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Effect of N_1-(2′-Tetrahydrofuryl)-5-fluorouracil and 5-Fluorouracil on Nucleic Acid and Protein Biosyntheses in Ehrlich Ascites Cells

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SUMMARY

N_1-(2′-Tetrahydrofuryl)-5-fluorouracil (FT-207) is a derivative of 5-fluorouracil (5-FU) and has been accepted as a new chemotherapeutic drug. The inhibitory effects of FT-207 and 5-FU on nucleic acid and protein biosyntheses in Ehrlich ascites cells were compared. Both drugs markedly inhibited the incorporation *in vivo* of the labeled precursors into nucleic acid and protein. The inhibitory effect of FT-207 on DNA and RNA synthesis lasted for a long period of time. Two hr after administration of 5-FU (250 µg/g body weight), the absolute size of uracil pool of liver increased by at least 50%. However, in an *in vitro* study, FT-207 at a concentration of 60 µg/ml produced no effect on the incorporation of precursors into DNA and RNA of Ehrlich ascites cells 3 hr incubation. If 5-FU was added to Ehrlich ascites cell suspension simultaneously with [5-^{3}H]uridine, the incorporation of the labeled precursor into RNA increased by 30 to 50%.

INTRODUCTION

FT-207[1] and 5-FU are fluorinated pyrimidines. FT-207, synthesized by Hiller *et al.* (8), differs from 5-FU by the addition of a furan ring at N-3 and has been of clinical value in the treatment of malignant tumors (1, 7, 12). 5-FU has for over a decade been accepted as a standard chemotherapeutic drug for the treatment of several common human neoplasms. These drugs are noticeably different in therapeutic efficacy in several experimental tumors, including solid Sarcoma 180, solid Sarcoma 37, ascitic hepatoma 130, rhodamine sarcoma, and Ehrlich ascites carcinoma in mice (13). The design of chemotherapeutic programs with FT-207 is still obscure because of our limited knowledge of its metabolism. This study was undertaken to compare the modes of action of 5-FU and FT-207 given p.o. and i.v.

MATERIALS AND METHODS

Chemicals. [^{3}H]TdR (24 Ci/mmole), [5-^{3}H]uridine (24 Ci/mmole), [4,5-^{3}H]leucine (38 Ci/mmole), and [6-^{3}H]-5-FU (1.08 Ci/mmole) were purchased from The Radiochemical Centre, Amersham, England. FT-207 was supplied by the courtesy of Taichō Pharmaceutical Company, Ltd., Tokyo, Japan. FT-207, which was dissolved in Tris buffer, was freshly prepared before administration. Calf thymus DNA and yeast RNA were purchased from Sigma Chemical Company, St. Louis, Mo.; bovine albumin, uracil, and uridine were from Wakō Junyaku-Kogyo Company, Osaka, Japan; UMP and UTP were from Boehringer-Mannheim GmbH, Mannheim, Germany; and UDP was from P-L Biochemicals, Inc., Milwaukee, Wis.

Animals and Ascitic Tumor. Male DD mice weighing 20 to 25 g were used. EA cells were transplanted at 7-day intervals by i.p. injection of 2 × 10^6 cells.

***In Vivo* Drug Incubation.** The effects of 5-FU and FT-207 *in vivo* were studied by treating EA-bearing mice with i.v. injection or p.o. administration through a stomach tube. The control group received the same volume of 0.9% NaCl solution. At prescribed intervals posttreatment, the mice were sacrificed by decapitation and the ascitic fluid was taken. After centrifugation twice in Hanks' solution, EA cells were suspended at a final concentration of 5 × 10^6 cells/ml in 0.9% NaCl solution containing [^{3}H]TdR, [5-^{3}H]uridine, or [4,5-^{3}H]leucine (1.0 µCi/ml). Pulse-labeling with radioactive precursors was carried out at 37° for 30 min in a shaking water bath. The reaction was stopped by immersion into ice water. An equal volume of 10% trichloroacetic acid was added to each suspension. DNA, RNA, and protein fractions were extracted using a slight modification of the method of Schmidt and Thannhauser (11). The radioactivity in each fraction was counted in 10 ml of Bray's solution in a Packard Tri-Carb liquid scintillation spectrometer. DNA contents were measured by the diphenylamine procedures (14) using calf thymus DNA as standard, RNA contents were measured by the orcinol procedures (14) using yeast RNA as standard, and protein contents were measured by the method of Lowry *et al.* (10) using bovine albumin as standard. Data were computed as percentage of the control values.

***In Vitro* Drug Incubation.** Aliquots of EA cell suspension (5 × 10^6 cells/ml) in 0.9% NaCl solution were incubated with the labeled precursor and either FT-207 or 5-FU at 37° with agitation. At the end of incubation, the suspensions were chilled and processed as above.

Analysis of Uracil Pool. [6-^{3}H]-5-FU (1 µCi/g body weight) plus unlabeled 5-FU (250 µg/g) were simultaneously administered i.p. into each mouse. After various periods of time,

[1] The abbreviations used are: FT-207, N_1-(2′-tetrahydrofuryl)-5-fluorouracil; 5-FU, 5-fluorouracil; [^{3}H]TdR, [6-^{3}H]thymidine; EA cells, Ehrlich ascites cells.

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mice were sacrificed by decapitation and the liver was quickly removed and homogenized in ice-cold 5% trichloroacetic acid. The supernatant was analyzed by descending chromatography on No. 51 Toyo filter paper. The paper was developed at room temperature for 18 hr in a solvent composed of isopropyl alcohol:HCl:water (65:16.7:18.3, v/v/v). The above procedures were generally found to be satisfactory for separation of uracil and of uridine and its nucleotides from others (R_F of uracil, uridine, UMP, UDP, UTP, and 5-FU was 0.76, 0.75, 0.76, 0.76, 0.76, and 0.78, respectively); 5-FU, uracil, and uridine and its nucleotide fraction were eluted with 0.1 N HCl. Although uracil, uridine, and UMP in liver were not detectable with butanol:concentrated NH_4OH:water (86:3:14, v/v/v) and ethyl acetate:formic acid:water (65:5:5, v/v/v), the ratio of the pool sizes of liver UTP and UDP determined by this method was approximately 4:1. Uracil pool sizes in liver were measured by UV absorption using UDP and UTP as standard and were determined to be 0.45 ± 0.08 μmole/g, wet weight. The fluorinated uracil pool was estimated by the dilution rate of the injected marker [6-^{3}H]-5-FU.

RESULTS

***In Vivo* Drug Incubation.** The time course of [^{3}H]TdR incorporation into DNA in EA cells is shown in Chart 1. In groups treated with 5-FU, a sharp increase in [^{3}H]TdR incorporation was observed compared to that in mice treated with FT-207. This increased activity by 5-FU ended much earlier than that observed in the group treated with FT-207. The incorporation rate by the i.v. injection of 5-FU or FT-207 reached a higher peak than that in the groups receiving p.o. administration, and it returned faster to the control value. On the other hand, in the p.o. groups (Chart 1*B*) a gradual increase in [^{3}H]TdR incorporation rate was recorded, which thereafter declined slowly to the control level.

The effect of 5-FU or FT-207 on [5-^{3}H]uridine incorporation into RNA was studied using p.o. and i.v. routes of administration. As shown in Chart 2, the extents of depletion of [5-^{3}H]uridine with 5-FU were about equivalent to those observed with FT-207. The delayed nadir and delayed return to control values were observed in cells treated with FT-207. In the p.o. groups, the incorporation rate of [5-^{3}H]uridine into RNA was inhibited less but longer than in the i.v. groups.

The incorporation of [4,5-^{3}H]leucine into protein is shown in Chart 3. In this case, too, the delayed nadir was observed in the group treated with FT-207. There was no noticeable difference in the protein biosynthesis between i.v. and p.o. groups.

***In Vitro* Drug Incubation.** The effects of FT-207 and 5-FU on incorporation into DNA of [^{3}H]TdR are illustrated in Chart 4*A*. By simultaneous addition of 5-FU (40 μg/ml) and [^{3}H]-TdR (1.0 μCi/ml) to the cell suspensions, [^{3}H]TdR incorpora-

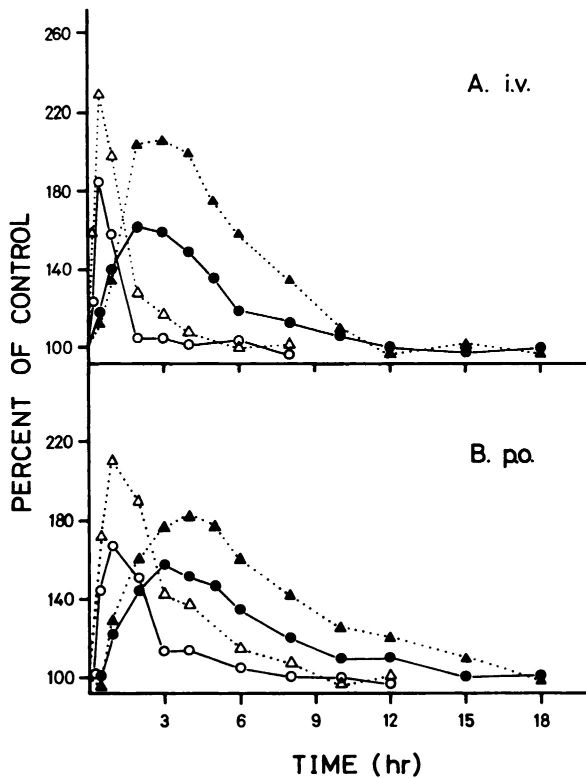

Chart 1. The effect of a single administration (*A*, i.v.; *B*, p.o.) of 5-FU or FT-207 on the incorporation of [^{3}H]TdR into DNA of Ehrlich ascites cells. At 0 time the drug was given either i.v. or p.o. into mice at 7 days after transplantation. The mice were killed at the various times indicated and pulse-labeling *in vitro* with [^{3}H]TdR was performed for 30 min. The average radioactivity of control mice was 4.04 ± 0.38 cpm × 10^5/mg DNA. ○, animals treated with 40 μg of 5-FU per g; △, animals treated with 160 μg of 5-FU per g; ●, animals treated with 60 μg of FT-207 per g; ▲, animals treated with 240 μg of FT-207 per g. Each *point* represents the mean of individual determinations from 3 animals.

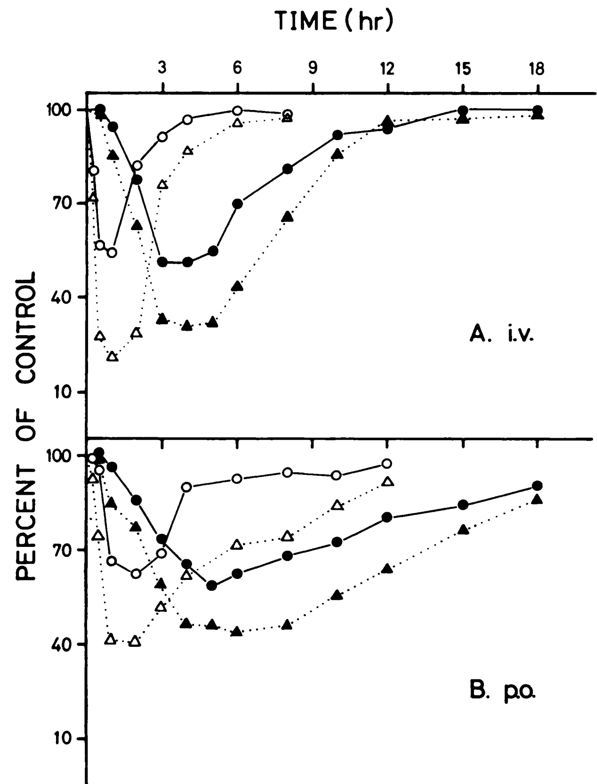

Chart 2. The effect of a single administration (*A*, i.v.; *B*, p.o.) of 5-FU or FT-207 on the incorporation of [5-^{3}H]uridine into RNA of Ehrlich ascites cells. ○, △, 5-FU; ●, ▲, FT-207. Details are given in the legend to Chart 1. The average radioactivity of control mice was 7.26 ± 0.45 × 10^4 cpm/mg RNA. Each *point* represents the mean of individual determinations from 3 animals.

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tion into DNA increased by 50%. Under the same conditions, FT-207 did not cause any inhibition of DNA synthesis (Chart 4*A*).

When 5-FU and [5-^{3}H]uridine were added simultaneously to EA cells, the incorporation of [5-^{3}H]uridine into RNA increased up to 30 to 50%. FT-207 had no inhibitory effect on [5-^{3}H]uridine incorporation into RNA (Chart 4*B*). The preceding incubation of 5-FU for 10 min decreased [5-^{3}H]uridine incorporation into RNA (Chart 4*C*). Under this condition, FT-207 also had no inhibitory effect on [5-^{3}H]uridine incorporation into RNA.

Analysis of Hepatic Uracil Pool. The time course of enlargement of both the original hepatic uracil pool and the fluorinated hepatic uracil pool is shown in Chart 5. These pools increased rapidly for 10 min after the administration of 5-FU. The UTP levels in the original pool reached a maximum at 10 to 60 min after 5-FU administration and returned to the normal value within 3 hr. The maximal increase in the fluorinated pool was attained at 30 min.

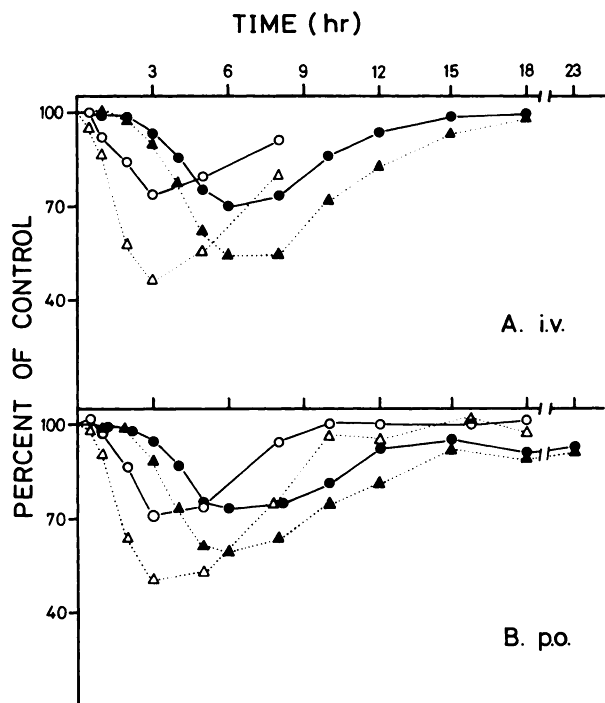

Chart 3. The effect of a single administration (*A*, i.v.; *B*, p.o.) of 5-FU or FT-207 on the incorporation of [4,5-^{3}H]leucine into protein of Ehrlich ascites cells. ○, △, 5-FU; ●, ▲, FT-207. Details are given in the legend to Chart 1. The average radioactivity of control mice was 2.41 ± 0.25 × 10^4 cpm/mg protein. Each *point* represents the mean of individual determinations from 3 animals.

DISCUSSION

It has been reported (5, 6, 13) that FT-207 is a masked compound of 5-FU and is converted to 5-FU in the liver, muscles, and other organs. The results in Chart 4 suggest that FT-207 may not be metabolized to 5-FU *in vitro*. Thus, FT-207 produced no inhibition of nucleic acid synthesis *in vitro*, a finding that is compatible with most of the previous observations (6). When [^{3}H]TdR, a precursor of DNA, was used, 5-FU and FT-207 stimulated its incorporation *in vivo* into DNA in EA cells. This agrees with the findings by Bosch *et al.* (2) and Danneberg *et al.* (4). The stimulation observed here can be expected, since an enhanced utilization of exogenously supplied thymidine owing to the inhibition of thymidylate synthetase may occur (2–4). On the other hand, the incorporation of [5-^{3}H]uridine was markedly decreased by FT-207 and 5-FU. The extent of the decrease was consistent substantially with the findings of Danneberg *et al.* (4) that the incorporation of [2-^{14}C]uracil into RNA was inhibited by 5-FU to a somewhat lesser extent than was its incorporation into DNA, particularly in EA tumor cells.

Inhibition by FT-207 of DNA, RNA, and protein synthesis was more prolonged than that produced by 5-FU. This may be due not to the slow absorption of the drug through the gastrointestinal tract but to a gradual conversion of the drug to 5-FU in the liver, resulting in the delay and prolongation of the effect. Furthermore, the present data on 5-FU or

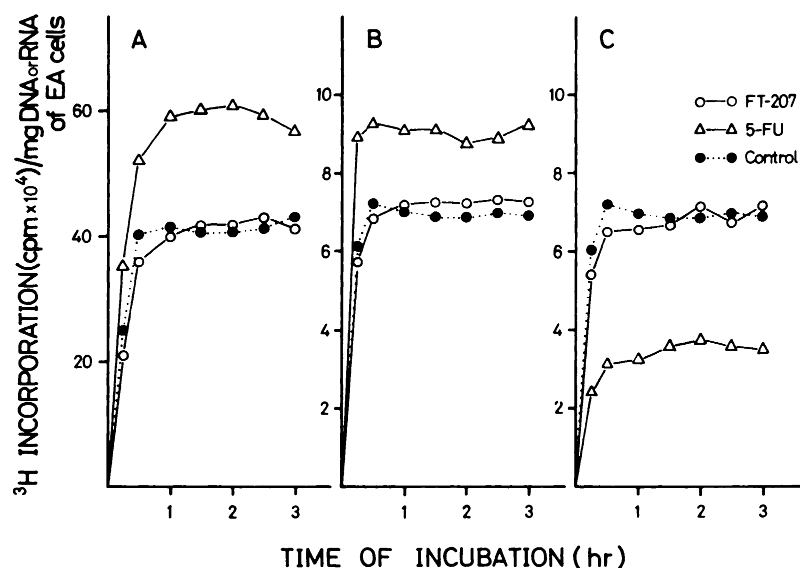

Chart 4. *In vitro* effect of FT-207 on the incorporation of [^{3}H]TdR (*A*) and [5-^{3}H]uridine (*B*, *C*) into DNA and RNA, respectively. The cell suspension of 5 × 10^6 cells/ml was incubated with FT-207 (60 µg/ml), or 5-FU (40 µg/ml) plus the labeled precursors at 37° for various times. In *A* and *B*, [^{3}H]TdR or [5-^{3}H]uridine was added at 0 time. In *C*, [5-^{3}H]uridine was added 10 min after incubation of the drugs and EA cells. The drug concentration of both drugs was calculated from their molecular weights. Each *point* represents the average of duplicate determinations from 3 animals.

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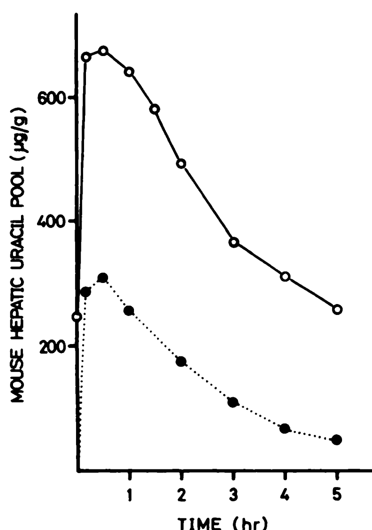

Chart 5. The effect of a single administration of 5-FU on the original hepatic uracil pool and the fluorinated hepatic uracil pool. At 0 time [6-^{3}H]-5-FU (1 μCi/g) plus unlabeled 5-FU (250 μg/g) were administered i.p. The mice were killed at the various times indicated, and acid-soluble fractions of the liver were chromatographed (the solvent used was isopropyl alcohol:HCl:water; 65:16.7:18.3, v/v/v) along with the marker 5-FU as standard. O, total uracil pool estimated by UV absorption; ●, fluorinated pool estimated by the dilution rate of [6-^{3}H]-5-FU. Each *point* represents the average of duplicate determinations from 3 animals.

FT-207 administered p.o. suggest that the time course of the delayed incorporation and delayed recovery of precursors into nucleic acids of EA cells that may also depend on the further absorption of unchanged FT-207 or 5-FU from the small bowel. In any event, from the data of Charts 1 to 3 we may reasonably conclude that the effects of FT-207 *in vivo* are similar to those of 5-FU but that they are delayed in time.

However, purine and pyrimidine analogs with antitumor activity, when used p.o., interfere with the metabolism of the liver. The contents of uracil and of uridine and its nucleotides in mouse liver were measured by paper chromatography. The contents of mouse liver found by this method are in general agreement with the data yielded by the enzymatic analysis (9). 5-FU in a large dose (250 μg/g) increases the hepatic uracil pool. Evidence for conversion of 5-FU to acid-soluble fluorouridine mono-, di-, and triphosphate and for incorporation of 5-FU into RNA of the mouse liver has been presented by Chaudhuri *et al.* (3) and that of EA cells has been given by Bosch *et al.* (2). As shown in Chart 4*B*, the incorporation of [5-^{3}H]uridine into RNA increased up to 30 to 50% by the simultaneous addition of [5-^{3}H]uridine and 5-FU (0.3 μmole/ml) to EA cells in suspension. The addition of 5-FU simultaneously with [5-^{3}H]uridine might have stimulated the metabolic process to synthesize RNA resulting in the increase of [5-^{3}H]uridine incorporation. By contrast, the decrease of uridine incorporation by addition of 5-FU 10 min before may be explained by the simple competitive inhibition by [5-^{3}H]uridine with 5-FU which has already been converted into fluorouridine, etc. This may correspond to the observed increase in the hepatic uracil pool of mice treated with 5-FU. The increase in the acid-soluble uracil pool may be due in part to an enhanced degradation of liver RNA.

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