Comparison of the Actions of 5-Fluorouracil and Ftorafur in Escherichia coli

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Received for publication 21 September 1976

The actions of 5-fluorouracil (FU) and Ftorafur (NCS 148958) were compared in Escherichia coli B3 to determine if Ftorafur can act in ways other than through the production of FU. Very high concentrations of Ftorafur relative to FU were necessary to achieve a comparable inhibition of cell growth. Inhibition by both agents could be reversed by uracil. An FU-resistant strain and an Ftorafur-resistant strain were isolated and found to be cross-resistant to Ftorafur and FU, respectively.

A derivative of 5-fluorouracil (FU), 1-(2-tritylhydrofuranyl)5-fluorouracil (Ftorafur [NSC 148958]), has been synthesized in the Soviet Union and shown to be an effective antitumor agent. The spectrum of antineoplastic activity of Ftorafur is similar to FU, but it appears to be less myelosuppressive (2). It has been suggested that the cytotoxic activity of Ftorafur in murine L1210 leukemia cells may reflect cleavage of the drug with the subsequent release of FU (5). In this report we describe experiments that were designed to compare the mechanisms of action of FU and Ftorafur in Escherichia coli B3. Three types of comparative experiments were carried out: (i) growth inhibition; (ii) reversal of growth inhibition by uracil; and (iii) isolation of drug-resistant strains and determination of cross-resistance.

MATERIALS AND METHODS

Chemicals. Ftorafur was obtained through T. L. Loo from the Drug Research and Development Branch of the National Cancer Institute, Bethesda, Md. All other chemicals were purchased from appropriate commercial sources.

Growth of cells. E. coli B3, a thymidine-requiring strain, was grown in a previously described minimal medium (7) that was supplemented with 2 μg of thymidine per ml. Growth curves were routinely carried out in 250-ml nephelometer flasks (19 by 130 mm, with side arms) containing 10 ml of medium and additions as described. Incubation was at 37°C in a New Brunswick G-77 water bath shaker. The media were inoculated with an overnight culture such that the absorption at 420 nm was 0.07 to 0.15. An absorption reading of 0.1 was equal to 4.2 × 10⁵ colony-forming units per ml. Thereafter, absorption readings were taken every 30 min at 420 nm with a Bausch and Lomb Spectronic 20 colorimeter.

Isolation of drug-resistant strains. Two sublines of E. coli B3, one capable of full growth in 10 μg of FU per ml of medium and a second exhibiting full growth in 200 μg of Ftorafur per ml, were obtained by serial cultivation of the parent strain in increasing concentrations of the respective drugs. Pure colonies were isolated by streaking onto nutrient agar. The stability of the phenotype was tested by carrying each strain for several transfers in the absence of drug. The susceptibility of each strain to varying concentrations of the agent was then tested as described above.

RESULTS AND DISCUSSION

For simplicity, E. coli B3 was chosen for these experiments to assure that thymidylate synthetase would not, in this case, be a primary target. (The organism requires thymidine.) Thus, any observed inhibition by FU would presumably reflect formation of FU ribonucleotides and incorporation into ribonucleic acid. In these forms, FU has been demonstrated to interfere with several cell functions in E. coli, including cell wall synthesis (8), ribosome maturation (1), and the fidelity of translation (6).

The growth curves of Fig. 1 and 2 show the comparative inhibitory activities of FU and Ftorafur toward E. coli B3. Very low concentrations of FU, 0.05 to 0.2 μg/ml, dramatically inhibited the cultures. Ftorafur, on the other hand, was active only at very high levels (25 to 200 μg/ml). It is possible that this inhibition arose from spontaneous cleavage of Ftorafur, resulting in the formation of FU, especially since very low levels of FU (0.05 μg/ml) can produce significant inhibition. The following experiments were performed to clarify this point.

Since the synthesis of deoxyribonucleic acid should not be affected by FU in this system, the inhibition produced should be reversible by uracil. When a culture of strain B3 was exposed...
Inhibition of growth of E. coli B3 by FU. Cultures were grown as described in minimal medium supplemented with thymidine and varying concentrations of FU. Symbols: * control; O, △, A, and△, 0.05, 0.08, and 0.20 μg of FU per ml in the culture medium, respectively.

Inhibition of growth of E. coli B3 by Furafur. The experiments were executed as described for Fig. 1. Symbols: O, control; ●, □, and △, 25, 75, and 200 μg of Furafur per ml in the culture medium.

Inhibition of growth by uracil. Cultures were grown as for Fig. 1 with the following additions: ●—●, control (no additions); ○—○, 10 μg of ml FU per ml (A) or 100 μg of Furafur per ml (B); △—△, 10 μg of FU per ml plus 20 μg of uracil per ml (A) or 100 μg of Furafur per ml plus 20 μg of uracil per ml (B).

Effect of preincubation on growth inhibitory properties of FU and Furafur. Portions of media containing Furafur (160 μg/ml) were preincubated for 18 h at 37 and 0°C. Each was then diluted 1:1 with minimal medium containing the inoculum. Cultures were grown and monitored as described for Fig. 1. Symbols: O, control; ●, Furafur at 0°C; △, Furafur at 37°C.

If the action of Furafur arises from a spontaneous cleavage that produces FU, it should be possible to enhance the inhibitory activity of the compound by preincubation. Figure 4 shows
the results of preincubation of Ftorafur in medium without cells. After preincubation for 18 h and subsequent incubation with cells, the inhibitory activity of Ftorafur was greatly enhanced. When a similar experiment was carried out with FU (not shown), there was no change in drug activity. Although this observation has no bearing on other possible modes of action of Ftorafur, it does substantiate the spontaneous cleavage hypothesis.

Another means of detecting similarities or dissimilarities in the mode of action of drugs is to isolate drug-resistant strains and determine the degrees of cross-resistance to potentially related agents. An FU-resistant strain was isolated from E. coli B3 as described in Materials and Methods. This strain could grow normally in the presence of at least 10 \( \mu \)g of FU per ml (Fig. 5C), a level which is extremely toxic to the parent strain, E. coli B3 (Fig. 5A). When the FU-resistant strain was challenged with a toxic concentration of Ftorafur (200 \( \mu \)g/ml) the strain was found to be equally resistant (Fig. 5C). Likewise, a Ftorafur-resistant strain was cross-resistant to a high concentration of FU (Fig. 5B). Although the mechanisms of the resistance demonstrated by these strains are not known, the observation that they are indeed cross-resistant suggests again that FU and Ftorafur share similar mechanisms of action in E. coli.

The comparative experiments described here indicate that Ftorafur acts only as a release form of 5-fluorouracil in this system. All attempts to observe inhibitory activity unattributable to FU have been negative. Repeated attempts were made to detect enzymatic cleavage of Ftorafur, both with crude cell-free preparations of bacteria and purified pyrimidine nucleoside phosphorylase; all were unsuccessful. Since the spontaneous cleavage of Ftorafur to form FU can occur at a very slow rate in solution (unpublished observation), the inhibition observed toward E. coli probably reflects the formation of FU in this way.

The antitumor activity and toxicity of Ftorafur in man may be attributable, in part, to the spontaneous cleavage reaction. It is, however, probable that the agent is metabolized in the liver. Definition of the products of this metabolism, their rates of formation, and their cytotoxic activity, if any, will clarify this question.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA 12429 and CA 11520 from the National Cancer Institute.

LITERATURE CITED


**Fig. 5.** Resistance of E. coli B3 (A), an Ftorafur-resistant strain (B), and an FU-resistant strain (C) to FU and Ftorafur. Cultures were grown as for Fig. 1. Symbols: Control cultures (○) contained no drug; FU concentration in each case was 10 \( \mu \)g/ml (△); Ftorafur concentration was 200 \( \mu \)g/ml (●).
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