[CANCER RESEARCH 44, 2251–2252, May 1984]

Letter to the Editor

Aspartate Transcarbamylase from Human Tumoral Cell Lines: Accurate Determination of Michaelis Constant for Carbamylphosphate by Intercept Replots[1]

Jean Baillon,[2] Marcelle Guichard, and Guy Hervé

Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette [J. B., G. H.], and Laboratoire de Radiobiologie Cellulaire, Institut National de la Santé et de la Recherche Médicale, U.247, Institut Gustave Roussy, 94805 Villejuif Cedex [M. G.], France

ATCase[3] catalyzes the second reaction of the *de novo* pyrimidine pathway that is the carbamylation of the amino group of aspartate by carbamylphosphate. The kinetic parameters of ATCase were investigated previously in the dialyzed cell-free extracts of 10 different human normal and tumoral cell lines (1). PALA, a transition state analogue of ATCase substrates (6), has been extensively used as an antitumor agent in clinical investigations (3, 7, 8, 17, 18). Previous studies showed that different normal and tumoral cell lines exhibit large differences in sensitivity to PALA which cannot be accounted for by their differences in ATCase specific activities (1, 10, 11, 13). In addition, it has been shown that these differences in sensitivity to PALA cannot be attributed to an intrinsic molecular property of ATCase (1). In particular, in contrast to what had been reported previously using human and rodent cells (2, 4, 9, 12, 14–16), no significant difference could be detected in the affinity for carbamylphosphate of the ATCase present in the extracts of 10 different human normal and tumoral cell lines (1). However, the value obtained in the case of a rectal adenocarcinoma cell line (HRT18) was beyond the S.D. of the results obtained with the 10 cell lines (10 μM compared to 5.9 ± 2.5 μM), and it was interesting to determine whether this difference was significant. The importance of that point derives from the fact that PALA competes with the substrate carbamylphosphate for binding to the catalytic site of ATCase (1, 6, 9). Consequently, the affinity for carbamylphosphate of HRT18 ATCase was accurately reinvestigated in comparison with that of a melanoma cell line (Bell) by the intercept replot method (5).

Saturation curves for carbamylphosphate were determined in the presence of varying concentrations of L-aspartate, and the K_m values obtained from the corresponding Lineweaver and Burk double-reciprocal plots were replotted against the inverse of aspartate concentration and extrapolated to the infinite concentration of this substrate (Chart 1). The variation of the apparent K_m for carbamylphosphate as a function of aspartate concentration is consistent with the previously reported indication of an ordered mechanism for human ATCase. The values of the ordinate intercepts were calculated using a computerized linear regression program. These values, 6.98 and 5.12 μM, obtained for the ATCases present in the dialyzed cell-free extracts of HRT18 and Bell cells, respectively, are not significantly different and fall into the S.D. calculated previously (1).

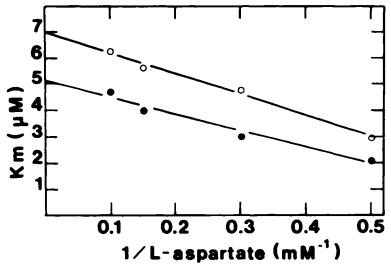

Chart 1. Intercept replots from the saturation curves of ATCase by carbamylphosphate at varying aspartate concentrations. The extracts (20-μl samples containing 25 μg of protein) of HRT18 and Bell cells were prepared, and their ATCase activity was tested as described previously (1). The saturation curves for carbamylphosphate were determined using 8 concentrations of this substrate from 0.5 to 20 μM in the presence of 2, 3.33, 6.66, and 10 mM aspartate. The K_m values obtained were replotted against the inverse of aspartate concentration. The intercepts were calculated using a computerized linear regression program. The correlation coefficients were 0.992 and 0.976 in the cases of HRT18 cells (○) and Bell cells (●), respectively.

In conclusion, it appears that, among all the human normal and tumoral cell lines tested (3 fibroblasts, 4 melanoma, and 3 colorectal carcinomas), the differences in sensitivity of the ATCase activity to PALA cannot result from a difference in affinity of this enzyme for carbamylphosphate. Since these differences cannot be attributed to any of the other tested enzymatic properties of ATCase (1), they might result from variations in the intracellular pools of carbamylphosphate. This hypothesis is currently under investigation.

ACKNOWLEDGMENTS

The authors are indebted to Dr. John Louis (Laboratoire d'Enzymologie du Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France) for reading and improving this manuscript.

REFERENCES

1. Baillon, J., Guichard, M., Malaise, E. P., and Hervé, G. Kinetic parameters of aspartate transcarbamylase in human normal and tumoral cell lines. Cancer Res., *43:* 2277–2282, 1983.
2. Bresnick, E. Feedback inhibition of aspartate transcarbamylase in liver and in hepatoma. Cancer Res., *22:* 1246–1251, 1962.
3. Carroll, D. S., Gralla, R. J., and Kemeny, N. E. Phase II evaluation of *N*-(phosphonacetyl)-L-aspartic acid (PALA) in patients with advanced colorectal carcinoma. Cancer Treat. Rep., *64:* 349–351, 1980.
4. Christopherson, R. I., and Jones, M. E. The overall synthesis of L-5,6,-dihydroorotate by multienzymatic protein *pyr-1-3* from hamster cells. Kinetic studies, substrate channelling and the effects of inhibitors. J. Biol. Chem., *255:* 11381–11395, 1980.
5. Cleland, W. W. Steady state kinetics. *In:* P. Boyer (ed.), The Enzymes, Vol. 2. New York: Academic Press, Inc., 1970.
6. Collins, K. D., and Stark, G. R. Aspartate transcarbamylase. Interaction with the transition state analog *N*-(phosphonacetyl)-L-aspartate. J. Biol. Chem.,

[1] This work was supported by the Centre National de la Recherche Scientifique and the Insitut National de la Santé et de la Recherche Médicale.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: ATCase, aspartate transcarbamylase; PALA, *N*-(phosphonacetyl)-L-aspartate.

Received October 21, 1983; accepted February 16, 1984.

246: 6599–6605, 1971.
7. Creagan, E. T., Ahmann, D. L., Ingle, J. N., Purvis, J. D., and Green, S. J. Phase II evaluation of PALA and AMSA for patients with disseminated malignant melanoma. Cancer Treat. Rep., *65:* 169, 1981.
8. Erlichman, C., Strong, J. M., Wiernick, P. H., McAvoy, L. M., Cohen, M. H., Levine, A. S., Hubbard, S. M., and Chabner, B. A. Phase I trial of *N*-(phosphonacetyl)-L-aspartate. Cancer Res., *39:* 3992–3995, 1979.
9. Hoogenraad, N. J. Reaction mechanism of aspartate transcarbamylase of mouse spleen. Arch. Biochem. Biophys., *161:* 76–82, 1974.
10. Jayaram, H. N., Cooney, D. A., Vistica, D. T., Kariya, S., and Johnson, R. K. Mechanism of sensitivity or resistance of murine tumors to *N*-(phosphonacetyl)-L-aspartate (PALA). Cancer Treat. Rep., *63:* 1291–1302, 1979.
11. Johnson, R. K., Swyryd, E. A., and Stark, G. R. Effects of *N*-(phosphonacetyl)-L-aspartate on murine tumors and normal tissues *in vivo* and *in vitro*, and the relationship of sensitivity to rate of proliferation and level of aspartate transcarbamylase. Cancer Res., *38:* 371–378, 1978.
12. Kensler, T. W., Mutter, G., Hankerson, J. G., Reck, L. J., Harley, C., Han, N., Ardalan, B., Cysyk, R. L., Johnson, R. K., Jayaram, H. N., and Cooney, D. A. Mechanism of resistance of variants of the Lewis lung carcinoma to *N*-(phosphonacetyl)-L-aspartic acid. Cancer Res., *41:* 894–904, 1981.
13. Leyva, A., Appel, H., Smith, P., Lankelma, J., and Pinedo, H. M. Inhibition of cell growth by *N*-(phosphonacetyl)-L-aspartate in human and murine cells *in vitro*. Cancer Lett., *12:* 169–173, 1981.
14. Mally, M. I., Grayson, D. R., and Evans, D. R. Catalytic synergy in the multifunctional protein that initiates pyrimidine biosynthesis in Syrian hamster cells. J. Biol. Chem., *255:* 11372–11380, 1980.
15. Smith, E. E., and Rutenburg, A. M. Aspartate transcarbamylase in normal and neoplastic human colon. Cancer Res., *27:* 1470–1473, 1967.
16. Tsuboi, K. K., Edmunds, H. N., and Kwong, L. K. Selective inhibition of pyrimidine biosynthesis and effect on proliferative growth of colonic cancer cells. Cancer Res., *37:* 3080–3087, 1977.
17. Valdivieso, M., Moore, E. C., Burgess, A. M., Marti, J. R., Russ, J., Plunkett, W., Loo, T. L., Bodey, G. P., and Freireich, E. J. Phase I clinical study of *N*-(phosphonacetyl)-L-aspartic acid (PALA). Cancer Treat. Rep., *64:* 285–292, 1980.
18. Van Echo, D. A., Diggs, C. H., Scoltock, M., and Wiernik, P. H. Phase II evaluation of *N*-(phosphonacetyl)-L-aspartic acid (PALA) in metastatic adenocarcinoma of the colon or rectum. Cancer Treat. Rep., *64:* 339–342, 1980.