Jpn J Clin Oncol 2006;36(1)55–59
doi:10.1093/jjco/hyi214

Case Reports

Intermediate Dose 5-Fluorouracil-Induced Encephalopathy

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Received August 1, 2005; accepted November 11, 2005; published online January 25, 2006

As an acute neurotoxicity, high dose 5-fluorouracil (5-FU)-induced encephalopathy is well-known, but encephalopathy associated with lower dose is rarely reported. Here, we report a case of a male with anal cancer who was treated with 5-FU 1000 mg/m^2, continuous infusion for 5 days q4 weeks. At the second and the fourth cycles of chemotherapy, sudden confusion, cognitive dysfunction and disorientation occurred during 5-FU infusion. They were accompanied by hyperammonemia in the absence of focal neurological deficits or structural abnormalities. These symptoms completely disappeared and the serum ammonia level returned to normal after discontinuation of 5-FU and conservative care. In order to investigate a possible deficit of dihydropyrimidine dehydrogenase (DPD), we checked its mRNA level before and after treatment using real-time PCR. The patient's pre-treatment level was 80% compared with reference group, and it was elevated up to 187% of initial after 5-FU treatment, implying that that his encephalopathy may be 5-FU catabolite type rather than DPD deficiency. In conclusion, we report that encephalopathy can develop even with the dose of 5-FU lower than ever reported, and it should be considered as a differential diagnosis for proper management.

Key words: 5-fluorouracil – encephalopathy – anal cancer – hyperammonemia

INTRODUCTION

Neurotoxicity is an uncommon side effect of 5-fluorouracil (5-FU) treatment (1,2). Two types of well-recognized neurotoxicity have been described, namely acute and chronic. Acute neurotoxicity, which manifests as a diffuse encephalopathy or a cerebellar syndrome, is known as dose-related and generally self-limiting. Delayed neurotoxicity, which appears with a few months interval, has been reported when 5-FU was given with levamisole. This form of subacute multifocal leukoencephalopathy is immune-mediated and responds to corticosteroids (3,4). Most cases of 5-FU induced encephalopathy are associated with infusion of high-dose 5-FU (>2200 mg/m^2 every week). However, encephalopathy associated with lower dose of 5-FU has been rarely reported. Here we report a case of transient encephalopathy related to intermediate-dose 5-FU infusion (1000 mg/m^2, continuous infusion for 5 days q4 weeks) in a patient with anal cancer. Additionally, in order to investigate a possible defect of dihydropyrimidine dehydrogenase (DPD), we checked its mRNA level in the patient before and after treatment using real-time PCR.

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CASE REPORT

A 68-year-old Korean male, who had been diagnosed of anal cancer with bladder and prostate invasion, was admitted to the hospital for a course of chemotherapy. He had no history of chronic alcohol or benzodiazepine addiction or any other significant medical history. His initial liver function test and abdomen-pelvis CT scan showed no abnormal findings other than locally infiltrated anal mass. Combination chemotherapy was planned with infusion of 5-FU (1000 mg/m^2, continuous infusion, D1–5) and cisplatin (70 mg/m^2, 2 h-infusion, D1), which was to be repeated every 4 weeks (Table 1). Other medications included dexamethasone 10 mg i.v. before chemotherapy, and two doses of ondansetrone.

He finished the first cycle of chemotherapy without signs of remarkable toxicity. However, in the second cycle, sudden mental change emerged on the fifth day of 5-FU infusion (109 h from infusion, total dose infused was 4500 mg/m^2). The blood pressure checked was 120/80 mmHg, the pulse rate 80/min,

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and the respiratory rate 14/min. Neurological examination revealed cognitive abnormalities in the form of confusion and disorientation to place and time. The patient had impaired memory, calculation, judgment and naming difficulty. Deep tendon jerks were all 2+ and symmetric. CT and MRI scan of the brain showed no evidence of metastasis or stroke. Laboratory examination showed elevated ammonia level to 354 μg/dl (0–64) and slightly elevated total bilirubin level to 1.6 mg/dl (0.2–1.2). Other laboratory findings, such as complete blood count, serum electrolytes including sodium, potassium, calcium and aminotransferases level, were all in the normal range (Table 2). Serum levels of glucose and creatinine were within normal range. Blood and urine culture were negative. 5-FU infusion was stopped immediately and i.v. hydration along with lactulose enema were performed. Patient's mental status was recovered completely 24 h after the discontinuation of 5-FU and the serum ammonia level fell down to 58 μg/dl.

Assuming that his neurotoxicity was drug-related, we decided to reduce the dose of cisplatin to 60 mg/m^2 without modifying 5-FU from the next cycle. On the admission for third cycle of chemotherapy, his pre-treatment serum ammonia level was 63 μg/dl. Although the level was slightly increased to 120 μg/dl in the course of 5-FU infusion, neurotoxicity was not documented with him. However, at the fourth cycle, another attack of neurotixicity developed on the second day of 5-FU infusion (37 h from infusion, total dose infused was 1500 mg/m^2). Neurological symptoms and signs were the same as those at the second cycle. Serum ammonia level was elevated up to 555 μg/dl without affecting other laboratory and radiological findings. After hydration and enema, the ammonia level was normalized down to 60 μg/dl after 24 h of 5-FU discontinuation, followed by complete recovery of the patient's mental status.

In this patient, we checked mRNA levels of three representative 5-FU metabolism-related genes including dihydropyrimidine dehydrogenase (DPD), thymidine synthase (TS) and thymidine phosphorylase (TP), using real-time PCR. Heparinized blood was obtained from the patient before treatment and at the second cycle of chemotherapy just after the neurological attack had occurred. As a control, 16 patients who received infusional 5-FU of the same dose and schedule as the case patient were collected and blood sampling was done before and after treatment. Total RNA of the peripheral blood mononuclear cells was isolated using Trizol Reagent (GibcoBRL, Grand Island, NY, USA). After confirming an rRNA band on an agarose gel, 1.6 μg of RNA was reversed-transcribed using a First Strand cDNA Synthesis kit (MBI Fermentas, Lithuania).

Two microliters of cDNA was used for the real-time PCR assay. The total volume of the reaction mixture was 20 μl, containing HotstarTaq DNA polymerase, QuantiTect SYBR Green PCR buffer, dNTP mix include dUTP, SYBR Green, 10 μl of QuantiTect SYBR Green PCR Kit including 2.5 mM $MgCl_2$ (QIAGEN, CA, USA), 2 μl of the cDNA and 20 pmol of each primer (Proligo Singapore Pty Ltd, Singapore). PCR was performed 1 cycle at 95 μl for 15 min and then for 35 cycles of amplification at; 95 μl for 20 s, 50 μl for 30 s, 72 μl for 45 s on a Rotor Gene 2072D real-time PCR machine (Corbett Research, Australia). The amplified fluorescence signal in each specimen was measured at the late extension step of each cycle. In order to quantify each gene, we used 10-fold serial dilution of human genomic DNA (Promega, Madison, WI, USA). The standard curve was drawn by plotting the measured threshold cycle versus the arbitrary unit of the copies/reaction according to the β-actin expression. The threshold cycle (Ct) value was determined as the cycle number at which the fluorescence exceeded the threshold value. The following human specific primers were used: for DPD, 5′-GTG

Table 1. Summary of chemotherapy schedule

	1st cycle	2nd cycle	3rd cycle	4th cycle
BSA (m^2)	1.48	1.55	1.64	1.64
5-FU (mg, 1000 mg/m^2)	1480	1550	1640	1640
Cisplatin (mg, 70 mg/m^2)	103	108	98*	98*

BSA, body surface area (m^2).
*Dose of the cisplatin was reduced from 70 to 60 mg/m^2 owing to encephalopathy after the second cycle.

Table 2. Major laboratory findings of the patient during encephalopathy

	Reference	Cycle 2			Cycle 3			Cycle 4		
		PreTx	D5*	D6	PreTx	D5	D6	PreTx	D2*	D6
NH_3 (μg/dl)	0–64	58	354	75	63	120	64	60	555	60
AST/ALT (IU/l)	13–34/5–36	19/22	24/23	19/20	17/22	23/26	26/25	18/11	17/21	35/18
T. bil (mg/dl)	0.2–1.2	0.6	1.6	0.5	0.5	0.7	0.5	0.8	1.8	0.7
BUN (mg/dl)	5–25	20.6	23.2	20.0	17.2	15.2	15.9	18.0	17.4	21.0
Cr (mg/dl)	0.5–1.4	1.2	1.2	1.1	0.9	1.2	1.3	1.1	1.3	1.2
Na (mmol/l)	135–145	141	139	138	142	140	139	141	139	143
K (mmol/l)	3.5–5.5	4.3	4.9	4.2	4.2	3.8	4.1	4.3	4.2	4.5

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Cr, creatinine.
*The sample was taken when neurotoxicity developed.

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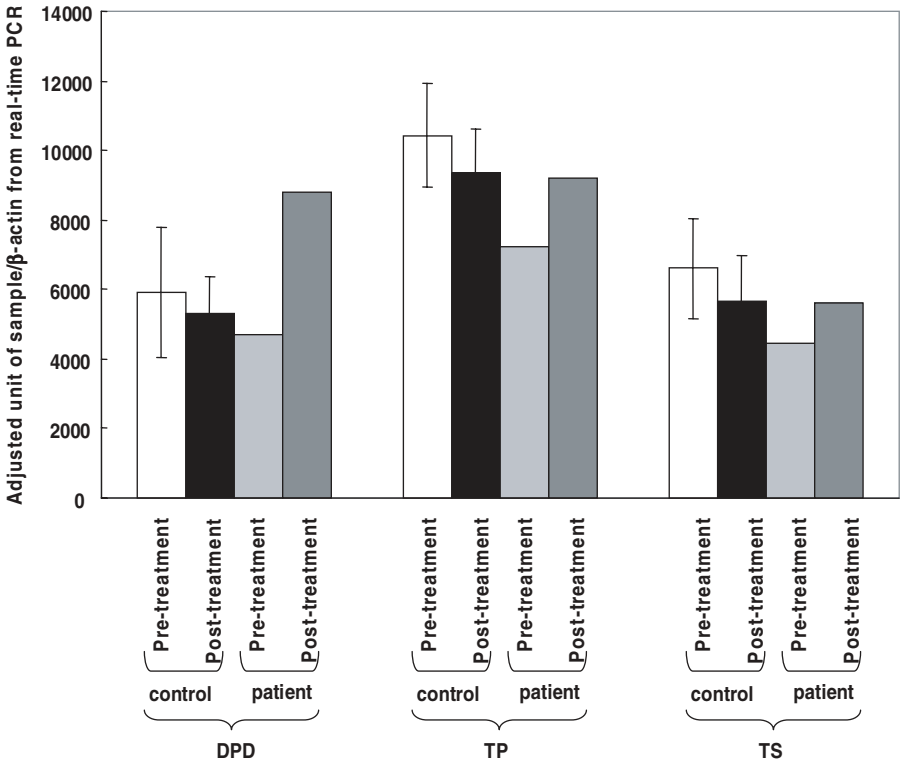

Figure 1. Comparison of the changes of patient's mRNA levels of 5-FU metabolism-related genes extracted from peripheral blood mononuclear cells before and after the 5-FU treatment using real-time PCR. Control means 16 patients who received infusional 5-FU of the same dose and schedule as the case patient. (DPD, dihydropyrimidine dehydrogenase; TP, thymidine phosphorylase; TS, thymidine synthetase).

AGA AGG ACC TCA TAA AAT ATT GTC-3′ (sense) and 5′-GAA TTG GAT GTT TAA ATA AAC ATT CAC CAA C-3′ (antisense); for TP, 5′-GGA TTC AAT GTC ATC CAG AG-3′ (sense) and 5′-CCT CCA CGA GTT TCT TAC TG-5′ (antisense), and for TS, 5′-TTT TTA AGG ATG TTG CCA CT-3′ (sense) and 5′-GTG TTA CTC AGC TCC CTC AG-3′ (antisense).

The total amount of RNA of DPD, TP and TS of the patient normalized with β-actin were compared with those of control. When pretreatment level of each value of control patients was considered as 100%, the patient's pre-treatment level of DPD was within the control level, while those of TP and TS were somewhat lower (69 and 67% of the control, respectively). After 5-FU treatment, the patient's DPD level was markedly elevated up to 187% of the pre-treatment level. The patient's post-treatment levels of TP and TS were also elevated up to 127% of initial level (Fig. 1).

DISCUSSION

The incidence of 5-FU neurotoxicity in patients undergoing high-dose 5-FU chemotherapy is estimated to be 5.7% (5,6). The risk factors are not well described, but it has been reported that the combination of 5-FU and interferon α–2a increased the incidence of neurotoxicity (7). The diagnostic criteria of the 5-FU induced acute neurotoxicity includes: (i) development of encephalopathy during or shortly after completion of 5-FU administration; (ii) exclusion of other metabolic factors that may affect consciousness and mental functioning, such as hypoglycemia, organ failure, electrolyte imbalance, sepsis and central nervous system metastasis and (iii) exclusion of an adverse effect by concomitant medications (1,8).

Although the biochemical basis for neurological toxicity of the 5-FU is not fully understood, there have been at least two distinct pathogenetic entities suggested for 5-FU-induced encephalopathy (9). First is the DPD deficiency type. DPD is the major enzyme to inactivate 5-FU, and its deficiency results in the failure of 5-FU catabolism that leads to drug accumulation. Approximately 85% of 5-FU is degraded by DPD and this process produces ammonia as the end product. In a population study, the prevalence of DPD deficiency, defined as <95% distribution range of normal of DPD activity, was reported to be 2.7% in cancer patients. Patients who have DPD deficiency have shown more severe and more generalized forms of 5-FU-related toxicity besides neurotoxcitiy (10–12).

The second is 5-FU catabolite type. In this type, although the major catabolic pathway of 5-FU remains intact, the relatively high rate of 5-FU infusion results in a transient accumulation of 5-FU catabolites in a short time period. Koenig et al. proposed a hypothesis that following large dose administration, a large amount of fluoroacetate, the intermediate product of 5-FU, directly inhibits the Kreb's cycle and consequently causes impairment in the ATP-dependent urea cycle (13). This results in a transient hyperammonemia,

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which could induce encephalopathy. Okeda et al. also suggested that 5-FU metabolites, monofluoroacetic acid and α-fluoro-β-alanine, could have a direct toxicity on myelin itself (14). Although its mechanism remains to be further elucidated, this form of neuropathy is milder than DPD deficiency type. It is generally reversible and is also known not to preclude a re-treatment with 5-FU at reduced dosages.

Most cases of 5-FU-related encephalopathy have been reported to be associated with weekly high-dose infusion of fluorouracil, which was enforced by high-dose folinic acid, using 2200–2600 mg/m^2/week of 5-FU, measuring 5-FU dose intensity >1600 mg/m^2/week. The patient received 5-FU with its actual dose intensity of 1170 mg/m^2/week, which is lower than weekly high-dose schedule. In our case, the patient's mental change emerged during 5-FU infusion without remarkable predisposing factors: malnutrition, infection or hypotriglyceridemia. Although we did not perform invasive study such as spinal tap, imaging study of the brain properly ruled out infection, stroke, and leptomeningeal seeding. There were no concomitant medications that could have affected his mental status. Cisplatin and dexamethasone can also cause encephalopathy, but ciplatin-related neurotoxicity has a feature of focal neurological deficit such as seizure, ocular symptom and peripheral feature. In addition, there has been no report that any of these agents caused mental change accompanying hyperammonemia. Moreover, significant toxicities that were related to chemotherapy other than neurotoxicity were not noted with the patient in the whole course of treatment. Thus, we suggest that the patient's mental change is attributed to 5-FU. We cannot, however, clearly explain why neurotoxicity was not noticed in the first and the third cycle, but some elevation of serum ammonia level was also documented at the third cycle, implying that baseline insufficiency in 5-FU catobolism persistently existed in the patient, although it may not be cumulative.

We did not determine the patient's enzymatic activity of DPD. Instead, we examined its mRNA levels with fluorouracil metabolism-related genes, TS and TP, from the peripheral blood mononuclear cells. Several reports demonstrated significant association between DPD enzyme activity and mRNA levels (15,16). The level was compared with a control from 16 patients who were treated with the same regimen. The pre-treatment level of DPD of the patient was in the control range, and its level was elevated up to 187% after 5-FU treatment, accompanying elevation of TS. The elevation of the latter after 5-FU administration was basically consistent with the previous reports. Because DPD is the primary enzyme responsible for metabolizing 5-FU, the induction of DPD as a catabolic enzyme for 5-FU would be expected although direct evidence remained to be established (17). It is worth pointing out that the patient's pre-treatment DPD level was not strikingly low compared with other patients and that inducibility of DPD with treatment was maintained. This suggests that there is little chance of the patient having DPD deficiency, and the transient stagnation of 5-FU catabolites would have played a rather important role in the development of neurotoxicity. The measurement of the level of 5-FU metabolites and DPD genotyping could offer further information on its precise role in the pathophysiology of encephalopathy.

Prognosis is good if diagnosis is established. Treatment includes: immediate discontinuation of 5-FU along with supportive care such as fluid supplement, lactulose enema and correction of predisposing factors (18). It is, therefore, important to recognize this condition at an early stage. In conclusion, we report here that encephalopathy can develop even with the lower dose of 5-FU. Despite its rarity, if a patient showed abrupt mental change during or right after continuous infusion of 5-FU, drug-induced encephalopathy should be considered in differential diagnosis in order for precise diagnosis to be made and proper management to be introduced.

Acknowledgments

This study was supported by the Korea Science and Engineering Fund through the Cancer Metastasis Research Center at the Yonsei University.

Conflicts of Interest: None identified.

References

1. Pirzada NA, Ali II, Dafer RM. Fluorouracil-induced neurotoxicity. *Ann Pharmacother* 2000;34:35–8.
2. Langer CJ, Hageboutros A, Kloth DD, Roby D, Shaer AH. Acute encephalopathy attributed to 5-FU. *Pharmacotherapy* 1996;16:311–3.
3. Cossaart N, SantaCruz KS, Preston D, Johnson P, Skikne BS. Fatal Chemotherapy-induced encephalopathy following high-dose therapy for metastatic breast cancer. *Bone Marrow Transplant* 2003;31:57–60.
4. Price RA, Jamieson PA. The central nervous system in childhood leukemia. II. Subacute leukoencephalopathy. *Cancer* 1975;35:306–18.
5. Moertel CG, Reitemeier RJ, Bolton CF, Shorter RG. Cerebellar ataxia associated with fluorinated pyrimidine therapy. *Cancer Chemother Rep* 1964;41:15–8.
6. Riehl J, Brown WJ. Acute cerebellar syndrome secondary to 5-fluorouracil therapy. *Neurology* 1964;14:961–7.
7. Kemeny N, Younes A, Seiter K, Kelsen D, Sammarco P, Adams L. Interferon alpha 2a and 5-fluorouracil for advanced colorectal carcinoma. *Cancer* 1990;66:2470–5.
8. Yeh KH, Cheng AL. High-dose 5-fluorouracil infusional therapy is associated with hyperammonaemia, lactic acidosis and encephalopathy. *Br J Cancer* 1997;75:464–5.
9. Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renee N, et al. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol* 1994;12:2248–53.
10. Takimoto CH, Lu ZH, Zhang R, Liang MD, Larson LV, Cantilena LR Jr, et al. Severe neurotoxicity following 5-fluorouracil-based chemotherapy in a patient with dihydropyrimidine dehydrogenase deficiency. *Clin Cancer Res* 1996;2:477–81.
11. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. *Cancer Res* 1993;53:5433–8.
12. Lazar A, Mau-Holzmann UA, Kolb H, Reichenmiller HE, Riess O, Schomig E. Multiple organ failure due to 5-fluorouracil chemotherapy in a patient with a rare dihydropyrimidine dehydrogenase gene variant. *Onkologie* 2004;27:559–62.
13. Koenig H, Patel A. Biochemocal basis for fluorouracil neurotoxicity. *Arch Neurol* 1970;23:166–60.
14. Okeda R, Karakama T, Kimura S, Toizumi S, Mitsushima T, Yokoyama Y. Neuropathologic study on chronic neurotoxicity of 5-fluorouracil and its masked compounds in dogs. *Acta Neuropathol* 1984;63:334–43.
15. Johnson MR, Wang K, Smith JB, Heslin MJ, Diasio RB. Quantitation of dihydropyrimidine dehydrogenase expression by real-time

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reverse transcription polymerase chain reaction. *Anal Bochem* 2000; 278:175–84.
16. Ishikawa Y, Kubota T, Otani Y, Watanabe M, Teramoto T, Kumai K, et al. Dihydropyrimidine dehydrogenase and messenger RNA levels in gastric cancer: possible predictor for sensitivity to 5-fluorouracil. *Jpn J Cancer Res* 2000;91:105–12.
17. Sakurai Y, Uraguchi T, Imazu H, Hasegawa S, Matsubara T, Ochiai M, et al. Changes in thymidylate synthase and its inhibition rate and changes in Dihydropyrimidine dehydrogenase after the administration of 5-fluorouracil with cisplatin to nude mice with gastric cancer xenografts SC-1-NU. *Gastric Cancer* 2004;7:110–6.
18. Liaw CC, Wang HM, Wang CH, Yang TS, Chen JS, Chang HK, et al. Risk of transient hyperammonemic encephalopathy in cancer patients who received continuous infusion of 5-FU with the complication of dehydration and infection. *Anticancer Drugs* 1999; 10:275–81.

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