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RESEARCH ARTICLE

Antioxidant Effect of Lycopene on Retinal Pigment Epithelial Cell Line

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ABSTRACT

Purpose: Diabetic retinopathy is a common cause of blindness among diabetics. Studies around the globe establish oxidative stress as one of the major players of diabetes and diabetic retinopathy. Epidemiological reports suggest that consumption of large quantity of fruits and vegetables of high carotenoid content decreases risk of diabetes-based complications including retinopathy. Lycopene is a potent antioxidant and a carotenoid family member having known health benefits. Thus, the present investigation was designed to evaluate the antioxidant property.

Methods: The antioxidant potential of lycopene was determined using D407 retinal pigment epithelial cell lines through lycopene incorporation studies and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell cytotoxicity assay.

Results: Lycopene showed good antioxidant effect in-vitro on retinal pigment epithelial cell line and it was devoid of any cellular toxicity in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Conclusion: The results suggest that lycopene can be a potential candidate to halt the progression of diabetic retinopathy due to its effective defense against oxidative stress and non-toxic nature on the retinal pigment epithelial cells.

Keywords: Diabetic retinopathy, oxidative stress, lycopene, carotenoids.

1. Introduction

Diabetes mellitus is a major public health problem in the Middle East and the third world.[1] Hyperglycemia is a major factor in the development of diabetic complications such as nephropathy, neuropathy, retinopathy, cardiovascular disease, peripheral vascular disease and so on.[2-3] Diabetic retinopathy is a microvascular complication of sustained hyperglycemia leading to blindness and visual impairment. In retinopathy, the microvasculature of the retina is damaged, blood vessels swell and leak fluid, new vessels start to grow, and ultimately lead to the detachment of the retina.[4,5] It is a duration-dependent disease that develops in stages; the incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 50% by 10 years, and to 90% by 25 years of diabetes.[6]

The high oxygen uptake and glucose oxidation level renders retina more susceptible to oxidative stress.[7] It has been suggested that the redox homeostasis and oxidative stress are the key events in the pathogenesis of diabetic retinopathy. Animal studies have demonstrated that oxidative stress contributes to the development of diabetic retinopathy.[8] The oxidative stress in retinopathy is highlighted by the decreased intracellular enzymatic and non-enzymatic antioxidants in the retina.[9-10] Thus, there is accumulating evidence from animal studies that oxidative stress is associated with the development of retinopathy in diabetes, and antioxidants have beneficial effects on the development of retinopathy.

Among the non-enzymatic antioxidants, carotenoids (β-carotene, lycopene and lutein etc.) plays a vital role, as they are efficient singlet oxygen quenchers and scavengers of reactive oxygen intermediates.[11] Carotenoids are a class of more than 600 natural pigments that are present in fruits and vegetables.[12] Epidemiological reports suggest that consumption of large quantity of fruits and vegetables of high carotenoid content have a decreased risk of age related macular degeneration (AMD).[13-14] Similarly, supplementation of β-carotene along with other antioxidants resulted in a significant reduction in the development of advanced AMD.[15] Lycopene is a potent antioxidant and member of the carotenoid family. It is a naturally occurring compound that gives the characteristic red color to tomato, watermelon, pink grapefruit, orange, and apricot. A number of studies have indicated health benefits of consuming lycopene. Identification of a diverse range of carotenoids including lycopene in ocular tissues, its ability to reduce the pathogenesis of diabetes[16] and above all its high oxygen-quenching capacity[17] makes lycopene, a potential candidate to explore its antioxidant effect on retinal pigment epithelial (RPE) cells. Thus, the present investigation was designed to evaluate the antioxidant property of lycopene on D407 RPE cell lines through lycopene incorporation studies and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay.

2. Materials and Methods

2.1 Chemicals

D407 RPE cells were procured from American Type Culture Collection (Rockville, MD). Cell culture materials and accessories were purchased from GIBCO, USA. Lycopene, Tween 40, antibiotics and all other chemicals were of analytical grade and were obtained from Sigma Chemical Company (St. Louis, MO).

2.2 Cell culture

D407 RPE cells were grown in high glucose Dulbecco's modified Eagle's medium (DMEM) containing sodium pyruvate(1 mM), penicillin(100 U/ml), streptomycin (100 mg/ml) and amphotericin (100 mg/ml) and supplemented with 10% fetal calf serum. Cells were grown in 25 cm^2 culture flasks for incorporation experiment and in 96-well culture plates for other parameters, under 5% CO_2/95% moist air atmosphere at 37° C.

2.3 Lycopene solution

Lycopene (1 mmol/L) stock was prepared in Tween 40 in a dark room just before use. Different amounts of lycopene stock solution (1 mmol/L) were added to culture tubes, resulting in final lycopene concentrations of 5 µM/L, 10 µM/L, and 15 µM/L. The highest amounts of Tween 40 used to dissolve lycopene were added to separate cultures to provide control populations of cells.

2.4 Lycopene incorporation studies

To evaluate the effect of lycopene on viability, cells were grown in 25cm^2 culture flasks until they reach the confluence of 70-80%. At this sub confluency, medium was removed and cells were treated with increasing concentrations of lycopene (5µM, 10µM, and 15µM) in DMEM using Tween40 vehicle. After different time periods (3, 6, 12 and 24 hours) of incubation with

lycopene, the cells were collected by trypsination with 0.05% trypsin in phosphate buffer saline (PBS), washed 3 times with phosphate buffered saline by vortex motion, centrifuged and PBS was removed. After the final wash, the cell pellet was treated with 2% cold Triton X100 solution and subjected to lycopene extraction with solvents. The extraction of lycopene was achieved by treatment with ethanol and hexane and was repeated twice. Hexane phases were combined and the concentration of lycopene was determined in cells by spectrophotometry at 472nm. The viability of cells was analyzed by trypan blue assay.[18] The results were expressed as the percentage of the control culture treated with vehicle (Tween 40) alone.

2.5 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell cytotoxicity assay

The MTT assay was carried out by the method of Edmonson et al.[19] Cells cultivated in 96 well plates were washed with phosphate-buffered saline (PBS) and replenished with fresh culture medium. Then the cells were preincubated with lycopene before the addition of chemical oxidant; H_2O_2 (500 µM). After 24 hours of incubation with the sub-lethal dose of H_2O_2, the cells were washed with PBS and 20 µl of 5 mg/ml MTT was added to each well. The control well had MTT but no cells. The contents were incubated for 3.5 hours at 37°C in culture hood and then media was removed carefully followed by addition of 150 µl MTT solvent. The absorbance was read 590 nm with a reference filter of 620 nm. Each experiment was performed at least three times, and multiple control subjects were included. For each concentration of lycopene, six wells were analyzed. Of these six wells, the cells in two were treated with the lycopene alone to determine the toxicity of the compound. The cells in the remaining four wells were treated with lycopene and H_2O_2. Background absorbance values consisted of blank wells (with no cells) into which medium, MTT dye, and MTT solvent buffer were added. The background readings were subtracted from the average absorbance readings of the treated wells to obtain an adjusted absorbance reading that represented cell viability. This reading was divided by the adjusted absorbance reading of untreated cells in control wells to obtain the percentage of cell survival. Results were expressed as the percentage of surviving cells relative to the control samples.

3. Results:

Figure 1 exhibits the effect of lycopene concentration on cell viability at different time intervals. The current result clearly reveals that lycopene (5–15 µM) treatment did not significantly affected the cell viability in trypan blue exclusion (cell counting) assays. The cell viability was neither increased nor decreased significantly when compared to that of control. This finding matches with the earlier findings that clearly establishes the nontoxic nature of lycopene.[20,21]

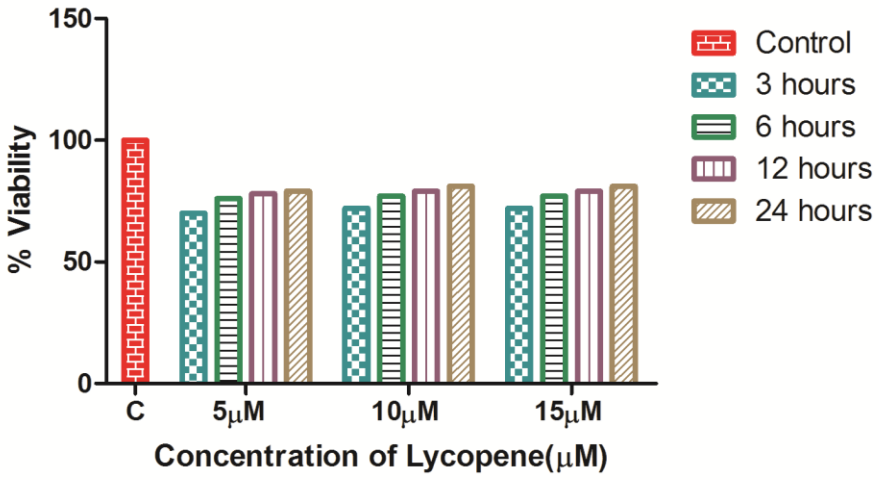

Figure 1: Effect of lycopene on cell viability

The pattern of lycopene incorporation to the cells is shown in Figure 2. The data reveals that the lycopene uptake was dose and time dependent. The lycopene incorporation values were significantly increased at a dose of 10 µM lycopene than 5 µM, when compared with the control. About 22% and 43.5 % of lycopene incorporation was achieved with 5 µM and 10 µM respectively. There was no further significant uptake of lycopene observed at higher concentrations of 15 µM. This may be due to the fact that at higher concentration lycopene forms more microcrystallines on the cell membranes that cannot be easily taken up by the cells. Similarly, the maximum incorporation of lycopene was achieved at 6 hours of incubation.

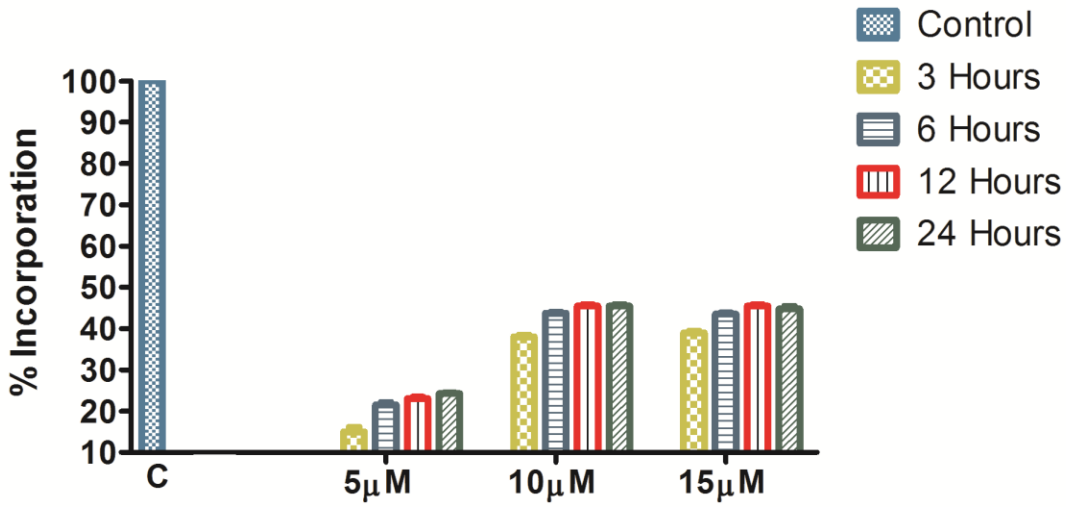

Figure 2: Time & dose dependent incorporation of lycopene on RPE cell line

4. Discussion:

A number of studies have indicated the health benefits of consuming lycopene in the diets of people in developed and developing countries.[22-23] Lycopene and a diverse range of carotenoids were identified and quantified in the human ciliary body and RPE/choroid.[24] The consumption of lycopene-rich foods has been demonstrated to prevent occurrence of a number of chronic diseases.[25-26]

The antioxidant property of lycopene was well established from the results of MTT cell cytotoxicity assay (figure 3). Oxidative damage to the RPE is a potential final common pathway for diabetic complications including retinopathy that depends on free radical injury and many other factors.[27] The administration of H_2O_2 exerts oxidative stress on the RPE cells through the production of Reactive Oxygen Species. These species react, for example, with nucleic acids, membrane lipids, surface proteins, and integral glycoproteins of RPE cells and mediates the damage.[27] The oxidative damage to the RPE cells results in the loss of viability (more than 60%) which was well documented in this study. However, lycopene pretreated cells significantly were more viable (80% to that of control). This function of lycopene is due to its antioxidant nature as mentioned earlier. Lycopene is effective antioxidant and able quencher of singlet oxygen and other ROS. The oxidative stress exhibited by the H_2O_2 was well scavenged by the lycopene and resulted in the maintenance of cell viability to the near normal value of control. The treatment of lycopene alone to the cells does not influence the cell viability in a significant way. This finding coincides with the results of the trypan blue assay and confirms nontoxic nature of lycopene. One of the underlying hypotheses for the protective role of carotenoids in age-related Macular Degeneration (AMD) and cataracts has been based on the ability of these carotenoids to act as antioxidants that can protect the human retina from photo-oxidation. The results of the present investigation uphold this hypothesis by establishing the antioxidant effect of lycopene on RPE cell line and needs further insight to explore the preventive effect of lycopene against the progression of diabetic retinopathy.

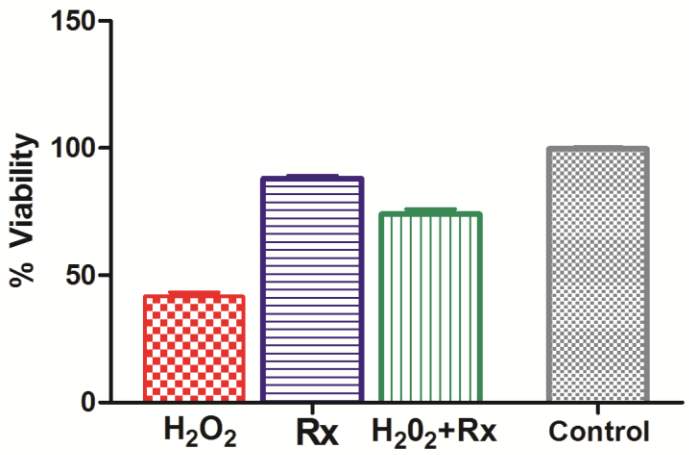

Figure 3: Antioxidant activity of Lycopene(Rx)

5. Conclusion

Diabetic retinopathy requires an immediate attention and needs a strategic plan to circumvent this complication. Lycopene, a natural carotenoid pigment, long known for its potent antioxidant nature provides significant health benefits against variety of diseases. This antioxidant present in diet gets widely distributed in different tissues and organs of the body including eye. In the present investigation it was found that lycopene was incorporated to the RPE cell lines effectively and does not exhibit any kind of toxicity to the cells. The presence of lycopene in the RPE cell lines offer a significant protection against the oxidative damage exerted by H_2O_2. Thus, lycopene is an able scavenger of free radical mediated stress and promising candidate to halt the progression of ocular damage in diabetic retinopathy.

6. References

1. Al Dawish MA, Robert AA, Braham R, et al. Diabetes Mellitus in Saudi Arabia: A Review of the Recent Literature. *Curr Diabetes Rev*. 2016;12(4):359-368.
2. Choby B. Diabetes Update: Prevention and Management of Diabetes Complications. *FP Essent*. 2017 May;456:36-40.
3. Valencia WM, Florez H. How to prevent the microvascular complications of type 2 diabetes beyond glucose control. *BMJ*. 2017 Jan 17;356:i6505.
4. Silpa-archa S, Ruamviboonsuk P. Diabetic Retinopathy: Current Treatment and Thailand Perspective. *J Med Assoc Thai*. 2017 Feb;100 Suppl 1:S136-47.
5. Das A. Diabetic retinopathy: Battling the global epidemic. *Indian J Ophthalmol*. 2016 Jan;64(1):2-3.
6. Liew G, Wong VW, Ho IV. Mini Review: Changes in the incidence of and progression to proliferative and sight-threatening diabetic retinopathy over the last 30 years. *Ophthalmic Epidemiol*. 2017 Apr;24(2):73-80.
7. Hurst J, Kuehn S, Jashari A, et al. A novel porcine ex vivo retina culture model for oxidative stress induced by H_2O_2. *Altern Lab Anim*. 2017 Mar;45(1):11-25.
8. Orhan C, Akdemir F, Tuzcu M, et al. Mesozeaxanthin Protects Retina from Oxidative Stress in a Rat Model. *J Ocul Pharmacol Ther*. 2016 Nov;32(9):631-637.
9. Echeverri-Ruiz N, Haynes T, Landers J, et al. A biochemical basis for induction of retina regeneration by antioxidants. *Dev Biol*. 2018 Jan 15;433(2):394-403.
10. González de Vega R, García M, et al. Protective effect of selenium supplementation following oxidative stress mediated by glucose on retinal pigment epithelium. *Metallomics*. 2018 Jan 24;10(1):83-92.
11. Xavier AA, Pérez-Gálvez A. Carotenoids as a Source of Antioxidants in the Diet. *Subcell Biochem*. 2016;79:359-75.
12. Yabuzaki J. Carotenoids Database: structures, chemical fingerprints and distribution among organisms. *Database (Oxford)*. 2017 Jan 1;2017(1).
13. Liu H, Liu W, Zhou X, et al. Protective effect of lutein on ARPE-19 cells upon H2O2-induced G2/M arrest. *Mol Med Rep*. 2017 Aug;16(2):2069-2074.
14. Widomska J, Subczynski WK. Mechanisms enhancing the protective functions of macular xanthophylls in the retina during oxidative stress. *Exp Eye Res*. 2019 Jan;178:238-246.
15. Rossi M, Puccini R, Romagnoli MC, et al Acute and subacute effect of rheopheresis on microvascular endothelial function in patients suffering from age-related macular degeneration. *Ther Apher Dial*. 2009 Dec;13(6):540-8.
16. Roohbakhsh A, Karimi G, Iranshahi M. Carotenoids in the treatment of diabetes mellitus and its complications: A mechanistic review. *Biomed Pharmacother*. 2017 Jul;91:31-42.
17. Icel E, Icel A, Uçak T, et al. The effects of lycopene on alloxan induced diabetic optic neuropathy. *Cutan Ocul Toxicol*. 2019 Mar;38(1):88-92.
18. Phillips, H.J. Dye exclusion tests for cell viability. In: TissueCulture, Method and Applications (P.F. Kruse and M.K. Patterson, eds.), pp. 406–408, Academic Press, New York, USA. 1973.
19. Edmonson JM, Armstrong LS, Martinez AO. A rapid and simple MTT-based assay for determining drug sensitivity in monolayer cultures. *J Tissue Cult Methods* 11;1988: 15–17.
20. Borawska MH, Czechowska SK, Markiewicz R, et al. Cell viability of normal human skin fibroblast and fibroblasts derived from granulation tissue: effects of nutraceuticals. *J Med Food*. 2009 Apr;12(2):429-34.
21. Burgess LC, Rice E, Fischer T, et al. Lycopene has limited effect on cell proliferation in only two of seven human cell lines (both cancerous and noncancerous) in an in vitro system with doses across the physiological range. *Toxicol In Vitro*. 2008 Aug;22(5):1297-300.
22. Farinetti A, Zurlo V, Manenti A, et al. Mediterranean diet and colorectal cancer: A systematic review. *Nutrition*. 2017 Nov - Dec;43-44:83-88.
23. Cheng HM, Koutsidis G, Lodge JK, et al. Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. *Atherosclerosis*. 2017 Feb;257:100-108.
24. Khachik F, de Moura FF, Zhao DY, et al. Transformations of selected carotenoids in plasma, liver, and ocular tissues of humans and in nonprimate animal models. *Invest Ophthalmol Vis Sci*. 2002 Nov;43(11):3383-92.
25. Mustra Rakic J, Liu C, Veeramachaneni S, et al. Lycopene Inhibits Smoke-Induced Chronic Obstructive Pulmonary Disease and Lung Carcinogenesis by Modulating Reverse

Cholesterol Transport in Ferrets. *Cancer Prev Res (Phila)*. 2019 Jul;12(7):421-432.

26. Thies F, Mills LM, Moir S, et al. Cardiovascular benefits of lycopene: fantasy or reality? *Proc Nutr Soc*. 2017 May;76(2):122-129.

27. Hammes HP. Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond. *Diabetologia*. 2018 Jan;61(1):29-38.