Macedonian pharmaceutical bulletin, 69 (Suppl 1) 275 - 276 (2023)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2023.69.03.134
Short communication

Predictive model for determination of relative *ABCB1* gene expression in different tissues

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Introduction

The *ABCB1* gene has 14 single nucleotide polymorphisms that are associated with the pharmacokinetic response to drugs (Wang and Sadée, 2006). Three of them (C1236T, C3435T and G2677T/A are subject of most intensive research and their influence on the expression and activity of *P*-glycoprotein in humans and the pharmacokinetics of drugs that are substrates of this transporter has been confirmed with the large number of studies evaluating the influence of haplotype inheritance (Fukui et al., 2007; Wang et al., 2005). The aim of this paper is to create a predictive model that will be able to determine the relative expression of the *ABCB1* gene in the blood and the correlation with the expression in the brain, liver, kidney, duodenum, jejunum, ileum and colon.

Materials and methods

The study was approved by Ethical committee of the Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, North Macedonia. All procedures were conducted in accordance with Declaration of Helsinki.

Tissues (blood, brain, liver, kidney, duodenum, jejunum, ileum and colon) from total of 30 subjects (24 men, 4 women, 2 unknown) with a mean age of (51.32 ±16.4 years) were provided by the Institute for Forensic Medicine and Criminalistics, Faculty of Medicine, Ss Cyril and Methodius University. Autopsy samples from subjects who died of a violent death (traffic accident, electric shock, fall from a height, drowning, suicide) collected between March 2011 and April 2013 were immediately frozen in liquid nitrogen and stored at -80 °C. DNA and RNA isolates obtained from tissues were stored under the same conditions.

Single nucleotide polymorphisms for the *ABCB1* gene for three polymorphisms C1236T [rs1128503], G2677A/T [rs2032582] and C3435T [rs1045642] and the relative expression of *ABCB1* gene in different tissues (blood, brain, liver, kidney, duodenum, jejunum, ileum and colon) from the same individuals were analyzed by Real-Time PCR method in Centre for Bimolecular and Pharmaceutical Analysis (CBPA) at Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, North Macedonia (Naumovska et al., 2016).

In the first stage of modeling, the relative gene expression of respective haplotypes (C1236T, G2677T and C3435T) in blood in correlation with patients` gender and age were considered. In the second phase, a model with appropriate accuracy was developed for predicting the expression of the *ABCB1* gene in other organs (brain, liver, duodenum, jejunum, ileum and colon). In order to obtain a more accurate, precise and reliable model, in addition to the haplotype, gender and age of the patients, the expression of the *ABCB1* gene in the blood was additionally included. The predictive model was built using OPLS (Orthogonal partial least squares) multivariate regression analysis (SIMCA 14.0, Umetrics AB, Sweden). In each analysis, the variance of each variable was appropriately scaled to 1/SD (UV reduced). The built OPLS model was validated by a permutation test that was performed for each ΔCt (blood, brain, liver, duodenum,

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ileum, jejunum, and colon) with 100 permutations performed.

Results and discussion

When modeling the relative expression of the *ABCB1* gene in the blood (ΔCtB), in addition to the haplotype, age and gender of the patients, the additional effects of their possible interactions such as the mutual effects of gender and age, gender and haplotype and age and haplotype were taken into account. The ΔCtB predictability model was characterized by 1+4+0 OPLS components, with this model explaining a total of 66.4% (R^2Y) of the variation in the obtained results with a satisfactory degree of predictability (Q^2) of 58%. The model pointed out that ΔCtB is most influenced by age, the interaction of age/male gender and age/haplotype (CT GT CT) with which ΔCtB is negatively correlated, as well as haplotype (TT TT TT), age/female gender, age/haplotype (TT TT TT) and age/haplotype (TT GG CC) with which ΔCtB is positively correlated.

After modeling ΔCtB, modeling of the relative expression of the *ABCB1* gene in other analyzed tissues (brain, liver, duodenum, jejunum, ileum and colon) was performed. In this model, in addition to haplotype, age and gender of patients and ΔCtB, the additional effects of interactions between gender and age, age and haplotype, gender and ΔCtB, age and ΔCtB, as well as quadratic functions of age and ΔCtB, and the cubic function of ΔCtB were taken into account. This model was characterized by 6+7+0 OPLS components and it explained a total of 68.7% (R^2Y) of the variation in the obtained results with a satisfactory predictability (Q^2) of 54.7%.

The linear function of ΔCtB was positively correlated with ΔCt of the duodenum and colon, and a positive correlation was determined of ΔCt of the colon with the quadratic function of ΔCtB, and ΔCt of the jejunum with the cubic function of ΔCtB. In all other cases, a negative correlation with the linear, quadratic and cubic function of ΔCtB was established. The linear function of ΔCtB had a statistically significant ($p<0.05$) association with brain ΔCt, while the quadratic function of ΔCtB with ΔCt of brain, liver, ileum and colon, and the cubic function of ΔCtB had a significant ($p<0.05$) association with ΔCt of ileum.

Validation of the model with a permutation of the test indicated a relative error between 4.67% (brain) and 11.70% (liver), which indicates that the created predicative model was acceptable.

Conclusion

A predictive model with an acceptable relative error (4.7–11.7%) was established. It is applicable for determination of relative expression of mRNA for *ABCB1* gene in different tissues (blood, brain, liver, kidney, duodenum, jejunum, ileum and colon) in the same subjects, based on results obtained for *ABCB1* gene polymorphisms and haplotypes for C1236T, G2677T/A and C3435T variants, gender, age and their interactions.

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