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A Non-Invasive Approach for Cataracts: Efficacy of Carnosine in the Treatment and Prevention of Crystallin Aggregation in vitro

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Abstract

This study investigated the effects of Carnosine on protein aggregation in cataract lenses of the eye using an in vitro model. Carnosine is a dipeptide of histidine and beta-alanine and has many powerful properties such as retarding cancer growth and preventing glycation. To simulate the protein aggregation in a cataract lens, Methylgloxal, a product known for causing advanced glycation end-products (AGE), was incubated with crystallin (CRY). A dialysis sac membrane was used to analyze the changes in the aggregated crystallin. Carnosine separates the aggregated crystallin by reducing the increased opacity of CRY, and Carnosine does not affect normal CRY. Carnosine has been shown to improve vision by partially reversing the development of the cataract, thus increasing the transmissivity of the lens to light. This research with Carnosine demonstrates that it is effective not only in preventing cataracts but also in treating them.

Introduction

Cataracts are the leading cause for blindness worldwide[1]. They are the clouding in the lenses of the eye and lead to a loss of vision. In third world countries where treatment is not as available and ready, cataracts lead to vision problems in almost 60% of the elderly[2]. One of the most prevalent causes for cataract formation is protein aggregation in the lens. As humans age proteins in the lens called crystallins clump together and cause blurriness and the formation of cataracts[3]. Cataracts may also be formed as a result of external damage such as debris or ultraviolet radiation (figure 1)[4]. After crystallin is exposed to harmful conditions such as oxidation and truncation, both the high and low molecular weight aggregates scatter the light coming into the lens, and lens opacification occurs[5,6]. This study presents results of using carnosine, a super-antioxidant, to prevent and treat crystallin aggregation[7].

Carnosine is an active antioxidant that can also reverse glycation and chelate divalent metal anions (figure 2). Furthermore, carnosine is known to reduce cloudiness in rat lenses that were exposed to guanidine, but products involving carnosine are not used due to their unknown efficacy and safety[9]. However, carnosine could be a powerful weapon in treating cataracts. In contrast to an elaborate eye surgery, a simple drop of carnosine might suffice to treat cataracts.

In this experiment, carnosine was tested against glycated crystallin in order to make a conclusion about its efficacy against treating cataracts. In order to examine the issue in an efficient manner, we wished to test the hypothesis that carnosine would disaggregate the glycated crystallin. A cataract was simulated through the process of adding methylglyoxal to the crystallin protein, causing glycation. Carnosine was added to the glycated crystallin (g-CRY) and CRY to see not only if carnosine will treat the clumped proteins but also if it would affect the normal proteins in cataracts. Quantitative results were gathered in the form of % opacity, which specifically showed how carnosine affected the different test groups of crystallin. Carnosine could treat the glycation or leave the glycated crystallin unaffected. We hypothesized that carnosine would not only treat the glycated protein but also prevent the process of glycation.

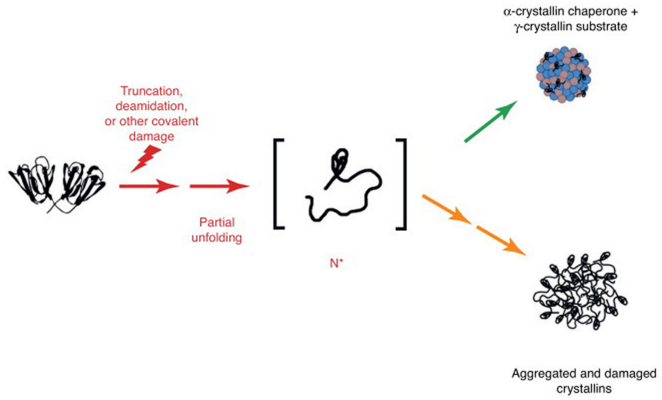

Figure 1. Accurate representation of the unfolding and aggregation of crystalline proteins in the lens. Due to external damage the crystallin proteins partially unfold, and polymerization occurs via domain swapping. In a young lens, crystalline proteins that have been damaged are put away in substrates (upper pathway), but as the lens ages and more substrates are gathered, the aggregated crystallin form[8].

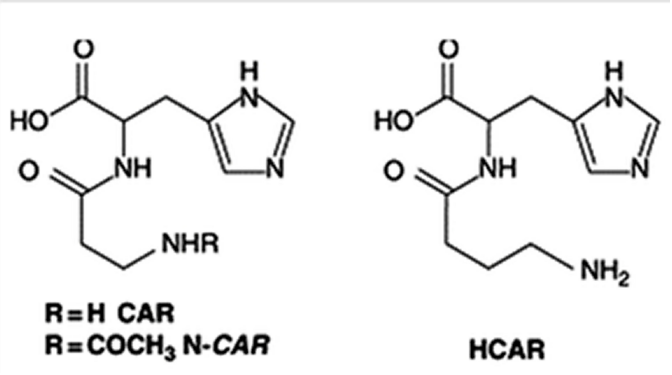

Figure 2. Molecular structures of different carnosine molecules: Carnosine (CAR), N-acetylcarnosine (N-CAR), and homocarnosine (HCAR)[10].

Materials and Methods

As this experiment was carried out using an in vitro model, the formation of the proteins in a cataract lens needed to be recreated. Methylglyoxal (Sigma Chemicals, St. Louis, MO) was incubated with CRY (from lens α-crystallin bought from Sigma Chemicals) to make glycated crystallin (g-CRY), which is highly similar to the protein arrangement in a cataract lens. The experiment was repeated three times under the same conditions. *Incubation conditions:* Various quantities of crystallin were incubated in a 0.1 M sodium phosphate buffer (available at lab) with different concentrations of methylglyoxal (MG) for 24 hours at 24–37* C prior to dialysis. This procedure was carried out in order to verify that the aggregated proteins could be analyzed under proper glycation. Aliquots were dialyzed against the 0.1 M sodium phosphate buffer using dialysis membranes. As a separate test, quantities of crystallin were first added to carnosine and put through the same process. Additionally, some of those aliquots with crystallin and carnosine (Sigma Chemicals) were incubated with MG, afterwards, and then dialyzed. The additional test was to see not only if Carnosine had any side effects on the normal protein but also if Carnosine could prevent glycation. The effects of carnosine on glycated crystallin (g-CRY) were examined as described below. *Finding the % Opacity:* The efficacy was determined by measuring the absorbance using a spectrophotometer at 350 nm. Aliquots containing 1 mg of CRY protein were incubated at room temperature. Aliquots were kept on ice for half an hour and centrifuged at 10,000 g for 2 minutes using a centrifuge. The pellet was resuspended in a 0.1 M Tris Buffer along with 10 M EDTA and 5 M Guanidine hydrochloride (Sigma Chemicals), and it was read at 350 nm using the spectrophotometer. The glycation-induced opacity was an indicator to measure the CRY aggregation. Opacity was calculated from measurements of light transmittance using a microplate reader. Data was normalized and transformed into percent opacity in transmittance. The measurements contained data for the normal CRY, the glycated CRY, the glycated CRY with carnosine, normal CRY with carnosine, and CRY with carnosine + MG.

Results

In this experiment, the % opacity was observed for normal crystallin (CRY), glycated crystallin (g-CRY), and the glycated crystallin with carnosine along with additional tests for crystallin with carnosine and crystallin with Carnosine + MG. Carnosine reversed the glycation induced increase in opacity levels (figure 3). Opacity levels initially increased following incubation with MG to 7.2%. When carnosine was added to these g-CRY samples the opacity levels decreased down to baseline levels, 6%. Carnosine promoted disaggregation and addition of carnosine had no effect on unmodified CRY. The control group with the glycated crystallin and the plain crystallin had average opacity numbers of 7.2% and 6.0%, respectively. The glycated crystallin with Carnosine had an opacity value of 6.1%. The standard deviation for CRY, g-CRY, g-CRY w/ Carnosine, CRY w/ Carnosine, and CRY w/Carnosine + MG data were 0.82, 0.67, 0.79, 0.85, and 0.73 respectively, indicating that the CRY w/ Carnosine data varied the most (figure 3).

An ANOVA test was carried out to determine the significance of the data. The null hypothesis for the ANOVA test was that the opacities of the different crystallin test groups should be the same. The ANOVA test found that the probability that the change in opacities was not merely due to chance. As the f-critical value was greater than the f-statistic with an alpha value of 0.05, the null hypothesis is rejected. The ANOVA test proves that the statistical data on carnosine's effect on crystallin is not due to chance and is statistically significant.

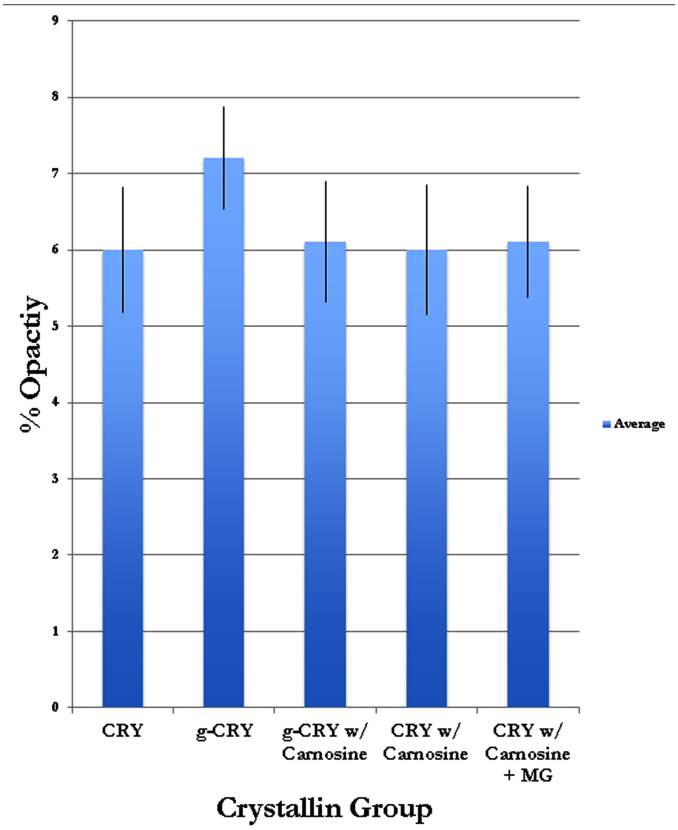

Figure 3. Comparison of the average numbers of % opacity of the five experimental crystallin groups.

Discussion

The data from the % opacity graphs strongly suggests: Carnosine reduces the increased opacity of g-CRY by separating the aggregated crystallin and does not affect the normal CRY. The additional data from carnosine with plain CRY and the data from carnosine w/ CRY + MG (later) demonstrates that it is effective not only in preventing cataracts but also in treating them. Carnosine has been shown to improve vision by partially reversing the development of the cataract, thus increasing the transmissivity of the lens to light. Many people cannot undergo surgery due to either health issues or the inaccessibility of surgical instruments and equipment. Carnosine has been proven to be useful in preventing the formation of cataracts. Carnosine could be very practical in applications that involve people who are faced with lots of ultraviolet radiation such as pilots and astronauts. Astronauts who fly into space are exposed to Galactic Cosmic Space Radiation (GCR) and their chances of getting a subcapsular cataract and a cortical cataract (peripheral) highly increase. This medicine can be used as

a method to prevent cataracts for not only people above the age of 45 who are more prone to cataracts, but also for situations in which people are exposed to this sort of hazardous environmental conditions or any conditions that would induce a cataract.

Carnosine will work against the lens directly, but its bioavailability is extremely low when applied topically, bordering around 5%[11]. When an ophthalmic drug is applied topically to the eye, only a small amount (< 5%) actually penetrates the cornea and reaches the internal anterior tissue of the eyes. Also, drainage by the nasolacrimal apparatus, non-corneal absorption and the relative impermeability of the cornea to both hydrophilic and hydrophobic molecules, all account for such poor ocular bioavailability. Some possible solutions to these problems are mucoadhesives, absorption promoters, and ophthalmic inserts. Mucoadhesives are not feasible because they can cause permanent damage to the cornea, and carnosine drugs currently have mucoadhesives. Adhesives attach themselves to the mucin layer near the retina. Absorption promoters (surface active agents, calcium chelators) act on the cornea to enhance the permeability of corneal epithelium by altering the cell membranes and loosening the tight junctions between superficial cells. Ophthalmic Inserts are not applicable for many people and are very costly.

In future studies, the use of fluorescent Cadmium Tellurium nanoparticles could be investigated as a possible solution. These particles have been used to study protein lens fibers and can pass through the cornea[12]. CdTe nanoparticles will be able to diffuse through the lens and serve as a messenger for cataracts due to a dipole-to-dipole reaction between each particle and the ability to rework themselves into nanowires[13]. Carnosine along with CdTe may prove to be able to prevent and treat cataracts, leading to a breakthrough for optical sciences[14]. Additionally, CdTe particles have low cytotoxicity and are relatively cheap[15], so could be an addition to treatment with Carnosine. Thus, the functionality of the nanoparticles along with carnosine could present a possible solution, to be tested in the future.

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