

SERUM GLUTATHIONE S-TRANSFERASE ALPHA (α -GST): A SUITABLE BIOMARKER FOR DRUG-RELATED LIVER INJURY?

S Eason¹, M Manghani², G Greig³, M Merz³

¹Novartis Horsham Research Centre, Horsham, United Kingdom, ²Arthritis Unit, North West London NHS Trust, United Kingdom, ³Novartis Pharma AG, Basel, Switzerland

Abstract

Drug related liver injury has been the most frequent cause for recalling marketed drugs and one of the predominant reasons for discontinuing otherwise promising drug development projects. The current standard battery of tests used to detect liver injury in clinical development lacks both sensitivity and specificity with respect to drug relatedness. In order to improve the detection of signals predicting possible liver toxicity of new drug candidates, alternative and/or additional tools are urgently needed. The characteristics and potential added value of serum α -GST levels in supporting earlier and more specific detection and monitoring of drug related liver injury were investigated across three exploratory clinical development studies involving three different drugs. The results show that α -GST may be more sensitive in detecting drug related liver injury compared with transaminases.

- An immediate drop of α -GST levels after discontinuation of a suspected drug could help to increase specificity of elevated standard liver function tests.
- Time-gain in terms of detecting liver injury earlier using α -GST elevations versus transaminase elevations may be rather marginal.

Based on these data, the most effective use of measuring α -GST in clinical trials may be to *take extra samples along with transaminase sampling*, and assay them if there is a need to further investigate potential hepatic effects. More large, prospective studies are needed to explore in more depth the benefits of α -GST in drug development.

Introduction

The standard biochemical assessment of drug related liver injury includes measurement of the transaminase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). For early detection and monitoring of hepatotoxicity in clinical drug development however, the sensitivity and specificity of these enzymes is often inadequate. Hence, there is a clear need for alternative biomarkers of drug related liver injury.

Glutathione S-transferase α (α -GST) is found in high concentration in the human liver, representing more than 2% of the soluble protein [1]. It is expressed uniformly in both the periportal and centrilobular zones [2]. Hepatocellular damage leads to the rapid release of α -GST into the bloodstream due to its smaller molecular size compared with the transaminases. The half-life is short (~1 hour) in comparison to ALT (47 hours) and AST (17 hours) and therefore α -GST concentration follows changes in hepatocellular damage more rapidly than that of the transaminases [3], which makes the enzyme an interesting candidate for early detection and monitoring of hepatic drug side effects. A quantitative enzyme immunoassay (HEPKIT™ Alpha, Biotrin International, Dublin, Ireland) is available for routine measurement of serum α -GST which may constitute a useful tool for assessment of liver cell integrity in drug development.

The potential added value of α -GST as a biomarker for drug related liver injury was evaluated across three unrelated development compounds analysing data from Exploratory Clinical Development studies in healthy subjects, in whom α -GST levels had been measured in addition to transaminases. The objectives were

- to analyse the correlation of α -GST levels with age, BMI, and standard liver function tests at baseline,
- to explore the sensitivity and specificity of α -GST compared to ALT and AST,
- to assess whether liver injury is detectable earlier using α -GST, as compared to ALT and AST, and
- to explore to which extent α -GST levels may be able to support causality assessments in the case of elevated transaminases.

Material and Methods

All compounds were in Phase 1 of clinical development. Studies included in the analysis:

Compound A: four week, double-blind, placebo-controlled, parallel-group, multiple dose study, 48 subjects at four different dose levels, 8/12 subjects on active treatment in each group.

Compound B: two week, double-blind, placebo-controlled, parallel-group, multiple dose study, 36 subjects at four different dose groups planned. Only two dose groups completed due to signs of intrinsic liver toxicity at the second dose level. 7/9 subjects in each group assigned to active treatment.

Compound C: two week, double-blind, placebo-controlled, parallel-group, multiple dose study in 52 (planned 54) subjects at six dose levels, 7/9 subjects in each group assigned to active treatment.

Baseline values were defined as the average of screening and first in-house day (before first drug/placebo administration). For compound A and C, only pre-dose values, but no screening values were available for α -GST. Baseline values for α -GST were therefore set to values at first in-house day for these two studies.

Analyses were done on normalized values to facilitate cross-study and cross-parameter comparisons.

Normalized values were calculated as $X_n = (\text{raw value} - \text{LLN}) / (\text{ULN} - \text{LLN}) * 100$.

For assessment of sensitivity and specificity, **enzyme increments** were calculated, defined as $\text{Inc} = (\text{normalized value during treatment}) / (\text{normalized value at baseline})$.

Increments < 0 resulting from raw values below LLN were set to 1 (indicating absence of any increase), since liver function tests below LLN were not considered to have clinically relevant diagnostic value.

Sensitivity was explored in subjects on active treatment in two of the three studies (compound A and B) in which α -GST was measured frequently and in parallel to transaminases. Enzyme profiles were compared between subjects assigned to active treatment and subjects assigned to placebo. Indicators of sensitivity evaluated were

- number of subjects with relevant enzyme elevations, defined as at least three consecutive elevated and increasing values, at least one of them more than or equal to two-fold baseline
- day of first enzyme elevation
- maximum extent of enzyme elevation.

Specificity was assessed by comparing distributions of all enzyme levels between subjects assigned to active treatment and subjects assigned to placebo.

To assess the potential use of α -GST to support **causality assessment in cases of elevated transaminases**, individual enzyme profiles were explored for any obvious differences between subjects assigned to active treatment and those assigned to placebo treatment.

Results

119 subjects were included in the analysis (Table 1).

Results of the correlation analysis are given in Table 2. Normalized α -GST levels (GSTn) show a significant correlation with normalized ALT (ALTn), AST (ASTn) and γ GT (GGTn), as well as with BMI. Age, APn, and TBn did not show significant correlations with GSTn.

Table 3 displays the number of enzyme elevations observed in the two studies with frequent measurement of α -GST in parallel with transaminase sampling. Compound B shows a stronger signal for liver toxicity than compound A. Subsequently, the majority of results presented focus on compound B.

Compound	Compound		
	A	B	C
N	48 + 1 repl.	18	52
Age	29.1 (25.9)	40.1 (19.3)	37.5 (21)
BMI	22.6 (9.82)	23.7 (12.5)	26.1 (11.8)

Table 2: Baseline correlations of GSTn (Spearman's rank correlation, one sided tests)

Treatment	Compound	
	A	B
Active	20/33 (61%)	11/14 (79%)
Placebo	8/16 (50%)	2/4 (50%)

	ALTn	ASTn	BMI	GGTn
r _s	0.57	0.45	0.37	0.29
p	0	0	0	0.0009

Glossary	
BMI	Body Mass Index
ALTn	Alanine amino transferase normalized to % ULN
ASTn	Aspartate amino transferase normalized to % ULN
GGTn	γ glutamyl transferase normalized to % ULN
GSTn	Glutathione-S-transferase α normalized to % ULN
APn	Alkaline phosphatase normalized to % ULN
TBn	Total bilirubin normalized to % ULN
LLN	Lower limit of normal
ULN	Upper limit of normal

Table 3: Number and percentage of any enzyme elevation* by compound and treatment

*only active treatment, only subjects showing at least three consecutive values elevated and increasing, at least one of them >= two-fold baseline

Figure 1 shows the distribution of days to first increase across ALTn, ASTn, and GSTn levels with compound B. Both ALTn and GSTn show elevations earlier than ASTn, and, with substantial variability, GSTn shows elevations slightly earlier than ALTn.

In terms of maximum increase from baseline, GSTn seems to be more sensitive to a drug effect in this study as compared to ALTn and ASTn (Figure 2).

Difference in enzyme level variation between subjects assigned to active treatment and those assigned to placebo is greater for GSTn as compared to ALTn (Figure 3).

Figures 4 and 5 display mean enzyme profiles with compound B during active treatment vs. placebo treatment. All three enzymes show a parallel increase during active treatment, with mean ALTn and GSTn levels being almost superimposable. All three mean enzyme profiles showed a further increase after discontinuation of study drug, reaching a peak around day 38.

However, looking at individual values for GSTn levels particularly in the high dose group (data not shown), 6 out of 7 subjects with GSTn levels increasing during active treatment showed a pronounced drop after discontinuation of study drug. In contrast, ALTn levels showed a similar drop in only one subject of the high dose group. All other subjects showed either plateauing or further increasing ALTn values after stop of treatment. Only one subject showed GSTn levels increasing further after stop of treatment.

Figure 5 demonstrates that the placebo group also showed enzyme elevations during the study period, predominantly for ALTn and GSTn.

Enzyme profiles in the study with compound A showed similar relationships between GSTn, ALTn and ASTn, however at lower enzyme levels, indicating the absence of a clear toxicity signal.

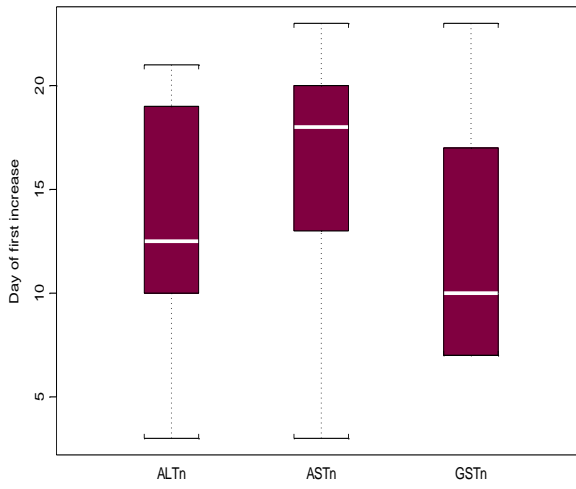


Figure 1: Day of first enzyme increase in actively treated subjects, compound B

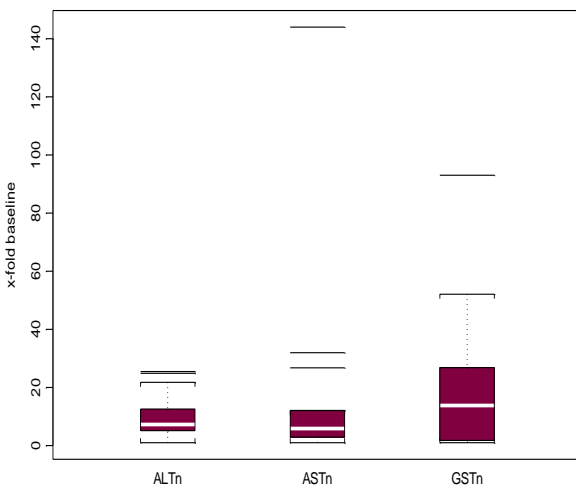


Figure 2: Maximum increment from baseline in actively treated subjects, compound B

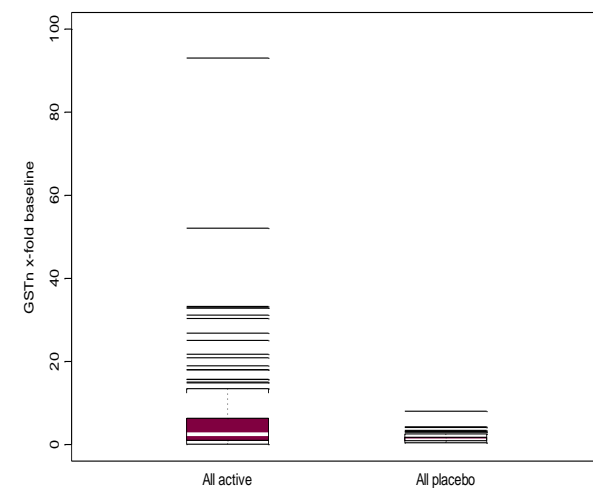


Figure 3: Distribution of GSTn and ALTn by treatment, compound B

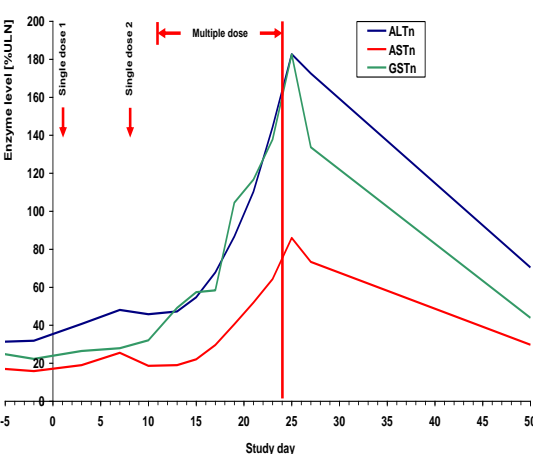


Figure 4: Enzyme profiles in active treatment group compound B (mean values)

Discussion

The analysis showed a significant correlation of α -GST to ALT, and an associated correlation to AST and γ GT. The correlation to ALT levels was expected since both enzymes are located in the cytosol of hepatocytes and if ALT is released into plasma, α -GST can be expected to be released as well, since its molecular size is substantially smaller than that of ALT. The observed correlation of α -GST to BMI may be related to hepatic lipid content and associated alterations in hepatocyte membrane stability.

Serum α -GST may be more sensitive to drug-related liver effects with respect to maximum increase from baseline as compared to both ALT and AST. Liver injury could possibly be detected slightly earlier using α -GST measurements as compared to ALT elevations. However, based on the data available, the time gain may be marginal, and variability of times to first elevation in the data analysed is substantial. The data do not yet justify a recommendation to routinely measure α -GST levels in clinical trials.

An interesting observation however relates to enzyme levels after stop of drug administration in the study analysed in more detail: for α -GST, 6 out of 7 subjects assigned to active treatment showed a pronounced drop of enzyme levels after discontinuation of study drug. Thus, with respect to effects on the liver, α -GST showed a higher sensitivity to loss of rather than onset of drug effect. These findings clearly differentiate α -GST from the transaminases and may be used to support causality assessment in cases of transaminase elevations, i.e. a sudden decrease of enzyme levels after stop of study drug administration indicating the existence of a drug effect on hepatocyte integrity. In order to make use of this characteristic of α -GST, it is not necessary to regularly measure the enzyme in parallel with transaminases but just sample additional serum along with the routine liver function tests and store those samples for retrospective analysis in case clinically relevant elevations of transaminases are observed in a study. However, the value of such a strategy may be limited to compounds with

- intrinsic, but not immunologically mediated hepatotoxicity,
- short half life of both parent and reactive metabolites

The presence of liver enzyme elevations, predominantly ALT, during placebo treatment, is an observation well known for phase 1 studies⁴⁻⁶ and thought to be due to the impact of diet⁷⁻⁸ and lack of physical exercise⁹ during domiciling. Although in the study with the clearest signal for drug-related liver injury, mean α -GST levels in the placebo group increased almost in parallel to ALT levels, the difference in variation between subjects assigned to active treatment and those assigned to placebo was much greater for α -GST as compared to ALT. This may point to superior specificity for drug related liver effects for α -GST versus ALT. However, these findings have to be interpreted with due caution taking into account the small sample analysed and the retrospective nature of the analysis. Larger, prospective studies including frequent sampling of α -GST along with standard liver function tests are needed to further explore and validate the diagnostic potential of α -GST for assessment of drug-related liver injury.

Conclusions

- α -GST levels at baseline showed expected correlations to ALT, AST, γ GT, and BMI
- Differences between α -GST and ALT in terms of sensitivity to drug related liver injury are related to both maximum extent and onset of enzyme elevations
- Clinical relevance of time gain with respect to first detection of drug related liver injury may be marginal using regular additional monitoring of α -GST
- Measurement of α -GST may help to improve specificity of elevated transaminases for drug related liver injury
- Routine sampling and retrospective measurement of α -GST in cases of clinically relevant transaminase elevations in a study may help to support causality assessment in terms of drug-relatedness without significantly increasing study costs
- More and larger studies employing frequent sampling of α -GST are needed to validate the diagnostic potential of α -GST for assessment of drug-related liver injury

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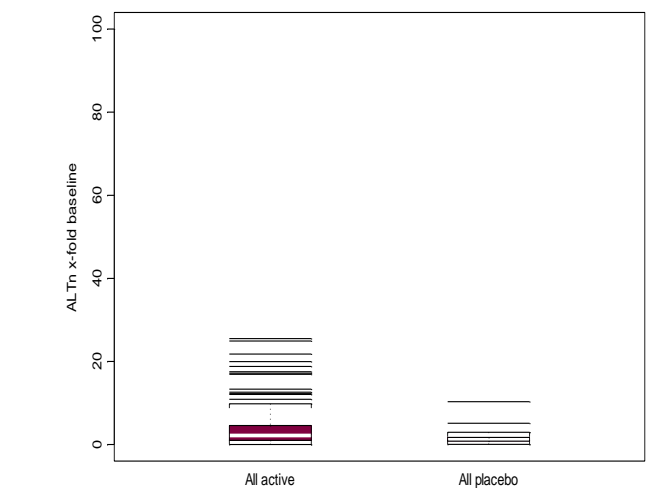


Figure 5: Enzyme profiles in placebo group compound B (mean values)