Mechanisms of toxicity and metabolism of thiopurines in non tumor human hepatic and intestinal cell lines

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Thiopurine antimetabolites, 6-mercaptopurine (6MP), its prodrug azathioprine (AZA) and 6-thioguanine (6TG) have cytotoxic properties for lymphocytes and leukemic blasts and, for this reason, are used in the treatment of acute lymphoblastic leukemia or as immunosuppressants. Despite their proven efficacy, the therapeutic utility of these medications is limited by significant interindividual differences in clinical response at standard doses, both in terms of efficacy and incidence of adverse events. Thiopurines undergo a complex intracellular metabolism mediated by several enzymes (Stocco et al., 2010). Anabolism involves the purine synthesis salvage pathway and leads to the formation of thioguanine nucleotides (6TGN), the main active metabolites. Catabolism is mainly catalyzed by xanthine oxidase (XO) in the liver and by thiopurine-S-methyl transferase (TPMT) in extra-hepatic tissues. Inosine triphosphate pyrophosphatase (ITPA) is another enzyme involved in reducing thiopurines toxicity, while GST-M1 may have a role in the conversion of AZA to 6MP (Stocco et al., 2007). Moreover, drug activation and some severe adverse effects of thiopurines occur in the intestinal and hepatic tissues and elucidation of the molecular events specific of these districts may be of interest to improve these medications' efficacy and safety. The aim of this study was therefore to investigate *in vitro* the sensitivity to thiopurines and the effects of altered expressions of these three enzymes (TPMT, ITPA and GST-M1) in stabilized non tumor human cell lines of hepatic (IHH) and intestinal (HCEC) origin. IHH and HCEC cells resulted more sensitive to AZA and 6TG cytotoxic effects, evaluated by the MTT assay, than 6MP. Evaluation of antiproliferative activity by ^{3}H-thymidine incorporation and Real Time Cell Analyzer assays, showed, in the hepatic cell line, a significant concentration-dependent antiproliferative effect only after AZA and 6TG exposure. This specific reduced sensitivity to 6MP may be due to peculiarities in thiopurines metabolism, such as the presence of the catabolic XO, which expression is higher in the hepatic cells. Indeed, co-treatment with allopurinol, a XO inhibitor, restores the IHH sensitivity to 6MP. On the contrary, in HCEC cells, where XO is less expressed, allopurinol co-treatment did not alter 6MP effects. Concerning thiopurines metabolism, basal levels of TPMT, ITPA and GST-M1 protein expression were determined by western blotting: IHH expressed high levels of all three enzymes, while HCEC expressed TPMT, higher ITPA and no GSTM1. To evaluate the effects of alterations of TPMT, ITPA and GST-M1 levels on sensitivity to thiopurines, expression of cDNAs for these proteins has been forced by transient transfection. So far, experiments have been completed in the IHH cell line: forced expression of wild-type TPMT increased sensitivity to all thiopurines (MTT assay), with a stronger effect on 6MP, as previously reported on other cell models (Dervieux et al., 2003), while GST-M1 induced an increased sensitivity to AZA but not to 6MP and 6TG. For ITPA, no significant difference was observed. In conclusion, the non tumor cells IHH can be used as a model to study the effects of the modulation of enzymes involved in thiopurines' sensitivity by heterologous expression. Particularly interesting seems the recapitulation *in vitro* of the effects of GST-M1 on AZA sensitivity. The current analyses should be fully extended to the intestinal HCEC cell line and integrated with measurements of drugs' metabolites and of activity of the enzymes studied. These analyses may contribute to the construction and optimization of a more complete *in vitro* system simulating complex physiological processes of drug absorption and metabolism.

Stocco et al. (2010). Exp Opin Drug Saf. 9: 23-37.
Stocco et al. (2007). Inflamm Bowel Dis. 13:57-64.
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