[2-^{14}C] 5-FLUOROURACIL METABOLISM TO [$^{14}CO_2$]

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SUMMARY

The rate of $^{14}CO_2$ production from 2-^{14}C-5-FU was measured in rats bearing the Novikoff ascites hepatoma. Tracer amounts were injected and $^{14}CO_2$ collected over a 6-hour period. As the tumor cells proliferated the rate of 2-^{14}C-5-FU oxidation decreased markedly over the approximately 12$\pm$ 2 days between implantation of tumor cells and death. On the day of innoculation of tumor cells, oxidation proceeded nearly linearly until almost 50% of the injected 2-^{14}C-5-FU was converted to $^{14}CO_2$ in about 4 hours. With time after tumor innoculation, the rate of $^{14}CO_2$ collection declined; ten days after innoculation only about 27% was oxidized by 4 hours, and at the terminal stage it declined to about 18% over 4 hours. Calculated from the time curves, the time oxidation of 25% of the injected trace dose increased from 88$\pm$ 27 min to 195$\pm$ 42 min after ten days, to 300 $\pm$ 59 min at the moribund state after 12 $\pm$ 2 days.

Similar decreases in oxidation rate of 5-FU were observed during growth of a solid implanted mammary carcinoma and for two other 2-^{14}C-labelled pyrimidines, uracil and thymine injected in trace quantities.

By comparing cancer subjects with themselves at different time-intervals from the onset of the disease and treatment, it might be possible to guage treatment dosages in the progress of the disease.

Key Words: Fluorouracil, Novikoff ascites, tumor treatment, fibrosarcoma.

INTRODUCTION

5-Fluorouracil (5-FU) is considered to be one of the most active anti-neoplastic agents for some cancers(1,2). Several parameters of its metabolism and pharmacokinetics have been studied in some animals bearing tumors, by the above investigators and others (3-9). Figure 1 shows the catabolic pathway of 5-FU, via reduction of the double bond, opening of the ring and conversion of carbon 2 to CO_2, ammonia and 2-fluoro-β-alanine. No quantitative data are presently available concerning the formation of CO_2 in-vivo from 5-FU during cancer growth.

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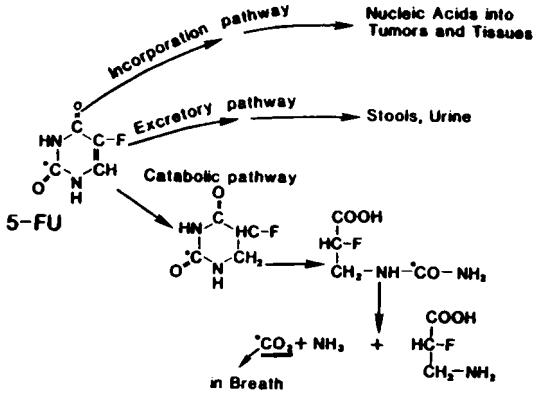
Figure 1



The commercial synthesis of [2-^{14}C] 5-Fluorouracil (Dupont NEN-109H)(10), presented the opportunity to study CO_2 formation from this pyrimidine as a measure of its catabolism in relation to the stage of the disease. The formation of CO_2 is an easy approach to information on 5-FU metabolism; but excretory mechanisms also occur and are hard to quantitate(7,11). Additional comparative information was obtained by use of other [^{14}C]-pyrimidines closely related to 5-FU, uracil and thymine.

Assay of $^{14}CO_2$ is a standard procedure in some Nuclear Medicine Laboratories (10). The feasibility of such an assay to help plan a treatment may be a possibility. It is suggested that by comparing the 5-FU metabolism in each subject at different time-intervals from the onset of the disease and treatment, it may be possible to vary the treatment in such a way as to obtain better results.

MATERIALS AND METHODS

Animals - Male Wistar rats, averaging 350g were kept quarantined for one week in air-conditioned rooms at 68-70 degree with light cycles of 12 hrs on and 12 hrs off.

Tumor - Novikoff ascites hepatoma or solid mammary fibrosarcomas were maintained by transplantation using 1 mm troquels inserted either intraperitoneally or subcutanously into the rats. The gained 10 $\pm$ 2g of weight during ten days of growth of the tumor to about 2 $\pm$ 1 cm. They were fed Purina rat laboratory chow, and tap-water was available ad libitum.

Radioactive Chemicals - All radioactive chemicals were purchased from Dupont-NEN Boston, MA. A single dose of 5 μCi (0.5 mg) [^{14}C] pyrimidine (3.84 umoles) was dissolved in 0.5 ml of saline solution and injected sub-cutaneously for each assay.

[^{14} CO_2] Collection and Assay - Using a modified Weinhouse glass metabolic cage(13) under a gentle air flush, the exhaled air of each individual rat was bubbled into a tube containing 10 ml of hyamine solution (0.1 M in methanol containing 1 drop of 1% phenolphthalein solution.) When the indicator changed color, an equivalent of CO_2 was trapped (the time required was recorded and a new tube inserted.) This method (12-14) has been well described and frequently used. The hyamine-carbonate solution was then assayed in a Packard Tri-Carb Spectrometer Samples of [$^{14}CO_2$] were collected continuously for 6 hours after injection of the labelled 5-FU. These assays were repeated at 1,2,3,6, and 10 days at approximately the same time each day.

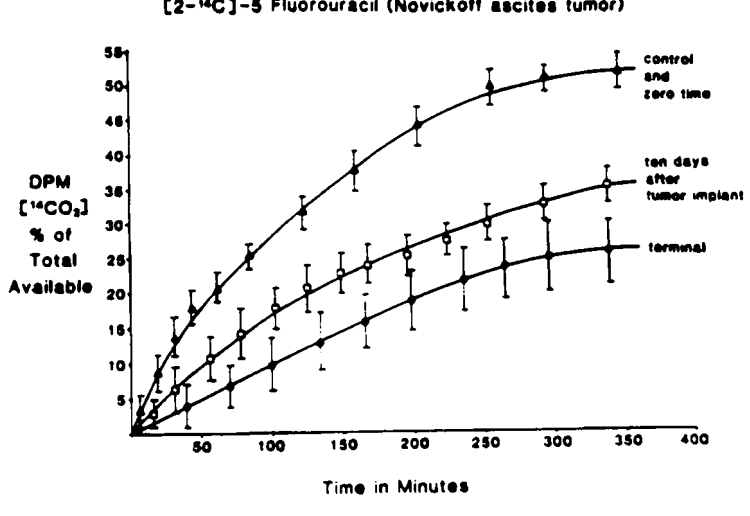
Treatment - Methotrexate sodium parenteral (Lederle) was used in a single 5 mg dose in 2 ml of saline given at the same time each day for five days. The first treatment dose was given on the fifth day after the allograft. A total of 5 rats were used for each of these studies.

Statistics - The Student t-test with number of degrees of freedom was used for the individual statistical evaluation (P) for each set of comparisons. The [t-0.25] was determined by calculation (by the least squares method) of the time when 25% of the [^{14} CO_2] obtained from carbon 2 was recovered from 5-FU or from the other pyrimidines.

RESULTS

The time course of $^{14}CO_2$ collection after injection of the tracer dose of 2-^{14}C-5-FU over the 10-day period of tumor growth in 14 rats is depicted in Figure 2. At the time of tumor injection the $^{14}CO_2$ output was at a rate to yield approximately 50% of the total $^{14}CO_2$ equivalent in 4 hours. The rate declined as the tumor grew and after 10 days when the tumor burden was nearly maximal, the rate declined to 34% after 4 hours, and at 11 days near

Figure 2



death the rate dropped further to 25% in 4 hours. A rough quantitave comparison by calculating t-0.25, the time for release of 25% of the injected 2-^{14}C-5-FU as $^{14}CO_2$, yielded figures of 88 min at 0 days, 165 min at 10 days, and 300 min at 11 days after tumor implantation.

To determine whether the rate of $^{14}CO_2$ release would be affected by chemotherapy, the experiment was repeated with 5 rats, while methotrexate was administered daily from the 5th to the 11th day. As shown in Table 1, the rate of oxidation as determined by t-0.25 was changed, and reverted to previous values for several days. With an implanted solid mammary fibrosarcoma the rate of $^{14}CO_2$ release over the 10-day period was similar to the results obtained for the untreated and/or treated Novikoff-bearing rats.

TABLE 1A

[t-0.25] Yield of $^{14}CO_2$ after Treatment Attempts

PYRIMIDINE	Days	Methothrexate treatment	[t-0.25]* min
Ascites Tumor			
[2-^{14}C]-5-FU	0	no	79 ± 30
-	3	no	151 ± 17
-	6	yes	181 ± 22
-	7	yes	100 ± 14
-	9	yes	118 ± 21
-	10	yes	180 ± 37
-	11	yes	219 ± 52

TABLE 1B

[t-0.25] Yield of $^{14}CO_2$ after Treatment Attempts

PYRIMIDINE	Days	Methothrexate treatment	[t-0.25]* min
Solid Tumor			
[2-^{14}C]-5-FU	0	no	89 ± 33
-	3	no	157 ± 21
-	6	yes	164 ± 30
-	7	yes	111 ± 41
-	9	yes	119 ± 36
-	10	yes	169 ± 42
-	11	yes	210 ± 53

* Legend Data are based on 5 rats ± standard error of the mean.

Labelled uracil and thymine were assayed in similar fashion, and both yielded $^{14}CO_2$ at 0 and 10 days after tumor implantation at rates similar to that of labelled 5-FU (Table 2).

TABLE 2

[t-0.25] Yield of $^{14}CO_2$ from Various Pyrimidines

PYRIMIDINE	Days after allograft	Ascites tumor	Solid tumor	[t-0.25]* min	Number of rats
[2-^{14}C]-5-FU	0	yes		88 ± 27	14
"	1	yes		96 ± 19	"
"	2	yes		109 ± 23	"
"	3	yes		145 ± 35	"
"	6	yes		180 ± 36	"
"	10	yes		195 ± 42	"
[2-^{14}C]-Uracil	0	yes		85 ± 31	5
"	10	yes		201 ± 38	5
[^{14}C]-thymine	0	yes		81 ± 27	5
"	10	yes		210 ± 38	5
[2-^{14}C]-5-FU	0		yes	75 ± 21	5
"	10		yes	217 ± 42	5
[2-^{14}C]-Uracil	0		yes	83 ± 31	5
"	10		yes	201 ± 39	5

*Standard error of the mean.

DISCUSSION

The decline in oxidation as a measure of 5-FU catabolism raises the question whether the efficacy of this agent is affected by this factor, and raises a second question whether other pathways may also be altered, such as its incorporation into nucleic acids and nucleotides and its excretion via other pathways (Figure 1). When each subject is compared to itself at different time intervals from the onset of the disease, it becomes possible to have an idea of the metabolic rate of utilization of 5-FU in this patient. This information may be very useful in the planning of the treatment of these patient.

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BIBLIOGRAPHY

1. Pinedo, H.M. & Peters, G.F.J.: "Fluorouracil: Biochemistry and Pharmacology" J. of Clinical Oncology 6 1653-1664 (1988).
2. Gustavsson, B. & Hafstrom, L.: "Adjuvant and palliative treatment of collateral cancer with fluorinated pyrimidines. A pharmacological and clinical review." Acta Chir. Scand. 504. 1-28 (1981).
3. Kremer, A.B., Makita, T. & Beardsley, G.F.: "Chemical consequences of incorporation of 5-Fluorouracil into DNA as studied by NMR." Biochem. 26, 391-397 (1987).
4. Chaudhuri, N.K., Mukherjee, K.L. & Hidelberger, C.: "Studies in Fluorinated Pyrimidines VII - Degradative Pathway." Biochemical Pharmacology 1, 328-341 (1958).
5. Mukherjee, K.L., Boohar, J., Wentland, D., Ausfield, F.J. & Heidelberger,C.: "Studies on Fluorinated pyrimidines XVI" Cancer Research 23, 49-66 (1963).
6. Wolf, W., Presant, C.A., Servis, K.L., El-Tahtawy, A., Albright, M.J., Barker, P.B., Ring III, R., Atkinson, D., Ong, R., King, M., Singh, M., Ray, J., Wiseman, C., Blayney, D., & Shani, J.: "Tumor trapping of 5-fluorouracil: ^{19}F NMR spectroscopic pharmacokinetics in tumor-bearing humans and rabbits." Proc. Natl. Acad. Sci. USA 87, 492-496. (1990)
7. Collins, J.M.: "Pharmacokinetics of 5-fluorouracil infusions of the rat: Comparison with man and other species" Cancer Chemother Pharmac 14, 108-111 (1985).
8. Fraile, R., Baker, L.H., Buroker, T.R., Horwitz, J. and Vaitkevicius, V.K.: "Pharmacokinetics of 5-Fluorouracil administered orally by rapid intravenous and slow infusion: Cancer Research 40, 2223-2228 (1980).
9. Heggie, G.D., Sommadossi, J.P., Cross, D.S., Huster, W.J. and Diasio, R.B.: "Clinical Pharmacokinetics of 5-Fluorouracil and its metabolites in Plasma: Cancer Research 47, 2203-2206 (1987).
10. Mandel, H.G. & Brown, C.L.: "Synthesis of 2-^{14}C-Fluorouracil." J Am Chem. Soc. 74, 2439 (1952).
11. Stevens, A.M., Morris, P.G., Lles, R.A., Sheldon, P.W., and Griffiths, J.R.: "5 Fluorouracil metabolism in vivo by 19-F NRM" Br. J. Cancer 50 113-117 (1984).
12. Rabinowitz, J.R., Lopez-Majano, V., Campbell, P.: "Clinical radioactive breath tests" in Rothfeld, B., (ed): Nuclear Medicine Hepatolinear, Philadelphia, Pa. Lippincott, pp. 222-227 (1980).
13. Chase, G. & Rabinowitz, J.L.: "Radioisotope Methodology" Burgess, Minn. pp. 471-473 (1990).
14. Rabinowitz, J.L.: "Apparatus for wet oxidation of organic samples and carbon-dioxide trapping for subsequent radioactive assay." Anal. Chem. 29, 982-984 (1957).