The phenotype genotype relationship biology essay

Science, Biology

The significant role of variation in transporter activity and expression in organs such as the liver has been established as a key determinant of inter-individual variability in drug response . Metrics which aim to monitor metabolic enzyme activity, in reality, act as a measure of hybrid parameters, including not only metabolic clearance, but also influx into the hepatocyte. As uptake transporters play an important role in regulating influx into the liver, this study set out to assess the misphenotyping which may arise when probe substrates used to determine metabolic enzyme activity are also substrates of hepatic uptake transporters.

Impact of findings

The metabolic activity of CYP2D6 was simulated in virtual patients, altering the intrinsic passive diffusion clearance of the probe substrate in order to vary influx into the hepatocytes. The % contribution of active uptake to total uptake was used to infer the permeability characteristics of the probe substrate for each of the simulations. Without any permeability limitations into the liver, a multimodal frequency distribution of CYP2D6 activity was established. When permeability restrictions were applied so that influx into the hepatocyte was mainly transport-mediated and not passive, the multimodal distribution of enzyme activity was altered to a unimodal frequency distribution, where the distinction between the poor metabolisers and extensive metabolisers was no longer apparent. The results from the simulations verify that decreased influx of the probe substrate into the liver can affect the analysis of enzyme activity, by causing phenotypic misclassification to occur. In the studied scenario, a contribution of more than 90% of active uptake to total uptake was required in order to cause a

distinctive change in modality. Therefore, this has clinical implications when phenotyping for enzyme activity, using a probe substrate that is predominantly actively transported into the hepatocyte. In these circumstances, the metrics used to phenotype will be largely influenced by influx into the hepatocyte, as opposed to the metabolic enzyme activity. The fact that hepatic uptake transporters are polymorphic highlights the capability for misphenotyping to occur. These findings therefore can be used to speculate that in situations where the contribution of active uptake to total uptake is significantly high, variation in transporter activity can significantly alter phenotypic classification. For instance if an individual possessed a polymorphism in OATP1B1 and was phenotyped with a probe substrate that was mainly transport-mediated, then the transporter polymorphism is much more crucial to the substrate in terms of determining the phenotype. Consequently the potential effect of transporter activity variation should be appreciated and caution should be taken when assigning enzyme activity if using probe drugs that are also substrates for polymorphic transporters.

Phenotype-genotype relationship

In vivo phenotyping aims to give an accurate indication of the actual metabolic activity of the CYP enzyme, using indirect metrics such as the MR. It is important to acknowledge the potential limitations of the indirect metrics used, as they may often act unknowingly as a measure of hybrid parameters for not only enzyme activity, but also transporter variation. As mentioned earlier, confounding factors such as the effect of renal function

and urinary pH can influence the measure of enzyme activity. This study has focused on the role of transporter activity as a confounding factor, and has found that the effect of transporter variation has the potential to discredit the validity of the metabolic ratio (metabolite/drug) as a measurement of enzyme activity. However, the effect of the transporter will only be seen in cases where the contribution of active uptake is significantly more prominent than passive diffusion. In comparison to the phenotyping, which looks at the manifestation of the metabolic enzyme activity, genotyping is restricted specifically to the genetic aspect of enzyme variability. Although phenotyping, involving administration of a probe drug, is still valid for assigning metabolic activity, nowadays genotyping of the CYP2D6 gene is a popular and widely used method to assess activity . It involves molecular genetic analysis of frequent functionally relevant variants of the CYP enzyme to predict individual phenotypes . However, an accurate prediction of a patient’s specific phenotype from the genotype is challenging, especially in the case of the CYP2D6 enzyme, which possesses vast genetic diversity . By analysing only frequent variants responsible for the polymorphisms, rare or novel mutations in the CYP2D6 gene resulting in loss of enzyme activity would not be identified . Therefore, genotyping cannot ultimately replace phenotyping, and in some cases, validation of the CYP genotype data with results obtained from phenotyping using metabolic ratio measurements is necessary .

Very commonly genotype fails to accurately predict phenotype in clinical practice for two main reasons. Firstly DNA sequence of a gene coding for a protein usually does not correlate with the amount of the actual protein expressed due to post-translational and post-transcriptional modifications. Secondly because of many environmental parameters that " fade" the genotype effect.

When assessing CYP2D6 activity, only two phenotypes are predominately detected, which are the poor metabolisers (PM) and extensive metabolisers (EM). However, the situation is not so simple, since each of the two phenotypic groups consists of individuals with allelic variants that correlate to different levels of enzyme activity . For instance the EM phenotype, incorporating the homozygotes and heterozygotes, includes genes that are partly deficient or more efficient . Therefore there is a range of enzyme activity within the phenotype. In addition, further variability within each allelic variant is present, which exists as a result of direct environmental effects on the enzyme or by factors affecting gene regulation . Figure 5-1 depicts the phenotype-genotype relationship along within the complex occurrence of variability within phenotype groups and allelic variants.

Figure 51| Frequency distribution highlighting the phenotype- genotype relationship

Figure displays the enzyme activity variability within the EM and PM phenotype. In addition, within the allelic variants (a-h) there is variance in enzyme activity shown from the horizontal bars, which are influenced by gene regulation and environmental regulation. Extracted from (Tucker et al., 1998)The variability in phenotype and genotype, caused by influencing

factors, has the potential to confuse the relationship between the two. The results from this study have certain implications on this phenotype-genotype relationship, by introducing further variability from changes in transporter activity. From a clinical point of view, it is important to accurately identify the genotype and phenotype in order to predict the drug response in individuals. The presence of transporter effect has the capability of distorting the relationship and creating further challenges in predicting phenotypes from genotypes.

Limitations

This study effectively illustrates the effect of transporter variation using a particular probe substrate to determine that under these specific conditions, bimodality is lost above 90% contribution of active uptake to total uptake. However, due to the specificity of the study, only a general application of the results can be made, as the incorporation of different probe substrates or drug metabolising enzymes may yield altered results.

Analysis of metrics used

The metric used to measure enzyme function must be an appropriate index for the particular pathway that is being measured . Indirect metrics such as the plasma AUC and urinary recovery metabolite/drug ratios were used, in this study. The use of inappropriate indices has been reported to result in skewed modality . For instance, a multimodal distribution is likely to yield a distorted unimodal distribution when using a less specific metric such as the AUC . However, this is less likely to be the case when using the AUC if the polymorphic pathway accounts for the majority of the total clearance and if

there is a clear difference in the enzyme activity of the various phenotypes . For this case, as the O-demethylation pathway is primarily dependent on CYP2D6, the AUC was an appropriate index.

Analysis of graphical methods

Although the graphical methods used to assess the modal distribution were successful in demonstrating the effect of transporter variation, it is important to recognize the limitations in both the histogram and NTV approach. In the frequency distribution histograms, the value for the antimode is difficult to distinguish in some of the simulation results; therefore a clear cut separation in phenotypes is not apparent. In addition, histograms are affected by factors such as sample size, which if insufficient can lead to flawed modal distributions . However, due to the simplicity and visual clarity of this approach, histograms are appealing in detecting multimodality. The NTV approach displays a bimodal distribution based on the presence of strongly negative values. To establish what constitutes as strongly negative, an arbitrary value of -0. 03 was assigned . Therefore, for the purpose of this study, the value of above 90% contribution representing the point at which transporter activity altered the modal distribution is purely an approximate value. In addition, the visual representation of the NTV plot is not as clear as the histogram in displaying bimodality. Although the histogram and NTV method possess limitations, it is important to appreciate that two different graphical methods were incorporated in the study. Therefore, the observations were not solely reliant upon one graphical approach .

Additional research

In this study, the passive diffusion clearance was altered to modify the permeability of the compound into the hepatocyte. In order to analyse specific transporter variations, additional work could be carried out introducing the effect of particular transporter genotypes on the enzyme activity. This would allow a more accurate understanding on the impact of transporter variation on phenotyping for activity of metabolic enzymes. In addition, it would be interesting to understand how altered CYP2D6 abundance or activity could affect the results. Therefore, further research creating scenarios with altered enzyme abundance would be valuable. Simulations were carried out using the genetically polymorphic enzyme, CYP2D6, which therefore exhibits a multimodal frequency distribution. Consequently, this study observed the alteration of a bimodal distribution into a skewed unimodal distribution, due to transporter interference. Research could be undertaken to examine what effect transporter variation might have on the activity of less polymorphic enzymes, such as CYP3A4, which is relatively well-conserved . This additional work would investigate whether transporter variation could alter the original unimodal distribution into skewed multimodality.

Conclusion

In conclusion, reduced influx of a compound into the hepatocyte has the potential to interfere with in vivo phenotyping via administration of probe substrates and consequently may cause phenotypic misclassification to occur. This study demonstrated a drastic change in the modal distribution

from bimodality to unimodality as a result of altered permeability into the hepatocyte, which therefore has significant implications on the phenotype-genotype relationship. The phenotype is influenced by hybrid parameters, including the influx into hepatocytes and the metabolic clearance from the enzyme. In cases where the influx of a drug into hepatocytes predominantly occurs by passive diffusion, the phenotype will represent the metabolic activity of the enzyme. However, in situations where influx is largely transporter mediated, the phenotype will be primarily controlled by the influx of the drug into the liver instead of the metabolic activity of the enzyme. As polymorphisms also exist in drug transporters, it can be speculated that transporter variation may produce a flawed perception of enzyme activity. Therefore, the potential for mis-phenotyping to arise from transporter polymorphisms is a fact that must be acknowledged.