EFFECTS OF 5-FLUOROURIDINE ON MODIFIED NUCLEOSIDES IN MOUSE LIVER TRANSFER RNA[1]

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SUMMARY. Administration of the pyrimidine antimetabolite, 5-fluorouridine, to mice was found to cause a marked specific reduction of the amounts of 5-methyluridine, pseudouridine, and dihydrouridine but not of 3-(3-amino-3-carboxypropyl)uridine in tRNA from the livers of the treated animals. The data presented indicate that this effect is not simply due to the incorporation of 5-fluorouridine into tRNA; the drug appears to interfere directly with the enzymic reactions involved in the modification of the 5-position of uridine. 5-Fluorouridine was found to have no effect on the modification of adenosine, guanosine, and cytidine in mouse liver tRNA.

The antimetabolite, 5-fluorouridine (FUrd[2]), has been reported to exhibit antitumor activity against several experimental (1, 2) and human (3) neoplasms. Incorporation of FUrd into the positions of Urd in RNA (5, 6) is thought to contribute significantly to the cytotoxicity of FUrd and its parent compound, FUra. In addition to incorporation, FUra has been reported to affect the posttranscriptional modification of Ura residues in tRNA. Thus, tRNA isolated from E. coli grown in the presence of FUra was shown to contain reduced amounts of 5-methyluridine (7, 8), pseudouridine (7 - 9), and dihydrouridine (10). Similar findings have been reported for yeast tRNA (11, 12). Administration of 5-fluoroorotic acid but not FUra to rats was reported to cause

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[2] FUrd, 5-fluorouridine; FUra, 5-fluorouracil.

incorporation of analog and a decrease in the pseudouridine content of liver tRNA (13). The effects of FUra and its derivatives on modified Urd derivatives in tRNA have been attributed previously to the incorporation of FUrd into positions normally occupied by modified Urd derivatives, which was thought to prevent physically the modification reactions at the 5-position of Urd (see review by Heidelberger (5)).

Since the fluorinated pyrimidines are extensively used clinically in the treatment of malignant disease their molecular effects on the structure of mammalian nucleic acids warrant careful investigation. The present study was undertaken to determine, in a mammalian system, the relationships between analog incorporation and the decrease in the modified Urd derivatives of tRNA after treatment with FUrd. To this end we have analyzed the base composition of liver tRNA obtained from mice treated with various doses of FUrd. The results presented show that, at a low level of incorporation of analog, the amount of FUrd incorporated is too small to account for the decrease in the modified nucleosides.

MATERIALS AND METHODS. Male Swiss mice (about 25 g) were used. FUrd was obtained from Calbiochem. Injection solutions of FUrd were prepared in sterile 0.9 % NaCl. Mice (5 groups, 4 animals per group) were given various doses of FUrd by intraperitoneal injection, once daily for 4 days, and were killed 24 hours after the last injection. Livers from each group of mice were pooled and kept frozen at -70° until extraction. Crude 4S RNA was prepared by phenol extraction at pH 4.5 and adsorption to DEAE-cellulose (14, 15). tRNA was further purified by polyacrylamide gel electrophoresis (16). The nucleoside composition of tRNA was analyzed by a chemical tritium derivative method (17, 18). (^{3}H)-labeled nucleoside derivatives were separated on cellulose thin layers (EM Laboratories No. 5502) in solvents A and B of ref. 18. The trialcohol derivative of FUrd was prepared by treatment of FUrd with $NaIO_4$ and $NaBH_4$ according to a published procedure (17). Its chromatographic behavior was found to be identical to that reported by Horowitz et al. (19) for this compound. Data were analyzed by Student's t-test.

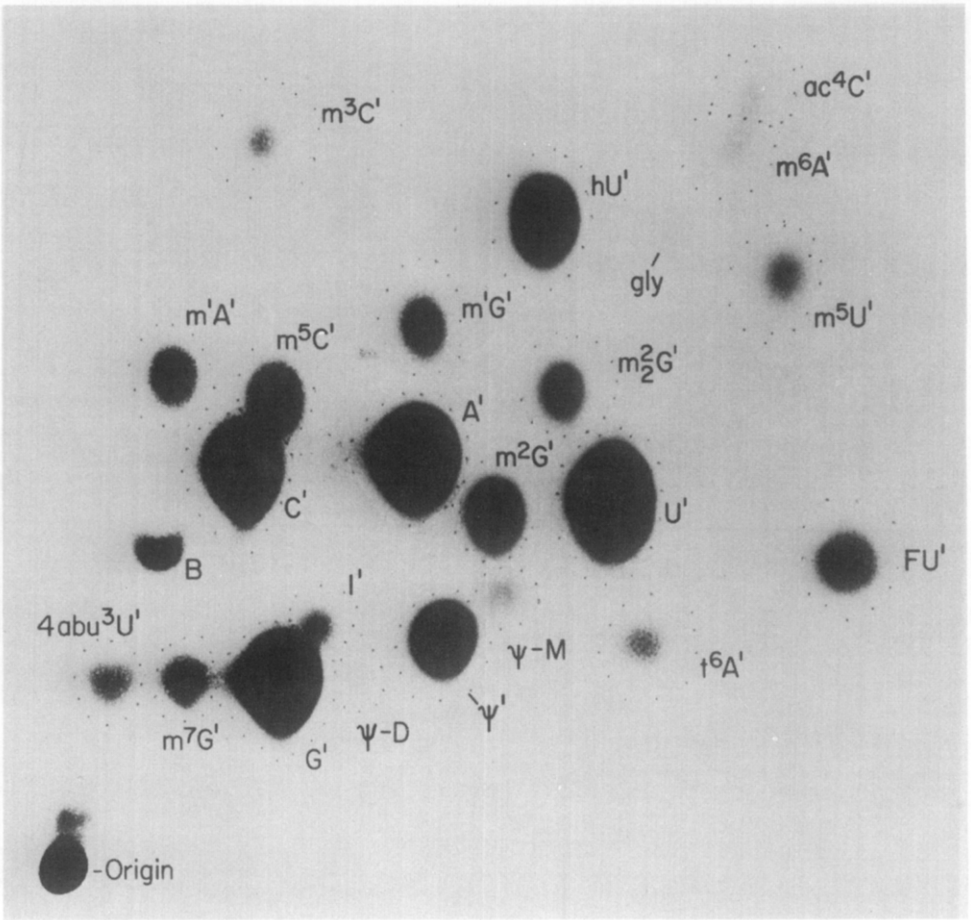

Fig. 1. Fluorogram of cellulose thin-layer map obtained by digestion to nucleosides and chemical tritium labeling (17, 18) of liver tRNA from mice that had received 100 mg/kg FUrd once daily for 4 days. N', a nucleoside trialcohol; FU', trialcohol derivative of FUrd; 4abu^{3}U, 3-(3-amino-3-carboxypropyl)uridine (27); ψ-D and ψ-M, traces of labeled products derived from pseudouridine; B, background spot (not from RNA).

RESULTS AND DISCUSSION. The fluorogram shown in Fig. 1 was obtained by two-dimensional cellulose thin-layer chromatography (17, 18) of a tritium-labeled digest of liver tRNA from a group of mice that had received 100 mg/kg of FUrd once daily for 4 days. The presence of the trialcohol derivative of FUrd (FU', see Fig. 1)

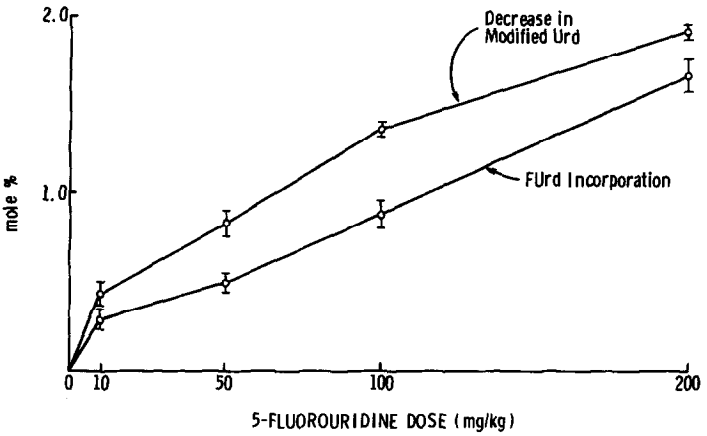

Fig. 2. Effects of FUrd dose on analog incorporation and modified Urd derivatives.

in the labeled digest provides evidence for the incorporation of FUrd into the tRNA of the treated animals. This material was found to co-chromatograph with authentic nonradioactive FUrd trialcohol. Incorporation was evident at all dose levels tested (Fig. 2). In addition, the amounts of 5-methyluridine, pseudouridine, and dihydrouridine but not of 3-(3-amino-3-carboxypropyl)uridine were found to be reduced after drug treatment (Fig. 3). These alterations were statistically highly significant (t-test, $P < 0.01$). Thus, administration of FUrd specifically affected reactions involving C5 of Urd in preformed RNA, being without influence on a modification occurring at N3 of Urd.

As shown in Figures 2 and 3, the extent of incorporation of analog into tRNA and its effects on the modified Urd derivatives were dose-dependent. Urd_{total} (= Urd + FUrd + $Urd_{modified}$), on the other hand, did not exhibit a dose-related change (data not shown) indicating specific replacement of Urd by FUrd during transcription. Accordingly, there was also no statistically significant change in the amounts of adenosine, guanosine, cytidine, and their

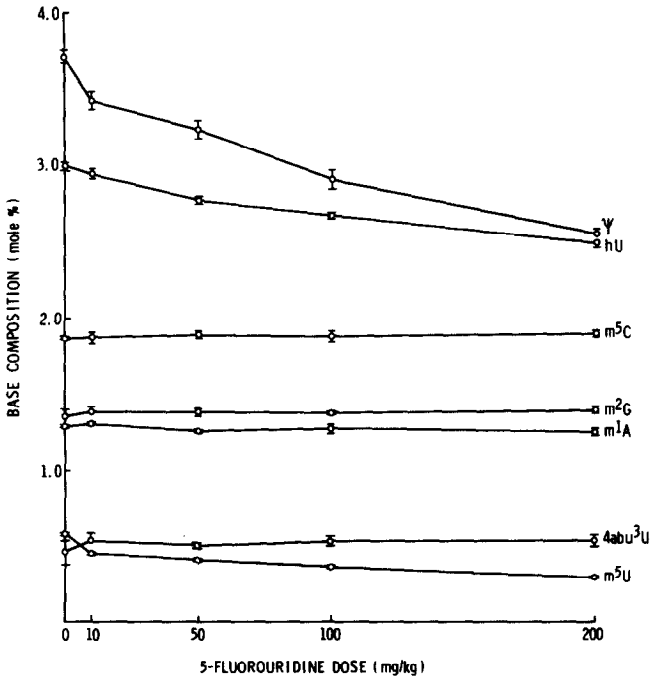

Fig. 3. Effect of FUrd treatment on the base composition of mouse liver tRNA. The data are the means ± S.D. of 4 chromatographic analyses.

modified derivatives following the administration of FUrd (data shown in part in Fig. 3).

As shown by a simple calculation, the decrease in the amounts of 5-methyluridine, pseudouridine, and dihydrouridine was not due to random incorporation of FUrd into the positions normally occupied by Urd and its modified derivatives. For example, at a dose of 100 mg/kg, the amounts calculated for random incorporation of 0.88 mole % FUrd (i.e. the found value) are 0.02, 0.15, and 0.12 mole % for 5-methyluridine, pseudouridine, and dihydrouridine, respectively. However, the actual decrease in these nucleosides was 0.21, 0.81, and 0.31 mole %, respectively (cf. Fig. 3).

Inspection of Fig. 2 reveals that, at all doses of FUrd used, the decrease in the modified Urd derivatives was greater than the

amount of analog incorporated. For example, at 50 mg/kg, incorporation was found to be 0.49 (± 0.06) mole % while the decrease in modified nucleosides amounted to 0.83 (± 0.07) mole %. It is clear from these data that the latter effect cannot be the result of preferential incorporation of FUrd into the positions of tRNA that are normally occupied by the modified Urd derivatives. Such a mechanism has been postulated to explain the reduction of 5-methyluridine in tRNA from FUra-treated E. coli (8). Our results suggest that the decrease in the modified Urd derivatives is caused by direct and specific inhibition, in FUrd-treated cells, of enzymic posttranscriptional modification reactions that normally convert Urd to 5-methyluridine, pseudouridine, and dihydrouridine during the maturation of tRNA. Studies directed at identifying the inhibitor(s) of these reactions are underway in our laboratory.

In the present study, the effects of FUrd on the major and modified nucleoside composition of mammalian tRNA have been examined in detail. While distinct albeit low incorporation of FUrd into tRNA was found our data indicate clearly that the drug exerts its major effects on the modification reactions involving C5 of Urd in preformed RNA. Similar results have been obtained also for FUra (W.-C. Tseng and K. Randerath, unpublished). The following reasons may explain why previous investigators did not detect the pronounced effect of FUrd (and FUra) on the formation of the 3 modified Urd derivatives. (i) Only total RNA was analyzed ((20), see also review by Mandel (6)). (ii) Treatment was such that a high level of replacement of Urd by FUrd in tRNA was obtained, which in turn masked the effects of the fluorinated pyrimidines on the comparatively small amounts of the modified nucleosides (7, 9, 10, 12, 19). (iii) The base composition of tRNA was not completely analyzed, e.g. modified nucleosides were not determined at all (20 - 22) or only

5-methyluridine (8), pseudouridine (9, 13), dihydrouridine (10, 12) or 5-methyluridine plus pseudouridine but not dihydrouridine (7) was determined. The tritium derivative method for analysis of RNA (17, 18) enabled us to compare directly the level of incorporation with the reduction in the amounts of the 3 Urd derivatives affected by FUrd.

The antineoplastic agent, 5-azacytidine, has been shown previously to interfere specifically with the formation of 5-methylcytidine in mouse liver tRNA (23); here we have presented evidence for related effects of another pyrimidine nucleoside analog. While the production of fraudulent nucleic acids, following treatment with purine or pyrimidine analogs, is usually presumed to be the consequence of incorporation of analog into RNA or DNA the data of this and a previous (23) communication suggest that major effects of antimetabolites on structure and function of mammalian nucleic acids may be mediated by alterations of the patterns of modified nucleosides. It appears that the modified constituents of tRNA may have both translational and regulatory functions (24). In connection with the results of the present work, it may be noted that, in prokaryotes, pseudouridine (25) and 5-methyluridine (26) have been implied in the latter.

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REFERENCES

1. Heidelberger, C., Griesbach, L., Cruz, O., Schnitzer, R.J., and Grugberg, E. (1958) Proc. Soc. Exp. Biol. Med. 97, 470-475.
2. Kessel, D., Bruns, R., and Hall, T.C. (1971) Mol. Pharmacol. 7, 117-121.
3. Currie, V.E., Burchenal, J.H., Sykes, M.P., Clarkson, B.D., and Krakoff, I.H. (1975) Proc. Amer. Assoc. Cancer Res. 16, 188 (Abstr. 751).
4. Chaudhuri, N.K., Montag, B.J., and Heidelberger, C. (1958) Cancer Res. 18, 318-328.

5. Heidelberger, C. (1975) in Handb. Exp. Pharmacol., vol 38/2 (Sartorelli, A.C., and Johns, D.G., eds.) pp 193-231. Springer Verlag, New York, Heidelberg, Berlin.
6. Mandel, H.G. (1969) Progr. Mol. Subcell. Biol. 1, 82-135.
7. Lowrie, R.J., and Bergquist, P.L. (1968) Biochemistry 7, 1761-1770.
8. Baliga, B.S., Hendler, S., and Srinivasan, P.R. (1969) Biochim. Biophys. Acta 186, 25-32.
9. Johnson, J.L., Yamamoto, K.R., Weislogel, P.O., and Horowitz, J. (1969) Biochemistry 8, 1901-1908.
10. Kaiser, I.I., Jacobson, M., and Hedgcoth, C. (1969) J. Biol. Chem. 244, 6707-6708.
11. Ebel, J.P., Weil, J.H., and Giege, R. (1972) Studia Biophys. 31/32, 165-173.
12. Kaiser, I.I. (1971) FEBS Lett. 17, 249-252.
13. Wagner, N.J., and Heidelberger, C. (1962) Biochim. Biophys. Acta 61, 373-379.
14. Roe, B.A. (1975) Nucleic Acids Res. 2, 21-42.
15. Anandaraj, M.P.J.S., and Cherayil, J.D. (1974) Anal. Biochem. 58, 190-194.
16. Chia, L.S.Y., Randerath, K., and Randerath, E. (1973) Anal. Biochem. 55, 102-113.
17. Randerath, E., Yu, C.-T., and Randerath, K.(1972) Anal. Biochem. 48, 172-198.
18. Randerath, K., Randerath, E., Chia, L.S.Y., and Nowak, B.J. (1974) Anal. Biochem. 59, 263-271.
19. Horowitz, J., Ou, C.-N., Ishaq, M., Ofengand, J., and Bierbaum, J. (1974) J. Mol. Biol. 88, 301-312.
20. Horowitz, J., and Chargaff, E. (1959) Nature 184, 1213-1215.
21. Andoh, T., and Chargaff, E. (1965) Proc. Natl. Acad. Sci. USA 54, 1181-1189.
22. Wilkinson, D.S., Tisty, T.D., and Hanas, R.J. (1975) Cancer Res. 35, 3014-3020.
23. Lu, L.W., Chiang, G.H., Medina, D., and Randerath, K. (1976) Biochem. Biophys. Res. Comm. 68, 1094-1101.
24. Littauer, U.Z., and Inouye, H. (1973) Annu. Rev. Biochem. 42, 439-470.
25. Singer, C.E., Smith, G.R., Cortese, R., and Ames, B.N. (1972) Nature New Biol. 238, 72-74.
26. Björk, G.R., and Neidhardt, F.C. (1975) J. Bacteriol. 124, 99-111.
27. Ohashi, Z., Maeda, M., McCloskey, J.A., and Nishimura, S. (1974) Biochemistry 13, 2620-2625.