

19. N. SHIMIZU and N. MORIKAWA, *Nature, Lond.* **184**, 650 (1959).
20. S. T. NACHMIAS, *J. Neurochem.* **6**, 99 (1960).
21. N. ROBINSON, *J. Neurochem.* **14**, 1083 (1967).
22. A. L. GREEN and T. M. HAUGHTON, *Biochem. J.* **78**, 173 (1961).
23. S. R. GUHA, *Biochem. Pharmac.* **15**, 161 (1966).
24. In *Manometric Techniques* (Eds. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER), p. 130, Burgess Publishing Co., U.S.A. (1957).
25. B. M. BRAGANCA, J. H. QUASTEL and R. SCHUCHER, *Archs Biochem. Biophys.* **52**, 18 (1954).
26. H. I. KOHN, *Biochem. J.* **31**, 1693 (1937).
27. S. R. GUHA and S. K. GHOSH, in preparation.
28. D. RICHTER, *Biochem. J.* **31**, 2022 (1937).

Biochemical Pharmacology, Vol. 19, pp. 2932-2934. Pergamon Press, 1970. Printed in Great Britain

#### Enzymatic determinants of responsiveness of the LPC-1 plasma cell neoplasm to fluorouracil and fluorodeoxyuridine\*

(Received 27 March 1970; accepted 15 May 1970)

THE MURINE plasma cell tumor LPC-1 has been examined as a possible model for human plasma cell disease. In a study conducted by Abraham *et al.*<sup>1</sup> 5-fluorouracil (FU) proved to be the most effective of 31 agents tested for anti-neoplastic response. 5-Fluoro-2'-deoxyuridine (FUDR) was much less active. This paper reports results of a study of the biochemical basis for this differential response.

**Tumor.** The LPC-1 tumor<sup>2</sup> was supplied by Dr. Morris Teller, Sloan-Kettering Institute and was maintained in CDF<sub>1</sub> mice by intraperitoneal or subcutaneous inoculations of 10<sup>5</sup> cells.

**Chemicals.** Radioactive substrates were purchased from Calbiochem and New England Nuclear Corp. Other chemicals were purchased from Mann Research Laboratories, Sigma Chemical Company and Calbiochem. Unlabeled fluorinated pyrimidines were provided by Hoffman-LaRoche.

**Enzyme preparations.** Nodules of tumor infiltrating the intestinal mesentery were collected and placed in 2 vol. of 0.05 M potassium phosphate at pH 7.0 containing 5 mM mercaptoethanol, 1 mM EDTA and 3 mM MgCl<sub>2</sub>. The preparation was homogenized in a Potter-Elvehjem homogenizer and centrifuged at 8000 *g* for 10 min. The supernatant fluid was decanted, then centrifuged at 100,000 *g* for 30 min. The resulting supernatant comprises fraction E<sub>1</sub>; the 100,000 *g* pellet forms fraction P. In an alternative procedure, the initial homogenate was subjected to three freeze-thaw cycles followed by brief sonic oscillation, then centrifugation at 30,000 *g* for 30 min. The supernatant fluid resulting from this process represents fraction E<sub>2</sub>. All operations described above were carried out at 4° or below.

**Enzyme assays.** Protein was determined by the method of Lowry *et al.*<sup>3</sup> Uridine phosphorylase (EC 2.4.2.3) was measured at 37° by the procedure of Friedkin and Roberts.<sup>4</sup> Incubation mixtures contained 0.05 M arsenate or phosphate at pH 7.5, 10 mM substrate [2'-deoxyuridine (UdR), uridine (UR), thymidine (TdR), 5-fluorouridine (FUR) or FUDR], 5 mM mercaptoethanol, and 5-6 mg of enzyme protein in a total volume of 0.5 ml. At intervals of 0, 10, 20, 30 and 60 min after addition of enzyme, samples of 100  $\mu$ l were removed from the mix and diluted with 100  $\mu$ l of 0.6 M HClO<sub>4</sub>. The protein was removed by centrifugation and a 100- $\mu$ l portion of the supernatant fluid was mixed with 100  $\mu$ l of 1 M KOH. After chilling to precipitate KClO<sub>4</sub>, the optical density of the solution was read at 290 m $\mu$  (substrates: UR and UdR) or 300 m $\mu$  (other substrates). Standards were run to determine extinction coefficients of the substrates and products.

Uridine kinase (EC 2.7.1.48) and thymidine kinase (EC 2.7.1.21) were determined by the DEAE disc method of Breitman.<sup>5</sup> Incubations were carried out at 37° using a mixture containing 5 mM

\* Supported by Contract NIH 69-39 with Chemotherapy, CCNSC, NCI, and Grant CA-11198-01 from the National Institutes of Health.

ATP, 6 mM MgCl<sub>2</sub>, 60 mM TES\* buffer at pH 7.2, and 1 mM of labeled TdR, UR, FUdR or FUR. The reaction was initiated by addition of 0.03–0.3 mg of enzyme protein to yield a final total incubation volume of 100  $\mu$ l. At intervals of 0, 3, 10 and 15 min, 20- $\mu$ l aliquots were removed from the mixture and allowed to soak into 2 cm discs of DEAE-impregnated filter paper (H. Reeve Angel DE81). With UR and TdR as substrates, the discs were washed for 2  $\times$  3 min in 1 mM ammonium formate and 2  $\times$  1 min in water, then immersed for 2 min in acetone and dried in air. When FU and FUdR were used as substrates, the discs were first washed for 2  $\times$  3 min in 10 mM citric acid, then in water and acetone as before. Radioactivity was determined by liquid scintillation counting of the discs using a toluene-based liquid phosphor.

Pyrimidine-5'-phosphoribosyltransferase activity was measured as described before,<sup>5</sup> using a DEAE disc method.

**Chemotherapy trials.** Experimental trials were carried out on CDF<sub>1</sub> animals implanted with 10<sup>5</sup> cells by intraperitoneal inoculation. Animals received subcutaneous injections of FU (15 mg/kg) or FUR (2 mg/kg) for 6 days/week over a 4-week interval. Ten animals were used in each group, including untreated controls.

**Enzyme levels.** Table 1 summarizes our data on enzyme levels in fraction E<sub>1</sub>. Rates represent initial values which were linearly dependent on the amount of enzyme protein added, and could be sustained for at least 10 min. With fraction E<sub>2</sub>, the kinase levels fell at enzyme levels above 1 mg/ml of protein. This effect could be reversed by addition of excess ATP, and was judged to represent the consequence of a particulate ATPase. Addition of fraction P to the E<sub>1</sub> preparation also inhibited kinase activity. Other transplantable murine leukemias examined did not appear to contain high levels of this particulate ATPase.<sup>6</sup>

TABLE 1. ENZYME LEVELS IN E<sub>1</sub> FRACTION OF THE LPC-1 TUMOR\*

Enzyme	Substrate	Activity†
Phosphoribosyltransferase	FU	31
	FUR	210
Uridine kinase	UR	875
	TdR	5
Thymidine kinase	FUdR	3
	FUR	6.5
Uridine phosphorylase	FUdR	5
	UdR	5
	TdR	2.7
	UR	9

\* Incubations were carried out as described in the text; values represent results of a typical experiment in which enzyme activity was linearly proportional to added enzyme protein, and the rate did not vary for at least 10 min. See text for substrate abbreviations.

† Activity =  $\mu$ moles product formed/g protein/hr.

**Chemotherapy.** Therapy with fluorouracil increased survival of animals bearing LPC-1 by 200 per cent. Values of 216 per cent have been reported.<sup>7</sup> The FUR dose used increased survival by only 33 per cent. Toxicity of the drug limited dosage; CDF<sub>1</sub> mice receiving 8 mg/kg/day died after 8 days. Therapy with FUdR was reported only 30 per cent as effective as FU in increasing the lifespan of animals bearing LPC-1<sup>4</sup>.

The observed low levels of uridine phosphorylase in LPC-1 indicate that interconversion of FU and FUR/FUdR by this pathway is limited. This could provide an explanation for the inability of FUdR to promote survival of this FU-responsive neoplasm. The limited capacity of LPC-1 for phosphorylation of FUdR could also contribute to the relative insensitivity to this drug.

Drug-induced resistance to FUdR has been attributed to low levels of thymidine kinase,<sup>8</sup> but natural resistance to the drug was correlated with high thymidine kinase levels.<sup>9</sup>

Since conversion of FU to FUR is limited by low levels of uridine phosphorylase in LPC-1, the phosphoribosyltransferase route<sup>6,10</sup> must provide the major capacity for conversion of FU to

\* TES = N-Tris (hydroxymethyl)-2-aminoethanesulfonic acid.

pharmacologically active<sup>11</sup> nucleotides. High levels of this enzyme were generally found in transplantable solid murine leukemias which were FU responsive.<sup>10</sup> The phosphoribosyltransferase level found in LPC-1 is higher than any value reported by Reyes and Hall.<sup>10</sup>

The LPC-1 cell line is not unique in its responsiveness to FU and resistance to FUdR; these characteristics are also shared by the lymphoma 4 neoplasm.<sup>8,10</sup> But the latter cell line demonstrated relatively high levels of both uridine phosphorylase<sup>8</sup> and the phosphoribosyl transferase.<sup>10</sup> The LPC-1 neoplasm has provided us with a model for demonstrating the association between remarkable FU responsiveness and high levels of phosphoribosyltransferase in a cell line with very low levels of uridine phosphorylase. The finding that FUdR is not an effective drug in LPC-1 provides additional evidence that uridine phosphorylase levels are low *in vivo*, and that activity of the enzyme was not lost during preparation of cell extracts.

Department of Pharmacology and  
Division of Oncology,  
University of Rochester School of Medicine,  
Rochester, N. Y. 14620, U.S.A.

RICHARD EVERSON†  
DAVID KESSEL\*  
THOMAS C. HALL

\* To whom requests for reprints should be directed.

† Bentley Fellow.

## REFERENCES

1. D. ABRAHAM, P. P. CARBONE, J. M. VENDITTI, I. KLINE and A. GOLDIN, *Biochem. Pharmac.* **16**, 665 (1967).
2. M. POTTER and E. L. KUFF, *J. natn. Cancer Inst.* **26**, 1109 (1961).
3. O. LOWRY, N. J. ROSENROUGH, A. L. FARR and R. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
4. M. FRIEDKIN and D. ROBERTS, *J. biol. Chem.* **207**, 245 (1954).
5. T. R. BREITMAN, *Biochim. biophys. Acta* **67**, 153 (1963).
6. D. KESSEL, T. C. HALL and P. REYES, *Molec. Pharmac.* **5**, 481 (1969).
7. M. N. TELLER, D. ABRAHAM, M. BOWIE and P. CARBONE, *J. natn. Cancer Inst.* **43**, 123 (1969).
8. D. KESSEL and I. WODINSKY, *Molec. Pharmac.* **6**, 251 (1970).
9. M. UMEDA and C. HEIDELBERGER, *Cancer Res.* **28**, 2530 (1968).
10. P. REYES and T. C. HALL, *Biochem. Pharmac.* **18**, 2587 (1969).
11. C. HEIDELBERGER, *Prog. nucleic Acid Res.* **4**, 1 (1965).

## Selective inhibition of monoamine oxidase in rat brain mitochondria

(Received 13 February 1970; accepted 14 May 1970)

MANY INHIBITORS of monoamine oxidase (MAO) have been found, and some have been reported to inhibit selectively the oxidation of particular substrates.<sup>1-9</sup> Previous studies have been done with only one substrate present at a time. We report here the effects of inhibitors on the simultaneous oxidation of two substrates, serotonin and tyramine, by MAO present in rat brain mitochondria.

Mitochondria were prepared from whole brain of male Wistar rats as described before.<sup>5</sup> The principle of the assay method was as used previously,<sup>5</sup> based upon the method of Wurtman and Axelrod.<sup>10</sup> Tyramine-H<sup>3</sup> (generally labeled, specific activity, 50  $\mu\text{C}/\mu\text{mole}$ ) and serotonin-2-<sup>14</sup>C (specific activity, 2.8  $\mu\text{C}/\mu\text{mole}$ ) were from New England Nuclear Corp. Each substrate was present at a 50  $\mu\text{M}$  concentration in a total volume of 0.3 ml. Sodium phosphate buffer (final concentration was 0.25 M with respect to P) of pH 7.4 was used. After 20 min incubation at 37°, the reaction was stopped