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PHARMACOGENETICS OF PURINE METABOLIZING ENZYMES IN CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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Background. Purine analogues, including 2-deoxycoformycin, fludarabine and 2-chloro-deoxyadenosine, are effective drugs in the treatment of chronic lymphoproliferative disorders (Johnson SA, 2000). Cellular uptake of chemotherapeutic agents is allowed by extra cellular dephosphorylation with ecto-5'-nucleotidase (ecto-5'-NT) followed upon their activation to cytotoxic metabolites by deoxy cytidine kinase (dCK), while drug inactivation occurs by dephosphorylation through endo-5'-nucleotidase (endo-5'-NT) (Resta R et al., 1998). Phosphorylation of 2'-deoxyformycin appears to be required for biological activity and is probably catalysed by adenine kinase (AK) rather than dCK. Furthermore, adenosine deaminase (ADA) inhibition by 2-deoxycoformycin is associated with cellular overload of deoxynucleotides, mainly deoxyadenosine-triphosphate (dATP) derived from a high level of deoxyadenosine and S-adenosyl-homocysteine (S-AHO) by inhibition of S-adenosyl-homocysteine-hydrolase (S-AHOH). An elevated plasma level of S-AHO seems to be responsible of cytotoxic effects on bone marrow cells. The presence of nucleoside metabolism enzymes in malignant cells is a critical determinant of their sensitivity to chemotherapeutic agents (Dumontet C et al., 1999; Moriwaki Y et al., 1999). In fact, deficiency of dCK and/or AK activity seems to be associated with resistance to these agents, whereas increased enzyme activity may be associated with high activation of such compounds to cytotoxic nucleoside triphosphate derivatives. **Aims** Since the enzymes of purine metabolism are critical determinants of chemosensitivity of malignant cells, a RT-PCR study has assessed the expression of dCK, endo-5'-NT, ecto-5'-NT, ADA, AK, S-AHOH in lymphoid cells from patients with lymphoproliferative disorders. **Methods** From April 1999 to March 2002, one hundred and thirty-eight blood samples have been examined: one-hundred and ten (63M; 47F, mean age 63) from patients with low-grade non-Hodgkin lymphomas (LG-NHLs, $n=62$; mean age 61), with B-chronic lymphocytic leukaemia (CLL, $n=39$; mean age 65) nine patients with HG-NHLs (mean age 60) and twenty-eight samples from blood donors. Gene expression was analysed on RNA extracted from mono-nucleated cells from peripheral blood (66 patients and 28 blood donors) or bone marrow (44), reverse-transcribed into cDNA and amplified by PCR, using specific primers for dCK, endo-5'-NT and ecto-5'-NT. The semi quantitative analysis of the amplification products of AK (500 bp), dCK (436 bp), endo-5'-NT (359 bp) ecto-5'-NT (391 bp), ADA (299 bp) and S-AHOH (50 bp) was performed by image analysis and normalized to beta-actin to obtain an index of relative gene expression. The ratios of dCK/endo-5'-NT and dCK+ecto-5'-NT/endo-5'-NT were evaluated in LG-NHL, CLL and blood donors samples and the differences were statistically analysed by Student's t-test. On the contrary, the ratios of endo-5'-NT/dCK and ADA/beta-actin were only calculated in a group of 31 patients (17 patients were affected by B-chronic lymphocytic leukaemia, 11 patients by low-grade NHLs, 2 subjects by Waldenström macroglobulinemia and one patient had high-grade NHL). Moreover, the ratios of ADA/beta-actin were assessed in the group of CLL, LG-NHLs and HG-NHLs patients. **Results.** DCK/endo-5'-NT ratio in CLL vs. LG-NHLs was 1.06 ± 0.22 vs. 0.63 ± 0.24 (mean \pm S.D.; $p < 0.005$), while dCK+ecto-5'-NT/endo-5'-NT in CLL vs. LG-NHLs was 2.22 ± 0.59 vs. 1.44 ± 0.27 ($p < 0.025$). DCK/endo-5'-NT ratio in blood donors vs. LG-NHLs was 0.98 ± 0.68 vs. 0.63 ± 0.24 ($p < 0.05$), while the ratio dCK/endo-5'-NT in blood donors vs. CLL and the ratio dCK + ecto-5'-NT/endo-5'-NT blood donors (1.85 ± 0.64) vs. both CLL and NHL-LG were not statistically significant. The group of 31 patients, in which has been evaluated endo-5'-NT/dCK and ADA/beta-actin ratio, displayed a distinctive pattern of gene expression; in those subjects with endo-5'-NT/dCK ratio < 1 , the ratio ADA/beta-actin was < 1 ($n=16$), while in patients with endo-5'-NT/dCK ratio > 1 , also ADA/beta-actin ratio was > 1 ($n=15$). In the former group, endo-5'-NT/dCK ratio was 0.66 ± 0.29 and ADA/beta-actin ratio was 0.59 ± 0.28 ; on the contrary, in the latter group, endo-5'-NT/dCK ratio was 1.92 ± 0.57 and ADA/beta-actin ratio was 1.43 ± 0.37 . ADA/beta-actin ratios in CLL vs. LG-NHLs was 0.6 ± 0.36 vs. 1.29 ± 0.46 ($p < 0.0005$), ADA/beta-actin ratios in CLL vs. HG-NHLs was 0.6 ± 0.36 vs. 1.6 ± 0.84 ($p < 0.0005$), while the ratios ADA/b-actin in LG-NHL vs. HG-NHL was significant only for $p < 0.2$. AK and S-AHOH analysis showed variable expression. **Conclusions.** The higher dCK/endo-5'-NT and dCK+ecto-5'-NT/endo-5'-NT ratios in CLL patients suggest a major likelihood of response to purine analogues as compared to LG-NHLs, possibly allowing the prediction of individual sensitivity or resistance to treatment. The progressive increase of ADA/beta-actin ratio seems associated with the rise of histological aggressiveness. In the group in which has been evaluated endo-5'-NT/dCK and ADA/b-actin ratio, the results

provide evidence that a lower expression of endo-5'-NT enzyme, as compared to dCK, is also associated with low transcription of ADA. On the contrary, a higher expression of endo-5'-NT, appears to be associated with ADA overexpression. These enzymatic patterns might be correlated with different drug effectiveness in chronic lymphoproliferative diseases, since the target of pentostatin is ADA enzyme and fludarabine must be activated by dCK or may be inactivated by endo-5'-NT enzyme. These results provide evidence of the feasibility of routine pharmacogenetic analysis in chronic lymphoproliferative disorders for prognostic purposes, in order to select patients who are likely to respond to nucleoside analogues.

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