before injection, no differences in colony numbers were shown. These results suggest that different AML progenitor cells can be defined in the whole AML population, dependent on the test system used. Moreover, the findings indicate a hierarchy in AML progenitors, including cells with short-term and long-term repopulating abilities, as has already been defined in the normal counterpart. This expanding knowledge on the biological behaviour of AML progenitors *in vitro* may be used in future clinical trials to optimise new therapeutic strategies, especially in the possible elimination of AML progenitor cells from the graft.

1. Herrmann F, Vellenga E. The role of colony-stimulating factors in acute leukemia. *J Cancer Res Clin Oncol* 1991, **116**, 275–282.
2. Vellenga E, Halie MR. Effects of colony-stimulating factors on myeloid leukaemia cells. In Mertelsmann R, Herrman F, eds. *Hematopoietic Growth Factors in Clinical Applications*. New York, Marcel Dekker, 1991; 363–378.
3. Young DC, Wagner K, Griffin JD. Autocrine secretion of GM-CSF in acute myeloblastic leukaemia. *Blood* 1986, **68**, 11–78.
4. Rambaldi A, Torcia M, Bettoni S, *et al.* Modulation of cell proliferation and cytokine production in acute myeloblastic leukaemia by interleukin-1 receptor antagonist and lack of its expression by leukemic cells. *Blood* 1991, **78**, 3248.
5. Furukawa Y, DeCaprio JA, Belvin M, *et al.* Heterogeneous expression of the product of the retinoblastoma susceptibility gene in primary human leukemia cells. *Oncogene* 1991, **6**, 1343–1346.
6. Demetri GD, Ernst TJ, Pratt ES, *et al.* Expression of *ras* oncogenes in cultured human cells alters the transcriptional and posttranscriptional regulation of cytokine genes. *J Clin Invest* 1990, **86**, 1261–1269.
7. Löwenberg B, Putten van WLJ, Touw IP, *et al.* Autonomous proliferation of leukemic cells *in vitro* as determinant of prognosis in adult acute myeloid leukemia. *N Engl J Med* 1993, **328**, 614–619.
8. Hunter AE, Rogers SY, Roberts IAG, *et al.* Autonomous growth of blast cells is associated with reduced survival in acute myeloblastic leukemia. *Blood* 1993, **83**, 899–903.
9. Lapidot T, Sirard C, Vormoor J, *et al.* A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994, **367**, 645.

Pergamon

European Journal of Cancer Vol. 30A, No. 10, pp. 1408–1411, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)00218-5

Predictive Value of Thymidylate Synthase and Dihydropyrimidine Dehydrogenase

G.J. Peters, C.L. van der Wilt and C.J. van Groeningen

INTRODUCTION

POTENTIATION OF inhibition of thymidylate synthase (TS) is considered to be the mechanism by which leucovorin modulates the antitumour activity of 5-fluorouracil (5FU) against colorectal cancer [1, 2]. Combination of leucovorin with 5FU has doubled the response rate of single-agent 5FU from approximately 10–15% to 20–40%, depending on schedule and dosing of both 5FU and leucovorin [1, 3]. However, this still means that the majority of patients does not benefit from this treatment in terms of response rate. In most of the studies, no major effect on survival time has been reported.

ROLE OF TS IN THE ACTION OF 5FU

Sensitivity and resistance

Several factors may be responsible for the lack of potentiation of 5FU by leucovorin. These factors are related to an aberration in the metabolism and disposition of either leucovorin or of 5FU. Besides the interpatient variation in pharmacokinetics of both drugs, there are a number of cellular or tumoral factors which determine whether leucovorin can modulate 5FU [1]. In order to be active, leucovorin has to be transported across the cellular membrane, a process mediated by a reduced folate carrier. For the folate antagonist, methotrexate, transport deficiency has been associated with resistance [4]. After transfer of the membrane, leucovorin has to be metabolised to 5,10-methylene-tetrahydrofolate (CH_2-THF), which is the one-carbon donor required for conversion of dUMP to dTMP, the reaction catalysed by TS. CH_2-THF is also essential for the formation of a stable ternary complex between FdUMP (the activated form of 5FU), TS and CH_2-THF. This complex is responsible for inhibition of thymidylate synthase. Although it has been reported that intermediates of the metabolic pathway of leucovorin to CH_2-THF can also support the formation of this ternary complex, CH_2-THF is the best substrate [5]. An even better inhibition is achieved in the presence of polyglutamates of CH_2-THF [6], which are formed by the action of folylpolyglutamate synthetase. A lack of polyglutamylation has been associated with resistance to 5FU [7]. For 5FU, transport deficiency has not been reported to be associated with resistance, but aberrations in its metabolism to FdUMP (the inhibitor of TS) or to FUTP (the metabolite for RNA incorporation) have been associated with resistance [1]. Considering the inhibition of TS, a number of possibilities exist, such as an increased activity of the enzyme (for instance, due to gene amplification) or altered kinetics of the enzyme (e.g. decreased affinity of either FdUMP or CH_2-THF to TS, leading to a diminished inhibition) [1]. Thus, it would be of interest to determine these parameters in patients who are receiving 5FU in combination with leucovorin for anti-cancer treatment. This information would also be

Correspondence to G.J. Peters.
The authors are at the Dept. of Oncology, Free University Hospital, PO Box 7057, 1007 MB Amsterdam, The Netherlands.
Received 10 May 1994; accepted 17 May 1994.

useful in designing alternative treatment schedules, bypassing potential resistance mechanisms, without enhancing toxic side-effects.

TS as a predictive parameter

When one considers TS as the major target for 5FU, it is a logical step to determine a relationship between the activity of TS and sensitivity to 5FU or to 5FU in combination with leucovorin. Consequently, one needs to know the minimal concentration of leucovorin required to achieve potentiation. Both aspects have been addressed repeatedly in the literature (see [8] for a recent review), but the answers are not clear, as has been pointed out by Beck and colleagues in this issue (pp. 1517–1522, 1522–1526). Traditionally, TS levels have been measured by determining the number of FdUMP binding sites or by measurement of the conversion of dUMP to dTMP. More recently, new methods for TS have become available, such as ELISA assays [9, 10], immunohistochemistry [11, 12] or semi-quantitative PCR [13]. However, for each of these methods, it has to be determined whether TS is really related to the sensitivity for 5FU. In a panel of 19 cell lines from various histological origins (five head and neck cancer, six breast cancer and eight from cancer of the digestive tract), Beck and colleagues (pp. 1517–1522) suggest they have observed such a relationship; however, the significance of this relationship is rather poor (r^2 of 0.22), possibly caused by a relatively large variation in crucial samples (considering either the sensitivity for 5FU or the TS activity), but more likely due to other factors determining sensitivity to 5FU. In a study from our laboratory, in which more parameters were included [14], we did not observe a relationship between 5FU sensitivity and TS activity, although the panel was smaller (six cell lines); sensitivity was mainly related to a balance in the activities of anabolic and catabolic enzymes, similar to other studies [15–17]. An update of our data on a possible relationship between TS activity and sensitivity to 5FU in an additional group of eight cell lines (colon cancer and head and neck cancer) also resulted in a similarly poor correlation ($r^2 = 0.27$). This means that more factors play a role in the sensitivity to 5FU, although TS seems to be one of the major factors.

Dosing of leucovorin

A major question in the modulation of 5FU activity is the dose of leucovorin. Several randomised clinical trials have been published [3, 18] in which patients received a low dose of leucovorin [20–25 mg/m^2, given either intravenously (i.v.) or orally], an intermediate dose (about 100 mg/m^2) or a high dose (500 mg/m^2). From preclinical *in vitro* studies, it was initially postulated that prolonged exposure to concentrations of leucovorin higher than 1 μM would be required [2, 8]. For most administration schedules of leucovorin, this plasma concentration was achieved [19, 20]; however, in patients receiving a low dose of leucovorin, this plasma concentration will not be achieved, although potentiation of the effect of 5FU has been observed [21]. This might be related to either a selective enhanced uptake of l-leucovorin, the active stereoisomer, in the tumour or by a low requirement of that specific tumour for leucovorin to potentiate the effect of 5FU [8, 10]. Indeed, in cell culture, very low concentrations of leucovorin (<0.1 μM) have been found to be sufficient to potentiate the growth-inhibitory effect of 5FU in certain cell lines [8]. However, at the concentrations of leucovorin usually employed in *in vitro* studies (1–10 μM), a considerable number of cell lines do not respond to

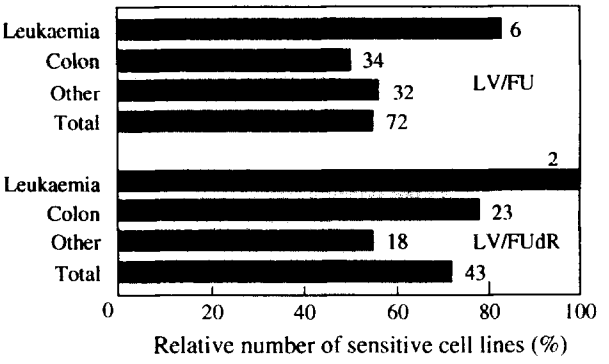

Figure 1. Summary of published modulation studies in cell lines. Data are from [8]. The total number of reported cell lines which were tested for modulation by leucovorin was set at 100%. The bars give the percentage of cell lines in which the sensitivity to 5FU could be modulated by co-incubation with leucovorin (the threshold potentiation factor was set at 1.5). The numbers in the figure indicate the number of cell lines tested for that specific tumour type.

potentiation by leucovorin (Figure 1). An interesting feature of the *in vitro* modulation studies was the observation that modulation of 5-fluoro-2'-deoxyuridine (FUdR) was observed in more cell lines than that of 5FU. Beck and colleagues (pp. 1522–1526) describe that modulation of 5FU by leucovorin, in apparently modulation-resistant cell lines, cannot be achieved by exposing the cells to either low or high concentrations of leucovorin. In the modulation-sensitive lines, the modulation is a saturable phenomenon related to the concentrations of leucovorin. Another interesting feature in the modulation of 5FU by leucovorin was the observation that cell lines with a low IC_{50} for 5FU could be modulated by leucovorin, but cell lines with a high IC_{50} could not. This was a pattern which was also found in more cell lines [8]. The concentration of leucovorin required for modulation was variable. In *in vivo* tumour models, we observed that administration of a very high dose of leucovorin (total 500 mg/kg) did not improve the modulating effect compared to lower doses (total 100–200 mg/kg), the latter comparable to the high doses used in patients [22].

Inhibition of TS

Most tumour cell lines are derived from one tumour, and one could consider a panel of cell lines being representative for several different tumours and thus for the heterogeneity observed among tumours. This could mean that certain tumours would respond to modulation by a low dose of leucovorin, but others would not. Indeed, when we compared the inhibition of TS in patients who received a test dose of 5FU (500 mg/m^2), in combination with either low-dose leucovorin (25 mg/m^2) or high-dose leucovorin (500 mg/m^2), inhibition of TS was significantly enhanced in patients receiving the high dose of leucovorin as compared with patients receiving 5FU alone [23]; in patients receiving the low dose of leucovorin, inhibition of TS was comparable with that of 5FU alone. When comparing patients who had received 5FU alone in a test dose and 5FU alone as treatment, a clear correlation was observed between the extent of TS inhibition and response to treatment, as well as between total TS and response to treatment [24]. Similar results were published recently in a preliminary form for a relation between the expression of TS-mRNA and response of patients with gastric cancer [25], as well as between immunohistochemical staining for TS and survival of patients with rectal cancer [26];

both groups of patients were treated with a 5FU-containing regimen.

The question remains whether those patients not responding to combination chemotherapy with 5FU and leucovorin would respond to a therapy with even higher doses of leucovorin. The answer is not straightforward. Possibly several patients would, but a number of patients might have a mutant form of TS which cannot be modulated by high-dose leucovorin. In several patients, the activity of TS may be too high to be inhibited sufficiently to block DNA synthesis. This activity can be high due to the large genetic heterogeneity which has been observed between non-treated patients [27] or as a result of prior treatment with 5FU. Even a 99% TS inhibition in the tumour of a patient with a basal TS activity of 3000 pmol of dTMP formed per h per mg protein, would still result in a residual activity of 30 pmol dTMP formed per h per mg protein. However, 99% inhibition in the tumour of a patient with a basal activity of 100 would lead to just 1 pmol of dTMP formed per h per mg protein. Thus, in the patient with the very high activity, it may be impossible to inhibit TS to the level which will result in cytotoxicity. Alternatively, it is also possible that, in the modulation-resistant patients, the major mechanism of action of 5FU is RNA directed, which cannot be modulated by leucovorin. However, it has been reported that the combination with leucovorin can cause a shift in the mechanism of action of RNA to TS directed [1, 8]. In order to bypass more mechanisms of resistance to 5FU, it seems worthwhile to use multiple modulators such as interferon-α, PALA (*N*-phosphonacetyl-L-aspartate), uridine and others [1, 8].

ROLE OF DIHYDROPYRIMIDINE DEHYDROGENASE

A novel aspect in the study of Beck and colleagues (pp. 1517–1522) is their observation of a relatively high activity of dihydropyrimidine dehydrogenase in the tumour cell lines studied. Although the presence of comparable levels of this enzyme has been previously reported in tumours [28], it is the first time that a relatively large panel of cell lines has been assayed for the activity of this degradation enzyme in cell extracts. For intact cells, using a flow-through system, we observed that the degradation pathway can have considerable activity [29]; however, no relationship could be demonstrated with sensitivity to 5FU. Beck and colleagues (pp. 1517–1522) observed a relatively low correlation coefficient ($r^2 = 0.27$), indicating that this enzyme may play a role in sensitivity to 5FU, but is not the major determinant. It should be noted that the balance between activating and inactivating enzymes will ultimately determine which is the predominant pathway. However, both in tumour samples from colon cancer patients and several cancer cell lines, in which we characterised the activity of the activation and inactivation pathways [14, 30], we observed that the activity of dihydropyrimidine dehydrogenase was 2–10-fold lower than that of the activation enzyme with the lowest activity ([30], unpublished observations). Under pharmacological conditions, especially with continuous infusions in which plasma concentrations are low (1–5 μM; [31]), the kinetic characteristics (K_m and V_{max} values) of the activation and inactivation pathways would favour activation of the drug. Recently, the same group also reported on the activity of dihydropyrimidine dehydrogenase in tumour samples from patients with head and neck cancer [32]; a large variation in the activity of this enzyme was observed, both in the group of patients responding to 5FU-based chemotherapy and in patients not responding. The mean activity of dihydropyrimidine dehydrogenase in the responding group was significantly lower, but there was a large overlap in enzyme activity. Currently, the meaning of these findings, although interesting, is not clear. The balance between activation/inactivation of 5FU metabolism was not measured in this study, but compared to reported activities of anabolic enzymes in squamous cell carcinoma, the degradation activity is still low [33]. Thus, based on the information currently available, tumour dihydropyrimidine dehydrogenase levels cannot (yet) be considered to be a predictive parameter for 5FU. However, high or low dihydropyrimidine dehydrogenase activities in normal tissues (e.g. liver, in which levels are much higher than in tumours) may be a major determinant in the pharmacokinetics of 5FU; low dihydropyrimidine dehydrogenase levels being clearly related to enhanced toxic side-effects [34].

CONCLUSIONS

Measurement of pretreatment TS levels with either an enzyme assay, immunohistochemistry or semi-quantitative PCR, may give sufficient information to predict whether a patient has a good chance of responding to 5FU-containing therapy. Considering the encouraging clinical data with new specific folate-based TS inhibitors, such as Tomudex (ZD-1694) which has shown an overall response rate of 27% in patients with advanced colorectal cancer [35], this information would also be valuable in order to determine a relationship between TS activity and response to this new class of anti-cancer drugs. The data on dihydropyrimidine dehydrogenase are too preliminary to be considered as predictive for the result of 5FU therapy, but are clearly indicative of aberrations in 5FU pharmacokinetics.

1. Peters GJ, Van Groeningen CJ. Clinical relevance of biochemical modulation of 5-fluorouracil. *Ann Oncol* 1991, **2**, 469–480.
2. Mini E, Trave F, Rustum YM, Bertino JR. Enhancement of the antitumor effects of 5-fluorouracil by folinic acid. *Pharmacol Ther* 1990, **47**, 1–19.
3. Advanced Colorectal Cancer Meta-analysis Project. Modulation of fluorouracil in patients with advanced colorectal cancer. Evidence in terms of response rate. *J Clin Oncol* 1992, **10**, 896–903.
4. Bertino JR. Ode to methotrexate. *J Clin Oncol* 1993, **11**, 5–14.
5. Van der Wilt CL, Pinedo HM, De Jong M, Peters GJ. Effect of folate diastereo-isomers on the binding of 5-fluoro-2′-deoxyuridine-5′-monophosphate to thymidylate synthase. *Biochem Pharmacol* 1993, **45**, 1177–1179.
6. Radparvar S, Houghton PJ, Houghton JA. Characteristics of thymidylate synthase purified form a human colon adenocarcinoma. *Arch Biochem Biophys* 1988, **260**, 342–350.
7. Wang FS, Aschele C, Sobrero A, Chang YM, Bertino JR. Decreased folylpolyglutamate synthetase expression: a novel mechanism of fluorouracil resistance. *Cancer Res* 1993, **53**, 3677–3680.
8. Van der Wilt CL, Peters GJ. New targets for pyrimidine antimetabolites for the treatment of solid tumors: I thymidylate synthase. *Pharmacy World Science* 1994, **16**, 84–103.
9. Johnston PG, Drake JC, Steinberg SM, Allegra CJ. Quantitation of thymidylate synthase in human tumors using an ultrasensitive enzyme-linked immunoassay. *Biochem Pharmacol* 1993, **45**, 2482–2486.
10. Aherne GW, Hardcastle A, Newton R. Measurement of human thymidylate synthase (hTS) in cell lines using ELISA. *Ann Oncol* 1992, **3** (suppl. 1), 77.
11. Johnston PG, Drake JC, Trepel J, Allegra CJ. Immunological quantification of thymidylate synthase using the monoclonal antibody TS 106 in 5-fluorouracil-sensitive and -resistant human cancer cell lines. *Cancer Res* 1992, **52**, 4306–4312.
12. Van der Wilt CL, Smid K, Aherne GW, Pinedo HM, Peters GJ. Evaluation of immunohistochemical staining and activity of thymidylate synthase in cell lines. In Ayling JE, Nair MG, Baugh CM, eds. *Chemistry and Biology of Pteridines and Folates, Adv Exp Med Biol* 338. New York, Plenum Publishing Cooperation, 1993, 605–608.

13. Horikoshi T, Danenberg KD, Stadlbauer THW, *et al.* Quantitation of thymidylate synthase, dihydrofolate reductase, and DT-diaphorase gene expression in human tumors using the polymerase chain reaction. *Cancer Res* 1992, **52**, 108–116.
14. Peters GJ, Laurensse E, Leyva A, Lankelma J, Pinedo HM. Sensitivity of human, murine and rat cells to 5-fluorouracil and 5'-deoxy-5-fluorouridine in relation to drug-metabolizing enzymes. *Cancer Res* 1986, **46**, 20–28.
15. Evans RM, Laskin JD, Hakala MT. Assessment of growth-limiting events caused by 5-fluorouracil in mouse cells and human cells. *Cancer Res* 1980, **40**, 4113–4122.
16. Laskin JD, Evans RM, Slocum HK, Burko D, Hakala MT. Basis for natural variation in sensitivity in mouse and human cells culture. *Cancer Res* 1979, **39**, 383–390.
17. Fernandes DJ, Cranford SK. Resistance of CCRF-CEM cloned sublines to 5-fluorodeoxyuridine associated with enhanced phosphatase activity. *Biochem Pharmacol* 1985, **34**, 125–132.
18. Buroker TR, O'Connell MJ, Wieand HS, *et al.* Randomized comparison of two schedules of fluorouracil and leucovorin in the treatment of advanced colorectal cancer. *J Clin Oncol* 1994, **12**, 14–20.
19. Trave F, Rustum YM, Petrelli NJ, Herrera L, Mittelman A, Frank C, Creaven PJ. Plasma and tumor tissue pharmacology of high-dose intravenous leucovorin in combination with fluorouracil in patients with advanced colorectal carcinoma. *J Clin Oncol* 1988, **6**, 1184–1191.
20. Schalhorn A, Kühl M. Clinical pharmacokinetics of fluorouracil and folinic acid. *Semin Oncol* 1992, **19** (suppl. 3), 82–92.
21. Hines JD, Adelstein DJ, Spiess JL, Giroski P, Carter SG. Efficacy of high-dose oral leucovorin and 5-fluorouracil in advanced colorectal carcinoma. Plasma and tissue pharmacokinetics. *Cancer* 1989, **63**, 1022–1025.
22. Van der Wilt CL, Pinedo HM, Smid K, Peters GJ. Elevation of thymidylate synthase following 5-fluorouracil treatment is prevented by the addition of leucovorin in murine colon tumors. *Cancer Res* 1992, **52**, 4922–4928.
23. Peters GJ, Hoekman K, Van Groeningen CJ, *et al.* Potentiation of 5-fluorouracil induced inhibition of thymidylate synthase in human colon tumors by leucovorin is dose dependent. In Ayling JE, Nair MG, Baugh CM, eds. *Chemistry and Biology of Pteridines and Folates, Adv Exp Med Biol* 338. New York, Plenum Publishing Cooperation, 1993, 613–616.
24. Peters GJ, Van der Wilt CL, Van Groeningen CJ, Meijer S, Smid K, Pinedo HM. Thymidylate synthase inhibition after administration of 5-fluorouracil with or without leucovorin; implications for treatment with 5-fluorouracil. *J Clin Oncol* 1994, in press.
25. Lenz HJ, Leichman C, Danenberg P, *et al.* Thymidylate synthase (TS) gene expression predicts response of primary gastric cancer (GC) to 5-fluorouracil (5FU)-leucovorin (LV)-cisplatin (DDP). *Proc Am Soc Clin Oncol* 1993, **12**, 199.
26. Johnston PG, Fisher E, Rockette HE, Fisher B, Wolmark N, Allegra CJ. Thymidylate synthase expression is an independent predictor of survival/disease free survival in patients with rectal cancer. *Proc Am Soc Clin Oncol* 1993, **12**, 202.
27. Peters GJ, Van Groeningen CJ, Laurensse EJ, Pinedo HM. Thymidylate synthase from untreated human colorectal cancer and colonic mucosa: enzyme activity and inhibition by 5-fluoro-2'-deoxyuridine-5'-monophosphate. *Eur J Cancer* 1991, **27**, 263–267.
28. Naguib FNM, El Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985, **45**, 5405.
29. Spoelstra EC, Pinedo HM, Dekker H, Peters GJ, Lankelma J. Measurement of *in vitro* cellular pharmacokinetics of 5-fluorouracil in human and rat cancer cell lines and rat hepatocytes using a flow-through system. *Cancer Chemother Pharmacol* 1991, **27**, 320–325.
30. Peters GJ, Van Groeningen CJ, Laurensse EJ, Pinedo HM. A comparison of 5-fluorouracil metabolism in human colorectal cancer and colon mucosa. *Cancer* 1991, **68**, 1903–1909.
31. Peters GJ, Schornagel JH, Milano GA. Clinical pharmacokinetics of antimetabolites. *Cancer Surv* 1993, **17**, 123–156.
32. Milano G, Etienne MC, Dassonville O, *et al.* Prediction of FU treatment resistance based on tumoral dihydropyrimidine dehydrogenase activity. A clinical study. *Proc Am Assoc Cancer Res* 1994, **35**, 208 (abstract 1242).
33. Peters GJ, Braakhuis BJM, De Bruijn EA, Laurensse EJ, Van Walsum M, Pinedo HM. Enhanced therapeutic efficacy of 5'deoxy-5-fluorouridine in 5-fluorouracil resistant head and neck tumours in relation to 5-fluorouracil metabolising enzymes. *Br J Cancer* 1989, **59**, 327–334.
34. Harris BE, Song R, Soong S, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res* 1990, **50**, 197–201.
35. Zalcberg J, Cunningham D, Van Cutsem E, *et al.* Good antitumour activity of the new thymidylate synthase inhibitor tomudex (ZD1694) in colorectal cancer. *Ann Oncol* 1994, **5** (suppl. 5), 133 (abstract 243).

Pergamon

European Journal of Cancer Vol. 30A, No. 10, pp. 1411–1413, 1994

0959–8049/94 $7.00 + 0.00

0959-8049(94)00265-7

Lonidamine: *In Vitro/In Vivo* Correlations

B. A. Teicher

LONIDAMINE, 1[(2,4-dichlorophenyl)methyl]-1H-indazole-3-carboxylic acid, was first prepared and studied in the mid-1970s as an anti-spermatogenic agent [1]. It was soon recognised that mitochondria and, consequently, cellular energy metabolism were targets for the observed anti-spermatogenic activity. The potential application of lonidamine to malignant disease was quickly realised, and preclinical development of lonidamine in cancer began [2]. Lonidamine is interesting as an anti-cancer agent for two reasons: (1) it has a unique mechanism of action and (2) it has a unique spectrum of normal tissue toxicities.

Numerous careful and elegant studies on the mechanism of lonidamine cellular effects focused on the mitochondria and oncellular energetics. These studies identified mitochondrial hexokinase as an enzymatic target for this drug [3–6]. Later studies, however, found that lonidamine alters properties of the inner surface of the plasma membrane of cells as well as damaging both the inner and outer mitochondrial membranes, resulting in

Correspondence to B. A. Teicher at the Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, U.S.A.
Received 19 May 1994; accepted 24 June 1994.