J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

A case of 5-fluorouracil toxicity

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KEY WORDS. 5-Fluorouracil, Dihydropyrimidine dehydrogenase, DPYD.

Abstract

Dihydrouracil (UH2)/Uracil (U) ratio in plasma was determinated as a surrogate marker for dihydropyrimidine dehydrogenase (DPD) activity. The purpose of the study was to investigate the relationship between the UH2/U plasma ratio and the variation of DPD gene (DPYD), associated with a deficiency of DPD activity, in a patient who developed a severe adverse reaction to 5-fluorouracil. Patients'plasma sample and those of 20 healthy volunteers, used as control, were analyzed. The plasma concentrations of UH2 and U were assayed by HPLC-UV. UH2/U plasma ratio of the patient was 4.31; the mean ± SD of UH2/U plasma ratios in controls was 5.26 ± 2.08. The UH2/U ratio of the patient was lower than the mean values recorded from a reference population suggesting a reduction of DPD activity according to haplotype of the patient, previously identified.

Introduction

5-Fluorouracil (5-FU) is a chemotherapeutic agent often administered in the treatment of cancers. Dihydropyrimidine dehydrogenase (DPD), encoded by the DPD gene (DPYD), is the initial and rate-limiting enzyme of 5-FU catabolism to dihydrofluorouracil (5-FDHU) [1]. Numerous genetic mutations have been identified in the DPYD with a few key variants having functional consequences on enzymatic activity. Deficiencies in DPD activity, reducing 5-FU catabolism, may be result in increased drug exposure and possible toxicity [2]. Since 5-FU and Uracil (U) are metabolized by the same pathways, with DPD as the key rate limiting enzyme, the measurement of plasma concentration of U and its metabolite, 5,6-dihydrouracil (UH2), expressed as UH2/U ratio, would be theoretically a sensitive marker for indirect evaluation of DPD enzyme activity and therefore for prevention of high risk toxicity [3].
In this study was reported a case of a 50-year-old woman with metastatic breast cancer treated with docetaxel and capecitabine, an oral pro-drug of 5-FU, who developed a severe adverse reaction to capecitabine (febrile pancytopenia with grade 4 leukopenia and grade 2 mucositis). U and UH2 plasma concentrations were determinated by HPLC in order to investigate the relationship between UH2/U ratio and the genetic variation associated with deficiency of DPD activity, previously identified in the patient.

Materials and Methods

Plasma sample was obtained from patient after withdrawal of treatment with capecitabine. Plasma samples provided by 20 healthy volunteers (11 male, 9 female) were used as controls. Written informed consent was obtained from all subjects included in this study.
Plasma extraction and HPLC method were an adaptation of that described by Gamelin [4]. DPYD mutation analysis was performed by Genetic Laboratory "E.O. Ospedali Galliera" Hospital, Genoa-Italy.

Results

The calibration curves of peak areas versus concentrations of U and UH2 were linear giving a correlation coefficient (r^2) of 0.9997 and 0.9977 respectively. Fig. 1 shows the chromatographic analysis of U, UH2 and 5-Bromouracil (Internal Standard) from patient plasma. The retention times were about 7.7 min for UH2, 8.3 min for U and 22.7 min for IS. No significant endogenous peaks that could interfere with the analysis were observed.
Tab. 1 shows patient data and reference values used for UH2/U ratio: UH2/U ratio in patient was 4.31; the mean ± SD of UH2/U ratio in controls was 5.26 ± 2.08 (CI 95% 4.35-6.17). The UH2/U ratio observed in the patient was below the lower bound of the 95% confidence interval of the mean estimated for the 20 controls. The values showed a normal distribution at the Kolmogorov-Smirnov test with the Lilliefors correction of the significance.

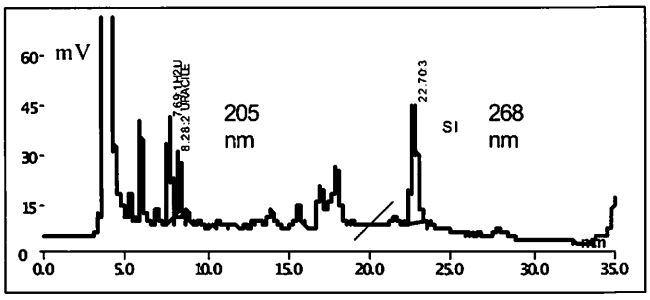

Figure 1. Chromatographic run of plasma patient

	U Conc. (ng/ml)	UH2 Conc. (ng/ml)	UH2/U ratio
Controls	20	20	20
Mean ± SD	83.87 ± 62.47	404.12 ± 253.31	5.26 ± 2.08
Median	67.74	336.74	4.58
Min. value	30.40	100.83	2.48
Max. value	285.35	1086.56	11.21
Patient value	**110.23**	**475.24**	**4.31**

Table 1. Patient and controls data

Discussion

In the present study a patient, suffering from severe toxicity after the administration of capecitabine, carriers of two haplotype, related to a deficiency of DPD activity [5], was tested for the determination of UH2/U ratio. The UH2/U ratio of the patient was lower than the mean values recorded from a reference population. This data showed a weakly correlation between UH2/U ratio and the haplotype of the patient and it could be explain the onset of toxic effects. However there is not a current consensus definition of risk-threshold levels for DPD activity [6]. According to literature, a large interpatient variation in UH2/U ratio was observed in our controls group; the reason for this high degree of interpatient variation was not been throughly explored and remains controversial. In conclusion an integrated approach based on quantification of UH2/U plasma ratio and DPYD genotyping may be a safer strategy to identify patient at risk of toxicity to 5-FU which remain major drugs used extensively in clinical oncology.

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