Goldin *et al.* (31). These experiments have led to the concept of "rescue", which refers to the utilization of a drug antidote which selectively salvages normal tissues. Toxicity of MTX *in vitro* can be reversed by TdR plus a purine. This was first shown by Hakala, who found that growth inhibition of Sarcoma-180 cells by 3×10^{-4} M MTX was completely reversed by TdR and hypoxanthine (Hyp) at a concentration of 3×10^{-5} M (36, 37). Partial protection was attained in certain cells with Hyp alone and in other cells with TdR alone. These observations were later confirmed by Borsa and Whitmore (7) and by Tattersall *et al.* (104), who demonstrated that in certain cell lines the cytostatic effects of MTX could be partially reversed by the addition of TdR to the culture medium, while in other cell lines both TdR and Hyp were required. Hryniuk studied the mechanism of action of MTX in L5178Y cells *in vitro* and found that MTX causes rapid depletion of reduced folates, thus producing a purineless and thymineless state (51, 52). This effect could be partially reversed by exogenous Hyp. With continuous exposure to MTX, the cells died a predominantly thymineless death, not rescued by Hyp. The purineless state may contribute initially to cell kill, but is reversible spontaneously after several hours of treatment, probably due to disintegration of nucleic acids, which replenishes the purine pools (53).

Grindey and Moran reported that allopurinol, a xanthine oxidase inhibitor, could reduce the therapeutic efficacy of MTX *in vivo* against L1210 leukemia but not against P388, without altering the toxicity of MTX to normal mice (32). This suggests that to some cells MTX exerts its toxic effects through inhibition of thymidylate synthesis while in other cells the cytotoxicity is determined by depletion of the purine pools.

Jackson and Weber found that MTX toxicity to rapidly growing hepatoma cells *in vitro* was due to an antipurine effect, as these cells could not be protected against MTX by TdR alone, while more slowly growing hepatoma cells maintained growth for 48 h in the presence of MTX when 200 μM TdR was added to the culture medium (56). The requirement for such large amounts of TdR indicated that the latter cells had retained the capacity of normal liver to catabolize TdR.

Pinedo *et al.* reported that, *in vitro*, in a culture system with dialyzed fetal calf serum (FCS) and dialyzed L-cell supernatant, MTX cytotoxicity to the mouse myeloid precursor cells (CFU-C) could be prevented by the presence of 10^{-5} M TdR with 10^{-5} M adenosine, inosine or hypoxanthine but not guanosine (85). When using whole medium, these authors were unable to block the proliferation of CFU-C with MTX, suggesting that salvage substances may be present in FCS. These findings indicate that the myeloid precursor cell has the capacity to utilize both the *de novo* and salvage pathways for synthesis of nucleotides (85).

Attempts have been made to correlate ribonucleoside (NTP) and deoxyribonucleoside triphosphate (dNTP) pools with inhibition of cell growth by MTX and MTX+TdR (53–55, 60, 104). Exposure of cultured cells to MTX alone was consistently followed by a fall in dTTP pools, while response of ribonucleotide pools to MTX varied according to the cell lines studied (53–55, 60, 104).

In all cases dATP and dCTP levels were either increased or not significantly altered. In L1210, Yoshida sarcoma and CCRF–CEM cells, the dGTP levels were unchanged or slightly increased by MTX (60, 104), while in N1S1 hepatoma, L and L5178Y cells the dGTP pools were greatly reduced (53, 55, 104), compatible with an antipurine activity of MTX in those cell lines. When both MTX and TdR were added to the culture medium, a rise in dTTP levels was observed in combination with a fall in other dNTP levels (60, 104).

This indicated that MTX exerts a predominantly antipurine effect in the presence of excess TdR.

Interestingly, Kinahan *et al.* found, that in L1210 leukemia cells the dTTP pools were depleted by MTX even in the presence of TdR (60). The dCTP levels were also reduced, but the purine dNTP pools remained unaffected, showing clearly that mammalian cells can respond in a differential fashion to metabolic perturbations, which in turn may play a role in determining drug selectivity (60). Jackson found that TdR reversed only the antithymidylate effect of MTX to N1S1 hepatoma cells *in vitro* when TdR was given after MTX (55). Pretreatment with TdR, however, appeared to antagonize both the antipurine and antithymidylate effect of MTX by diminishing cellular dUMP pools via inhibition of deoxycytidylate deamination and ribonucleotide reduction. The explanation for this observation was that the decrease in dUMP pool may have had the effect of switching off thymidylate synthetase and thus maintaining FH_4 cofactor levels and purine biosynthesis in the presence of MTX (55). This has been borne out by the data of Moran *et al.*, who found that when *de novo* thymidylate synthesis was blocked by 5-fluorodeoxyuridine (3 μM), a much higher degree of inhibition of dihydrofolate reductase *in vitro* could be endured without damage to the cell (76). These authors also observed that folinic acid-induced reversal of MTX *in vitro* was much more effective in the presence of 5-fluorodeoxyuridine and TdR than in their absence (76).

The observations that MTX cytotoxicity to normal mouse CFU-C could be reversed with exogenous TdR and/or one or more purines, while in other studies MTX cytotoxicity to some tumor cells could not be reversed, has led to the speculation that combining MTX with TdR and/or one or more purines might improve the therapeutic selectivity of the antifolate.

Nederbragt *et al.* reported that optimal reversal of MTX-induced inhibition of colony growth of L1210 leukemia cells *in vitro* (concentrations up to 10^{-4} M) required 10^{-5} M TdR and 3×10^{-4} M inosine (81). With the same inosine concentration but lower TdR concentrations (10^{-6} M) colony formation of DBA/2 mouse bone marrow cells in the presence of 10^{-6} M MTX was more than 60% of control, whereas this TdR–purine combination did not salvage L1210 cells. These authors suggested that in a murine *in vivo* system the combination of high concentrations of purines and low concentrations of TdR might lead to selective protection from MTX toxicity of myeloid precursor cells over L1210 cells (81).

Tattersall *et al.* reported that in L1210-bearing BDF_1 mice, TdR injected i.p. 3 times/day for 3 or 4 days could prevent MTX cytotoxicity to normal host tissues without affecting the antitumor action (102). In these experiments a bolus of MTX was given i.p. at a dose that was lethal to 13/16 animals when TdR was not co-administered. The fact that TdR was given in repeated i.p. bolus injections of 500 mg/kg 3 times/day could theoretically have contributed to the observed antitumor effect, because by this mode of administration millimolar (growth inhibitory) plasma concentrations of TdR would be obtained (67, 88). With only a 4-fold higher dose of TdR, injected i.p., Reiter showed an improved survival of C57Bl/6 mice with EL-4 tumors (88). However, the EL-4 tumor is reported to be among the most sensitive to TdR *in vitro* (90).

Semon and Grindey reported an increased therapeutic index for MTX against L1210 leukemia in DBA/2J and C57Bl/6 mice when TdR was co-administered (97). MTX and TdR were given as a continuous i.v. infusion, in order to obtain steady state plasma levels similar to cell culture conditions. These data agree with the observations of Kinahan *et al.* (60) who showed that in L1210 cells *in vitro* the intracellular dTTP pools were depleted by

MTX even in the presence of TdR, and also with Grindey and Moran (32) who claimed that allopurinol, injected i.p. prior to MTX, could protect L1210 leukemia cells but not the normal (DBA/2) mouse tissues against MTX. It should be stressed that the increased therapeutic effect reported by Semon and Grindey was observed only when low doses (5 g/kg/day) of TdR were administered, which did not completely protect the normal tissues of the mouse. Using higher doses (15 g/kg/day) of TdR they found that the toxicity of MTX was almost completely reversed but also that the antitumor effect was nullified (97).

Straw *et al.* were not able to protect non-tumor-bearing mice ({C57Bl × DBA/2}F1 and C57Bl/6) from the acute toxicity of MTX with TdR alone: both TdR and Hyp were required (100). However, in tumor-bearing mice (ascitic L1210 leukemia) TdR rescue in the absence of Hyp was successful, probably as a result of increased purine availability from MTX-induced tumor cell lysis. Drugs were given as i.p. bolus injections. In agreement with Hryniuk's observations (52, 53) these authors observed the existence of a purine deficiency, which was apparent earlier in the gut than in the bone marrow (100). Similar results were reported by Harrap *et al.* from the same institute (38). These authors confirmed in L1210 tumor-bearing mice (DBA/2 and DBA/2 × C57Bl hybrid) that a combination of purine and TdR gives protection from MTX toxicity while maintaining antitumor effect. In this study a combination of Hyp, allopurinol and TdR, given as i.p. injections following MTX (300 mg/kg i.p.), appeared to be superior to MTX and folinic acid rescue in terms of antitumor effect (38).

Grindey and coworkers found that when inosine (4 g/kg/day) was infused concurrently with MTX or with MTX + TdR (5 g/kg/day), both the toxicity to normal tissues and the antitumor activity of MTX were completely reversed (33, 97). However, when TdR + inosine in the same doses were administered following MTX, they were as effective as folinic acid in rescuing mice from toxicity with maintenance of antitumor effect. The concurrent infusion of MTX + TdR (5 g/kg/day) appeared to be much more effective than the two former regimens (33).

Clinical studies with methotrexate and thymidine

Concurrent infusion of MTX and TdR is referred to as "prevention" or "protection", while delayed administration of TdR has been termed "rescue". The first clinical study investigating the first of the two concepts has been reported by Ensminger and Frei from the Sidney Farber Cancer Institute (24). MTX was administered in progressively increasing doses up to 2 g/m^2/day for 72 h while TdR 8 g/m^2/day was simultaneously infused for 96 h. Three of 5 patients treated with the highest doses had mucositis and myelosuppression, which was reversible in 2 patients. With lower dose levels of MTX (80–1300 mg/m^2 in 24 h and 1300–3000 mg/m^2 in 48 h infusion) combined with TdR (8 g/m^2/day for 72 h) no toxicity was observed in patients with normal MTX clearance. TdR infusions resulted in increased plasma TdR levels from 0.19 to 1.5 μM. There was some evidence of antitumor activity in 4 of 12 patients treated (24). We have confirmed these findings in 10 patients with head and neck cancer, who received (MTX 0.6 g/m^2 in 24 h) with concurrent or delayed infusion of TdR (8 g/m^2/day) (96). We found no effect of TdR on MTX pharmacokinetics. With a dose of 8 g/m^2/day continuous infusion, plasma TdR levels reached 2×10^{-6} M, which were sufficient to protect bone marrow and gastrointestinal mucosa.

TdR "rescue" of high-dose MTX was initially described by Howell *et al.* (44). In that particular study MTX was given as a 24-h infusion at doses up to 8.5 g/m^2 followed by TdR infusion of 8 g/m^2/day until plasma MTX levels were below 5×10^{-8} M. These investigators also showed that plasma MTX concentrations of 1.5×10^{-5} M could be maintained for up to 40 h if followed by TdR 8 g/m^2/day until plasma was sufficiently cleared of MTX. Plasma TdR levels increased from 1.5×10^{-7} M pretreatment to 1.0×10^{-6} M during TdR infusion. The major toxicities were mucositis and myelosuppression, which were not life-threatening. Two of 16 evaluable patients achieved partial responses (44). Cytokinetic comparison of human bone marrow exposed *in vivo* to either MTX followed by folinic acid or MTX followed by TdR revealed that recovery of DNA synthesis was more rapid and more complete with TdR than with any dose of folinic acid (47). A striking observation in this study was that TdR could initiate DNA synthesis in human marrow cells in the presence of 1×10^{-4} M MTX consistent with the separate non-competitive membrane transport for both drugs (47). This is also consistent with earlier *in vitro* observations (7, 85). Evidently, TdR is more reliable and effective than folinic acid for rescuing patients who develop acute renal failure with persistent high plasma MTX levels.

The rapid marrow recovery with TdR would allow more frequent administration and higher doses of MTX (47). This was shown by Bruno *et al.*, who infused MTX 2 g/m^2/day for 2–7 days continuously, with protection by concurrent infusion of TdR 8 g/m^2/day plus folinic acid 1 mg/m^2/day up to 2 days beyond the end of the MTX infusion (9). Toxicity included mucositis in 15 of 22 courses and reversible myelosuppression in 5/22 courses. An antitumor effect was noted in 5 of 10 patients (9).

Recently, a Phase I clinical trial was conducted by Howell *et al.* (45), which demonstrated that the minimal requirements of TdR for rescue are in the range of 1 g/m^2/day, approximately one-eighth of the doses used in initial clinical studies (9, 24, 44, 47, 96). With a daily dose of 1 g/m^2 a less than 2-fold increase in TdR plasma levels compared to the mean pretreatment level of 2×10^{-7} M appeared to provide effective rescue for most patients, indicating a very steep dose–response relationship for TdR rescue of marrow (45). Subsequently, Howell and Tamerius described the achievement of long duration MTX exposure with concurrent TdR infusion at a dose of 2 g/m^2/day (50). With TdR plasma levels of only 5×10^{-7} M during the infusion, 72-h exposure to 5×10^{-5} M MTX was tolerated without major toxicity. However, the elimination half-life from the plasma increased linearly with the duration of MTX infusion, suggesting that prolonged exposure to these MTX levels resulted in storage of significant amounts of the antifolate in tissue reservoirs. None of the patients in the study, however, had any effusions which could function as a third space. The authors' hypothesis is that MTX may be stored in the form of polyglutamates which can be synthesized in liver, kidneys and red blood cells. Further studies are required to examine this theory. Despite the low dose of TdR, no antitumor effect was observed.

The same pharmacokinetic changes were observed by Creaven *et al.*, who maintained MTX plasma levels of 4.4×10^{-5} M for up to 7 days with TdR and folinic acid protection (9, 19). After cessation of MTX a triphasic decline of MTX was found in all patients receiving 7-day infusions. The $T_{\frac{1}{2}\beta+\alpha}$ were 5, 20–30 and 40 to more than 180 h respectively. Chromatography of plasma from 1 patient showed trace amounts of 7-OH-MTX (19). This is surprising and seems to be in contrast with data from Lankelma *et al.*, who reported a stepwise rise in plasma concentrations of 7-OH-MTX during 3 sequential MTX infusions (65).

In a current Phase II study of head and neck cancer patients who were treated with 0.6

g/m^2 MTX in a 24-h infusion with concurrent or delayed low-dose TdR (1.5 g/m^2/day for 72 h), we observed only minor responses in 3 of 12 patients, while toxicity was minimal. Further details of this study will be published separately. Thus it seems that in humans MTX + TdR does not offer any therapeutic advantages over MTX + folinic acid rescue and in fact it may even be inferior.

Howell *et al.* have investigated the minimal requirements of TdR and Hyp for the protection against MTX for normal and malignant human cells *in vitro* (48). They found that all the human cell types tested in their study required both TdR and Hyp for protection against MTX; although for the protection of marrow CFU-C 5–7-fold lower concentrations of TdR were required than for the protection of the malignant cells studied *in vitro*. This difference was regarded to be small due to the wide variability of plasma TdR concentrations *in vivo*. These authors also measured Hyp concentrations in 14 freshly aspirated bone marrow samples from patients undergoing orthopedic procedures and cancer patients with no marrow involvement and found a mean level of 11 μM, while Hyp levels in plasma were 0.5 μM for normal subjects and 0.9 μM for cancer patients. They also found that *in vitro* 10 μM Hyp was sufficient to allow CFU-C formation, dependent only on the availability of TdR (49). This disparity between marrow and plasma explains why TdR alone has shown a good rescue effect to marrow in man. Data on differences in Hyp concentrations between mouse bone marrow and plasma have not been reported. However, differences in pyrimidine and purine concentrations in plasma of different species have been shown (82) (A. Leyva, pers. comm.). Endogenous TdR levels in mouse plasma were found to be much higher than in human plasma. (2.8 and 0.5 μM respectively). In contrast, plasma Hyp in the mouse was much lower than in man, which can be explained by the high levels of xanthine oxidase that have been found in mouse plasma (2, 80). These interspecies differences in circulating purine and TdR levels may explain why MTX cytotoxicity to the mouse is mainly the result of a purineless state, whereas in man thymineless state seems to be the predominant mechanism of antifolate toxicity. However, those differences do not completely explain why an improved antitumor effect of MTX plus TdR has been observed in the mouse and not in man. It must be stressed, however, that in all the *in vivo* experiments that showed improvement of MTX antitumor effect, when TdR was co-administered, L1210 leukemia cells have been used (33, 38, 97, 100, 102).

Kinahan *et al.* have found a good correlation between the size of the dTTP intracellular pool and growth rate inhibition *in vitro* (60). These authors also observed an MTX-induced depletion of dTTP pools in L1210 cells even in the presence of exogenous TdR. This may well explain the improvement of therapeutic index for MTX + TdR that has been observed in the L1210 leukemia studies. Thus, the discrepancy between animal data and clinical results of the MTX–TdR combination may be related to unique properties of a tumor cell rather than to interspecies differences in endogenous purine and pyrimidine plasma concentrations.

Thymidine and 1-β-D-arabinofuranosylcytosine

The role of 1-β-D-arabinofuranosylcytosine (ara-C) in the treatment of acute leukemia is well established (16). Its cytostatic action has been attributed to its triphosphate, ara-CTP, which inhibits DNA polymerase by competition with the normal substrate dCTP, resulting in a block in DNA synthesis, defects in chain termination and chromosome aberrations. The conversion of ara-C to ara-CTP is mediated by deoxycytidine kinase (14). There is

evidence that dCTP inhibits the intracellular formation of ara-CTP probably by inhibiting deoxycytidine kinase (86). However, most of the growth-inhibitory effect of ara-C depends on the amount of intracellular ara-CTP in relation to the cellular content of dCTP (103, 39). As TdR inhibits dCTP formation by inhibition of ribonucleotide reductase (54), a combination of TdR with ara-C might theoretically enhance the sensitivity of the cells for the latter drug. Following this hypothesis, Harris *et al.* could show an increased sensitivity of 2 lymphoid cell lines to ara-C *in vitro* when combined with 10 μM TdR (39). These findings have been confirmed by other investigators (8, 21, 89, 101).

Another aspect of ara-C action is its inhibition of repair of single-strand DNA breaks induced by ultraviolet irradiation, which has been shown by Johnson and Collins and by Dunn and Regan (23, 57).

Woodcock *et al.* have presented evidence that ara-C can induce reinitiation of DNA synthesis in DNA segments which had been replicated earlier in the same S phase (110, 111). Thus, under the influence of ara-C under certain conditions some DNA segments undergo double replication during a single S phase. This is probably the direct cause of ara-C induced chromosome aberration and it may also have a role in the cytotoxicity of ara-C (110, 111).

Breitman and Keene reported synergism of TdR and ara-C in the treatment of melanomas and leukemia *in vitro* (8) and Reiter found increased sensitivity of TdR sensitive tumor cells to the combination TdR-ara-C *in vitro* (89). Combinations with ara-C, TdR and hydroxyurea, also an inhibitor of ribonucleotide reductase, appeared to have greater synergistic effect *in vitro* than any of the 2-drug combinations (101).

Martin *et al.* investigated the *in vivo* effect of a combination of ara-C with TdR and N-(phosphonoacetyl)-L-asparate (PALA), an inhibitor of *de novo* pyrimidine synthesis (72). Thus by modulating the activity of ara-C with two agents which deplete dCTP, these authors found improved activity of the combination in CD8F1 mice bearing breast tumors.

Danhauser and Rustum showed that following a 24-h infusion of TdR in rats, but not after i.v. bolus, the concentration of circulating dCyd dropped from 21·8 to 4·4 μM (21). They also observed an enhanced intracellular activation of ara-C to ara-CTP following infusion with TdR (21). A clinical Phase II trial with TdR + ara-C has been performed in 20 patients with relapsed acute leukemia (107). Simultaneous i.v. infusion of TdR 8 g/m^2/day with doses of ara-C escalating from 100 to 250 mg/m^2/day appeared to have activity even in patients refractory to ara-C alone. Toxicity consisted of vomiting, mucositis, diarrhoea, skin rash and hyperbilirubinemia in addition to bone marrow hypoplasia (107). More recently the same group reported the results of a Phase III trial in which ara-C (250 mg/m^2/day) and TdR (8 g/m^2/day) were given simultaneously as a continuous infusion until bone marrow aplasia occurred (usually 7–12 days) (106). Complete and partial responses were obtained in patients with ANLL (47%) and in 1 patient with blastic CML but not in ALL. Two patients developed severe enteritis with 1 therapy-related death (106). These data warrant further studies of the combination of TdR and ara-C in acute leukemia.

Concluding remarks

Despite a large amount of published data, there is as yet no defined role for TdR in anticancer treatment. TdR alone appears to be largely cytostatic, rather than cytotoxic (5, 15, 46, 62, 79). In clinical trials HD-TdR was shown to arrest tumor growth, but did not

lead to partial or complete remissions (5, 15, 62). One may conclude that single agent TdR has no role as an antineoplastic agent.

The use of TdR in modulating the mode of action or the pharmacokinetics of other antimetabolites is more promising. Although the clinical results of 5FU–TdR were disappointing (61, 83, 108, 112), further studies of combinations with TdR and one of the fluorinated pyrimidines may still be warranted. The observation that TdR altered the pharmacokinetics of 5FU (61, 83, 112) has once more shown the limitations of *in vitro* systems.

The reversal of MTX cytotoxicity with TdR has generated considerable interest. Relatively low doses of TdR appeared to afford almost complete protection against MTX cytotoxicity in man. However, the therapeutic ratio of MTX was not improved by TdR (48, 50). An increased antitumor activity of MTX+TdR has been shown in L1210-bearing mice (33, 38, 97, 100, 102) and this difference in antitumor effect between animal and human studies has been attributed to interspecies differences with regard to circulating purine and TdR levels (48). However, it seems more appropriate to relate it with properties of the L1210 cells, in which the dTTP pools are uniquely depleted by MTX even in the presence of exogenous TdR (53–55, 60, 104).

The reversal of MTX toxicity to human bone marrow was shown to be non-competitive and superior to folinic acid (9, 47), which makes TdR a useful drug for salvage of patients who develop acute renal failure during high-dose MTX treatment. The combination of TdR with ara-C in the treatment of acute leukemia is promising (106, 107) and may be also applied to the treatment of solid tumors (72).

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