HEPASIL DTX™ INCREASES BOTH ANTIOXIDANT AND DETOXIFICATION CAPACITY BY BOOSTING GLUTATHIONE AND VITAMIN C LEVELS

Brian Dixon, Adam Dern, Erik Schneider, Toni McKinnon, Tim Wood, John Cuomo
USANA Health Sciences, Inc; 3838 West Parkway Blvd; Salt Lake City, UT 84120

INTRODUCTION

The liver is the major detoxifying organ in the body as it is the primary site of Phase I and Phase II detoxification enzymes. Phase I detoxification enzymes consist of the cytochrome P450 family of enzymes. These enzymes serve as a first line of defense by first oxidizing toxins. Unfortunately, this initial oxidation can make some compounds more toxic. Phase II detoxification enzymes can further modify Phase I metabolites by conjugating them with water soluble molecules, ultimately making them less toxic and able to be excreted from the body. Because many Phase I metabolites can go on to damage biomolecules if not acted upon by Phase II enzymes, it is essential that this detoxification system remain in a balance favoring Phase II reactions. As such, it is imperative that the levels and efficiency of Phase II detoxification enzymes, as well as the pool of conjugation substrates, remain high to help maintain optimal liver detoxification capacity. Two important antioxidant and conjugation substrates involved in these detoxification pathways include glutathione and vitamin C.

Glutathione is the most abundant antioxidant in our bodies and plays a major role in the detoxification process. Unfortunately, glutathione is inefficiently absorbed from the diet and must be synthesized in the body by specific Phase II enzymes. Under normal conditions, these enzymes produce low levels of glutathione; however, under times of toxicological insult, the body increases production of glutathione by upregulating the Phase II enzymatic machinery. Interestingly, a number of phytochemicals including broccoli extract, milk-thistle, and alpha-lipoic acid have also been shown to upregulate Phase II enzymes including those that synthesize glutathione[1-5].

Vitamin C is an important first line of defense in protecting biomolecules from oxidative damage, especially in circulation. Moreover, recent scientific evidence suggests that vitamin C may also play a role in the removal of toxins[12-17]. However, the concentration of vitamin C in the body is tightly regulated by intestinal absorption from the diet and recycling by the kidneys[18,19]. Because of this tight regulation, it had previously been thought that circulating vitamin C levels could only be increased by supplementing with vitamin C. However, it has recently been shown that both circulating and tissue levels of vitamin C can be increased by certain phytochemicals even in the absence of vitamin C supplementation[18-19].

While individual nutrients and phytochemicals have been shown to increase both vitamin C and glutathione levels, to date, it has never been shown that the combination of these nutrients and phytochemicals can synergistically boost both glutathione and vitamin C levels, simultaneously. Thus, the purpose of this study was to assess the effectiveness of Hepasil DTX™ in increasing both glutathione and vitamin C levels.

MATERIALS AND METHODS

Study Design, Subjects, and Materials

This was a double-blind, placebo controlled study. Fifteen healthy volunteers between the ages of 25 and 45, completed the study (N=7 for the treatment group and N=8 for the placebo group). Hepasil DTX and placebo were provided by USANA Health Sciences, Inc and subjects instructed to take the recommended dose of 3 tablets per day.

Determination of Plasma Glutathione and Vitamin C

Glutathione analytes were separated by injecting 1uL of the prepared sample into an Agilent (Series 1200) HPLC using a Phenomenex C18 column. Method conditions were: 0-7 minutes at 0.5 mL/min 0.03% formic acid in water and 8-15 minutes 0.5 mL/min 2-propanol. Both reduced glutathione (GSH) and oxidized glutathione (GSSG) were detected on an Agilent tandem mass spectrometer (Series 6410, Model G6410A) using an electrospray (ES) source. Glutathione concentrations were determined relative to authentic standards and expressed as total soluble glutathione (GSH + 2GSSG) relative to placebo group.

Vitamin C was separated by injecting 7uL of the prepared sample into an (Agilent Series 1200) HPLC using an Agilent C18 column. Method conditions were: 0-5 mL/min 0.03% formic acid in water and 8-15 minutes 0.5 mL/min 2-propanol. Vitamin C was detected on a Hewlett-Packard UV detector (254nM; Series 1050). Vitamin C concentrations were determined relative to authentic standards and expressed relative to the placebo group.

RESULTS

Glutathione

- **Hepasil DTX acutely, chronically, and acute-on-chronically increased plasma total glutathione levels (Figure 3).**
- **Hepasil DTX increased plasma glutathione 2 hours following the first treatment and significantly increased plasma glutathione 8 hours after supplementation (p<0.05; Figure 3A).**
- **A chronic 18.3% increase in plasma glutathione was observed, but did not reach statistical significance (Figure 3B).**
- **Hepasil DTX significantly increased plasma glutathione 8 hours after supplementation, during the Acute-on-Chronic Phase. (p<0.05; Figure 3C).**
- **Plasma glutathione levels increased 74% by the end of the study.**

Experimental Design

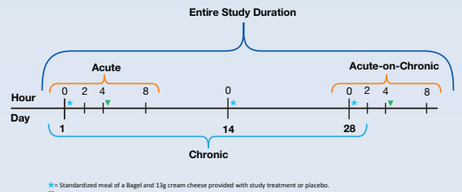

Figure 1. Experimental design. The current study was broken down into three Phases: Acute, Chronic, and Acute-on-Chronic. During the Acute Phase, effects of Hepasil DTX were monitored during the first eight hours following the intial treatment (Day 1, hours 0-8). During the Chronic Phase, changes in baseline effects were monitored over the 28-day study (Day 1, hour 0 versus Day 28, hour 0). The Acute-on-Chronic Phase was designed to monitored Acute effects that occurred in addition to those seen Chronically (Day 28, hours 0-8).

Glutathione and Vitamin C are Involved in Detoxification

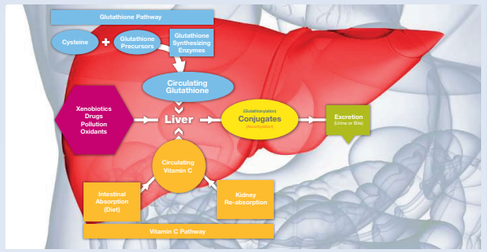

Figure 2. Glutathione and vitamin C are involved in detoxification reactions. In the liver, glutathione synthesizing enzymes combine cysteine with other glutathione precursors to ultimately form glutathione (blue pathway). Circulating vitamin C levels are determined by intestinal absorption (diet) and recycling in the kidneys (orange pathway). Toxins in the body can be conjugated with either glutathione or vitamin C, ultimately rendering them less toxic and able to be excreted in the urine or bile.

Hepasil DTX Increases Circulating Glutathione Levels

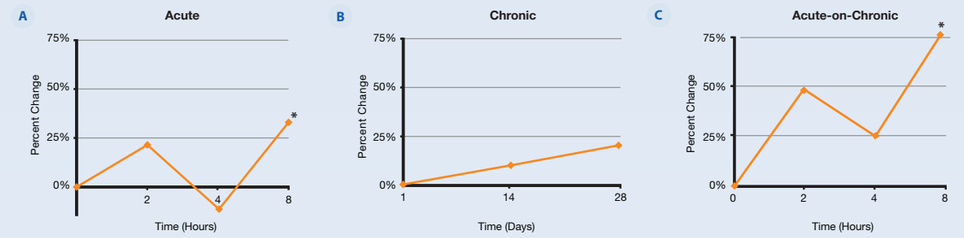

Figure 3. Hepasil DTX increases plasma glutathione levels. (A) Acute Phase changes in plasma glutathione. (B) Chronic Phase changes in plasma glutathione. (C) Acute-on-Chronic Phase changes in plasma glutathione. Data represents placebo-adjusted means. Astericks (*) denote statistical significance versus the placebo group (p<0.05).

Hepasil DTX Increases Circulating Vitamin C Levels

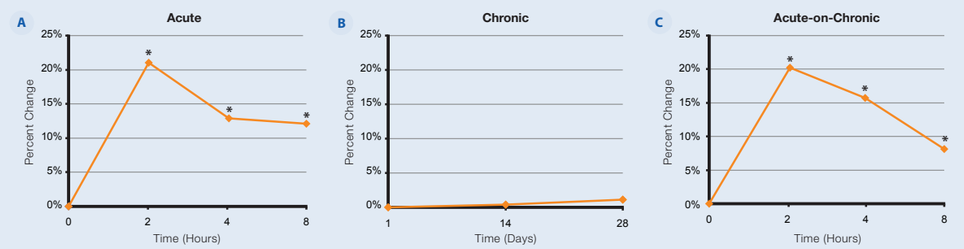

Figure 4. Hepasil DTX increases plasma vitamin C levels. (A) Acute Phase changes in plasma vitamin C. (B) Chronic Phase changes in plasma vitamin C. (C) Acute-on-Chronic Phase changes in plasma vitamin C. Data represents placebo-adjusted means. Astericks (*) denote statistical significance versus the placebo group (p<0.05).

RESULTS (Cont.)

Vitamin C

- **Hepasil DTX significantly increased plasma vitamin C as soon as 2 hours following the first treatment and was maintained during the entire Acute Phase (0-8 hour time points; p<0.05; Figure 3A).**
- **A chronic increase in vitamin C was seen, but did not reach statistical significance (Figure 3B).**
- **Hepasil DTX significantly increased plasma vitamin C concentrations during the Acute-on-Chronic Phase of the study (p<0.05; Figure 3C).**

CONCLUSIONS/DISCUSSION

The purpose of this study was to assess the effectiveness of Hepasil DTX in increasing both antioxidant and detoxification capacity as measured by plasma glutathione and vitamin C levels. The study design was organized into three Phases: Acute, Chronic, and Acute-on-Chronic (Figure 1). During the Acute Phase, the effects of Hepasil DTX were monitored during the first eight hours following the initial treatment (Day 1, hour 0). The Chronic Phase measured the effect of Hepasil DTX relative to baseline glutathione and vitamin C levels after 28 days of supplementation. The Acute-on-Chronic phase was designed to monitor any Acute effects that occurred in addition to the 28 day Chronic Phase. Our results show that both circulating glutathione and vitamin C levels increased following treatments with Hepasil DTX and to our knowledge is the first time a combination of essential nutrients and phytochemicals have been shown to increase both plasma vitamin C and glutathione levels, simultaneously.

Glutathione plays a major role in the overall antioxidant network and also acts as an intermediate/conjugate in the detoxification process. Interestingly, we found a biphasic response in both the Acute and Acute-on-Chronic Phases of the study (see Figure 3). This can be explained by two distinct mechanisms. The first mechanism is likely due to the N-acetyl-L-cysteine provided by Hepasil DTX. Cysteine is the rate limiting substrate in glutathione synthesis and has been shown that providing cysteine alone will increase glutathione levels. As such, we found a concurrent significant increase in plasma cysteine levels following treatment with Hepasil DTX (data not shown). The second mechanism is likely due to an increase in Phase II glutathione synthesizing enzymes over time. Alpha-lipoic acid, milk thistle, and broccoli extract have been shown to up-regulate Phase II enzymes, and likely accounts for the steady increase in glutathione observed during this study. The large Acute-on-Chronic increase in glutathione, relative to the Acute Phase, is likely due to this steady increase in glutathione over the course of the study in addition to the effects seen during the Acute Phase (p<0.05; Figure 3).

Vitamin C is a well known antioxidant but has also been recently shown to act as an important substrate for detoxification reactions. Relatively recent studies have shown that vitamin C binds to, and likely helps facilitate the removal of toxins. The increase found in vitamin C levels is intriguing because the current Hepasil DTX formulation does not contain vitamin C. Moreover, study subjects were neither taking any nutritional supplements prior to enrollment in the study nor was there vitamin C in the provided meal (nutritional data not shown). While this result is surprising, there is a scientific rationale for this effect. It has been shown that supplementation with phytochemicals can increase vitamin C transporter levels and ultimately increase vitamin C levels even in the absence of vitamin C supplementation. To this end, studies are currently underway to examine this effect further.

Taken together, these results show that the recommended dosage of Hepasil DTX™ is able to boost both glutathione and vitamin C levels—essential molecules utilized for both antioxidant defenses and substrates for endogenous detoxification reactions.

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