ABSTRACT

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Title of Diploma Thesis: Thiopurine S-methyltransferase – clinical importace of genotyping and phenotyping.

Thiopurine S-methyltransferase catalyzes S-methylation thiopurine´s drugs such as 6-mercaptopurine and thioguanine. TPMT genetic polymorphisms represent an important role in clinical pharmacogenetics. The differences in TPMT activity result from mutations in gene for TPMT. The polymorphisms are important factor in efficacy of treatment by thiopurine drugs. Patients inheriting low activity of enzyme TPMT have mutated allels, patients inheriting high activity of TPMT are usualy wild types.

TPMT gen was genotypized by method real-time PCR in volunteers (n=55) with autoimmune diseases. The average of patient´s age was 16,7 years. From blood collected into EDTA DNA was isolated by using QIAmp Mini Kit (Quiagen, Germany) and it was used for genotyping of TPMT. Genotyping was carried out by real-time PCR in LightCycler (Roche, Germany). TPMT was phenotypized in Hradec Králové in Medical Faculty of Charles University in Department of Pharmacology. The lysate of suspension of erythrocyte was used for phenotyping (The blood was collected into Li-heparinized tubes) and for phenotyping RP-HPLC with gradient was used.

The activity of enzyme TPMT depends on genotype of TPMT. The low activity of TPMT was observed in mutated samples (TPMT*3A), the high activity was observed in wild type allels (TPMT*1). Retention time of 6-MMP was 8,5 minutes and the activity of TPMT was shown like concentration 6-MMP in 1 hour in 1 ml of suspension of erythrocytes.