Fig. 2 and confirms that gallbladder emptying was the mechanism involved.

In conclusion, the feasibility and practicality of using iodinated analogs of tyrosine-containing small peptides to study *in vivo* drug disposition has been demonstrated. The modes of clearance and biliary excretion are similar for both A and ^{131}I-A in the rat, and ^{131}I-A behaves similarly in both rat and dog.

The advantages of non-invasive technology such as external gamma scintigraphy are many, including its applicability to human studies involving peptide drugs or drug candidates. The relatively straightforward process of preparing gamma-emitting iodinated peptide analogs, and performing the necessary control studies, may in many instances result in a convenient means to study the complex problems of polypeptide drug disposition and elimination. The unusual observation with the dog model that A may effect biliary retention is the subject of a follow-up study to be presented in another report.

Acknowledgement

The authors wish to thank Ms. S. Yonts for her skillful assistance with the external gamma scintigraphy and data processing. This work was supported in part by an INTERx-MSDRL research grant to the University of Kentucky College of Pharmacy.

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Effects of 5-Fluorouracil Prodrugs on the Central Nervous System in Mice and Rats

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Received: July 2, 1984; accepted: November 2, 1984.

Abstract: The effects on the central nervous system (CNS) of mice and rats were determined for the 5-fluorouracil prodrugs, 1-(2-tetrahydrofuranyl)-5-fluorouracil (FT), a combination of FT and uracil in a molar ratio of 1:4 (UFT), and 1-hexylcarbamoyl-5-fluorouracil (HCFU). Both FT and UFT failed to produce a significant prolongation of hexobarital sleeping time in mice, while HCFU, at the same dose levels, caused a significant ($P < 0.01$) prolongation of hexobarbital sleep. FT, UFT, and HCFU produced a slight suppression of coordinating ability in mice, but the effect of HCFU was more pronounced than that of FT and UFT. There were no significant changes in 5-hydroxytryptamine contents in the cerebral cortex and only small insignificant changes of dopamine contents in the corpus striatum by any of the drugs examined. Furthermore, HCFU was more potent than FT and UFT in potentiating the actions of ethanol. These results suggest that HCFU is more toxic to the CNS than are FT and UFT.

5-Fluorouracil (5-FU)[3] has been widely used for the treatment of cancer. In addition to its antitumor activity, however, 5-FU possesses various side effects such as gastrointestinal (GI) and hematological toxicity (1).

1-(2-Tetrahydrofuranyl)-5-fluorouracil (FT) (Fig. 1) was synthesized as a derivative of 5-FU by Hiller et al. (2) and is now commonly used as an oral antitumor agent in Japan. Because FT is slowly converted to 5-FU (3), its toxicities in bone marrow and GI tracts are lower than those of 5-FU (4). However, FT passes easily through blood brain barrier and produces occasionally side effects in the central nervous system (CNS), including lethargy, ataxia, confusion, dizziness, and hallucination (5, 6). 5-FU occasionally also causes reversible cerebellar ataxia (7).

It was found that coadministration of uracil with FT increased 5-FU level in tumor and blood, possibly because uracil inhibits the degradation of 5-FU formed from FT in the liver (8). There-

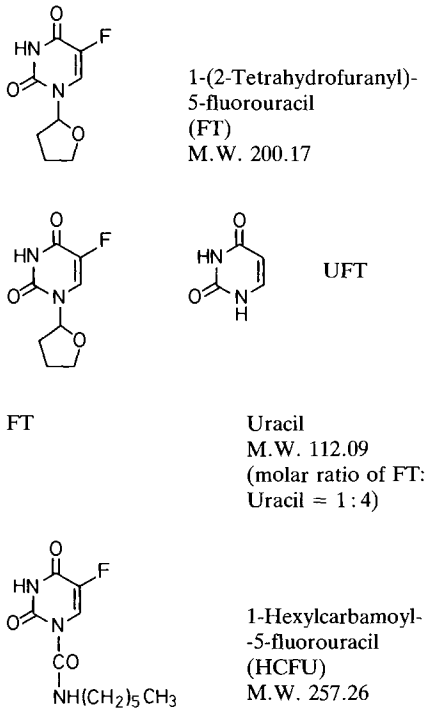

Fig. 1 Chemical structures of FT, UFT and HCFU.

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[3] Abbreviations
UFT: a fixed drug combination of 1-(2-tetrahydrofuranyl)-5-fluorouracil (FT) and uracil in a molar ratio of 1:4
FT: 1-(2-tetrahydrofuranyl)-5-fluorouracil
5-FU: 5-fluorouracil
HCFU: 1-hexylcarbamoyl-5-fluorouracil
5-HT: 5-hydroxytryptamine
DA: dopamine
p.o.: per os
i.p.: intraperitoneally

fore, UFT (Fig. 1), a new type of antitumor agent consisting of FT and uracil in a molar ratio of 1:4, has been developed for the purpose of increasing the antitumor activity of FT without producing the severe toxicity as 5-FU (9), and there are no reports concerning the CNS side effects of UFT so far (10).

Recently, 1-hexylcarbamoyl-5-fluorouracil (HCFU) (Fig. 1) has been developed as a derivative of 5-FU. Because of its lipophilicity, this compound is easily taken up into tumor tissues, and it is converted non-enzymatically to the active substance 5-FU (11), whereas FT is mainly converted enzymatically to 5-FU in the liver. HCFU was shown to be an effective anti-tumor agent in clinical trials (12). However, the characteristic subjective symptoms such as heat sensation and pollakisuria were reported at high degrees in clinical trials with HCFU (12). Moreover, patients treated with HCFU experienced consciousness disturbances following the ingestion of alcoholic beverages (13, 14).

In the present study it was attempted to investigate the CNS side effects of 5-fluorouracil prodrugs and to examine the interaction of these antitumor agents and ethanol.

Materials and Methods

Animals. Male ddY strain mice weighing 20 to 25 g and male Wistar strain rats weighing 150 to 180 g were used. They were housed at 22 ±1 °C and 55 ±5 % relative humidity under controlled lighting conditions.

Chemicals. The following agents were used; FT (Taiho Pharmaceutical Co., Ltd.), uracil (Wako Pure Chemicals, Ltd.), HCFU (Mitsui Pharmaceutical Co., Ltd.), hexobarbital sodium (Sigma), ethanol (Wako Pure Chemicals, Ltd.), dopamine (Nakarai Chemicals, Ltd.), 5-hydroxytryptamine creatinine sulfate complex (Sigma) and chlorpromazine hydrochloride (Shionogi Pharmaceutical Co., Ltd.).

Sample preparation and drug treatment. FT, UFT and HCFU were suspended in 5 % gum arabic solution and given per os (p.o.) at a volume of 1 ml/100 g body weight. Ethanol (25 % v/v solution) was given p.o. at a volume of 0.1 to 0.2 ml/10 g body weight.

Chlorpromazine was given intraperitoneally (i.p.). Before drug administration, the animals were starved for 16 h but given water *ad libitum*.

Analytical and pharmacological methods

1. Effect on hexobarbital-induced sleeping time in mice.
At 1 h after test drugs administration (p.o.), all mice were injected i.p. with 70.0 mg/kg hexobarbital, and the duration of sleep, measured as the time from loss to restoration of righting reflex, was recorded. The mean sleeping time of each group was calculated and compared with that of the control group treated with 5 % gum arabic.

2. Effect of hypnosis induced by ethanol in mice.
Mice were treated orally with test drugs 60 min prior to administration of ethanol (4000 mg/kg, p.o.), and the effect of drug was regarded as positive when a mouse showed a loss of righting reflex over 20 min.

3. Effect on coordinating ability in mice.
Mice capable of staying on a rotation rubber rod (3.0 cm diameter, 15 rpm) for longer than 1 min were selected and were trained for three more days. Groups of 8 mice were tested each time after drug administration (p.o.). When a mouse slipped off the rod within 1 min, the test was considered positive.

4. Effect on brain monoamines in rats – determination of 5-hydroxytryptamine (5-HT) and dopamine (DA).
For the determination of 5-HT and DA, animals were killed by microwave irradiation (4.5 kW, 1.0 sec.). The brain was rapidly removed, and cerebral cortex and corpus striatum were separated by the method of Glowinski and Iversen (15). 5-HT in the cerebral cortex was measured spectrofluorimetrically by the method of Curzon and Green (16). DA in the corpus striatum was extracted by the method of Anton and Sayre (17) and measured spectrofluorimetrically by the method of Chang (18).

Statistical analysis

Differences between control and experimental values were analyzed by Student's t-test.

Results

Effect on hexobarbital-induced sleep

Both FT (90.0 and 270.0 mg/kg, p.o.) and UFT (291.6 and 874.8 mg/kg, p.o.) did not produce a significant prolongation of hexobarbital sleeping time. In contrast, HCFU (90.0 and 270.0 mg/kg, p.o.) caused a significant ($P < 0.01$ and $P < 0.05$) prolongation of hexobarbital sleeping time (Fig. 2). Chlorpromazine also produced a significant prolongation of hexobarbital sleeping time ($P < 0.001$).

Effect on coordination ability

Within 1 h after administration, FT (90.0 and 270.0 mg/kg, p.o.) and UFT (291.6 and 874.8 mg/kg, p.o.) did not

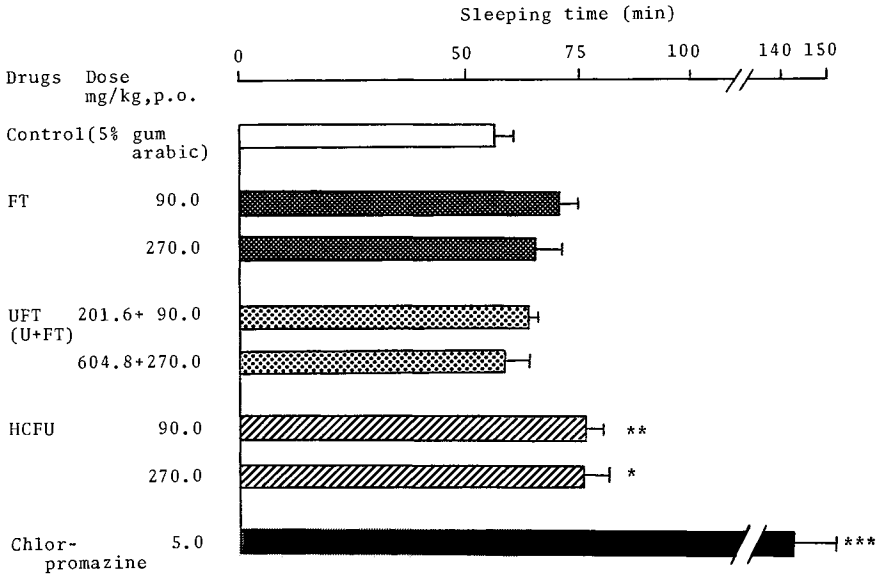

Fig. 2 Effect of FT, UFT and HCFU on hexobarbital sleeping time in mice.
Hexobarbital (70.0 mg/kg, i.p.) was injected 60 min after each drug administration. Values are means ± S.E. for 8 mice.
Significance of difference from control: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table I. Effect of FT, UFT and HCFU on motor coordination (rotarod method) in mice

Drugs	Dose mg/kg, p.o.	No. of animals	No. of animals falling from rotarod within 1 min Time (h) after administration 1	2	4	8	24
Control (5% gum arabic)		8	0	0	0	0	1
FT	90.0	8	0	0	0	0	1
	270.0	8	0	0	0	0	0
UFT (U + FT)	201.6 + 90.0	8	0	0	1	0	0
	604.8 + 270.0	8	0	1	2	1	2
HCFU	90.0	8	0	0	0	0	2
	270.0	8	2	1	2	1	3
Chlorpromazine	5.0	8	4	3	3	2	1

produce impairment of coordinated motor activity in mice. In contrast, HCFU (90.0 and 270.0 mg/kg, p.o.) produced ataxia in two mice. The total number of mice that failed to remain on the rod within 24 h after HCFU was 11, while 1 and 7 animals failed to remain after FT and UFT, respectively (Table I). Four animals failed to remain on the rod within 1 h after chlorpromazine (Table I).

Interaction of antitumor agents with ethanol

According to clinical reports (13, 14), patients treated with HCFU experienced consciousness disturbances after ingesting alcoholic beverages. In general, a combination of tranquilizer and alcohol strongly interferes with abilities of coordination and judgement more than alcohol alone (19, 20). Two laboratory tests (righting reflex and rotarod) were used to examine each drug's ability to potentiate the sedative effect of ethanol.

a. Effect on righting reflex (hypnosis action) in mice. The results in Table II indicate that treatment with FT (10 to 90 mg/kg, p.o.) or UFT (32.4 to 291.6 mg/kg, p.o.) plus ethanol (4000 mg/kg, p.o.) did not produce a loss of righting reflex. At 270.0 mg/kg, p.o. of FT and 874.8 mg/kg, p.o. of UFT, only 4 and 3 out of 8 mice produced a loss of righting reflex, respectively. In contrast, pretreatment with HCFU produced a dose-related (doses of more than 60.0 mg/kg, p.o.) loss of righting reflex. At 2.5 and 5.0 mg/kg of chlorpromazine, 6 and 7 mice displayed a loss of righting reflex, respectively.

b. Effect on coordinating ability in mice. In the rotarod performance study, trained mice were treated orally with test drugs 30 min prior to administration of ethanol (2000 mg/kg, p.o.). The results in Table III indicate that after treatment with FT (10.0 to 90.0 mg/kg, p.o.) or UFT (32.4 to 291.6 mg/kg, p.o.) plus ethanol all mice remained on the rod. AT 270.0 mg/kg FT and 874.8 mg/kg UFT, only 4 and 2 out of 8 mice showed an impairment of coordination. In contrast, pretreatment with doses of more than 30.0 mg/kg of HCFU resulted in a dose-related enhancement of the ethanol-induced impairment of coordination. At 270.0 mg/kg HCFU, all mice failed to remain on the rod within 2 h. After chlorpromazine (2.5 to 5.0 mg/kg, p.o.) plus ethanol (2000 mg/kg, p.o.), 5 and 6 mice failed to remain on the rod. Therefore, the sedative effect of ethanol was potentiated most potently by pretreatment with HCFU, followed by FT and UFT. The effective ratio calculated from their effective doses was HCFU : FT : UFT = 30 : 3 : 1. Thus, these results further indicate that HCFU could interfere with abilities such as coordination and judgement in humans when combined with alcoholic beverages.

Table II. Combined effect of FT, UFT or HCFU and ethanol on righting reflex in mice

Drugs	Dose mg/kg, p.o.	No. of animals	No. of animals showed loss of righting reflex
Control + Ethanol	4000	8	1
FT + Ethanol	10.0 + 4000	8	0
	30.0 + 4000	8	0
	90.0 + 4000	8	0
	270.0 + 4000	8	4
UFT (U + FT) + Ethanol	22.4 + 10.0 + 4000	8	0
	67.2 + 30.0 + 4000	8	0
	201.6 + 90.0 + 4000	8	0
	604.8 + 270.0 + 4000	8	3
HCFU + Ethanol	10.0 + 4000	8	1
	30.0 + 4000	8	1
	60.0 + 4000	8	4
	90.0 + 4000	8	7
	270.0 + 4000	8	8
Chlorpromazine + Ethanol	2.5 + 4000	8	6
	5.0 + 4000	8	7

Ethanol was administered 60 min after each drug administration.
Ethanol: 25% solution.

Effect of FT, UFT and HCFU on rat brain monoamine contents

Our previous studies have shown that fluoropyrimidine anti-tumor drugs affected the concentration of brain monoamines, and these effects may be related to their side effects in the CNS (21, 22). Accordingly, we attempted to investigate whether the 5-FU derivatives affect monoamines concentration in the CNS. Monoamine contents were determined for 1, 2 and 4 h after treatment of FT (270.0 mg/kg, p.o.), UFT (874.8 mg/kg, p.o.) and HCFU (270.0 mg/kg, p.o.).

a. 5-HT content in rat cerebral cortex. There were no significant changes in 5-HT content in cerebral cortex.

b. DA content in rat corpus striatum. Our results showed a slight increase of DA levels after both FT and UFT treatment and a slight decrease after HCFU treatment.

Table III. Combined effect of FT, UFT or HCFU and ethanol on motor coordination test in mice

Drugs	Dose mg/kg, p.o.	No. of animals	No. of animals falling from rotarod within 1 min. Time (h) after administration		
			Pre.	1	2
Control (5 % gum arabic)		8	0	0	0
Control + Ethanol	2000	8	0	0	0
FT + Ethanol	10.0 + 2000	8	0	0	0
	30.0 + 2000	8	0	0	0
	90.0 + 2000	8	0	0	0
	270.0 + 2000	8	0	4	1
UFT (U + FT) + Ethanol	22.4 + 10.0 + 2000	8	0	0	0
	67.2 + 30.0 + 2000	8	0	0	0
	201.6 + 90.0 + 2000	8	0	0	0
	604.8 + 270.0 + 2000	8	0	2	2
HCFU + Ethanol	10.0 + 2000	8	0	0	0
	30.0 + 2000	8	0	3	0
	60.0 + 2000	8	0	5	4
	90.0 + 2000	8	0	6	6
	270.0 + 2000	8	0	8	8
Chlorpromazine + Ethanol	2.5 + 2000	8	0	5	2
	5.0 + 2000	8	0	6	4

Ethanol was administered 30 min after each drug administration.
Ethanol: 25 % solution.

Discussion

Neuropsychiatric side effects of antitumor agents were reviewed by Peterson and Popkin (23). In the present work, the CNS side effects of the fluorinated pyrimidine derivatives, FT, UFT and HCFU, were studied.

According to general pharmacological studies of FT, UFT and HCFU, these drugs produced similar effects such as the prolongation of hypnotic drug-induced sleeping time in mice, suppression of spontaneous motor activity in mice, an arousal pattern on spontaneous EEG in cats and the drowsy EEG pattern in rabbits (24, 25, 26). However, as seen in Fig. 2 and Table I, prolongation of sleeping time and impairment of coordinating ability in mice were more pronounced with HCFU as compared with FT and UFT. Of particular significance is the fact that HCFU produced a dose-related potentiation of ethanol-induced loss of righting reflex and impairment of coordination (Tables II and III), although the mechanism of this action of HCFU is not clearly understood at present. Similarly, patients treated with HCFU experienced consciousness disturbances after ingesting alcoholic beverages (12, 13). FT and UFT, at higher doses, also potentiated the action of ethanol, but these drugs exhibited a much greater margin between therapeutic (9) and sedative doses than HCFU, in agreement with the interaction studies with ethanol.

It has been reported that CNS side effects induced by HCFU are relieved by major tranquilizers in clinical trials; furthermore, HCFU affects hypothalamic neurons (27, 28). Our results indicate that neither 5-HT in cerebral cortex nor DA in corpus striatum were changed by treatment with either drug. However, measurements of monoamine metabolites to study their turn-over are required to clarify the CNS side effects in further detail.

So far, our results indicate that the CNS side effects induced by HCFU were higher than those of FT or UFT. Further experiments are also needed to clarify the mechanism by which these antitumor agents, especially HCFU, potentiate ethanol-induced CNS side effects.

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