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Biochemical Pharmacology, Vol. 18, pp 2587–2590. Pergamon Press. 1969. Printed in Great Britain

Synthesis of 5-fluorouridine 5′-phosphate by a pyrimidine phosphoribosyltransferase of mammalian origin—II. Correlation between the tumor levels of the enzyme and the 5-fluorouracil-promoted increase in survival of tumor-bearing mice*

(*Received* 30 *January* 1969; *accepted* 9 *May* 1969)

IT IS now generally agreed that 5-fluorouracil (FU), the pyrimidine analog introduced by Duschinsky *et al.*,[1] must first be converted to 5-fluoro-2′-deoxyuridine 5′-phosphate (F-dUMP) before it can exert its carcinostatic effect. The latter compound has been shown to be a potent inhibitor of the enzyme thymidylate synthetase.[2,3] The inhibition of this enzyme interferes with DNA synthesis because of the resulting deficiency of available thymidylate. Current knowledge relating to the fluorinated pyrimidines has been summarized in a recent review by Heidelberger.[4]

Previous studies dealing with the development of tumor resistance to FU have indicated that more than one biochemical mechanism may be responsible for this phenomenon. Thus, insufficient levels of uridine phosphorylase,[5] uridine kinase[6] and orotidylate decarboxylase,[7] as well as the presence of an altered thymidylate synthetase[8] insensitive to inhibition by F-dUMP, have all been implicated as being determinants of resistance in a variety of transplantable mouse tumors.

This paper reports that cell-free extracts from a wide spectrum of transplantable mouse tumors catalyze the formation of 5-fluorouridine 5′-phosphate (F-UMP) from FU and 5-phosphoribosyl 1-pyrophosphate (PP-ribose-P). Furthermore, there is a statistically significant correlation between the specific activity of the enzyme responsible for this reaction in extracts from the various tumors

*These studies were supported in part by Research Grants C-6516 from the National Cancer Institute and FR-05526 from the Division of Research Facilities and Resources, and by Contract PH 43-66-541 from the Cancer Chemotherapy National Service Center, National Institutes of Health.

(solid form) and the FU-promoted increase in survival of mice bearing these tumors in the ascitic form. Studies reported elsewhere[9] identified the product as F-UMP and served to demonstrate that the properties of the enzyme are those of a pyrimidine phosphoribosyltransferase. Although its natural substrate(s) is not known with certainty at the present time, for purposes of convenience, hereafter the enzyme will be referred to as FU phosphoribosyltransferase. The possible chemotherapeutic importance of this enzyme activity, whose presence in mammalian tissues appears to be more widespread than was previously considered,[4] is discussed.

For the preparation of cell-free extracts, the mouse solid tumors were excised 6–8 days after subcutaneous inoculation of at least six mice with approximately 10^6 ascites tumor cells. The excised tumor from each mouse was rinsed with ice-cold 0·9% NaCl, blotted dry, weighed and minced with a sharp blade. The tumor mass was then homogenized in 3 vol. of ice-cold 10 mM Tris Buffer, pH 7·5, with a ground-glass tissue grinder. After the removal of an aliquot for the determination of protein[10], the homogenate was centrigufed at 30,000 *g* for 30 min and the resultant supernatant fraction was assayed immediately for FU phosphoribosyltransferase activity.

The enzyme assay, described in detail elsewhere,[9] measured the conversion of FU-2-^{14}C to F-UMP-2-^{14}C. This conversion requires the presence of the supernatant fraction, PP-ribose-P, and a divalent cation; ribose 1-phosphate does not substitute for PP-ribose-P. Chromatography of reaction mixtures on DEAE-cellulose paper strips separated residual FU-2-^{14}C from the newly synthesized F-UMP 2-^{14}C which remained at the origin. Product formation was quantitated by punching out the origin from the dried paper strips and measuring the radioactivity by liquid scintillation techniques. Blanks consisted of reaction mixtures in which the enzyme was immediately denatured by the presence of 6% trichloroacetic acid. Enzyme specific activity is expressed as mμmoles of F-UMP synthesized per hr per mg protein.

The transplantable mouse tumors used in the present study were kindly provided by Isidore Wodinsky, Arthur D. Little, Inc., Cambridge, Mass. The morphology and source of most of these tumors have been reported previously.[11]

Figure 1 illustrates the relationship between the specific activity of FU phosphoribosyltransferase in

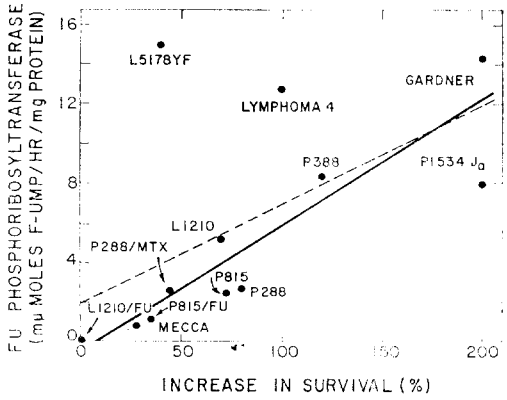

FIG. 1. Correlation between the specific activity of FU phosphoribosyltransferase in cell-free extracts from mouse solid tumors and the FU-promoted increase in survival of mice bearing these tumors in the ascitic form. FU was injected intraperitoneally at the maximally effective dose (in the range of 5–20 mg/kg) from day 1 through day 10 into mice that had received 10^6 tumor cells. The FU-promoted increase in survival of the tumor-bearing mice was calculated from the equation: $[(T - C)/C]$ 100, where T is the mean survival time of FU-treated mice and C is the mean survival time of untreated mice. The enzyme activity of at least six different cell-free extracts was determined for each of the transplantable mouse tumors; the mean of these values is reported in the figure. Regression lines were fitted to the experimental data by the method of least-squares.[13] The dashed regression line was calculated on the basis of all experimental points, whereas the L5178YF data were eliminated when calculating the solid regression line.

cell-free extracts from mouse solid tumors and the FU-promoted increase in survival of mice bearing these same tumors in the ascitic form. The coefficient of correlation, calculated[12] on the basis of all experimental points in Fig. 1, was 0·59 (dashed regression line). This value indicates the existence of a significant correlation between enzyme specific activity and increase in survival, with $0{\cdot}05 > P > 0{\cdot}01$. It is evident, however, that the L5178YF tumor does not lie close to the regression line. One possible explanation for this result may be the observation[14] that this particular tumor line possesses relatively high levels of the target enzyme, thymidylate synthetase. Such enzyme levels may have negated the carcinostatic effect of F-dUMP, resulting in a lower than expected increase in survival. In any event, elimination of the L5178YF data yielded a correlation coefficient of 0·82, with $P < 0{\cdot}01$ (solid regression line).

The above results not only demonstrate that FU phosphoribosyltransferase activity occurs in a broad spectrum of transplantable mouse tumors but also show that the specific activity of this enzyme in extracts from the various tumors is correlated with the FU-promoted increase in host survival. Thus, F-UMP synthesized via this pathway is chemotherapeutically very effective. These findings, therefore, do not agree with the currently accepted view that ribonucleotide formation from FU proceeds in large measure by way of the uridine phosphorylase–uridine kinase pathway.[15] This apparent anomaly may be due to intracellular compartmentation of F-UMP synthesized via the two pathways into separate metabolic pools. It is conceivable that F-UMP in one such pool could be more efficiently converted to F-dUMP. This may be the case for F-UMP synthesized by the FU phosphoribosyltransferase pathway.

In addition, it would appear that the pyrimidine phosphoribosyltransferase responsible for F-UMP formation is not required for nucleic acid synthesis *de novo*, since it is virtually absent from L1210/FU tumor cells (Fig. 1). This indicates that the enzyme may normally catalyze a "salvage" pathway[16] for the utilization of preformed pyrimidine bases such as uracil.[17,18] A discussion of the probable natural substrate(s) for this enzyme appears elsewhere.[9]

The significance of the present results is further strengthened by the earlier observation[14] that the level of uridine kinase, thymidine kinase and thymidylate synthetase in various tumor cells did not correlate with the FU-promoted increase in survival of tumor-bearing mice.

It must be emphasized that, whereas solid tumors were used to obtain the above enzymic data (Fig. 1), the survival data were acquired from studies with mice bearing the ascitic form of these tumors. However, our finding that the specific activity of FU phosphoribosyltransferase in cell-free extracts of a number of ascites tumors did not differ greatly on a relative basis from those values reported here for the corresponding solid tumors* indicates that this comparison is permissible and therefore supports the validity of the above correlation. Ascites tumors were used in the survival studies because the relationship between drug lethality, the kinetics of cellular proliferation, and host survival is better documented for this type of tumor than for the corresponding solid form (see Skipper[19] and references therein). Also, it was reasoned that variations in survival due to possible differences in blood supply, hence drug supply, to the solid tumor tissue would be reduced by use of the corresponding ascites tumors. On the other hand, FU phosphoribosyltransferase activity was determined on solid tumors because of our desire to utilize a biological sytsem which more closely resembled FU-sensitive human tumors.

In view of the present results, it is quite possible that the objective responses produced by FU in patients with cancer of the breast or gastrointestinal tract[4] may be due primarily to high levels of FU phosphoribosyltransferase in these human tumor cells. Accordingly, the FU phosphoribosyltransferase activity in cell-free extracts of a variety of human tissues, both normal and neoplastic, has been determined.† Although this enzyme activity was found to be present in all of these tissues, in general, enzyme specific activity values were substantially lower than those observed for the mouse tumors (Fig. 1). Thus, only further investigation will reveal whether a correlation exists between high levels of the enzyme and human tumor response to FU therapy. If this is indeed found to be so, FU phosphoribosyltransferase activity may prove useful as a predictive index of FU-promoted response in human cancer patients.

*The values for the ascites tumors, expressed as mμ moles of F-UMP synthesized per hour per mg of protein, are as follows: Lymphoma 4, 15·1; P1534Ja, 10·6; P388, 8·0; L1210, 6·9; P288, 5·1; P815, 3·1; Mecca, 1·9; and L1210/FU, 0·6. In general, these values are somewhat greater than those for the solid tumors reported in Fig. 1.

†P. Reyes and T. C. Hall, unpublished data.

Laboratories of Pharmacology,
The Children's Cancer Research Foundation and
Departments of Pathology and Medicine,
Harvard Medical School,
Boston, Mass. 02115, *U.S.A.*

PHILIP REYES*
THOMAS C. HALL†

*Requests for reprints should be addressed to this author at The Children's Cancer Research Foundation, 35 Binney Street, Boston Mass. 02115.
†Present address: Department of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, N.Y. 14620.

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Biochemical Pharmacology, Vol. 18, pp 2590–2595. Pergamon Press. 1969. Printed in Great Britain

Action of acetylcholine in the presence of organophosphates on single axons of the lobster*

(*Received* 26 *November* 1968; *accepted* 25 *April* 1969)

THE GIANT axon of the circumesophageal connectives of the lobster is a suitable preparation for studying the effects of acetylcholine (ACh) on axonal conduction. It was shown previously that ACh depolarizes the conducting membrane, causing repetitive firing, then block of conduction. These actions seemed similar to those seen at the neuromuscular junction. However, not all of the findings

*This work was supported in part by United States Health Service Grants NB07070, NB07337 and NB03304 and by The National Science Foundation Grant GB7149.