PWE-018

A BIOCHEMICAL MECHANISM FOR THE ROLE OF ALLOPURINOL IN TMPT INHIBITION

doi:10.1136/gut.2011.239301.281

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Introduction Hypermethylation of thiopurines has been associated with drug toxicity and non-response to treatment. In such patients the use of low dose thiopurines with concomitant allopurinol is advocated[1 2]. Allopurinol is observed to cause a reduction in methylated metabolites of thiopurines; however the biochemical mechanisms remain incompletely understood. Using an intact erythrocyte model we propose a novel pathway of allopurinol mediated thiopurine-*S*-methyltransferase (TPMT) inhibition, through the production of 2-hydroxy-6-thiopurine (2OH6MP).

Methods EDTA whole blood was obtained from healthy volunteers; the plasma and top 5th were removed and the red cells washed with 0.9% saline. 150 μL of Earl's balanced salt solution was added to 100 μL of packed red cells. Cells were incubated with 250 μM of 6-mercaptopurine (6-MP) for 0, 2, 4 and 6 h at 37°C. In the second experiment cells were pre-incubated for 2 h with 280 μM of 2OH6MP prior to the addition of 250 μM 6-MP for either 2 or 4 h. At the end of the incubation period, the media was removed and the red cells lysed with 15% perchloric acid after the addition of dithiotreitol. Methylated thiopurine-metabolites were reduced to the base by boiling at 100°C for 1 h. 75 μL of the red cell lysates and supernatant media were separated by reverse phase HPLC to detect the methylated metabolites of 6-MP.

Results In packed red cells there was an increase in the concentration of 6-methylmercaptopurine (6-MeMP) detected at each time point. However, the rate of 6-MeMP production remained constant (mean 0.825 pmol L^{-1} h^{-1}, SEM ± 0.038). The concentration of 6-MeMP observed in the media was up to 7-fold lower than the concentration in red cells (mean 0.133 pmol L^{-1} h^{-1}, SEM ± 0.009). In red cells pre-incubated with 2OH6MP prior to the addition of 6-MP there was a significant reduction in the rate of 6-MeMP production at both 2 (0.878 pmol L^{-1} h^{-1} vs 0.135 pmol L^{-1} h^{-1}, $p < 0.0001$, two-sided T-test) and 4 h (0.732 pmol L^{-1} h^{-1} vs 0.096 pmol L^{-1} h^{-1}, $p<0.0001$, two-sided T-test).

Conclusion The data suggests that 6-MP enters red blood cells, where it undergoes methylation to 6-MeMP. The presence of 2OH6MP leads to a reduction in the rate of 6-MeMP production, most likely through direct inhibition of TPMT. We propose that 6-MP undergoes preferential oxidation via aldehyde oxidase, producing 2OH6MP, which leads to feed-back inhibition of TMPT and thereby a reduction in methylated thiopurine-metabolites. This mechanism may explain why patients treated with a combination of thiopurine and allopurinol have dramatically decreased methylated metabolites.

Competing interests None.
Keywords Allopurinol, Thiopurine.

REFERENCES

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