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Comparative Studies on the Metabolism of New Fluorinated Pyrimidine Drugs in the Liver by *in vivo* ^{19}F Magnetic Resonance Spectroscopic Observation

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1-Ethoxymethyl-5-fluorouracil (EM-FU) is a fluorinated pyrimidine derived from 5-FU, and 3-cyano-2,6-dihydroxypyridine (CNDP) is a chemical modulator which suppresses the catabolism of 5-FU by inhibiting dihydrouracil dehydrogenase in the liver. In this study, the metabolism of EM-FU and the suppression of 5-FU catabolism by CNDP were observed by *in vivo* ^{19}F magnetic resonance spectroscopy in comparison with other similar drugs, because it is considered that the most effective mode of therapy using 5-FU is to suppress the catabolism of 5-FU in the liver and so to maintain for longer an effective blood level of 5-FU. The metabolism of EM-FU was very slow and the production of fluoro-β-alanine was very low as compared to the case of tegafur. The catabolic suppression by CNDP was much stronger than that of uracil. Therefore co-administration of EM-FU and CNDP should suppress catabolism and maintain an effective blood level of 5-FU for a long period of time.

Key words: Fluoropyrimidine — Liver — Rat — ^{19}F-MRS — Chemical modulator

Fluorinated pyrimidine drugs are usually decomposed in the liver, being converted into inactive fluoro-β-alanine (FBAL), which is excreted in urine. Therefore the drug level in the blood and tumor tissues is lowered, so that an effective level is not maintained, and also the adverse effect on the central nervous system increases.[1] The most effective method of chemotherapy with fluorinated pyrimidine is currently considered to be administration of 5-fluorouracil (5-FU) via continuous drip infusion.[2] The blood level of 5-FU is maintained by continuous infusion, but the total amount of FBAL is markedly increased compared to a single intravenous injection. A more effective approach may be to prevent the decomposition of the drug to FBAL.

The metabolism of fluorinated pyrimidines may be altered by other drugs; for example, the anti-tumor effect of a fluorinated pyrimidine drug was increased by methotrexate pretreatment.[3] Uracil and thymidine are decomposition-suppressing substances,[4] and such chemicals, which change the metabolism of other drugs, are termed "chemical modulators." In the present study, we used 3-cyano-2,6-dihydroxypyridine (CNDP), a stronger fluorinated pyrimidine decomposition-suppressor than uracil,[5] and by using the metabolic profile of 5-FU in the liver as an index, the effect of CNDP was compared with that of uracil. The metabolism of 1-ethoxymethyl-5-fluorouracil (EM-FU) and CNDP has been examined by chromatographic and radioisotopic methodology by Fujii *et al.*[6] Our study is the first to examine comparative changes of FBAL in the liver by using *in vivo* ^{19}F-magnetic resonance spectroscopy (MRS). Our aims were to compare the metabolism of EM-FU and other fluorinated pyrimidine drugs, 5-FU and tegafur, and to compare the suppressive effects of CNDP and uracil upon 5-FU decomposition.

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MATERIALS AND METHODS

Chemicals 5-FU and tegafur were purchased from Kyowa Hakko Co. Ltd., and Taiho Pharmaceutical Ind., Co., Ltd., respectively. EM-FU and CNDP were synthesized at our laboratory according to the methods described in the literature.[5]

Animals Male Wistar rats, 8–15 weeks old, were purchased from Japan SLC Inc., Shizuoka. Before the ^{19}F-MRS measurement, they were anesthetized with pentobarbital, 50 mg/kg i.p., and then a catheter was installed in the femoral vein for drug administration. Rats were grouped as follows.

1) Comparative study of fluorinated pyrimidine metabolism: 5-FU group (n=4), tegafur group (n=3), and ME-FU group (n=4). The dose of each was 100 mg/kg, administered over 30 s duration via the femoral vein, after which ^{19}F-MRS measurement was immediately initiated.

2) Comparison of the metabolism of 5-FU in the presence of various chemical modulators: Uracil combined group (n=3) and CNDP combined group (n=4) were

compared with 5-FU alone group (n=4). Uracil or CNDP, at a dose of 100 mg/kg p.o., with 5 ml of purified water was administered 30 min befor NMR measurement, and 5-FU at 100 mg/kg was administered via the femoral vein, after which ^{19}F-MRS measurement was immediately initiated.

^{19}F-MRS measurement The instrument was an Otsuka Electronics (USA) BEM 140/200 (^{19}F resonance frequency 187.4 MHz, magnetic field 4.7 tesla). Under anesthesia, the abdomen of the rat was opened and a coil of 20 mm in diameter was attached to the surface of the liver. Measurements were performed with a pulse width of 100 μs, repetition time of 2.5 s, and sum of 240 FIDs (one spectrum required 10 min) and continued for 120 min.

Statistical significance was tested by using the *t* test and the Wilcoxon test.

RESULTS

Preliminary measurement Chemical shifts were measured for 5-FU and EM-FU, and an EM-FU peak at 3.0 ppm lower than the corresponding peak of 5-FU was observed.

Comparison of the metabolism of fluorinated pyrimidines Spectra of 5-FU, tegafur and EM-FU groups are shown in Fig. 1. The peaks shown by the rats in the 5-FU group are those of 5-FU, FBAL and fluoronucleotides. In the tegafur and EM-FU groups, peaks of fluoronucleotides were not identified, and only the peaks of fluorinated pyrimidine and FBAL could be found. The FBAL peak was the highest in the 5-FU group and the lowest in the EM-FU group, but the signal intensity valuse are relative, and cannot be compared directly. Therefore, the signal intensities were normalized with respect to the

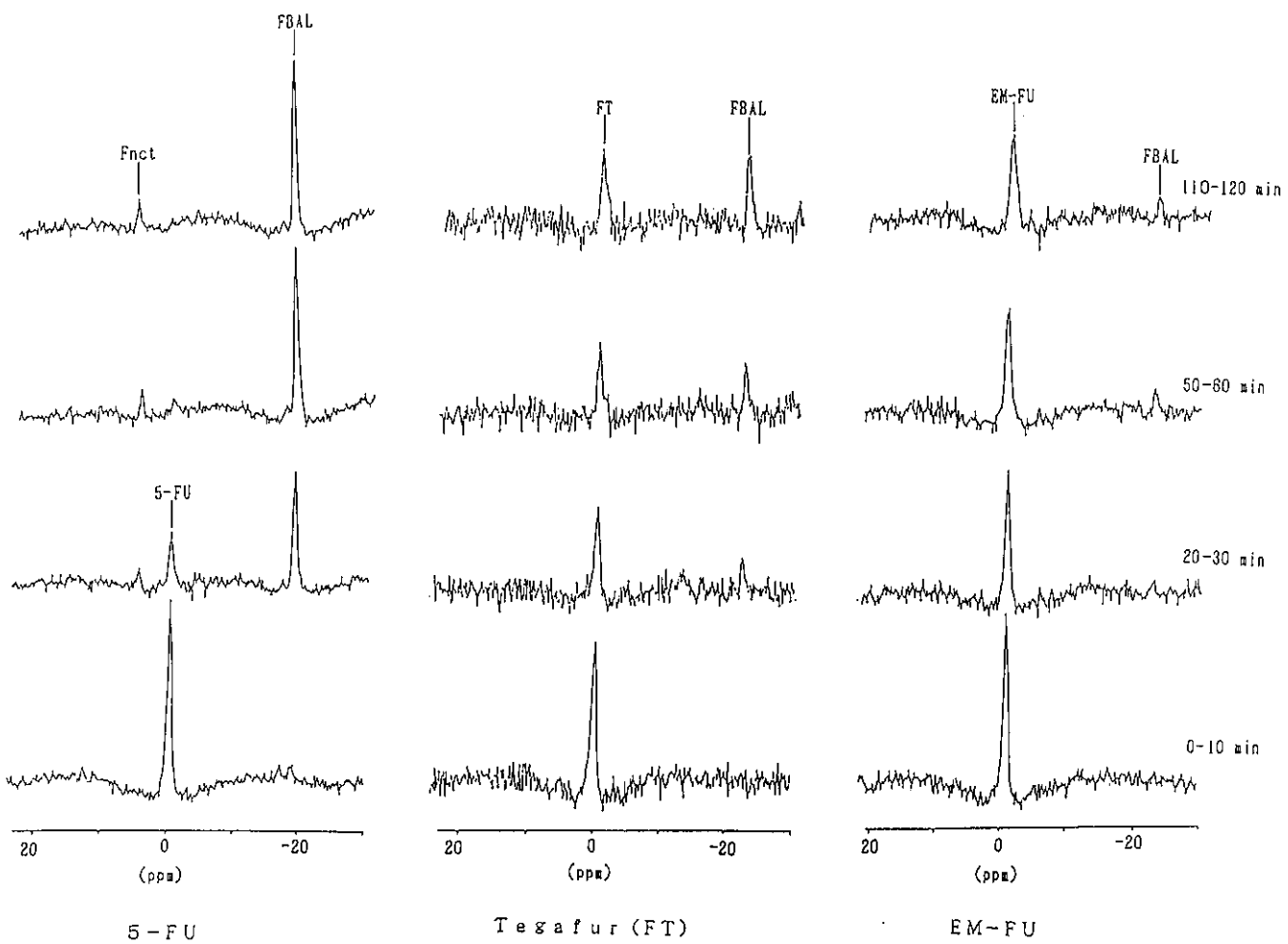

Fig. 1. ^{19}F-MR spectra showing metabolism of 5-FU, tegafur (FT) and EM-FU in rat liver. Measurement conditions were as follows; TR=2.5 s, pulse width=100 μs, sum of 240 FIDs (10 min). Fnct means fluoronucleotides.

signal intensity of fluorinated pyrimidine at 0–10 min.

Normalized fluorinated pyrimidine
=fluoro-pyrimidines(t)/fluoro-pyrimidines(0–10 min)
normalized FBAL
=FBAL(t)/fluoro-pyrimidines(0–10 min)

where fluoro-pyrimidines(t) and FBAL(t) are the signal intensities (peak heights) of fluoro-pyrimidines and of FBAL at time t, respectively, and fluoro-pyrimidines(0–10 min) is the signal intensity obtained during the first 10 min after i.v. injection in the group.

Decreases of 5-FU, tegafur and EM-FU are shown in Fig. 2. The decrease in 5-FU was very rapid, while those of tegafur and EM-FU were similar to each other. The differences in FBAL formation among the 3 groups are shown in Fig. 3. FBAL appeared rapidly after i.v. administration of 5-FU, reaching a maximum in 80–90 min. On the other hand, appearance of FBAL was slow in the tegafur group, and was even lower in the EM-FU group, in which only a slight FBAL formation was observed.

Differences in the metabolism of 5-FU in the presence of chemical modulators Spectra obtained in the 5-FU alone group, uracil combined group, and CNDP combined group are shown in Fig. 4. The peak of 5-FU in the 5-FU alone group rapidly disappeared, but in the uracil combined and CNDP combined groups, the peak of 5-FU still remained after 2 h. The difference in the rate of disappearance of 5-FU between the uracil combined and CNDP combined groups was not marked. A notable difference between the uracil combined and CNDP combined groups was seen in the peak of FBAL; almost no FBAL peak was seen in the CNDP combined group. For the purpose of comparison, the following normalization was attempted:

normalized 5-FU=5-FU(t)/5-FU(0–10 min)
normalized FBAL=FBAL(t)/5-FU(0–10 min)
normalized fluoronucleotides
=fluoronucleotides(t)/5-FU(0–10 min)

where 5-FU(t), FBAL(t), and fluoronucleotides(t) show the signal intensity of 5-FU, FBAL and fluoronucleotides at time t, respectively, and 5-FU(0–10 min) shows the signal intensity of 5-FU during the first 10 min immediately after i.v. administration of 5-FU.

The decrease profiles of 5-FU in the 3 groups are shown in Fig. 5. The decrease in 5-FU was the most rapid in the 5-FU alone group, and there was no significant difference in the decrease of 5-FU in the uracil combined and CNDP combined groups.

The FBAL formation in the 3 groups is shown in Fig. 6. As is clearly shown by the spectra, almost no FBAL formation was found in the CNDP combined group. In the uracil combined group, FBAL formation was intermediate between those of the 5-FU alone and CNDP

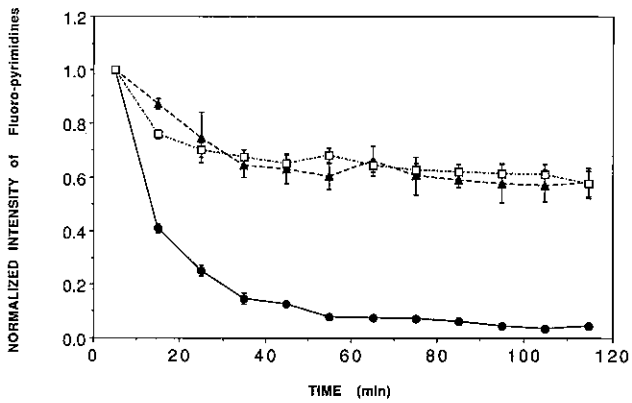

Fig. 2. Time courses of decrease of fluoro-pyrimidines in the 5-FU group, tegafur (FT) group and EM-FU group. ● 5-FU, ▲ tegafur(FT), □ EM-FU.

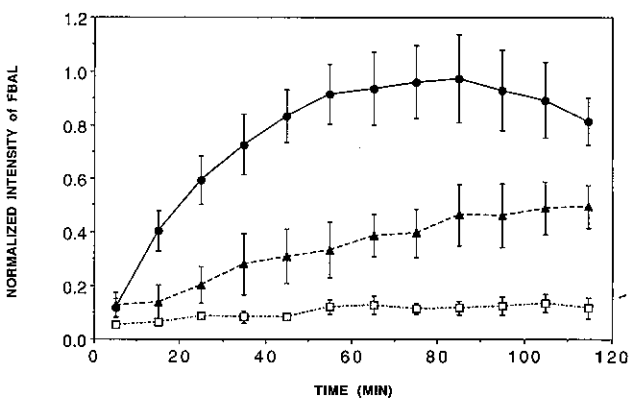

Fig. 3. Time courses of FBAL formation in the 5-FU group, tegafur (FT) group and EM-FU group. ● 5-FU, ▲ tegafur (FT), □ EM-FU.

combined groups. In the 5-FU group, FBAL formation was decreased after 90 min, and in the uracil combined group, a plateau was seen after 40 min.

The rates of fluoronucleotides formation are shown in Fig. 7. The peaks of fluoronucleotides tend to be a little higher in both the uracil combined and CNDP combined groups as compared with that in the 5-FU alone group, and a clear difference was observed in the late phase of 5-FU administration. However, there was no distinct difference between the uracil combined and CNDP combined groups.

DISCUSSION

We performed experiments to observe the effect of uracil on the metabolism of tegafur in the rat liver using ^{19}F-MRS. Comparison of the tegafur and 5-FU concen-

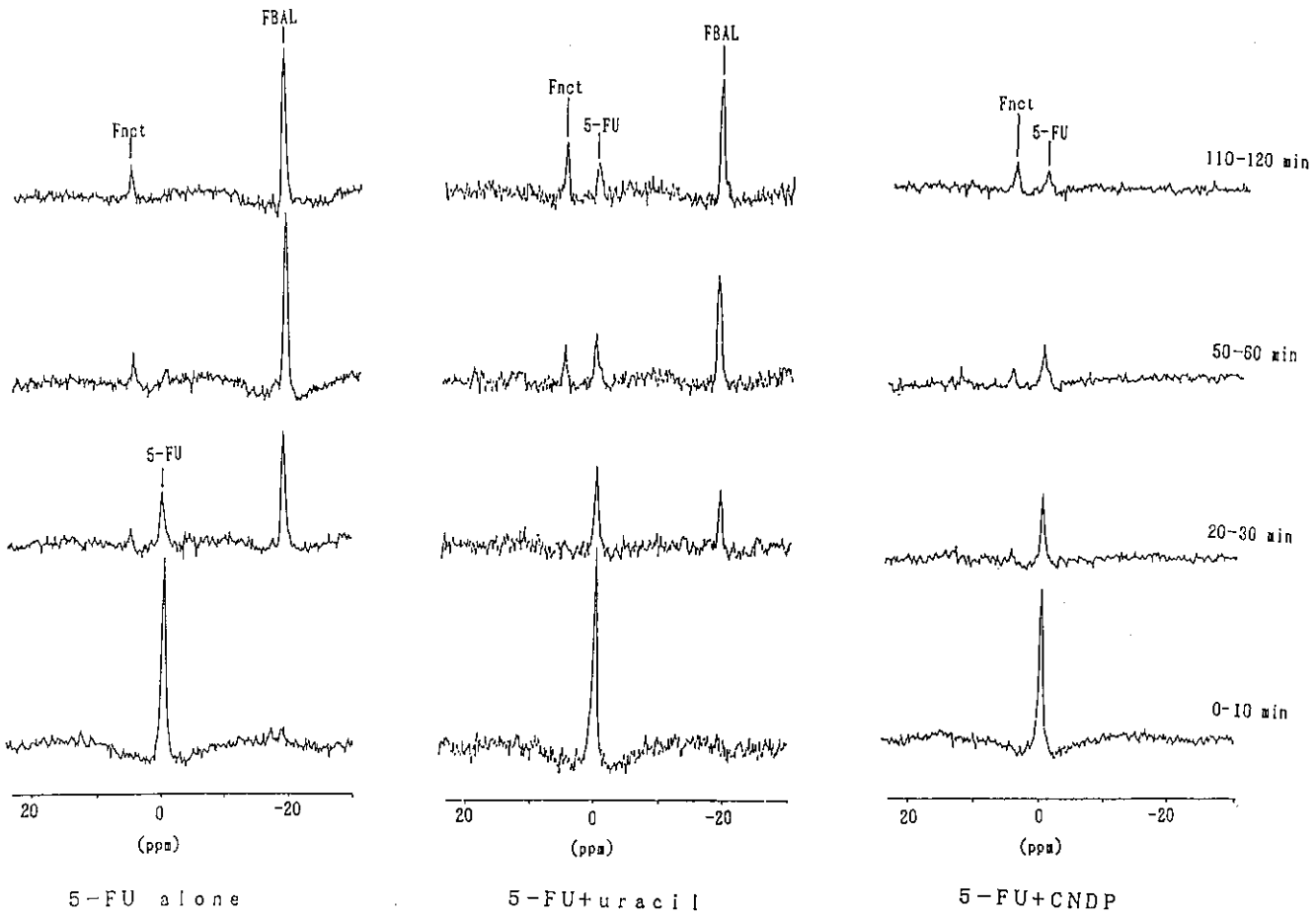

Fig. 4. ^{19}F-MR spectra of metabolites of 5-FU in rat liver after combined administration with uracil or CNDP compared to that after 5-FU alone. Fnct means fluoronucleotides.

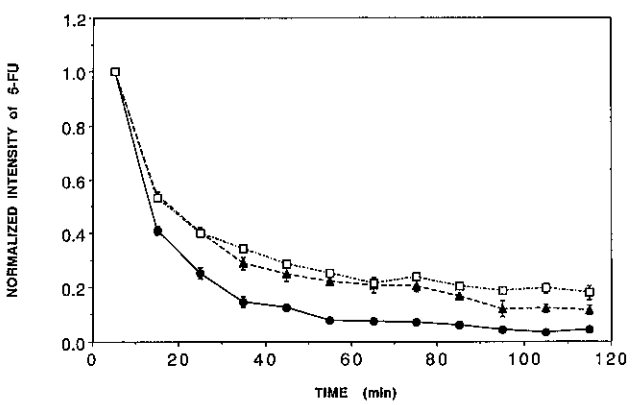

Fig. 5. Time courses of decrease of 5-FU in the 5-FU alone group, uracil combined group and CNDP combined group. ● 5-FU alone, ▲ 5-FU+uracil, □ 5-FU+CNDP.

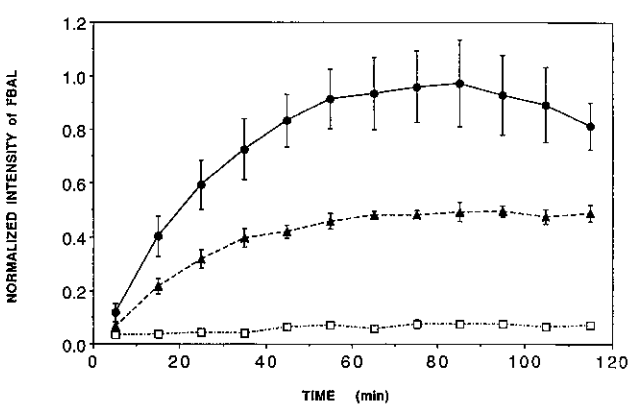

Fig. 6. Time courses of FBAL formation in the 5-FU alone group, uracil combined group and CNDP combined group. ● 5-FU alone, ▲ 5-FU+uracil, □ 5-FU+CNDP.

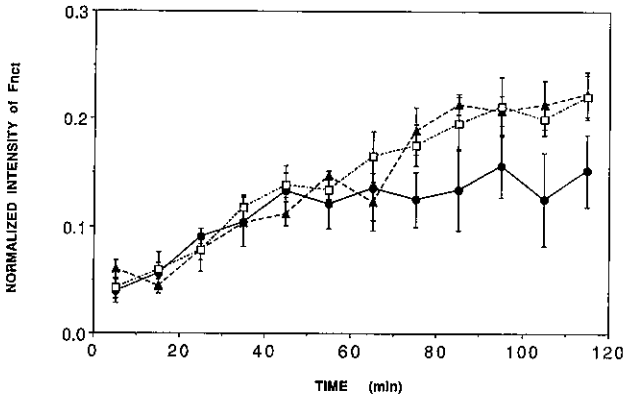

Fig. 7. Time courses of fluoronucleotides (Fnct) formation in the 5-FU alone group, uracil combined group and CNDP combined group. ● 5-FU alone, ▲ 5-FU+uracil, □ 5-FU+CNDP.

trations obtained by chromatography and by ^{19}F-MRS showed a good correlation,[7] suggesting the usefulness of the ^{19}F-MRS method for the present purposes. Sensitivity and quantitative character of ^{19}F-MRS were inferior to those of chromatography, but the animals can be observed non-invasively when ^{19}F-MRS is used. Furthermore, quantitative evaluation of FBAL by chromatography is technically difficult. On the other hand, direct evaluation of FBAL is possible by ^{19}F-MRS with much less error, so ^{19}F-MRS seems to be very useful.

EM-FU and CNDP have been reported to supppress the decomposition of 5-FU in the liver much better than the known fluorinated pyrimidine drugs and to maintain the effective blood level longer.[5,6] Comparison of the behaviors of EM-FU, 5-FU and tegafur in the present study showed significant slower decomposition of EM-FU to FBAL, though the rates of decrease of EM-FU and of tegafur in the liver were similar to each other. EM-FU and tegafur are predominantly metabolized to 5-FU within the hepatic cells by the enzyme cytochrome P-450,[6] and since the decomposition of EM-FU to FBAL is slow, the metabolism of EM-FU to 5-FU might also slow. On the other hand, the rate of decrease of EM-FU in the liver is not much different from that of tegafur, so EM-FU might be excreted into the bile or blood without being metabolized to 5-FU. Since the amount of drug used in the present study was approximately 5 times the clinical dose, the latter might be more likely.

The decomposition-suppressing activity of CNDP is very strong, and almost no formation of FBAL was observed during the 120-min period after 100 mg/kg 5-FU was administered. The suppression of decomposition of 5-FU by uracil was intermediate between that of the 5-FU alone and CNDP combined groups, but the rate of decrease of 5-FU from the liver was similar in the uracil combined and CNDP combined groups. This result is similar to that for the rate of decrease of tegafur and EM-FU. It was considered that the small difference between the uracil combined group and the CNDP combined group, in spite of the major difference in catabolic suppression, might reflect a limitation on the amount of drug accumulation in the liver. There was little difference in the rate of formation of fluoronucleotides between the uracil combined and CNDP combined groups, but both were somewhat different from that in the 5-FU alone group.

A marked decrease in total fluorine compounds from the liver was observed in the spectra in the CNDP group. It might be assumed that 5-FU did not decompose in the liver to FBAL and was excreted intact from the liver in the CNDP group. This would be consistent with the reported result that the blood level of 5-FU was higher in the presence of CNDP.[6] Thus, although the administered 5-FU might be accumulated in the liver, there is practically no decomposition, so the 5-FU is excreted into the blood at a higher level.

It may be suggested from the above results that EM-FU maintains the level of fluoro-pyrimidine in the liver, as in the case of tegafur,[8] and the decomposition of EM-FU to FBAL is less compared to that of tegafur. Thus, the blood level of fluorinated pyrimidines may be maintained higher and the increase of FBAL may be suppressed in the case of the administration of EM-FU.

Comparison of the effectiveness of the present method and the continuous i.v. drip infusion of 5-FU (which is currently considered to be the best method of administration) should be made in the future. We speculate that the co-administration of EM-FU and CNDP would suppress the catabolism of 5-FU and maintain an effective blood level for a long period of time. EM-FU can be administered orally, which is clinically desirable.

^{19}F-MRS should be a useful technique for the development and screening of fluoro-pyrimidine drugs, and also for choosing the most effective method for tumor therapy.

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