Human glycine N-acyltransferase (GLYAT) and inborn errors of metabolism

Daniel Schulke¹, Jörn Oliver Sass¹

¹ Research Group Inborn Errors of Metabolism, Department of Natural Sciences & Institute for Functional Gene Analytics (IFGA), Bonn-Rhein-Sieg University of Applied Sciences, Rheinbach, Germany
daniel.Schulke@h-brs.de
jörn.oliver.sass@h-brs.de

Background

GLYAT is involved in hepatic phase II detoxification (Fig. 1) of various xenobiotics by amino acid conjugation (Fig.2) [4]. Beyond that, it contributes to removal of toxic intermediates accumulating in certain inborn errors of metabolism [1, 3]. GLYAT activity is required for conjugation of these compounds, thus providing alternative detoxification pathways. Therefore, GLYAT sequence variants might affect the efficacy of disease specific treatment approaches. Sequence variant p.(Asn156Ser) has the highest homozygous genotype frequency and relative enzyme activity in all populations studied [5,6]. Annotation mistake in gene databases might have been occurred and p.(Asn156Ser) should be considered as the authentic human GLYAT wild-type. In an African Caucasian cohort – where isovaleric acidemia is of high frequency [2] - GLYAT sequence variant p.(Gln61Leu) was described as prevalent with 12% allele frequency due to a founder effect [7].

Figure 1. Amino acid conjugation as phase II reaction of human biotransformation. Phase II reactions of human biotransformation increase hydrophilicity of compounds and facilitate excretion via the urine. GLYAT catalyzes amino acid conjugation.

Figure 2: GLYAT catalyses acyl group transfer from benzoyl-coA to glycine to form benzyolglcyine. After activation of benzoic acid with CoA benzoyl-CoA donates the acyl group to glycine, the acyl group acceptor. Thus, benzyolglcyine is formed, which is the more hydrophilic compound eliminated via the urine.

Aims

• Set-up overexpression system for human GLYAT wild-type and sequence variants
• Development of GLYAT activity assay for purified proteins and cell homogenates
• Elucidation of intracellular localization of human GLYAT
• Deriving pharmacogenetic implications for certain inborn errors of metabolism from enzyme activity studies
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Methods

• Immunoblotting for verification of GLYAT overexpression
• Affinity chromatography for protein purification from E.coli Origami 2(DE3)
• Ellman’s assay for GLYAT enzyme activity detection
• Confocal microscopy for determination of intracellular localization of human GLYAT

Results

Overexpression and enzyme activity tests of human GLYAT N-terminally fused with thioredoxin (Trx) - His in E.coli Origami 2(DE3)

Human GLYAT has been overexpressed in E.coli Origami 2(DE3) and p.(Asn156Ser) variant is threefold more active than wild-type, while p.(Gln61Leu) variant shows only 20% of wild-type activity.

Figure 3. A: Western Blot for analysis of 20 µg total protein homogenate and 1.5 µg purified proteins from E.coli Origami 2(DE3) overexpressing wild-type human GLYAT and sequence variants as Trx-His fusion proteins and corresponding enzyme kinetics (B). A: Western Blot was treated with GLYAT specific antibody. B: Sequence variant p.(Asn156Ser) shows threefold increased activity compared to wild-type, whereas p.(Gln61Leu) variant shows only 20% of wild-type activity.

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Overexpression and enzyme activity tests of human GLYAT in HEK293 cells

Figure 4. A: Western Blot for analysis of 30 µg total protein and GLYAT enzyme kinetic (B) from HEK293 cell homogenates overexpressing human GLYAT wild-type. A: Western Blot was treated with GLYAT and α-tubulin specific antibody. B: Enzyme kinetic of GLYAT wild-type demonstrates substrate dependent specific activity of the overexpressed enzyme.

Human GLYAT wild-type has been overexpressed in HEK293 cells and enzyme activity is detected
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Intracellular localization of human GLYAT

Figure 5. Confocal microscopy of stable transfected HEK293 cells with eGFP and GLYAT-eGFP. HEK293 cells transfected with eGFP-pcDNA3.1(+) or GLYAT-eGFP-pcDNA3.1(+) monitored for eGFP (488 nm), DAPI (405 nm) and Mitotracker (561 nm) filters to stain eGFP protein, nuclei and mitochondria, respectively.

Mitochondrial localization of human GLYAT
Conclusions

1. GLYAT sequence variant p.(Gln61Leu) demonstrated decreased enzyme activity, which may result in pharmacogenetic impacts for patients with isovaleric acidemia
2. GLYAT sequence variant p.(Asn156Ser) enhanced enzyme activity compared to wild-type confirming literature data [5,6]
3. Overexpressed wild-type GLYAT in HEK293 homogenates observed sevenfold lower activity compared with purified wild-type GLYAT
4. GLYAT is localized within mitochondria
5. Missense mutations of GLYAT may affect enzyme activity and consequently human phase II conjugation

References

[7] van der Sluis et al. Gene 2015;571;126-34

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