Report for ASEA on Experimental Results – In Vitro Antioxidant Enhancement and Oxidative Stress Reduction

2009

Purpose:

This document reports to ASEA the results of the in vitro tests run from February to March, 2009. Live cells in culture dishes were exposed to ASEA and the bioactivity regarding antioxidant activity of Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) as well as the increase in the native production of these antioxidants inside cells was measured. In addition, tests were run to determine if oxidative stress was reduced in the cells and that cell viability was enhanced by exposure to ASEA.

Experimental Methods for Antioxidant Activity:

Cells were cultured in several dishes with a bovine serum growth medium. As a primary measure, mouse epithelial-like cells were cultured (these cells react similarly to human cells) and later human endothelial cells were used to obtain relevant quantitative results.

In the antioxidant enhancement tests, some of the cell cultures were exposed to ASEA and others cultures to the same amount of an inert phosphate buffered saline solution (PBS). The antioxidant activity of the cells in each was measured by a purchased kit, Array Design Stressgen kit (#900-158 for GPx activity and #900-157 for SOD activity). The chemical reagents inside these kits measure the ability of the antioxidants in the cell extracts to reduce oxidant activity that occurs naturally when certain oxidizing biological chemicals are added.

Due to the fact that some of the reactive molecules in ASEA might react and interfere with some of the chemical agents in these kits, several preliminary experiments were done to examine the accuracy of the results based against known standards of antioxidant activity and adjustments were made.

Results of Antioxidant Activity Tests:

The first results obtained showed large, well-defined effects. The cell extracts exposed to ASEA exhibited eight (8) times the antioxidant efficiency for GPx than those exposed to the inert PBS. The SOD antioxidant efficiency was slightly less, with about 5 times enhancements in efficiency. Of note, this efficiency was evident especially at low level concentrations of ASEA, tested down to 2.5% of full strength. Increasing the concentration of ASEA at high concentrations did not notably increase the antioxidant efficiency; thus there appears to be a very low saturation threshold at low concentrations of GPx. More experimentation would need to be done at very low concentrations of ASEA in order to understand the concentration dependence fully.

It is safe to say that at least a 500% improvement in the overall antioxidant efficiency was seen during these preliminary in vitro tests due to ASEA exposure.

Experimental Methods for Antioxidant Up-regulation:

In these experiments, some cultured human endothelial cells were exposed to ASEA and others only to an inert phosphate buffer solution (PBS). Standard Western Blot analysis on all cells was done to determine if exposure to ASEA activated the nucleus to call for increased production of antioxidants, such as GPx. The concentrations of transcription factors (messengers) in the nucleus that call for up-regulation of antioxidants were also measured in human endothelial cells and compared to cells that had not been exposed to ASEA.

The movement of the transcription factors into the nucleus can be seen with certain dyes under a microscope and thus offers a way to see the call for up-regulation of antioxidants without some of the obstacles presented by the use of endothelial cells.

Since the production of antioxidants can also be up-regulated by exposure of the cells to certain low levels of inflammatory toxins, tests were done to make sure that ASEA was not provoking the cells to undergo this low-level inflammatory or toxic response.

Results for Antioxidant Up-Regulation

The results for these tests were extraordinary in several regards. First, there was a real up-regulation of anti-oxidant production in cells exposed to ASEA. This effect was temporary, lasting only about 120 minutes but was clearly visible. The most interesting result, however, is that exposure to ASEA at any concentration did not invoke the normal inflammatory transcription factor (NF-kappaB) and yet did invoke the antioxidant transcription factor (NRF2). Stimulating the production of antioxidants without stimulation of low-level inflammation is very rare and has stirred some interest in the scientific community.

The tests that measured the movement of transcription factors were controlled tests, they had “positive controls” that showed the test was working. For example, a small amount of a toxin that is known to cause the inflammatory response (movement of the NF-kappaB transcription factor) was tested side by side with the ASEA, a positive response was very clearly seen with the toxin and no response was seen with ASEA. With the antioxidant up-regulation transcription factor NRF2, positive movement of this transcription factor was seen in both ASEA and in the positive control. Hundreds of cells were observed in order to obtain these results.

These results were also verified by the Western Blot analysis showing clear responses in the increase of antioxidants upon exposure to ASEA relative to the saline control.