Liposomal glutathione demonstrates enhanced cellular uptake in human cells and works synergistically with Liposomal PureWay-C® to enhance cell viability.

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Introduction

Glutathione (GSH) is considered the master antioxidant and is ubiquitous in the body found in all tissues, cells and cellular compartments (1). Energy capture by respiration of oxygen occurs in all cells and involves oxidation of glucose metabolites for energy, however respiration also leads to the oxidation of cellular molecules such as lipids, proteins and DNA leading to damaged membranes, toxicities and cancers (1- 5). As the master antioxidant, glutathione scavanges or neutralizes oxidants (reactive oxygen species) by binding to them as they form making them water soluble before these reactive oxygen species (ROS) bind and damage cellular membranes, proteins, and DNA (6 - 8). Further, glutathione plays an important role in the detoxification functions of the liver P450-monooxygenase system. Glutathione scanvanges ROS metabolites of foreign pollutants, toxins and pharmaceuticals generated by the P450-monoxygenase (8, 9). Indeed, increasing liver glutathione levels has been shown to protect patients from toxic and lethal doses of acetaminophen, alcohol and other drugs by quickly binding to and making the metabolites of toxins water souble and suitable for excretion (6-10).

Due to the wide ranging benefits of glutathione, people have tried to boost cellular levels through dietary ingestion, however, glutathione is easially degraded in the gut and not well absorbed (11). Glutathione is a hydrophillic tripeptide made of the three amino acids; glutamate, glycine and cysteine (12). All three amino acids are transported into cells and while glutamate and glycine are abundantly available in the diet, cysteine can be somewhat more limited (11 – 13). Further, glutathione transporters do not bring glutathione into cells across the membrane intact and instead have peptidase activity that breaksdown exogeous glutathione into the three component amino acids which the cell must reassemble into glutathione in the cytosol (14). Indeed, supplementation with glutathione and the cysteine precursor, N-acetyl cycteine, both boost cellular glutathione synthesis and ultimately cellular glutathione levels (15). N-acetyl cysteine treatment is used to boost liver glutathione leveles and prevent liver damage after ingestion of toxins (15, 16). Further, N-acetyl cysteine is a popular supplement because it can get into cells and boost cellular glutathione levels and provide health benefits just as effectively as supplementing with glutathione (15 – 17). N-acetyl cycteine and glutathione protection of cells from toxins and oxidative stress is best demostrated by the ability of glutathione to protect mamaliamn cell cultures from hydrogen peroxide toxicity. If the glutathione transport system could be bypassed and glutathione could be delivered intact to cells this enhanced activity could be demonstrated in cell cultures by enhanced protection from hydrogen peroxide toxicity.

Liposomes are small phospholipid lamallae with hydrophyllic compartments that can fuse with cells and deliver contents directly and rapidly by diffusion and bypass the need for cellular transport systems (18, 19). In the case of glutathione, liposomal delivery to cells bypasses the need to re-synthesize the glutathione in the cytosol (10 – 15). Indeed, here we show that LiposoMax™ Liposomal Glutathione® prepared by One Innovation's LiposoMax™ Technology enters cells more quickly and subsequently protects human endothelial cells from hydrogen peroxide toxicity in agreement with the literature (20). Further, when combined with Liposomal PureWay-C®, an addative effect was seen beyond that with glutathione alone suggesting two different mechanisms of antioxidant protection (21).

Methods and Results

Liposomal Glutathione demonstrates enhanced uptake into human epithelial cells: The huma human kidney 786-O epithelial cell line was maintained in DMEM containing 10% FBS and grown in a water-jacketed incubator with 5% CO2 and at 37oC. In order to measure cellular uptake of glutathione, human kidney epithelial cells (786-O) were harvested by tyrpisinization, the trypsin was neutralized with 10% FBS and then the cells were washed three times with PBS using centrifugation at 1000 rpm. Cells were then seeded at 0.5 x 10^6 cells/well in wells of 12 well tissue cluster plates (one plate for each time point) in 0.5 ml of PBS containing 300 μM buthione sulfoximine (BSO) and incubated for one hour at 37 ^oC in a water-jacketed CO_2 incubator. After one hour the BSO was rinsed out of each well three times using PBS by aspiration and 0.5 ml fresh PBS was applied to each well along with 10 mM liposomal GSH, 10 mM GSH, or no addition. An aliquot of the harvested cells were kept in normal culture medium (DMEM with 10% FBS) and were never starved or GSH-depleted with BSO (never depleted). Immediately after addition of liposomal GSH and GSH (time 0) and after 1, 2, 3 or 4 hours, all treatment groups, including never depleted, were assayed for total GSH content, by rinsing each well three times by aspiration with PBS and lysing the cell layer only (no medium or PBS) by placing the 24 well plate for three cycles of freeze-thaw at -80 oC. After three freez-thaw cycles, the total cellular GSH was measured using the Ellmans reaction directly in each well using the reduced GSH assay kit from InVitroGen and measuring OD at 405 nm.

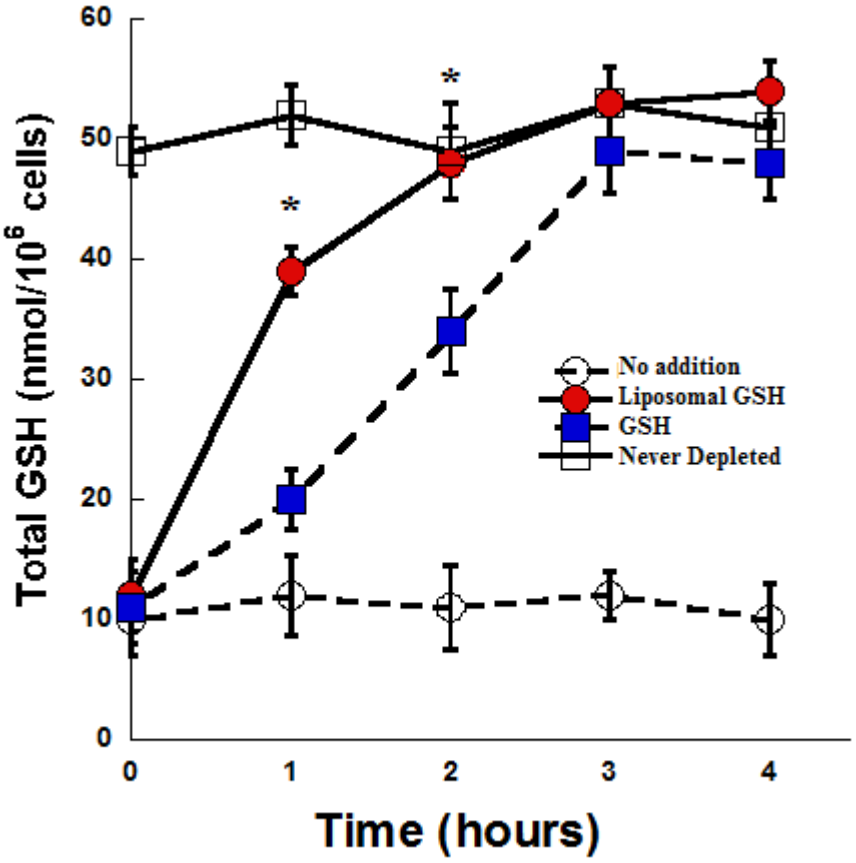

Figure 1. LiposoMax™ Liposomal Glutathione® restores depleted cellular glutathione levels at twice the rate as nonliposomal glutathione. 786-O cell glutathione levels were depleted with BSO and then restored with 10 mM nonliposomal or LiposoMax™ Liposomal Glutathione® . The asterisks shows that within one hour and continuing to hour two, LiposoMax™ Liposomal Glutathione® is more rapidly restoring cellular glutathione levels comepare to nonliposomal glutathione to a statistically significant extent ($p \leq 0.05$)

Table 1. LiposoMax™ Liposomal Glutathione® restores cellular glutathione levels better than nonliposomal glutahione

	Cell Treatment			
	Never Depleted	**GSH**	**LipoGSH**	**No Addition**
AUC:	912	618	780	188
Tmax:	1 hr	3 hr	3hr	1hr
Cmax:	52 mM	49 mM	51 mM	12 mM

Data are taken from figure 1. AUC is area under the curve, Tmax is the time to achieve the maximum concentration and Cmax is the maximum concentration achieved.

LiposoMax™ Liposomal Glutathione® demonstrates enhanced protection from oxidative damage: In order to test the ability of LiposoMax™ Liposomal Glutathione® alone and in combination with Liposomal PureWay-C® to protect endothelial cells from hydrogen peroxide toxicity, cell viability was measured using the MTT assay. Cells were harvested and washed free of serum and resuspended in DMEM that was both phenol-free and serum-free at then seeded at 0.5 x 10^5 cells/well in wells of 12-well tissue cluster plates. At the time of seeding cells were either untreated or treated with nonliposomal glutathione, LiposoMax™ Liposomal Glutathione®, Liposomal PureWay-C® and LiposoMax™ Liposomal Glutathione® in combination with Liposomal PureWay-C® for two hours at 37^0C. After two hours cells were treated with 300 μM hydrogen peroxide for three hours with the glutathione and vitamin C treatments pretreatments continuing to be present after which the medium was aspirated and 200 ml of serum-free and phenol red-free DMEM was added to each well that the MTT assay was performed to determine the percent viable cells. Cells were lysed by three cycles of freeze-thaw at -80^{o}C as described above and the MTT assay was performed directly in the wells, and the OD was read at 570 nm and the total glutathione in the 0.5 x 10^5 cells was determined and converted to nmol/10^6 cells. The results show in Figure 2 that 300 μM hydrogen peroxide will reduce 786-O cell viability to approximately 20%. When the cells are pretreated/treated with glutathione the cell viability is reduced to only 60%, however with pretreatment/treatment with LiposoMax™ Liposomal Glutathione® 85% viability is observed. Interestingly, when combined with Liposomal PureWay-C®, cell viability is returned to nearly 100% in the presence of 300 μM hydrogen peroxide. The data suggest not only that the liposomal forms of Liposomal PureWay-C® and LiposoMax™ Liposomal Glutathione® are able to work through two mechanisms in concert with one another to achieve a maximal benefit neither one alone can (Figure 2).

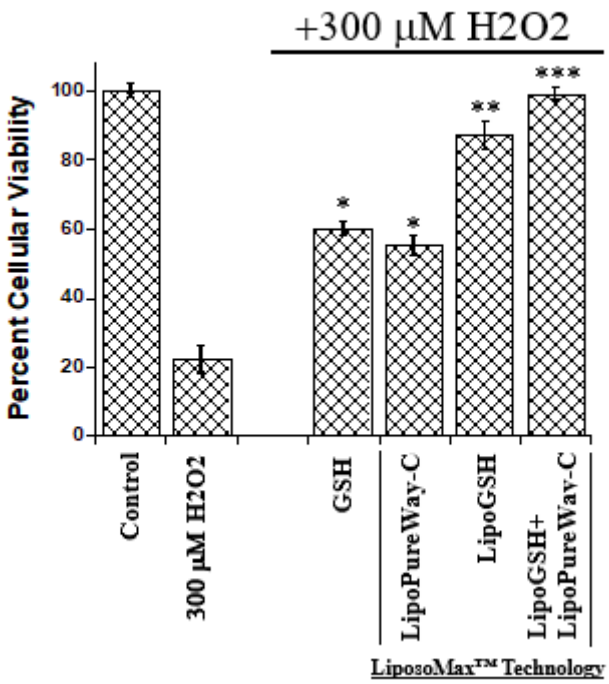

Figure 2. LiposoMax™ Liposomal Glutathione® protects human endothelial cells better than nonliposomal glutathione and acts synergistically with Liposomal PureWay-C® for a greater protective benefit. Cells were pretreated with 10 mM liposomal or nonliposomal glutathione or 5 mM Liposomal PureWay-C® for two hours and then treated with 300 µM hydrogen peroxide for an additional three hours after which the cells were lysed and the MTT assay was performed at the OD was read at 570 nm and percent cell viability was determined.

Discussion and Conclusions

These data show that LiposoMax™ Liposomal Glutathione® is more readily absorbed into cells and restores depleted pools of glutathione more rapidly compared to nonliposomal glutathione. These data also show that LiposoMax™ Liposomal Glutathione® is more able to protect cells from oxidative stress compared to nonliposomal GSH. Lastly, like LiposoMax™ Liposomal Glutathione®, Liposomal PureWay-C® also protects cells from oxidative stress and synergizes with LiposoMax™ Liposomal Glutathione to give a greater effect with both combined than either can achieve maximally alone. LiposoMax™ Liposomal Glutathione and Liposomal PureWay-C® have a chemical interplay at the cellular level for antioxidant/free radical scavenging actions. They regenerate each other during a process called the Redox Couple. This process and the historical research discovering this relationship, where a decline in one can be regenerated by the action of the other. By combining them in a supplement, this provides both halves of this cascade simultaneously, for optimal activity at the cellular level. Therefore, combinations of LiposoMax™ Liposomal Glutathione® and Liposomal PureWay-C® are likely to prove a uniquely effective antioxidant combination.

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