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Effect of Coadministration of Uracil on the Toxicity of Tegafur

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Abstract □ The cardiotoxic and neurotoxic effects of tegafur, an anticancer agent, were compared with those of uracil plus tegafur (4:1 mol/mol) in mice, rats, rabbits, cats and dogs. Uracil plus tegafur was shown to be less toxic than the drug alone in all the species, and uracil was found to decrease the toxicity of tegafur. α-Fluoro-β-alanine, a catabolic metabolite of the drug, had toxic effects similar to tegafur. The results suggest that administration of uracil with tegafur prevents the side effects of the drug on the heart and CNS by inhibiting the degradation of 5-fluorouracil.

Keyphrases □ Tegafur—coadministration with uracil, effect on toxicity, species comparison □ Uracil—coadministration with tegafur, effect on toxicity, species comparison □ Chemotherapeutic agents—coadministration of tegafur and uracil, effect on toxicity, species comparison

Tegafur [5-fluoro-1-(tetrahydro-2-furyl)uracil], synthesized by Hiller *et al.* (1), has been widely used clinically as an anticancer agent because it is a masked form of the pyrimidine antimetabolite, 5-fluorouracil. Jato and coworkers (2, 3) and Mukherjee and Heidelberger (4) reported that the antitumor activity and toxicity of fluorinated pyrimidines are increased by coadministration of pyrimidine. Fujii *et al.* (5–7) found that coadministration of uracil with tegafur increased the 5-fluorouracil level in tumors and the antitumor activity of tegafur, possibly because it inhibits the degradation in the liver of 5-fluorouracil formed from the drug. Moreover, it has been found that uracil plus tegafur (4:1 mol/mol) has stronger antitumor activity than the drug alone, both clinically and in animals (8–12).

Side effects of fluorinated pyrimidines on the heart and CNS have been observed clinically (13–15). But from the above findings, we suggested previously that uracil might prevent the cardiotoxic and neurotoxic effects of tegafur (16). To test this possibility, we compared the cardiotoxic and neurotoxic effects of the drug alone with those of uracil plus tegafur in mice, rats, rabbits, cats, and dogs.

EXPERIMENTAL

Drugs—The following drugs were used: tegafur[1], 5-fluorouracil[2], uracil[3], and α-fluoro-β-alanine hydrochloride[4]. Tegafur, uracil plus tegafur, and 5-fluorouracil were administered intravenously as their sodium salts and orally as suspensions in 5% gum arabic.

Animals—The following animals were used: male ddY mice (18–23 g)[5], male Wistar rats (160–200 g)[5], male Japanese White rabbits (2.5–3.5 kg)[6], mongrel cats of both sexes (2.3–3.4 kg)[6], and male Beagle dogs (9.5–10.0 kg)[7]. The animals were used for experiments after a period of acclimatization of at least 7 d in the laboratory at a controlled temperature of 23 ± 1°C and relative humidity of 55 ± 10% with a 12-h light/dark cycle. They were fasted for 18 h before oral administration of drugs.

Methods—Previously, we demonstrated that tegafur at high doses produces clonic convulsions in mice and rats, cardiac fibrillations in rabbits, and vomiting in dogs (16). 5-Fluorouracil has been reported to have a neurotoxic effect in cats (17). These cardiotoxic and neurotoxic effects of tegafur were compared with those of uracil plus the drug in the five species. The acute toxicity of tegafur in these animals was also compared with those of uracil plus tegafur and α-fluoro-β-alanine, a catabolic metabolite of the drug.

Convulsant Effects in Mice and Rats—Groups of 10 mice and rats

[1] Taiho Pharmaceutical Co., Ltd.
[2] Sigma Chemical Co.
[3] Wako Pure Chemical Industries.
[4] Tokyo Kasei Chemicals.
[5] Tokushima Laboratory Animals Co.
[6] Kearly Co.
[7] Laboratory Research Enterprise, Kalamazoo, Mich.

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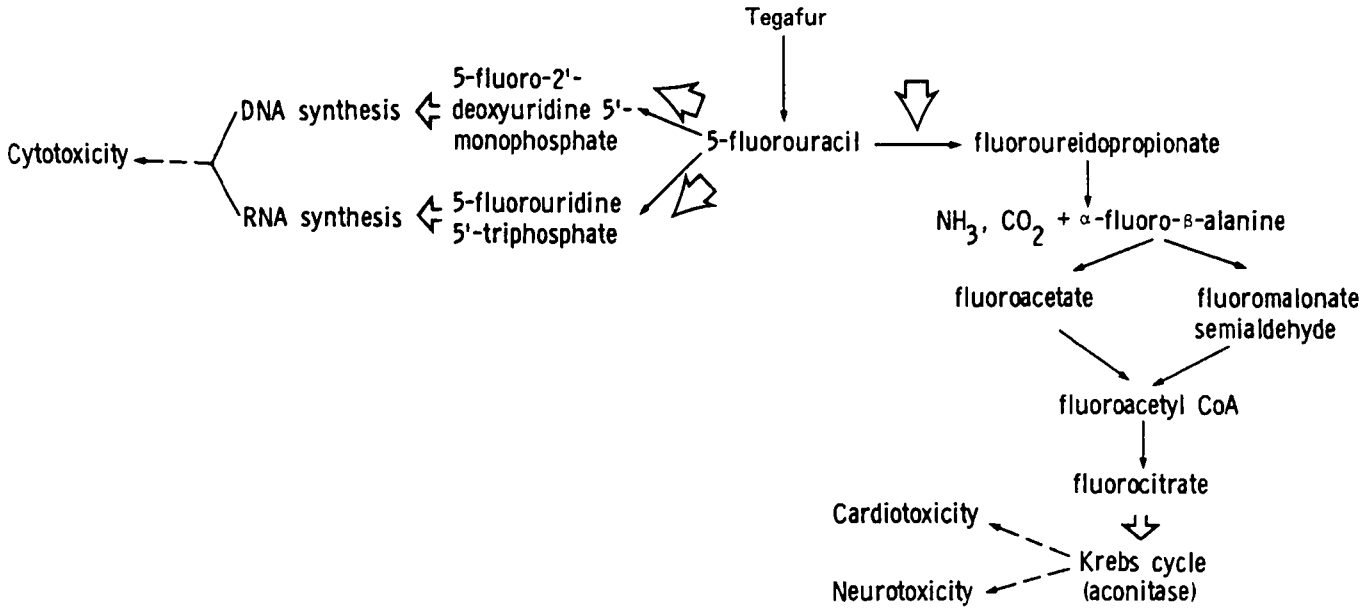

Figure 1—*Proposed mechanism of cyto-, cardio-, and neurotoxicity of tegafur and effect of uracil. Key: (⇦) coadministration of uracil.*

were observed for clonic convulsions after oral administration of the drugs. The CD_{50} values (doses producing clonic convulsions in 50% of the animals) were calculated by the method of Litchfield and Wilcoxon (18).

Cardiotoxic Effect in Rabbits—Groups of 8 rabbits were used. Cardiac fibrillations were determined from the ECG (A-B leads) using a polygraph[8] after intravenous administration of the drugs. The FD_{50} values (doses producing cardiac fibrillations in 50% of the animals) were calculated by the method of Litchfield and Wilcoxon (18).

Neurotoxic Effect in Cats—Cats were examined for gross behavioral changes after oral administration of drugs; tremors or abnormal gait was taken as an index of a neurotoxic effect. The ND_{50} values (doses producing neurotoxic effects in 50% of the animals) were calculated by the up and down method (19).

Neurotoxic Effect in Dogs—Vomiting was observed in dogs after administration of drugs. The VD_{50} values (doses producing vomiting in 50% of the animals) were calculated by the up and down method (19).

Acute Toxicities in Mice, Rats, Rabbits, Cats, and Dogs—Tegafur and uracil plus tegafur were administered orally to mice, rats, rabbits, cats, and dogs. The animals were then kept in cages with food and water, and all deaths within 21 d were recorded. The LD_{50} values in mice and rats were calculated by the method of Litchfield and Wilcoxon (18) and those in rabbits, cats, and dogs by the up and down method (19). In addition, α-fluoro-β-alanine was administered intraperitoneally to mice, rats, rabbits, cats, and dogs, and the LD_{50} values were calculated by the up and down method (19).

RESULTS

CD_{50} and LD_{50} in Mice and Rats—Tegafur at high doses caused two types of death in mice and rats: one following clonic convulsions ~4 h after treatment and the other by cytotoxicity, such as damage of the hematopoietic system, ~2 weeks after treatment. The CD_{50} values for uracil plus tegafur in mice and rats were 1.33 and 1.56 times those for the drug alone, respectively. On the other hand, the LD_{50} values for uracil plus tegafur in mice and rats were 0.28 and 0.34 times those for the drug alone, respectively (Table I). These results clearly demonstrated that coadministration of uracil decreased the convulsant effect of tegafur, but increased its acute toxicity.

FD_{50} and LD_{50} in Rabbits—Tegafur at high doses increased the voltage of the T-wave and decreased that of the ST-wave in the ECG after ~8 h, and caused cardiac fibrillation after ~15 h. Uracil plus tegafur induced cardiac fibrillation ~10 h later than the drug alone and had a weaker effect. 5-Fluorouracil induced more marked cardiac fibrillation than tegafur. The FD_{50} and LD_{50} values for uracil plus tegafur were 1.43 and 1.51 times those for the drug alone, respectively (Table I). Thus, coadministration of uracil decreased the cardiotoxic effect and acute toxicity of tegafur in rabbits.

ND_{50} and LD_{50} in Cats—Tegafur at high doses caused vomiting, tremors, and abnormal gait within 24 h. Uracil plus tegafur had similar, but weaker, effects which developed later after treatment. The effect of 5-fluorouracil was greater than that of tegafur. The ND_{50} and LD_{50} values for uracil plus tegafur were 1.18 and 1.20 times those for the drug alone, respectively (Table I). Therefore, coadministration of uracil decreased the neurotoxic effect and acute toxicity of tegafur in cats.

VD_{50} and LD_{50} in Dogs—Tegafur at high doses induced vomiting in dogs. Uracil plus tegafur had a weaker effect than the drug alone, which developed later, while 5-fluorouracil had a stronger effect than tegafur. The VD_{50} and LD_{50} values for uracil plus tegafur were 1.52 and 1.85 times those for the drug alone, respectively (Table I). Therefore, coadministration of uracil decreased the vomiting and acute toxicity induced by tegafur in dogs. In rabbits, cats, and dogs, no deaths were observed within 2 weeks after treatment, unlike mice and rats.

Toxic Effects of α-Fluoro-β-alanine in Mice, Rats, Rabbits, Cats,

Table I—Cardio- and Neurotoxic Effects and Acute Toxicities of Tegafur and Uracil plus Tegafur in Various Species

Species	Tegafur, mg/kg po	Uracil + Tegafur, mg/kg po[a]	Ratio[b]
Mice			
CD_{50}[c]	1850 (1480–2294)[h]	2470 (2266–2692)	1.33
LD_{50}[d]	1420 (1302–1547)	401 (371–474)	0.28
Rats			
CD_{50}	1340 (971–1849)	2100 (1826–2415)	1.56
LD_{50}	1410 (1308–1520)	487 (447–531)	0.34
Rabbits			
FD_{50}[e]	54.0[i] (40.9–71.3)	77.7[i] (65.8–91.7)	1.43
LD_{50}	71.0	107.7	1.51
Cats			
ND_{50}[f]	29.7	35.3	1.18
LD_{50}	35.5	42.7	1.20
Dogs			
VD_{50}[g]	30.9	47.2	1.52
LD_{50}	34.2	63.5	1.85

[a] In 4:1 mole ratio; dose is in terms of tegafur. [b] Uracil plus tegafur/tegafur. [c] Dose producing clonic convulsions in 50% of the animals, measured 3 d after drug treatment. [d] Lethal dose in 50% of the animals, measured for 21 d after drug treatment. [e] Dose producing cardiac fibrillation in 50% of the animals, measured 3 d after drug treatment. [f] Dose producing neurotoxic effects in 50% of the animals, measured 3 d after drug treatment. [g] Dose producing vomiting in 50% of the animals, measured 3 d after drug treatment. [h] Values in parentheses are 95% confidence limits. [i] Administered intravenously.

[8] San'ei-Sokki, 142-8.

Table II—LD_{50} Values for α-Fluoro-β-alanine in Various Species

Species	LD_{50}[a] mg/kg ip
Mice	167
Rats	218
Rabbits	27.4
Cats	9.4
Dogs	<24.5

[a] Measured for 21 d after drug treatment.

and Dogs—α-Fluoro-β-alanine, like tegafur, induced clonic convulsions in mice and rats, cardiac fibrillations in rabbits, and neurotoxic effects in cats and dogs. However, unlike tegafur, it did cause any deaths within 2 weeks after its administration. The LD_{50} values for α-fluoro-β-alanine in the five species are listed in Table II; rabbits, cats, and dogs were more sensitive than mice and rats to α-fluoro-β-alanine, as in the case of tegafur.

DISCUSSION

Previously, we suggested that uracil plus tegafur might have less cardio- and neurotoxicity than the drug alone (16). To test this possibility, in the present study we compared the pharmacological effects of tegafur alone and with uracil in mice, rats, rabbits, cats, and dogs, because tegafur has different effects in different species (16).

From our results on the effects of tegafur, 5-fluorouracil, and α-fluoro-β-alanine, we concluded that tegafur has two reactions, as illustrated in Fig. 1. One is due to the conversion of 5-fluorouracil to the phosphorylated anabolic metabolites, 5-fluoro-2′-deoxyuridine 5′-monophosphate and 5-fluorouridine 5′-triphosphate, which inhibit DNA and RNA syntheses. This is the mechanism of its antitumor activity (20). This action also results in damage of the hematopoietic system of mice and rats ~2 weeks after administration of tegafur. The other action is to induce clonic convulsions in mice and rats, cardiotoxicity in rabbits, and neurotoxicity in cats and dogs. These effects, which appear within 24 h after administration, were also observed after administration of α-fluoro-β-alanine.

The different effects in different species are very similar to those of methyl fluoroacetate or sodium fluoroacetate, which induce fluorocitrate intoxication (21). Thus, it has been suggested that the cardiotoxic and neurotoxic effects of 5-fluorouracil are due to fluorocitrate poisoning, probably as a result of catabolism of 5-fluorouracil (17, 22). Therefore, the second action of tegafur is caused by catabolic metabolites of 5-fluorouracil. Some metabolites may be converted to fluorocitrate, which inhibits the Krebs cycle, although no fluorocitrate has yet been detected in animals after treatment either with tegafur or 5-fluorouracil.

Rabbits, cats, and dogs are more sensitive to tegafur than mice and rats. This species difference in sensitivity might be due to species differences in the effects of fluorocitrate, because on clinical evaluation of tegafur (14), humans were found to be less sensitive than rabbits, cats, and dogs. Moreover, Meldrum and Bignell (23) reported that the sensitivity of humans to fluorocitrate is weaker than that of these animals. We think that the cardiotoxic and neurotoxic effects of fluorinated pyrimidines in clinical use (13–15) might also be caused by fluorocitrate.

On coadministration of uracil, Ikenaka *et al.* (24) found that uracil inhibited the degradation of 5-fluorouracil much more than its phosphorylation. Moreover, Unemi *et al.* (12) found that the antitumor activity of uracil plus tegafur is 5 times that of the drug alone, because uracil inhibits the degradation of 5-fluorouracil. Furthermore, Jato and coworkers (2, 3) and Mukherjee and Heidelberger (4) reported that coadministration of pyrimidine increased both the antitumor activity and the toxicity of fluorinated pyrimidines in mice.

In the present study, coadministration of uracil decreased the lethal dose of tegafur in mice and rats, but also increased the convulsant dose in these animals and the cardiotoxic, neurotoxic, and lethal doses in rabbits, cats, and dogs. These results can be explained by the fact that uracil inhibits the degradation of 5-fluorouracil, and so the effects of 5-fluoro-2′-deoxyuridine 5′-monophosphate and 5-fluorouridine 5′-triphosphate, anabolic metabolites of 5-fluorouracil, were increased while the effect of α-fluoro-β-alanine, a catabolic metabolite of 5-fluorouracil, was decreased. The extent of these effects probably depended on the relative inhibitions in degradation and phosphorylation of 5-fluorouracil caused by uracil (24). That the LD_{50} for tegafur in mice and rats was decreased and the LD_{50} in rabbits, cats, and dogs were increased by uracil can be explained by supposing that the acute toxicity in the former animals depends on anabolic rather than catabolic metabolites of 5-fluorouracil, whereas the acute toxicity in the latter animals depends on catabolic rather than anabolic metabolites.

The coadministrations of (aminooxy)acetate and acetamide were reported to reduce the toxicities of α-fluoro-β-alanine and fluoroacetate, respectively (25, 26). Furthermore, coadministration of malonate reduced citrate accumulation caused by fluoromalonate, which showed similar toxocity to fluorocitrate (27). Therefore, the inhibitory effect of uracil on the cardiotoxic and neurotoxic effects of tegafur may be explained not only by competitive inhibition of uracil with 5-fluorouracil, but also by competitive inhibition of the degradation of fluoroureidopropionate by ureidopropionate and of α-fluoro-β-alanine by β-alanine. Consequently, the actual concentration of fluorocitrate may be very low or the rate of formation of fluorocitrate may be very slow.

The present findings show that the side effects of tegafur on the heart and CNS are reduced by coadministration of uracil. Therefore, combination therapy with uracil and tegafur should be better than treatment with the drug alone.

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