Evaluation of bioavailability of antioxidants in EpiCor® at the cellular level and in clinical studies, using the cell-based antioxidant protection of erythrocytes (CAP-e) bioassay.

Kimberlee A. Redman, Steve G. Carter, Kathleen F. Benson, Gitte S. Jensen.
NIS Labs, 1437 Esplanade Avenue, Klamath Falls Oregon.

Purpose of the study

The objective of this study was to evaluate EpiCor® antioxidant bioavailability. One aspect of bioavailability is to examine whether compounds are capable of crossing the cell membrane and entering live cells. Performing cell-based testing for antioxidant protection involves the dynamics of living cells, their enzymes, and membrane properties.

An improvement in serum antioxidant protection capacity after ingestion of a food or natural product may reflect the content of easily absorbed antioxidants existing in the native product, as well as antioxidant compounds released or generated as a result of normal digestive processes. Unknown metabolites may contribute significantly to the *in vivo* antioxidant protection. By using the CAP-e assay to evaluate the serum capacity for antioxidant protection, such metabolites do not need to be chemically identified; as long as they are able to enter and protect living cells from oxidative damage they contribute to the CAP-e results. This is in contrast to other types of bioavailability studies, where chemical analysis is performed on serum to examine the sample for metabolites known to have come from the digestion of a natural product.

EpiCor®

EpiCor® is an immunogenic metabolite-rich product from *Saccharomyces cerevisiae*. Based on its high ORAC value1 it was of interest to evaluate whether antioxidants in the product provide a biologically meaningful protection of cells in vitro and of humans in vivo. EpiCor® has anti-oxidant and anti-inflammatory properties in vitro [Honzel], and has been studied in several clinical studies. Consumption of EpiCor® supports mucosal immune protection by increasing secretory IgA production [3-5], and reduces incidence and duration of cold/flu [4] and allergic rhinitis [5].

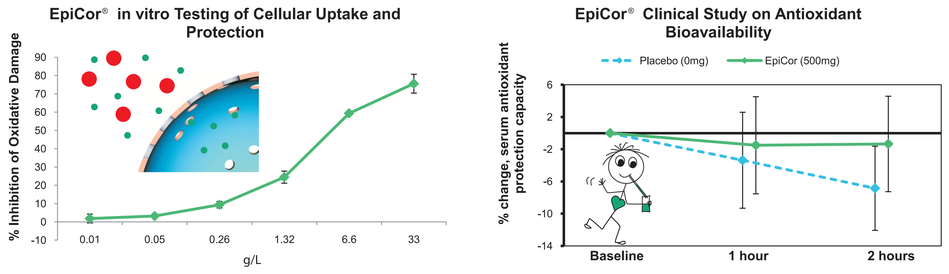

Figure 1. A) The *in vitro* CAP-e testing showed that EpiCor® contained antioxidants capable of entering into and protecting live cells from oxidative damage in a dose-dependent manner. B) EpiCor® consumption provided statistically significant improvement in serum antioxidant capacity ($P<0.04$). This was tested using a double-blinded randomized cross-over design in 11 healthy people. On test days where Placebo was consumed, a decline in serum antioxidant protective capacity was observed over the 2 hours where blood draws were taken (blue dashed line). This reflects normal metabolic processes resulting in depletion of antioxidant status. On test days where EpiCor® was consumed, no reduction was seen (green line), indicating that the consumption of a single 500 mg dose of EpiCor® provided sufficient bioavailable antioxidant to counteract the depletion seen if no antioxidants were consumed.

Method for testing of antioxidant bioavailability

The ***CAP-e*** cell-based antioxidant protection assay is a useful step in evaluation of foods, natural products, and purified compounds with antioxidant potential. The ***CAP-e*** assay is used for testing of both the *in vitro* and *in vivo* antioxidant protection capacity of the high-metabolite nutritional yeast product EpiCor®.

The ***e*** in ***CAP-e***: The importance of the ***CAP-e*** assay is the choice of cells. The ***CAP-e*** cell-based bioassay uses ***erythrocytes***, or red blood cells. There is a growing recognition of the role of RBC above and beyond the oxygen-transporting function. The ability of RBC to scavenge reactive oxygen and nitrogen species represents a direct antioxidant and anti-inflammatory protection of the body. However, in disease states, oxidatively damaged RBC are more prone to aggregate and adhere to the vessel walls, thus contributing to compromised circulation and vascular damage.
[diagram of method with umbrella]

In vitro: The CAP-e testing on EpiCor® was done by applying an aqueous extract of the product to the CAP-e bioassay, where live cells are exposed to product, un-absorbed antioxidants removed from the cells, and oxidative stress induced to the cells; a reporter dye becomes fluorescent upon oxidative damage, and allows quantification of damage versus protection.

In vivo: The antioxidant protection provided by EpiCor® was evaluated both the CAP-e assay *in vitro* and *in vivo* antioxidant uptake in a randomized, double-blind, placebo-controlled cross over study including 11 healthy volunteers. The antioxidant uptake was assessed by testing changes in serum antioxidant protection capacity before and after consumption. Each study participant, recruited upon informed consent, came to the clinic on two separate days, at least two weeks apart. After a baseline blood draw, one dose of 500 mg of either placebo or EpiCor® was fed to each person. Two more blood draws were performed, at 1 and 2 hours after consumption, respectively. Blood was drawn into serum separator tubes, serum was harvested and frozen at -80˚C until testing in the CAP-e assay.
The serum samples were tested in the accelerated CAP-e assay using fresh erythrocytes, where serum was added instead of an aqueous extract of the natural product. Each serum sample was tested in quadruplicate. The data from each personís baseline serum sample was indicative of this personís antioxidant protection capacity before consumption of either EpiCor® or placebo. The level of oxidative damage in samples where cells were treated with post-consumption serum samples was compared to the oxidative damage in samples exposed to baseline serum. Each personís start level was set to “0” and the relative change (increase, decrease) in serum antioxidant protection capacity was calculated.

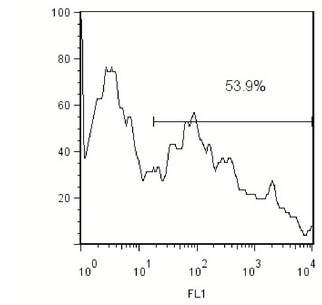

Figure 2. PBMC were incubated overnight for 18 hours and stained with AnnexinV-FITC for apoptotic effects and analyzed by flow cytometry. The figure above depicts the total apoptotic effect occurring during the incubation period. Upon analysis it was observed that 53.9% of the cells in the population were engaged in various stages of apoptosis, in the absence of EpiCor®.

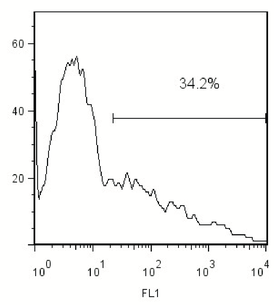

Figure 3. EpiCor® (0.4g/L) was incubated with PBMC during the course of the 18 hour incubation. After analysis it was determined that EpiCor® reduced the total apoptotic effects of PBMC from 53.9% (UT) down to 34.2% (EpiCor® 0.4g/L), this data was found to be statistically significant ($P<0.04$).

Conclusion

EpiCor® contains bioavailable antioxidants. A single daily dose has sufficient amounts to provide biologically important protective functions, both at the cellular level in vitro, and after consumption in vivo.

The antioxidants and anti-inflammatory compounds in EpiCor® have significant downstream biological effects, focused around protection from oxidative stress-induced damage and cell death, and reduction of inflammation.

EpiCor® Quality Assurance

EpiCor® undergoes CAP-e testing as part of quality assurance, to ensure that similar levels of bioavailable antioxidants are present in all lots.

References

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Acknowledgements

This study was conducted at NIS Labs, an independent contract research laboratory specializing in natural products testing. The work was sponsored in part by NIS Labs, and in part by Embria Health Sciences Inc.

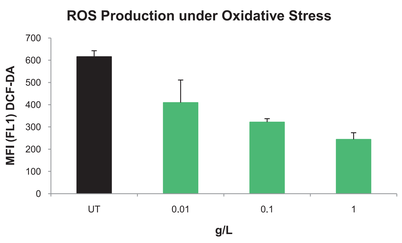

Figure 4. When EpiCor® was used to evaluate the Reactive Oxygen Species (ROS) production of PMN cells after a 20 minute incubation, it was observed that EpiCor® reduced the production of ROS in a dose dependent manner.

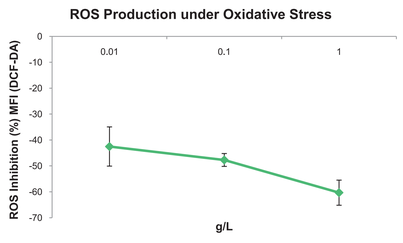

Figure 5. The figure above represents the percent inhibition of the production of ROS by PMN's. It was observed that the lowest concentration of EpiCor® inhibited ROS production past 40% while the higher concentrations of EpiCor® were of a greater value. All data points were found to be statistically significant ($P<0.05$).

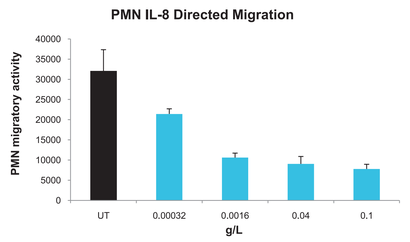

Figure 6. When EpiCor® was incubated overnight with PMNís it was observed that EpiCor® inhibited the migration of the cells towards the chemokine Interleukin-8 (IL-8). All dilutions tested elicited an anti-inflammatory effect and the data was found to be statistically significant ($P<0.05$) on the 3 highest concentrations.

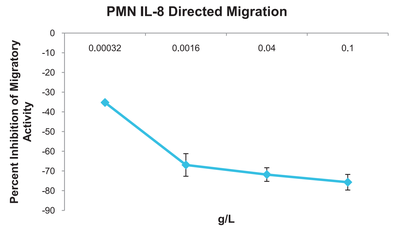

Figure 7. The above figure shows the inhibitory effect of EpiCor® calculated as percent inhibition of the migratory activity of PMN cells towards the inflammatory chemokine Interleukin-8.

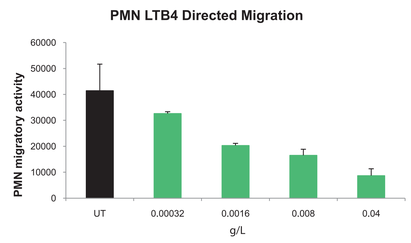

Figure 8. EpiCor® was shown to inhibit the migratory pattern of PMN cells towards the inflammatory chemoattractant Leukotriene B4 (LTB4). All dilutions of EpiCor® tested were shown to inhibit the migratory effect and the data was found to be statistically significant at the two highest doses.

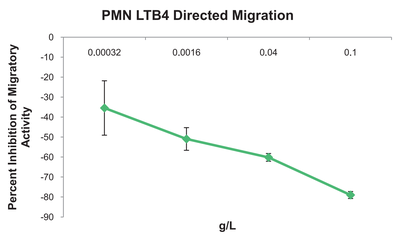

Figure 9. The above figure shows the inhibitory effect of EpiCor® calculated as percent inhibition of the migratory activity of PMN cells towards inflammatory chemokine Leukotriene B4 (LTB4).