Pergamon

Biochemical Pharmacology, Vol. 48, No. 2, pp. 233–236, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00272-X

5-ETHYNYLURACIL (776C85): PROTECTION FROM 5-FLUOROURACIL-INDUCED NEUROTOXICITY IN DOGS

Stephen T. Davis*†, Suzanne S. Joyner*, David P. Baccanari* and Thomas Spector#

*Division of Molecular Genetics and Microbiology and #Division of Experimental Therapy, Wellcome Research Laboratories, Research Triangle Park, NC 27709, U.S.A.

(Accepted 27 May 1994)

Abstract -- 5-Ethynyluracil (776C85) is a potent mechanism-based inactivator of dihydropyrimidine dehydrogenase (DPD), the enzyme that catalyzes the rapid catabolism of 5-fluorouracil (5-FU). Because catabolism is the major route for 5-FU clearance, we studied the effect of 5-ethynyluracil on the pharmacokinetics and toxicity of continuous i.v. 5-FU infusion in the dog. 5-FU at 40 mg/kg/24 hr resulted in a steady-state plasma 5-FU concentration of 1.3 μM and was fatal with dogs dying from apparent neurotoxicity. 5-Ethynyluracil lowered the total clearance of 5-FU from 9.9 to 0.2 L/hr/kg and enabled 1.6 mg/kg/24 hr 5-FU to achieve a steady-state plasma 5-FU concentration of 2.4 μM with no apparent toxicity. 5-FU at 4 mg/kg/24 hr achieved a steady-state plasma 5-FU concentration of 5.3 μM and produced only mild gastrointestinal disturbances in 5-ethynyluracil-treated dogs. Thus, a catabolite of 5-FU appears to be responsible for the 5-FU-induced neurotoxicity in dogs.

(Key words: α-fluoro-β-alanine; dihydropyrimidine dehydrogenase; continuous i.v. infusion)

Although 5-FU§ is widely used against solid tumors in human cancer patients (1), its use in dogs has been limited because these animals are particularly sensitive to 5-FU-induced neurotoxicity (2-5). This dose-limiting neurotoxicity differs from the typical limiting bone marrow suppression and gastrointestinal disturbances that occur both in human patients (6) and in tumor-bearing rodents treated with 5-FU (7). Some studies (2,8,9) implicate α-fluoro-β-alanine, the major catabolite of 5-FU (10), as the neurotoxic agent.

DPD (EC 1.3.1.2) catalyzes the rate-limiting reduction of 5-FU in the pathway to form α-fluoro-β-alanine (11). 5-Ethynyluracil (776C85), a potent, irreversible, mechanism-based inactivator of DPD *in vitro* (12) and *in vivo* (13), prevents the catabolism of 5-FU in mice and rats (14). In the present study, we predosed dogs with 5-ethynyluracil to determine whether the hypersensitivity to 5-FU-induced neurotoxicity could be circumvented by blocking the formation of α-fluoro-β-alanine, and if neurotoxicity was circumvented, whether higher nontoxic and potentially more therapeutic plasma levels of 5-FU could be achieved.

MATERIALS AND METHODS

5-FU (50 mg/mL) was obtained from Adria Laboratories (Columbus, OH) and was diluted with saline prior to dosing. 5-Ethynyluracil was synthesized at Wellcome Research Laboratories. A sterile 0.66 mg/mL solution was prepared in pH-adjusted (pH 10.0) saline. Both 5-FU and 5-ethynyluracil were determined to be >99% pure by reverse-phase HPLC.

Compound administration, whole blood collection, and toxicity evaluation were performed in the Division of Toxicology, Burroughs Wellcome Co. Male beagle dogs (9-10 kg) were maintained on a 6:00 a.m. to 6:00 p.m. light cycle and had free access to water and chow. These studies were performed in accordance with BW Co. procedures for animal care and handling. Animal body weight, animal activity, food consumption, and the condition of both feces and mucous membranes were monitored closely following drug administration.

†Corresponding author: Dr. Stephen T. Davis, Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709. Tel. (919) 315-0225; FAX (919) 315-0656.

§Abbreviations: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; and 776C85, 5-ethynyluracil.

5-FU was infused i.v. at a rate of 1 mL/hr via a catheter in the left external jugular vein. 5-Ethynyluracil (1 mg/kg) was administered s.c. 30 min prior to the start of 5-FU infusion, and every 6 hr thereafter.

Whole blood was collected from the left cephalic vein into EDTA-containing tubes at least every 6 hr during the infusion. Plasma was isolated by centrifugation (3000 *g* for 10 min, 4°) and was deproteinized by Centrifree ultrafiltration. Less than 10% of 5-FU was bound to dog plasma proteins. Plasma was analyzed for uracil and 5-FU by reverse-phase HPLC (14).

RESULTS AND DISCUSSION

Continuous i.v. infusion of 5-FU appears to result in higher efficacy and less toxicity than bolus i.v. 5-FU dosing in the clinic (15). Therefore, we dosed 5-FU by continuous infusion for pharmacokinetic and toxicity studies in the dog. Three dogs dosed with 5-FU at 40 mg/kg/24 hr had steady-state plasma 5-FU concentrations of approximately 1.3 μM. No toxicity was observed during the infusion. However, one dog experienced seizures, muscle tremors, and ataxia. The other two dogs were found dead, presumably from neurotoxicity, 24 hr after 5-FU infusion (Table 1). These observations are consistent with earlier reports demonstrating that dogs are hypersensitive to the neurotoxic effects of 5-FU (2-4).

We next studied the effect of 5-ethynyluracil on the pharmacokinetics and toxicity of 5-FU. 5-FU at 1.6 mg/kg/24 hr with 5-ethynyluracil resulted in a 1.8-fold greater mean plasma 5-FU concentration than produced by 5-FU alone at 40 mg/kg/24 hr (Table 1). 5-Ethynyluracil decreased the total clearance of 5-FU by a factor of 50 (from 9.9 L/hr/kg (5-FU alone) to 0.2 L/hr/kg). More importantly, although 5-ethynyluracil-treated dogs experienced a greater plasma 5-FU exposure, they did not show signs of CNS toxicity during the 2-week observation period. Thus, 5-ethynyluracil protected against 5-FU-induced neurotoxicity.

Table 1. Plasma 5-FU and uracil levels and toxicity following 26-hr 5-FU infusion in the dog

5-FU dose* (mg/kg/24 hr)	776C85	Mean plasma 5-FU† (μM)	Mean plasma uracil† (μM)	Toxicity
40	-	1.3 ± 0.3	0.6 ± 0.1	Severe CNS
1.6	+	2.4 ± 0.4	60 ± 10	None
4	+	5.3 ± 0.4	60 ± 2	Mild G.I.#
16	+	21 ± 2	70 ± 10	Severe G.I.

*Three dogs for each group.

†Plasma 5-FU and uracil values are means ± SD. Data were taken from the 12- to 26-hr portion of individual plasma concentration-time curves.

#Two of the three dogs had transient emesis.

Table 1 also shows that steady-state plasma uracil levels were elevated approximately 100-fold during 5-FU infusion in 5-ethynyluracil-treated dogs. Because maximum elevation is associated with >95% inactivation of DPD in mice and rats (13,16), it was used as a surrogate marker for DPD inactivation in dogs. In a preliminary study, 0.3 mg/kg 5-ethynyluracil also produced maximal elevation (to 60 μM) of plasma uracil in dogs*. Accordingly, we dosed with 1 mg/kg 5-ethynyluracil every 6 hr to ensure complete inactivation of DPD.

To test if 5-ethynyluracil-treated dogs could tolerate even higher 5-FU plasma concentrations without experiencing fatal seizures, we infused 5-FU at 4 mg/kg/24 hr. As shown in Table 1, the mean plasma 5-FU concentration was 5.3 μM (4-fold higher than that observed when 5-FU was administered alone at 40 mg/kg/24 hr). However, only mild, transient gastrointestinal disturbances were observed.

To determine the dose-limiting toxicity of 5-FU in 5-ethynyluracil-treated dogs, we infused 5-FU at 16 mg/kg during a 24-hr period. The mean plasma 5-FU concentration was 21 μM, and animals did

* D. Nelson, Burroughs Wellcome Co., personal communication. Cited with permission.

not show any signs of CNS toxicity (see Table 1 and Fig. 1). However, these animals had severe gastrointestinal toxicity and were euthanized 3 days after dosing.

We previously observed a linear relationship between plasma 5-FU concentration and oral 5-FU dose in 5-ethynyluracil-treated rats (14). Figure 1 shows a similar linear response (correlation coefficient = 1.0) with continuous i.v. 5-FU in 5-ethynyluracil-treated dogs.

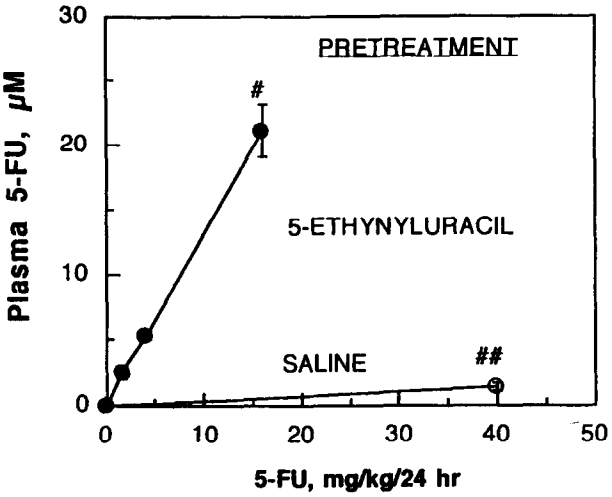

Fig. 1. Relationship between mean plasma 5-FU concentration and 5-FU dose during continuous i.v. infusion of 5-FU. The closed circles represent 5-ethynyluracil-treated dogs and the open circle represents saline-treated dogs. Key: (#) dose causing severe G.I. toxicity; (##) dose causing severe CNS toxicity. Values are means ± SEM, N=3. (Standard error bars are smaller than the data symbols in all but one case.)

In conclusion, 5-ethynyluracil protected dogs from dose-limiting neurotoxicity of 5-FU. Since 5-ethynyluracil prevents 5-FU catabolism (14), these findings support the earlier reports (2,8,9) suggesting that 5-FU catabolites are responsible for the neurotoxicity in dogs and cats. However, we cannot rule out the less likely possibility that the 100-fold increase in plasma uracil induced by 5-ethynyluracil also could play a role in antagonizing the neurotoxic effects of 5-FU. Finally, because 5-ethynyluracil enables considerably higher and more predictable plasma levels of 5-FU to be maintained without toxicity, it may be a useful modulator of 5-FU chemotherapy in these animals.

Acknowledgements -- The authors wish to thank David Melich for technical assistance and Dr. Herb Amyx and Nelson Johnson for their assessment of animal toxicity.

REFERENCES

1. Kovach JS and Beart RW, Cellular pharmacology of fluorinated pyrimidines *in vivo* in man. *Invest New Drugs* **7:** 13-25, 1989.

2. Okeda R, Karakama T, Kimura S, Toizumi S, Mitsushima T and Yokoyama Y, Neuropathologic study on chronic neurotoxicity of 5-fluorouracil and its masked compounds in dogs. *Acta Neuropathol* (Berl) **63:** 334-343, 1984.

3. Harvey HJ, MacEwen EG and Hayes AA, Neurotoxicosis associated with use of 5-fluorouracil in five dogs and one cat. *J Am Vet Med Assoc* **171:** 277-278, 1977.

4. Theilen G, Adverse effect from use of 5% fluorouracil. *J Am Vet Med Assoc* **191:** 276, 1987.

5. Okeda R, Shibutani M, Matsuo T and Kuroiwa T, Subacute neurotoxicity of 5-fluorouracil and its derivative, carmofur, in cats. *Acta Pathol Jpn* **38:** 1255-1266, 1988.

6. Petrelli N, Herrera L, Rustum Y, Burke P, Creaven P, Stulc J, Emrich LJ and Mittleman A, A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* **5:** 1559-1565, 1987.

7. Martin DS, Stolfi RL, Sawyer RC, Spiegelman S and Young CW, High-dose 5-fluorouracil with delayed uridine "rescue" in mice. *Cancer Res* **42:** 3964-3970, 1982.

8. Okeda R, Shibutani M, Matsuo T, Kuroiwa T, Shimokawa R and Tajima T, Experimental neurotoxicity of 5-fluorouracil and its derivatives is due to poisoning by the monofluorinated organic metabolites monofluoroacetic acid and α-fluoro-ß-alanine. *Acta Neuropathol* **81:** 66-73, 1990.

9. Koenig H and Patel A, Biochemical basis for fluorouracil neurotoxicity. *Arch Neurol* **23:** 155-160, 1970.

10. Heggie GD, Sommadossi J-P, Cross DS, Huster WJ and Diasio RB, Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res* **47:** 2203-2206, 1987.

11. Naguib FMN, el Kouni MH and Cha S, Enzymes of uracil catabolism in normal and neoplastic human tissues.*Cancer Res* **45:** 5405-5412, 1985.

12. Porter DJT, Chestnut WG, Merrill BM and Spector T, Mechanism-based inactivation of dihydropyrimidine dehydrogenase by 5-ethynyluracil. *J Biol Chem* **267:** 5236-5242, 1992.

13. Spector T, Harrington JA and Porter DJT, 5-Ethynyluracil (776C85): Inactivation of dihydropyrimidine dehydrogenase *in vivo*. *Biochem Pharmacol* **46:** 2243-2248, 1993.

14. Baccanari DP, Davis ST, Knick V and Spector T, 5-Ethynyluracil (776C85): A potent modulator of the pharmacokinetics and antitumor efficacy of 5-fluorouracil. *Proc Natl Acad Sci USA* **90:** 11064-11068, 1993.

15. Hansen RM, 5-Fluorouracil by protracted venous infusion: A review of recent clinical studies. *Cancer Invest* **9:** 637-642, 1991.

16. Spector T, Porter DJT, Nelson DJ, Baccanari DP, Davis ST, Almond MR, Khor SP, Amyx H, Cao S and Rustum YM, 5-Ethynyluracil (776C85), a modulator of the therapeutic activity of 5-fluorouracil. *Drugs Future*, in press.