P-15/17 INVESTIGATION ON MTHFR, DYPD AND TSER POLYMORPHISMS IN 5-FLUOROURACIL TOXICITY: A CASE REPORT

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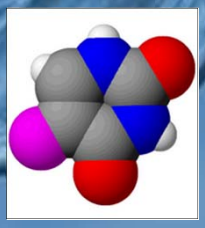
INTRODUCTION



5-Fluorouracil (5-FU) is a chemotherapic drug belonging to the fluoropyrimidine family, broadly used either alone or in combination with other agents. 5-FU indications include palliative and adjuvant treatment of many cancers, including colorectal, breast, head and neck cancers. 5-FU requires enzymatic conversion to the nucleotide floxouridine monophosphate (FdUMP) in order to exert its cytotoxic activity. The interaction between the FdUMP and the thymidylate synthase (TS) blocks the synthesis of thymidine triphosphate. The folate cofactor 5-10 methylenetetrahydrofolate (MTHF) and FdUMP form a covalently bound complex with TS. It should be underline that the MTHF intracellular levels are regulated by the enzyme MTHFR (MethyleneTetraHydroFolate Reductase). Enzymes involved in the 5-FU mechanism of action as well as those involved in its metabolism have shown polymorphisms at the genetic level, that influence the structure and function of the encoded protein. Based on this, it has been suggested that the presence of polymorphisms could be one of the reason for the significant interindividual variability in the safety profile reported in patients undergoing 5-FU therapy. Thus, the knowledge of the 5-FU-related pharmacogenomic profile may help to predict the response outcome and the chemotherapy toxicity in patients treated with this drug. In this work, we described a case report of two patients with relevant systemic toxicity following 5-FU therapy. The 5-FU related pharmacogenomic profile revealed polymorphisms in the target genes that may explain the clinical findings.

PATIENTS AND METHODS

Informed written consent was obtained from the two patients who experienced acute toxicity following 5-FU administration. An aliquot of routinarly collected peripheral blood was used for DNA extraction. Genomic DNA was extracted using the Qiagen Blood & Cell Culture DNA kit (Qiagen, Milano, Italy). The 5-FU pharmacogenomic profile was performed with the "fluoropyrimidines response" kit (Diatech, Jesi, AN, Italy) that evaluate the following genetic markers:

- **MTHFR** (Methylenetetrahydrofolate reductase) **C677T**,
- **MTHFR** (Methylenetetrahydrofolate reductase) **A1298C**,
- **DPYD** (DihydroPYrimidine Dehydrogenase)
- **TSER** (ThymidylateSynthasePromoter) **28bpVNTR**

RESULTS AND DISCUSSION

The systemic toxicity of the two patients was G4 mucositis and pancytopenia in one patient (A); while the other (B) developed incoercible vomiting, epatotoxicity and paresthesias.
The *genetic analysis* revealed that:

Patient A presented:

- ✓ heterozygosity (C/T) at MTHFR C677T gene marker and heterozygosity (A/C) at MTHFR A1298C, both associated with a reduced enzyme activity resulting in increased homocysteine levels and altered distribution of intracellular folate;
- ✓ mutation (2R/2R) at TSER 28bp VNTR, associated with an significant increase in the incidence of adverse events in a fluoropyrimidines - based therapy;
- ✓ the DPYD profile was wild-type (G/G).

Patient B presented:

- ✓ heterozygosity (A/C) at MTHFR A1298C;
- ✓ heterozygosity (2R/3R) at TSER 28bp VNTR gene marker. This is associated with an increased enzyme expression and activity;
- ✓ analysis of the other genetic markers (MTHFR C677T and DPYD) revealed a wild-type genotype.

Both MTHFR polymorphisms are associated with a reduced enzyme activity; both patients are heterozygous for the MTHFR A1298C allele and this gene profile may be responsible, at least in part, of the clinical findings. Furthermore, patient B, that expressed only A1298C polymorphism, developed severe diarrhoea, in line with previous published clinical findings in patients affected by metastatic colorectal cancer.
The tandem-repeat sequences identified in the TS promoter is involved in the 5-FU clinical response. It has been demonstrated that patients possessing the 2R variant allele show a significantly higher risk of severe toxicity to chemotherapy and the risk of toxicity significantly increased with the number of 2R allele. The rationale of this observation is that 2R/2R genotype, giving rise to a low copy number of TS, did not protect normal cells against the 5-FU-induced toxicity. Our patients were, respectively, patient A homozygous 2R/2R and patient B heterozygous 2R/3R, thus both exposed to a higher risk of 5-FU induced toxicity.

Polymorphism	Patient A	Patient B
MTHFR C677T	Heterozygotes C/T	Wild-type C/C
MTHFR A1298C	Heterozygotes A/C	Heterozygotes A/C
DPYD IVS14+1 G>A	Wild-type G/G	Wild-type G/G
TSER 28bp VNTR	homozygotes 2R/2R	Heterozygotes 2R/3R

CONCLUSIONS

The prediction of response or toxicity and therapy individualization are becoming very important tools in cancer chemotherapy. There have been, indeed, numerous studies on the relationship between genotypes and the response to chemotherapeutic agents. By identification of polymorphisms associated with drug metabolism and clearance and with drug targets, personalized therapy could be designed, i.e. dose modification, use of equivalent therapies for those at risk, or avoidance of a particular therapy if the individual risk outweighs the benefits. Potentially useful pharmacogenomic markers of the response to chemotherapeutic agents are now available. Here we reported two patients with severe systemic toxicity following 5-FU therapy, that may be linked to polymorphisms found in the MTHFR and TSER genes, whit a wild-type expression of the metabolizing enzyme DPYD. Taken together, the 5-FU gene profile of our patients strongly suggested that polymorphisms present in the target genes examined contribute to the adverse effects shown during the 5-FU therapy. To which extent these polymorphisms induced the adverse effects it cannot be established at the present, however, our results strengthen the relevance of the 5-FU-related pharmacogenomic profile to predict the response outcome and the chemotherapy toxicity in patients treated with this drug.

References

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Acknowledgement

This work was supported by Fondazione Guido Berlucchi per la Ricerca sul cancro Onlus (Borgonato, Bs) and by Diatech Pharmacogenetics srl (Jesi, An).