Biochemical Determinants for Antileukemia Drug Treatment

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The success or failure of chemotherapy for leukemia is determined biochemically by a combination of biochemical aberrations in the leukemic cells relative to their non-malignant counterparts, and biochemical perturbations created by antileukemic drugs. Examples of recent developments in our understanding of these factors include studies on the metabolism of cytosine arabinoside, the use of high-dose methotrexate, the relationship between "de novo" and "salvage" nucleic acid synthesis in leukemic cells, and the development of new drugs such as the adenosine deaminase inhibitor, 2'-deoxycoformycin.

1-β-D-arabinofuranosyl cytosine (Ara-C) requires phosphorylation by kinases to the triphosphate Ara-CTP, in order to exert its antileukemic effect by inhibiting DNA polymerase. The deamination of Ara-C, catalysed by cytidine deaminase, produces the inactive metabolite 1-β-D-arabinofuranosyl uracil. Early studies on the mechanism of action of Ara-C in man concentrated on enzymatic determinations of the kinases and deaminase, to reflect the potential for Ara-CTP formation in any given cell population. Steuart and Burke (1971) demonstrated an inverse relationship between cytidine deaminase activity and overall response to Ara-C, and that the development of resistance to this drug was associated with increased cytidine deaminase activity. Conversely Tattersall et al. (1974) claimed a determining role for deoxycytidine kinase activity, and Smyth et al. (1976) found no direct correlation between the ratio of kinase-to-deaminase activities with clinical response. It is likely that variation in experimental procedure accounts for some of the controversies in these various studies, but there are inevitable limitations inherent in indirect enzymatic studies of this nature. More recently attention has been focused on the direct measurement of intracellular levels of Ara-CTP. In a study of patients with acute and chronic leukemias, Chou et al. (1977) found that blast cells from patients with acute myeloid leukemia who were clinically responsive to Ara-C, produced two-fold more Ara-CTP than did cells from chronic myelocytic, acute or chronic lymphocytic leukemia, or normal subjects. Significantly increased levels of Ara-CTP were produced in the presence of tetrahydrouridine, a potent inhibitor of cytidine deaminase, but the development of acquired resistance to Ara-C was not associated with diminished formation of Ara-CTP. These findings indicate that the mere production of high levels of Ara-CTP is not of itself sufficient to determine therapeutic response to Ara-C. Recent studies by Rustum et al.

(1978) measured not only the formation of Ara-CTP in human leukemic cells, but also the time for which adequate levels were retained in the cells. Thus in 8 of 9 patients who achieved a complete clinical remission, high initial levels of Ara-CTP were produced, and greater than 9.7 pmoles/10^7 cells were retained at 4 hours incubation. In contrast, in 8 patients whose cells retained $<0{,}71$ pmoles Ara-CTP/10^7 cells at 4 hrs, only 4 attained complete remission, and the durations of the latter were shorter than with the former groups of patients. Further work on Ara-CTP retention is clearly indicated, but these results are encouraging in their potential for more accurate prediction of response to Ara-C treatment, than has previously been shown with the indirect enzymatic approach.

Methotrexate has long been known to be an active agent in the treatment of acute lymphocytic leukaemia. Recent developments in understanding the pharmacokinetics and mechanism of action of this drug, have led to its use in sufficiently high dosage to require "rescue" of vital host tissues following methotrexate infusion – either with folinic acid, or more recently with nucleosides. The rationale behind the administration of high concentration infusions of methotrexate is based on the concept of enhancing perfusion into "sanctuary" sites such as the testes and central nervous system (Stoffel et al., 1975) and enhancing the free intracellular methotrexate concentration – a factor recently shown to contribute towards maximal cytotoxic effect (Goldman, 1975; Bender and Makulu, 1976). Wang et al. (1976) have monitored the pharmacokinetics of methotrexate administered as a 24 hr intravenous infusion to patients with ALL, at doses of 500 mg/m^2, followed 24 hrs later by folinic acid. Methotrexate in the cerebrospinal fluid reached 1.2×10^{-7} M at 30 min, and remained constant for 24 hrs. Preliminary results indicate that methotrexate used systemically in this way may be effective in the prevention of central nervous system leukemia. Further results of these studies are presented elsewhere in this workshop (Freeman, 1978).

From the biochemical standpoint recent research has focused on alternative – more selective – rescue techniques following methotrexate infusions, than that provided by folinic acid. Evidence is accumulating to suggest that vital host tissues may be able to utilise pyrimidine and purine nucleosides preferentially over tumour tissues thus bypassing the metabolic lesion created by methotrexate, and restoring nucleic acid synthesis in the normal bone marrow and gastrointestinal tract, with less "rescue" of the tumour cells. Tattersall et al. (1975) demonstrated that the delayed administration of thymidine to BDF_1 mice bearing the L1210 leukemia was superior to folinic acid in preventing lethal methotrexate toxicity, whilst maintaining antitumour efficacy. Semon and Grindey (1976) using the L1210 system in DBA/2J mice, infused thymidine simultaneously with methotrexate and confirmed enhanced therapeutic selectivity. Extending these studies to man, Ensminger and Frei (1977) have shown that continuous thymidine infusion during and up to 48 hrs after methotrexate infusions, can prevent toxicity from doses of the antifolate of up to 6 g/m^2, although very large doses of the nucleoside were required. The above studies relate only to replenishment of thymidylate synthesis, but methotrexate is known also to inhibit de novo

purine synthesis. Thus the consequences of methotrexate depletion of reduced folate cofactors in any given target organ or disease will depend on the relative dependence of those tissues on pyrimidine and purine nutrition. Harrap et al. (1977) have recently published a study showing that the addition of a purine source (hypoxanthine) to thymidine rescue of methotrexate treated L1210-bearing mice, was superior to rescue with thymidine alone or to folinic acid. These studies indicate that nucleoside rescue is effective in preventing methotrexate toxicity in both animals and man, but selective rescue of host tissues versus tumour awaits confirmation in man. We are currently conducting a Phase I evaluation of pyrimidine-purine rescue in man, for future therapeutic comparion with conventional folinic acid.

Critical to the ability of host or tumour cells being able to utilise exogenous nucleosides is the activity of the "salvage" pathways for purine and pyrimidine re-utilisation. The "de novo" synthesis of purine and pyrimidine nucleotides – the immediate precursors of nucleic acid – involves multiple energy-consuming reactions starting from small molecular weight compounds such as glycine and aspartate, and resulting in the formation of inosinate from which the purine nucleotides dATP and dGTP can be produced, and uridylate the precursor of pyrimidine dCTP and TTP. The so-called "salvage" pathways refer to the reutilisation of pre-formed purine or pyrimidine nucleosides or bases, which by direct phosphorylation can yield the corresponding ribonucleotides and hence deoxyribonucleotides, with energy conservation to the cell. Controversy has existed for some time as to the reliance of human leukaemic cells on one or both of these pathways, but recent studies by Rustum and Higby (1978) and Rustum and Takita (1978) provide useful information on this issue.

Rustum has measured radiolabelled precursor incorporation and the ribonucleotide pools in cells from patients with chronic lymphocytic or myelocytic leukemia, during different phases of the diseases. A low ratio of ATP/IMP indicative of reliance on the salvage pathway was found in patients with stable, chronic leukemia, whereas the development of blast crisis was associated in the same patients with a shift to high ATP/IMP ratios indicating a change in metabolic dependence to the "de novo" synthetic route. Such information has a bearing on the choice of therapy for different stages of leukemia, and contributes to our knowledge of possible targets for the design of new antileukemic drugs. Interest in the design of inhibitors of salvage pathway enzymes has been stimulated in particular, by recent work on adenosine metabolism in malignant lymphocytes and the role of the enzyme adenosine deaminase.

A specific association between the activity of adenosine deaminase (ADA) and lymphocyte metabolism first became apparent with the description of severe combined immunodeficiency disease arising in children born with a congenital absence of this enzyme (Dissing and Knudsen, 1972; Giblett et al., 1972). Although ADA is normally present in all mammalian tissues, activity is highest in the lymphoid system and increases in antigenically stimulated lymphocytes (Hall, 1963; Hovi et al., 1976). The demonstration of greatly increased ADA activity in malignant lymphocytes (Smyth and Harrap, 1975;

Smyth, 1976; Smyth et al., 1978b) led to a search for effective inhibitors of this enzyme, which might by analogy with the severe lymphoid depletion associated with genetic deletion of the enzyme – exert a specific antilymphocytic effect of value in the treatment of lymphocytic leukemia. 2′-deoxycoformycin (DCf) is the most potent inhibitor of ADA, with a Ki of 1×10^{-12} M (Agarwal et al., 1977; Johns and Adamson, 1976). Toxicity studies in normal animals confirmed the hypothesis that effective inhibition of ADA results in severe lymphoid depletion (Smyth et al., 1978a) and led to a Phase I clinical trial which is currently in progress. The preliminary data from this toxicological evaluation indicates that inhibition of ADA with DCf exerts a selective antilymphocytic effect in man. In 8 patients with non-haematological malignancies and normal pre-treatment peripheral blood counts, administration of DCf at 0.25 mg/kg as a single dose resulted in greater than 50% decrease in circulating peripheral lymphocyte counts in 3 out of 4 patients, and greater than 90% decrease in all of 4 patients treated daily $\times 5$. Recovery to normal differential counts was seen by day 14. 4 patients with relapsed ALL having failed all conventional therapy have been treated with DCf at 0.25 mg/kg daily $\times 5$. In one of these heavily pre-treated patients there was no response, and in another the peripheral blast count had decreased by 70% on day 5 with respect to the pre-treatment value. However in the other two patients there was a complete clearing of peripheral blasts from pre-DCf values of 644×10^9/l and 82320×10^9/l respectively. In the latter case this dramatic change in peripheral activity was reflected by shrinkage of previous splenomegally and clearing of blasts from the marrow. Full details of this Phase I study will be published shortly.

In summary, recent research into the mechanism of action of existing antileukemic agents, and their biochemical consequences within the host, has yielded information that may improve the use of drugs such as cytosine arabinoside, and enhance the therapeutic selectivity of methotrexate. Studies of alternative metabolic pathways for the synthesis of nucleic acid by leukemic cells indicates that "de novo" synthesis or "salvage" reutilisation may operate at different stages in the leukemic process, and suggests possible targets for future development of antimetabolite drugs. Ongoing clinical trials with 2′-deoxycoformycin demonstrate that inhibition of the salvage enzyme adenosine deaminase results in selective lymphocytotoxicity, of potential value for immunosuppression and the treatment of lymphoid malignancies.

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