Effect of Experimentally Altered Thyroid States on 5-Fluorouracil Metabolism in the Rat

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Introduction

The metabolism of a number of drugs has been found to be influenced by the thyroid condition of the animal (2, 3, 6, 7). The toxicity and therapeutic actions of these drugs were found to be enhanced in the hyperthyroid condition. The enhanced drug action has yet to be explained.

Since chemotherapeutic drugs, such as 5-fluorouracil (FU), must be administered at near toxic doses to be effective, any alteration in toxicity and effectiveness are particularly important. This study was conducted to determine if experimentally altered thyroid states affect the metabolism of 5-fluoro(2-^{14}C)uracil (2-^{14}C-FU) in adult female rats.

Methods

Animals

Sprague-Dawley descendent female rats (Laboratory Supply Co., Indianapolis, Indiana) weighing 160-180 g were used throughout this investigation. With the exception of the time during which urine was being collected in the metabolism cages, the animals were housed individually in conventional metal cages with food and water being allowed *ad libitum*. The rats used in the preliminary toxicity study were acclimated for a period of 2 days before the experiment was begun. Those used in the metabolism study were acclimated for 1 day.

Induction of Altered Thyroid States

The hypothyroid state was induced with 6-*n*-propyl-2-thiouracil (PTU) prepared fresh daily. The daily dose was 2.0 mg PTU/0.5 ml/rat given by ip injection. The hyperthyroid state was induced with L-thyroxine (T_4) prepared fresh daily. The daily dose was 20 μg L-thyroxine sodium pentahydrate/0.5 ml/rat by ip injection. Euthyroid rats received 0.5 ml of normal saline by ip injection during the 15-day pretreatment period. These doses have been shown to produce the desired thyroid states (4).

Preliminary Toxicity Study

To determine the dose of FU that could be safely administered to the rats having the altered thyroid state, three groups of eight animals were pretreated for 15 days. On day 15, each treatment group was subdivided into four dosage groups of two animals each. Each dosage group was given either 0, 50, 75, or 100 mg FU/kg. The animals were observed and weighed daily for a period of 15 days after dosing.

Radionuclide and Counting

The 2-^{14}C-FU was determined to be radiochemically pure by thin layer chromatography and autoradiography. It was added to carrier FU to give a dosing solution which contained 18.5 μCi/ml and 10 mg FU/ml. Each rat was given 50 mg/kg of the dosing solution (approximately 18 μCi) by ip injection. An aliquot of the dosing solution was prepared for a standard so that the total activity injected into each rat could be determined.

Radioactivities in the samples and standard were measured in an Isocap 300 liquid scintillation counter (Amersham/Searle Corp., Arlington Heights, Illinois). The counting data were converted to disintegrations per minute by internal standardization using ^{14}C-labeled toluene as the standard.

Metabolism Study

A total of 18 female rats, purchased in three lots, was used. Three replicates, each consisting of three groups of two animals, were run on different days. The rats were randomly assigned to treatment groups and were pretreated for the 15-day period to establish the desired thyroid states. On day 15 all rats received ^{14}C-labeled FU. They were then placed in individual glass metabolism cages where CO_2 and urine were collected over a period of 24 hr (5). The CO_2 trapping solution consisted of a solution of 2-ethoxyethanol and 2-aminoethanol (2:1) and was changed at 1, 3, 6, 12, and 24 hr. Urine was collected at 12 and 24 hr. Aliquots of the trapping solutions and urine were counted in the liquid scintillation counter. The urine was then concentrated by lyophilization to reduce the time for autoradiography exposure.

Thin Layer Chromatography and Autoradiography

^{14}C-Labeled FU and urea were chosen as indicators of metabolism in the urine and were separated by thin layer chromatography. Plates precoated with a mixture of Adsorbosil 1 and Adsorbosil P-1 (3:1) (Applied Science Laboratories, State College, Pennsylvania) were used for separation of the urine samples. The plates were developed in a solution of *n*-butanol, water, and acetic acid (80:20:10). 5-Fluorouracil spots were visible under short-wave ultraviolet light in a viewing cabinet (Chromato-Vue, Ultraviolet Products, San Gabriel, California). Urea could be visualized as bright yellow spots by lightly spraying with a solution of 1 g of p-dimethylaminobenzaldehyde in 57 ml of 95% ethanol and 3 ml of HCl. After development the plates were sprayed with a protective polymeric coating of Neatan-new (Brinkman Instruments, Inc., Westbury, New York) and exposed to Kodak No-Screen medical x-ray film to determine where activity was located on the plates. The spots were scraped and counted in the liquid scintillation counter.

Results and Discussion

Preliminary Toxicity Study

Weight loss and diarrhea were evident in all groups after dosing. The severity of the symptoms appeared to be less in the group of

animals which received the smallest dose of FU. All animals improved after the fourth day past FU injection. They all survived and gained weight until the experiment was terminated on day 29. The 50 mg/kg dose was chosen for the main metabolism study.

Metabolism Study

The percentages of the 2-^{14}C-FU in the administered dose excreted in the CO_2 and urine were calculated from the radioactivities in the trapping solutions and urine and the total radioactivity injected. The percentages of the two metabolites in the urine were calculated from the radioactivities of the metabolites and the total radioactivity of the urine. The results are shown in Tables 1 and 2.

TABLE 1. *Percentage of administered dose excreted in expired CO_2 and urine in 24 hr by rats receiving 2-^{14}C-FU.*

Replicate	Route of excretion	Hypothyroid	Euthyroid	Hyperthyroid
1	CO_2			
	0-1 hr	41.3 ± 1.1[a]	40.2 ± 1.7	40.4 ± 0.56
	0-3 hr	57.6 ± 1.3	56.2 ± 1.4	56.3 ± 0.72
	0-6 hr	60.3 ± 1.4	59.8 ± 0.42	60.2 ± 1.4
	0-12 hr	63.9 ± 1.5	63.6 ± 1.0	63.0 ± 1.1
	0-24 hr	66.5 ± 1.3	66.1 ± 0.81	64.0 ± 1.1
	Urine			
	0-12 hr	18.0 ± 0.60[b]	18.4 ± 0.98	19.0 ± 0.08
	12-24 hr	3.1 ± 1.1	3.0 ± 1.5	3.2 ± 1.0
2	CO_2			
	0-1 hr	40.2 ± 0.71	39.2 ± 1.9	39.7 ± 1.1
	0-3 hr	57.3 ± 1.6	57.6 ± 1.6	56.5 ± 1.2
	0-6 hr	59.0 ± 1.3	59.4 ± 0.95	60.9 ± 0.51
	0-12 hr	62.2 ± 0.26	62.2 ± 0.53	64.3 ± 0.20
	0-24 hr	63.4 ± 0.90	65.2 ± 0.16	66.1 ± 2.3
	Urine			
	0-12 hr	21.9 ± 0.21	19.1 ± 1.0	18.0 ± 0.10
	12-24 hr	3.9 ± 1.4	3.1 ± 0.87	3.3 ± 0.43
3	CO_2			
	0-1 hr	39.9 ± 0.29	41.3 ± 0.19	38.0 ± 0.51
	0-3 hr	55.2 ± 1.2	55.8 ± 1.9	56.9 ± 1.8
	0-6 hr	60.8 ± 1.0	59.4 ± 0.48	59.6 ± 0.17
	0-12 hr	62.2 ± 0.78	63.6 ± 0.05	63.5 ± 1.1
	0-24 hr	67.1 ± 0.84	66.0 ± 0.56	66.4 ± 1.3
	Urine			
	0-12 hr	19.2 ± 0.35	18.8 ± 0.80	19.1 ± 0.79
	12-24 hr	3.0 ± 1.1	3.2 ± 0.28	3.2 ± 1.7

[a] Cumulative mean ± standard deivation for two rats.
[b] Values for urine are not cumulative.

Each route of excretion and each metabolite were then statistically analyzed separately. The Foster-Burr test for homogeneity of variance (1) was performed first. Homogeneity of variance was obtained for CO_2 and the urinary metabolites without transformation. The 1/y transformation was needed for homogeneity of variance in the whole urine data.

TABLE 2. *Percentages of 2-^{14}C-FU metabolites in rat urine separated by thin layer chromatography.*

Replicate	Time and metabolite	Hypothyroid	Euthyroid	Hyperthyroid
1	0-12 hr			
	FU	78.0 ± 1.2[a]	77.0 ± 0.50	78.2 ± 1.2
	urea	4.4 ± 1.0	4.2 ± 0.73	4.7 ± 0.48
	12-24 hr			
	FU	77.1 ± 0.18	76.0 ± 1.3	76.6 ± 1.1
	urea	4.4 ± 1.5	4.2 ± 0.70	4.6 ± 1.4
2	0-12 hr			
	FU	76.0 ± 1.0	77.1 ± 1.2	76.4 ± 0.58
	urea	4.8 ± 1.6	4.3 ± 1.8	4.6 ± 0.92
	12-24 hr			
	FU	76.1 ± 0.64	77.2 ± 1.4	77.2 ± 1.9
	urea	4.5 ± 0.19	4.7 ± 1.6	4.2 ± 0.46
3	0-12 hr			
	FU	76.1 ± 0.69	77.1 ± 1.1	77.2 ± 0.46
	urea	3.9 ± 1.4	3.9 ± 0.60	4.5 ± 0.37
	12-24 hr			
	FU	77.8 ± 0.16	76.0 ± 0.77	76.3 ± 2.2
	urea	4.1 ± 0.62	4.5 ± 0.76	4.1 ± 0.20

[a] Percentages shown are for the urine sample chromatographed and do not refer to the percentage of the dose administered. Mean ± standard deviation.

The results were further analyzed by analysis of variance (1). A three-way analysis of variance, with replication, time, and treatment as variables, was run separately for the cumulative CO_2, urine, and metabolite data. No significant ($p > 0.05$) replication effect was seen in any of the data groups. Time was highly significant for CO_2 and whole urine excretion as was expected, but had no effect on the fractionated components of the urine. The treatment effect was not significant ($p > 0.05$) for any of the data.

The breakdown and excretion of 2-^{14}C-FU in this investigation compared closely with results obtained in a previous study by Meeks *et al.* (5). They found that control rats exhaled 68% of the administered dose as $^{14}CO_2$ in 24 hr as compared to approximately 66% in the present study. The rats in this study excreted approximately 24% of the administered dose in the urine over the 24-hr period. This percentage compares favorably with the Meeks *et al.* study (5) which found 30% to be eliminated by this route in 24 hr with control rats. In all treatment groups, unchanged 2-^{14}C-FU accounted for the highest percentage of urine activity. Labeled urea accounted for approximately 4% of the total activity in the urine.

The treatment effect was not significant in any of the parameters measured. Thus, there was no effect of the altered thyroid states on the metabolism of 2-^{14}C-FU.

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