CLINICAL STUDY

(S-ACETYL-L-GLUTATHIONE) AND L-GLUTATHIONE COMPARATIVE SINGLE DOSE CROSSOVER STUDY IN HEALTHY VOLUNTEERS

BACKGROUND

Reduced glutathione (GSH) is the most powerful anti-oxidizing agent in all living organisms. Chronic oxidative stress, some pathologies and the aging process can reduce GSH level in the organism. Restoring GSH level is a good tool in the treatment of these states. Direct oral supplementation with GSH is not very efficient because of poor absorption of GSH and because of its hydrolytic degradation.

A valid alternative to direct supplementation is the administration of S-acetyl glutathione (SAG) which is more efficient in raising glutathione levels compared to GSH. SAG is a precursor of glutathione in which and acetyl group is present on the thiol function, the acetyl moiety stabilizes the molecule from the oxidation and protects it from enzymatic hydrolization allowing its transport across the intestinal mucosa as is, where, once internalized in cells, is de-acetylated by cytoplasmic thioesthrases to produce reduced glutathione. SAG has a more rapid dissolution rate used in this study.

OBJECTIVES

The aim of this clinical study was to compare the bioavailability of GSH and SAG in a single dose oral absorption study in healthy volunteers. Both pharmacokinetic and pharmacodynamic parameters were evaluated.

EXPERIMENTAL DESIGN

The present clinical trial was carried out according to the general principles of: “ICH Harmonized Tripartite Guidelines for Good Clinical Practice” ICH Topic E6,CPMP/ICH/135/95, July 1996. Before being admitted to the clinical study, subjects expressed their consent to participate. This was a single center, single dose, randomized, open-label, two-sequence, tow-period, cross-over bioavailability study. Subjects involved in the study were instructed to avoid food containing high levels of GSH or stimulating the production of GSH and any intense physical activity from 3 days before the screening visit until the end of the study. Eighteen subjects were enrolled in the study, even if one volunteer retired voluntary from the trial, so the study population was 17 subjects.

The subjects received a single oral dose of 3.5 g (50mg/kg body weight) of SAG and 3.5 g of GSH under fasting conditions in two subsequent periods separated by a wash-out interval of 7 days, according to a randomized cross-over design (Table 1). Blood was withdrawn at the following time points: -1 h, -30 and -5 minutes (pre-dose), 15 and 30 minutes, 1h, 1.5h, 2h, 3h, 4h, 8h, and 12h (Day 1), 24 h (Day 2) after dosing. The maximal care was taken in order to avoid hemolysis of blood samples during and after collection. Blood samples were transferred into tubes containing EDTA anticoagulant and centrifuged in order to obtain plasma. Samples were kept at -80° C pending analysis. Plasma samples were processed as described by Park et al. (Park E.Y. et al., J. Agric. Food Chem. 2014, 62, 6183-6189). The residual red blood cell fraction (RBC)

was immediately frozen and stored at -80°C pending analysis. All samples were analyzed in UPLC (Waters Acquity H-Class), equipped with a Mass spectrometry detector (QDA). The elution was carried out with a gradient elution utilizing water (eluent A) and 0.1% aqueous solution of formic acid (eluent B).

TABLE 1

TREATMENT	PERIOD 1	WASH OUT	PERIOD 2	BLEEDING TIMES (h)
SAG (3.5 g)	Subj. 1-9	7 days	Subj. 10-18	Pre-dose, 0.25, 0.5, 1,1.5,2, 3, 4, 8, 12, 24
REDUCED (3.5 g)	Subj 10-18	7 days	Subj 1-9	Pre-dose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24

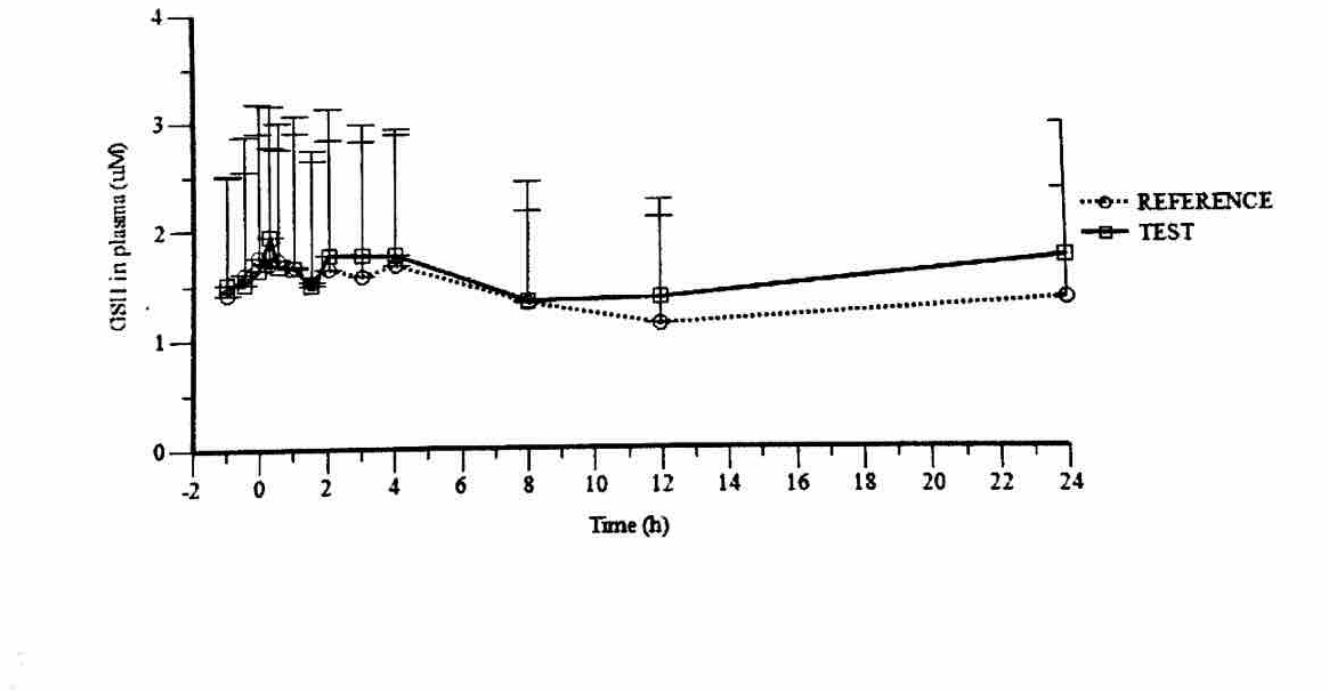
END POINTS OF THE STUDY

The levels of both GSH and SAG were measured in plasma and in the erythrocyte cell fraction (RBC) for all the time points. The main pharmacokinetic parameters were calculated ($C_{max,}$ $t_{max,}$ $AUC_{o\text{-}24h}$). Furthermore, the amount of reduced (GSH) and oxidized (GSSG) forms of glutathione were measured in RBC fraction at pre-dose and 4 h, 12 h, 24 h after dosing and the ration reduced/total glutathione (GSH/G_{tot}) was calculated. This ratio is considered as a pharmacodynamic endpoint indicating the long term GSH status and the anti-oxidizing power of individuals. The ratio of reduced to total glutathione is an indicator of cellular health, with reduced GSH constituting up to 98% of cellular glutathione under normal conditions. However, the GSH/G_{tot} ratio is reduced in neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease. Measuring the GSH/G_{tot} ratio in pathological tissues and experimental models in comparison to the results in controls is an excellent way to assess potential therapeutics efficacy in maintaining cellular redox potential.

RESULTS

No SAG was detected in any of the samples of subjects treated with SAG either in plasma or in the cell fraction. Only glutathione was found in plasma or in the cell fraction, both after the treatment with (SAG) and after the treatment with (GSH). This result confirms that S-acetyl-glutathione is soon de-acetylated and converted in its active metabolite, glutathione. All the Pharmacokinetic parameter were calculated on the GSH profiles. The data documented in this trial and the parameters measured were described using classic statistics, i.e. geometric mean (PK data only), arithmetic mean, SD, CV (%), minimum, median and maximum values for quantitative variables, and frequencies for qualitative variables. The statistical analysis of PK parameters was performed using Phoenix WinNonlin version 6.3 (10) and SAS version 9.3(TS1M1). In

Fig. 1 the mean plasma PK profiles for GSH are reported. PK parameters $AUC_{0\text{-}24h}$ and C_{max} were analyzed using analysis of variance (ANOVA).



SAG powder
GSH capsules

FIGURE 1- PK profile reporting the GSH level in plasma of subjects supplemented with SAG (test) or GSH (reference).

Main PK parameters for plasma GSH are presented in Table 2. C_{max} and final AUC values are also reported in FIG. 2 and 3 respectively. On average, GSH plasma concentrations showed higher C_{max} and $AUC_{0\text{-}24h}$ after single dose of SAG than the reference GSH formulation (point estimates of 133.40 and 189.84 respectively). Median t_{max} was 1.5 h post-dose for both (p-value=0.5276, Wilcoxon test).
In detail, when comparing GSH plasma levels, SAG induced a C_{max} and $AUC_{0\text{-}24h}$ 57.4% and 68.8% higher than GSH respectively.

TABLE 2

	N	C_{MAX}(uM)	t_{max}(h)	AUC_{0-24h} (uMxh)
SAG	17	0.74+/-0.41*	1.50 (0.25-24)	3.90+/-4.07*
GSH	17	0.47+/-0.24	1.50 (0.50-24)	2.31+/-3.38

Mean+/- SD is reported except for t_{max} for which median (range) is shown

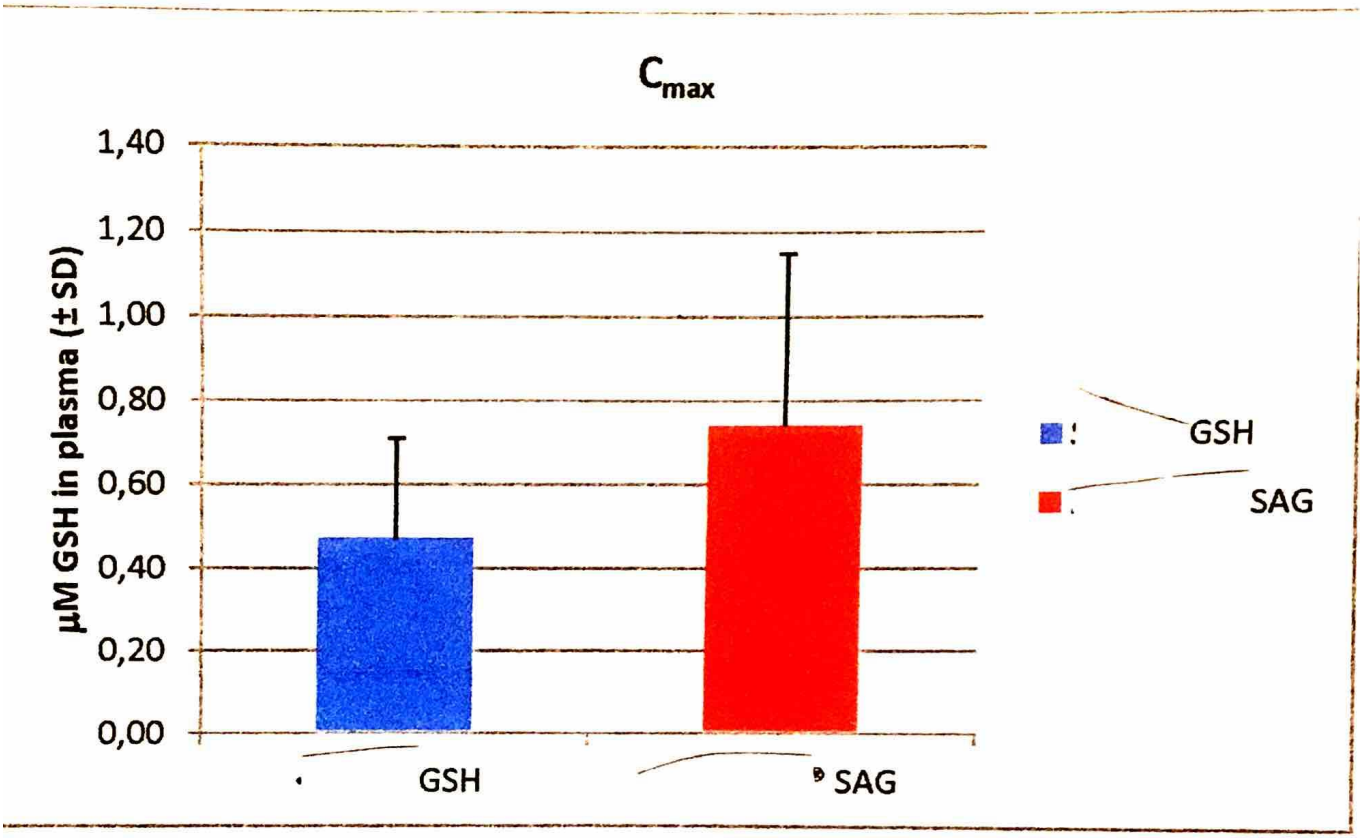


Figure 2- Maximal plasma concentration (C_{max}) reached in healthy volunteers after SAG or GSH oral supplementation. C_{max} is expresses in uMol/l(+/-SD).

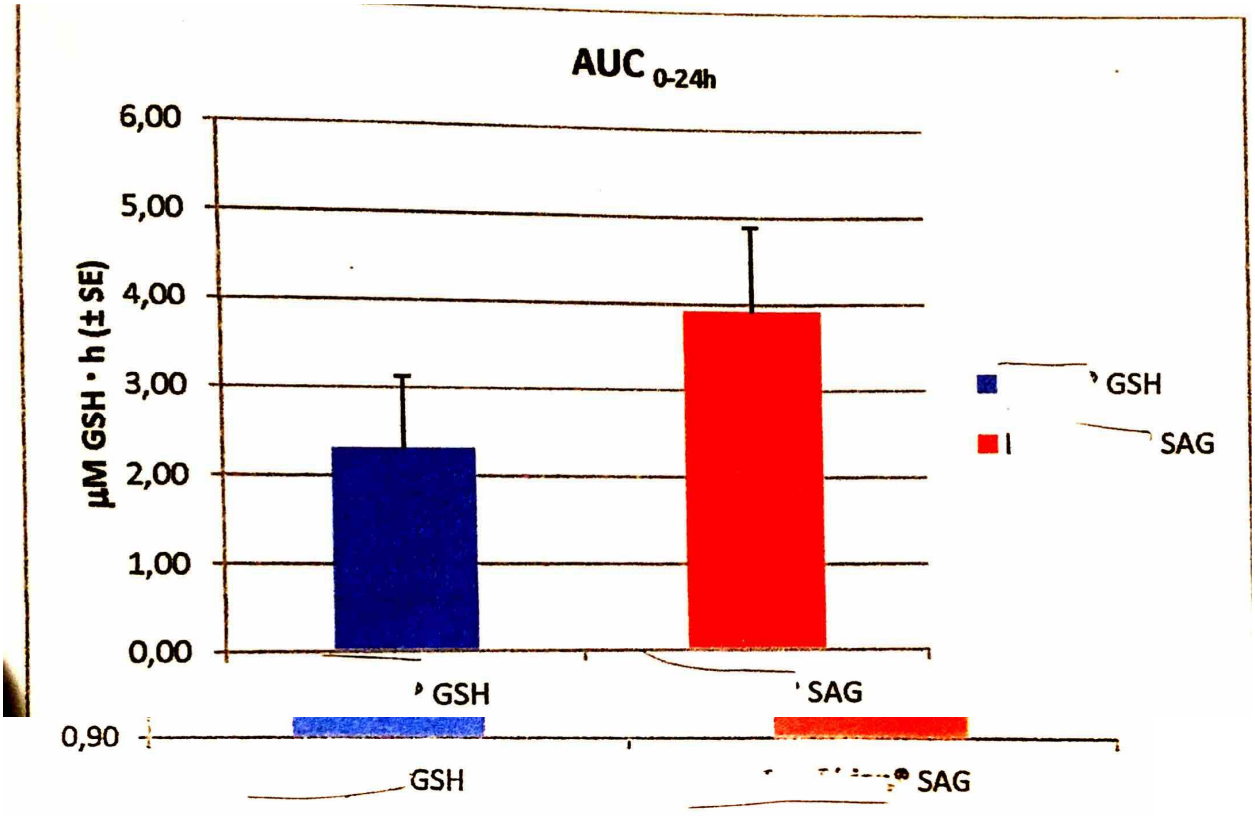


Figure 3- Final AUC (AUC_{0-24}) in healthy volunteers receiving SAG or GSH oral supplementation. AUC values are expresses in uMol/l h(+/- SE).

GSH/G_{tot} ratio in RBC was calculated, and SAG supplementation determined a higher value than reference GSH at 24 hours from administration. In Figure 5 normalized versus basal values are reported for both supplementations.
This ratio is considered an index of GSH status, cellular health and of the anti-oxidant power in the organism. Noteworthy, GSH/G_{tot} ratio is fairly stable under basal conditions and its variations following antioxidant administration may serve to assess potential therapeutic efficacy in preserving cellular redox potential(Owen 2010). IN our study, GSH/G_{tot} ratio increased significantly from basal level ($p< 0.01$) in subjects supplemented with SAG, up to 24 hours after supplementation, while remaining unchanged in the group supplemented with GSH.

Figure 4- GSH/G_{tot} ratio in RBC of healthy volunteers following SAG or GSH supplementation-normalized values at 24 h from dosing.

The safety and tolerability profile of SAG powder was excellent. In particular, only 3 unrelated adverse events were reported. No significant change in subject's baseline

conditions (vital signs, BW, clinical laboratory assays) was observed after single dose of either formulation.

CONCLUSIONS

GSH plasma concentrations showed an increase and a low peak followed by a decline up to 24 h post-dose, in a similar manner after single dose of SAG. GSH had significantly higher rate (C_{max}): 57.4% and extents (AUC_{0-24h}): 68.8%, of absorption in plasma after supplementation with SAG.

A greater GSH C_{max} may be helpful to counteract GSH consumption and enhance antioxidant defenses during particular stressful conditions such as infections, inflammation, physical exercise or postprandial phase. Conversely, a higher GSH AUC_{0-24h} may be considered as a marker of 24-hour tissue exposure to GSH pool and is reasonably correlated to enhanced anti-oxidant enzyme profile, natural killer cell activity, cellular and DNA protections from lipid per oxidation and S-glutathionylation.

The ratio between the reduced from and the total glutathione (GSH + GSSG) was significantly higher in the subjects that received a single dose of SAG at long term determination (24 h after dosing). This is an important pharmacodynamic parameter influenced by SAG treatment indicating a meaningful enhancing effect of the anti-oxidant bodily reserve. A long term and significative increase of the parameter has to be considered a good result, provided that the GSH/G_{tot} ratio is a very stable parameter not easily modified by any treatment in living organisms. This ration presents a diurnal variation (Blanco 2007) and is altered by dietary fat intake (Perez-Herrera 2013).

PUBLICATION

Methodologies and procedures utilized in this trial are reported in a detailed GCP compliant report describing volunteer enrollment, inclusion and exclusion criteria, biochemical methods for GSH and SAG determination in plasma and RBC, safety markers evaluation and reporting the informed consent form, quality assurance statements, statistical procedures and investigator's curricula (CROSS RESEARCH study report CRO-PK-16-310).

NOTE ABOUT SCIENTIFIC COLLABORATION REFERENCES

CROSS RESEARCH SA, who performed this trial, an independent full-service Contract Research Organization (CRO) based in Arzo, Switzerland, provides Customers with services in the early clinical development of drugs or nutraceutical products for either out licensing or further development: Phase I- Phase III. CROSS RESEARCH has deep knowledge of different and specific clinical areas. CROSS RESEARCH services are devoted to national and multi-national pharmaceutical or biotech companies. Since 1998 CROSS RESEARCH has delivered 60 scientific publications.

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