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Research paper

Protective effect of antioxidants against retinal injury induced by infrared radiation

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Abstract

The aim of the work is to evaluate the effect of infrared radiation on retinal rabbit rhodopsin and the protecting role of different antioxidants. NewZealand albino rabbits were exposed to infrared radiation 10 minutes direct or fractionated into 5 settings (2 minutes every day), with and without omega-3, Ginko Biloba extract and Grape seed extract as antioxidants supplementation. The rhodopsin has been extracted after decapitation of rabbits, total antioxidant capacity (TAC), and Fourier infrared spectroscopy (FTIR) of bleached rhodopsin was investigated. The data indicated that very high significant decrease ($p<0.001$) in the total antioxidant capacity after exposure to IR for 10 minutes direct or fractionated and changes in the spectral fingerprint region. After treating the rabbits with antioxidants, there were improvement in TAC and matched spectral fingerprint region with control. It may be concluded that the antioxidants omega-3, Ginko Biloba extract and Grape seed extract can protect retinal rhodopsin from oxidative stress that takes place after exposure to infrared radiation. The direct dose of infrared radiation leads to more damage than fractionated dose on retinal rhodopsin.

Keywords: *Infrared Radiation; Rabbits; Rhodopsin; Ginko Biloba; Grape Seed; Omega3.*

1. Introduction

Infrared radiation (IR) is defined as electromagnetic radiation with longer wavelengths than visible light, starting with a red spectrum of 700 nm with a frequency of 430 THz to 1 mm at 300 GHz. IR had three sub-ranges: IR-A (from 780 to 1400 nm); IR-B or far infrared (1400 to 3000 nm); IR-C (3000 to 10000 nm). Most of the thermal radiation in the room temperature range released by objects is infrared. In industrial, scientific and medical applications, this type of radiation is used. Mead (2008) [1] reported that the atmosphere of the earth absorbs and scatter most the harmful wavelengths which is shortest and had higher energy. Also, the different structures of the eye posses many filtering and absorption types. Mervat (2013) [2] found that exposure to IR radiation may cause damage to retina in the form of scotoma that lead to loss of vision in a portion of the visual field. Even low level in absorption can cause signs such as simple redness of the eye, but higher level can initiate swelling, hemorrhaging and lesion. Aly and Mohamed (2011)[3] examined the effect of IR radiation on the lens of the rabbit and the activity of Na^+–K^+ ATPase of lens membrane and found a change in the molecular weight of crystalline lens along with changes in protein secondary structure and decrease in the activity of Na^+–K^+ ATPase. Kohen et al (2018) [4] created a model of an ischemic retina with reperfusion (IR) and examined the possible antiapoptotic effect. It was observed injury due to IR lead to increasing apoptosis and after bevacizumab that used as antioxidant reduces the death and apoptosis of retinal cells.

Omega-3 ($C_{60}H_{92}O_6$) is fat in marine oils and plant oils. It is long chain polyunsaturated fatty acids with a double bond (C=C) from the end of carbon chain after the third carbon atom [5]. Ramchani et al., (2015) [6] tested omega-3 supplement in an in-vivo model of retinal degeneration induced by light and omega-3 protect retina from degeneration.

Ginkgo Biloba (GBE) is an extract of phytomedicine from the ginkgo tree leaves. It is thought to be favorable as a dietary supplement to treat various cognitive deficiencies associated with age. Scavenge free radicals by GBE lead to decrease the accumulation of oxygen free radicals [7]. Gamal et al., (2011)[8] investigated the effect of GB administration for up to 10 weeks on the retina of rabbits using Fourier transform infrared spectroscopy (FTIR). The results showed that the administration of GBE was associated with various positive affect on the retinal tissue in particular the secondary structure components of the amide I protein backbone and the NH-OH region.

The richest sources of proanthocyanins are Grape seeds $C_{32}H_{30}O_{11}$ a class of biologically active flavonoids found throughout the plant kingdom. Grape seed extract (GSE) has received a great deal of interest due to its many biological actions, such as antioxidant effects [9]. The power of administration of grape seeds as an antioxidant on retinal sensitivity induced by blue light was examined by Elawady et al., (2011)[10]. The results achieved showed that blue light had a clear effect on both a- and b-waves as a reduction in their amplitudes and an increase in implicit time. The grape seeds can therfore have a neuroprotective effects to protect the retinal neurons alongside photo-chemical damage which may induce death of the retinal cell.

The aim of the present study is to investigate the role of omega-3, Ginkgo Biloba and grape seed extract as antioxidants for protecting the retinal rhodopsin from the hazards of exposure to infrared radiation.

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2. Materials and methods

2.1. Animals

Forty five NewZealand albino rabbits weighing 2-2.5 kg were used in the present study from animal house of Research Institute of Ophthalmology, Giza-Egypt. The animals comprised both sexes and were fed on balanced diet. The temperature inside the animal house varied between 20° to 25°C. Lighting conditions were natural light for the period of the day and darkness during the night. The ARVO statement was applied for the use of animals in ophthalmic and vision research. The animals were classified into 3 groups I, II and III according to the following:

Group I: contain five rabbits and used as control group.

Group II: contain 20 rabbits subdivided into four subgroups (II-a, II-b, II-c and IId). subgroup II-a was supplemented with 10 mg/Kg omega-3, subgroup II-b was supplemented with 2 mg/Kg GBE and subgroup II-c was supplemented with 8 mg/Kg body weight Grape seed extract (GSE). All rabbits were supplemented with antioxidant two weeks before exposed to IR. Rabbits of subgroup II-d were exposed to IR without antioxidant supplementation. All the rabbits were decapitated after IR exposure.

Group III: contain 20 rabbits subdivided into four subgroups (III-a, III-b, III-c and III-d). As the previous group, subgroup III-a was supplemented with 8 mg/Kg omega-3, subgroup III-b was supplemented with 2 mg/Kg GBE and subgroup III-c was supplemented with 8 mg/Kg body weight GSE. All rabbits were supplemented with antioxidant two weeks before exposed to IR for 10 minutes fractionated into 5 settings (2 minutes every day). Rabbits of subgroup III-d were exposed to IR radiation without antioxidant supplementation. All the rabbits were decapitated 24 hours after the last IR sitting.

2.2. Infrared (IR) exposure

IR was supplied from a General Electric Lamp, model 250R 50/10, which was placed 20 cm from the rabbit and aimed at each eye. The wavelengths emitted by General Electric Lighting Division (Cleveland, OH, USA) from the anterior surface of the IR lamp were 0.34-0.4 µm (UV light), 0.4-0.76 µm (Visible light), 0.76-3.0 µm (IR-A, IR-B light 83%) and 3.0-7.0 µm (IR-C 10%) [11].The lamp has been calibrated at photometry Department, National Institute of standards, Giza, Egypt. The total IR percentage emitted was 93%. The irradiance of IR lamp at 20 cm was 0.2 W/cm^2. After 5 minute of IR exposure, the heat flux reaching the animal cornea was 88 J/cm^2. The heat flux therefore reached the cornea after 2 minutes and was 17.6 J/cm^2.

2.3. Extraction of rhodopsin

At the end of each period, the Albino rabbits maintained for 12 hours in dark and were decapitated; the eyes were enucleated and then the cornea and lens were removed and the posterior of the eye containing the retina was used for preparation of the rhodopsin sample. Extraction of rhodopsin was done by the method of Smith 1992. All operations were carried out either in dim red light or in total darkness.

2.4. Fourier transform infrared spectroscopy (FTIR)

200 µl of rhodopsin were lyophilized and then mixed with KBr powder to prepare the KBr disks for FTIR analysis. FTIR spectra were measured using Shimadzu infrared spectrometer. Hundred sample interferograms were recorded for each spectrum. Removing interference was done using a continuous purge of dry nitrogen gas and Savitzky-Golay to eliminate the noise. The average of three spectra for each group was obtained using Origin Pro 7.5 software.

2.5. Total antioxidant capacity

A calorimetric method was used to determine the total antioxidant capacity of retinal rhodopsin using a kit purchased from Biodiagnostic Co., Egypt, according to Koracevic et al. (2001) method [12]. The principle of this method depends on the reaction of antioxidants to a hydrogen peroxide (H_2O_2) amount in the tissue. The sample's antioxidant eliminates a defined hydrogen peroxide amount. The residual hydrogen peroxide is colorimetrically determined by an enzyme reaction involving the conversion of 3, 5 dichloro-2-hydroxy benzensulphonate into colored product.

Statistics

The data was expressed as the mean ± SD. Comparison between multiple groups was carried out using analysis of variance (ANOVA); commercially available statistical software package (SPSS-11 for windows) was used when the level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Total antioxidant capacity

Living organisms have complex antioxidant systems to work against reactive oxygen species (ROS) and to decrease their damage. The summations of endogenous and food-derived antioxidants represent the total antioxidant activity of the system. Table (1) and histogram in figure (1) illustrated total antioxidant capacity of rabbit's eye rhodopsin to all the studied groups compared to control. The data indicated a significant decrease ($p<0.001$) in the total antioxidant capacity after exposure to IR for 10 minutes. Argawal and Malik (1959) [13] found that retinal burns from exposure to industrial sources, such as xenon lamps, infrared and metal arc inert gas welding. After the supplementation with omega-3 and GBE, there was a significant decrease ($p<0.01$) in total antioxidant capacity. Rotstein et al., (2003) [14] found that in rat retinal cells cultured in a serum-free medium, the addition of Docosahexaenoic acid (DHA) to the cultures avoid the apoptotic death of photoreceptors. Politi at al., (2001)[15] mention that DHA was not only the most effective in supporting photoreceptor survival, but also the only fatty acid to minimize the number of nuclei suffered from apoptosis. After supplemented with GSE, there was a significant decrease ($p<0.01$) in total antioxidant capacity. In the same context, Elawady and El-hansi, (2011) [10] concluded that the grape seeds have a neuroprotective property that help in defensive the retinal neurons against photo-chemical damage which may lead to retinal cell death. So, the administration of grape seeds can assist in protecting the retina. Yanga et al., (2012) [16] concluded the same results of our study that GSE had a neuroprotective effect against oxidative stress-induced apoptotic death in Staurosporine-differentiated RGC-5(ssdRGC-5) cells. When rabbits were exposed to 10 minutes fractionated IR, there was significant decrease ($p<0.05$) in total antioxidant capacity. Low linear energy-transfer radiation is less efficient at low doses than at high doses indicating that cells can accumulate a certain sublethal damage before losing their reproductive integrity. The extent to which sublethal damage is repaired is illustrated by the failure of successive doses to be fully additive in their lethal effects if separated by several hours [17]. After the supplementation with omega-3, a significant decrease ($p<0.05$) in total antioxidant capacity was observed. Dyall and Michael (2008) [18] mention that the n-3 fatty acids work as an energy source and an important unique cell part, especially in cellular membranes. DHA is mostly rich in retinal photoreceptor outer segments. And there was non-significant decrease in case of supplementation with GBE but high significant decrease ($p<0.01$) in case of supplementation with GSE.

Table 1: Effect of Infrared Radiation with and without Antioxidants (Omega-3, GSE, GBE) on Total Antioxidants Capacity

	Direct		Fractionated	
	Mean ± SD	%change	Mean ±SD	%change
Control	2.82 ± 0.309		2.82 ± 0.309	
IR	1.42 ± 0.430***	-49.64 %	1.89±0.305***	-32.97%
IR+ Omega-3	2.29 ± 0.339**	-18.79 %	2.52 ± 0.427*	-10.63 %
IR + GBE	2.27 ± 0.344**	-19.50 %	2.65 ± 0.379	-6.020 %
IR + GSE	2.02 ± 0.324***	-28.36 %	2.23 ± 0.430**	-20.92 %

*Statistical significant p<0.05.
** High significant p<0.01.
*** Very high significant p<0.001.

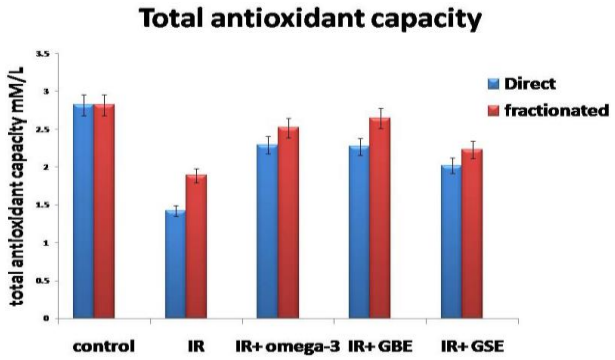


Fig. 1: Effect of Infrared Radiation with and without Antioxidants (Omega-3, GSE, GBE) on Total Antioxidant Capacity.

Fourier Transform Infrared (FTIR) Spectra of bleached Rhodopsin

Fourier transform infrared spectroscopy (FTIR) is a powerful tool for investigation of both conformational and functional changes in rhodopsin. Figure (2) illustrated the infrared spectra of final decay product of rhodopsin (bleached) extracted from normal rabbit's retina. Analysis of normal pattern revealed the presence of 9 bands in the fingerprint region at: 1678 cm^{-1} , 1634 cm^{-1} , 1630 cm^{-1} ,1547 cm^{-1} ,1460 cm^{-1} , 1393 cm^{-1} , 1248 cm^{-1} , 1139 cm^{-1} , 1068 cm^{-1} (Table 2). The band at 1630 cm^{-1} assigned for amide I, the band at 1547 cm^{-1} originated from the amide II vibration of peptide group. According to Susi and Byler (1983)[19] the presence of amide I components at 1691, 1681, 1639, and 1630 cm^{-1} and the amide II component at 1530 cm^{-1} strongly suggests that some β-structure is also present and that a portion of it forms antiparallel strands. The weak amide I component at 1681 cm^{-1} cannot be unequivocally assigned, as absorption near this frequency has been reported from β-structure. Also, the peak at 1544 cm^{-1} is due to Amide II bands (arises from C-N stretching & CHN bending vibrations [20]. The band at 1460 cm^{-1} assigned for CH_2 bending according to Baker et al., (2008) [21] and the band 1393 cm^{-1}assigned for CH_3 bending. In agreement with Agarwal et al, (2006) the band at 1405 cm^{-1} is due to CH_3 asymmetric deformation. The band at 1248 cm^{-1} related to PO_2^- asymmetric and PO_2^- symmetric at 1068 cm^{-1}. In the spectrum of the malignant tissue, there is a strong peak at 1076 cm^{-1} which was present only as shoulder at 1097 cm^{-1} in the spectrum of the normal tissues. The absorption was attributed to symmetric phosphate (V_s PO^{-2}) stretching vibration modes of phosphate (PO^{-2}) groups [22], contributed largely by nucleic acids in the breast tissues. The strong anti-symmetric phosphate (V_{as} PO^{-2}) stretch vibration evident at 1238 cm^{-1}. The band at 1075 cm^{-1} is due to C-N stretching absorption of aliphatic amines is appeared weak and it is the (PO^{-2}) symmetric phosphate stretching modes originate from the phosphodiester groups in nucleic acids and suggest an increase in the nucleic acids [23].

Table 2: Assignments of All Peaks in the Fingerprint Region of the FTIR Spectra to All Groups Compared to Control

Groups	Peaks											
	Amide I			Amide II		CH2 Bending		CH3 Bending	PO-2 asymmetric	C–OH Stretching	PO-2 symmetric	
Control	1678±8	1643±7	1630±6	1547±8		1460±5		1393±7	1248±8	1139±6	1068±7	
	56±1	47±2	79±2	59±2		130±3		135±4	85±3	92±3	59±4	
IR	1694± 2	1642±3		1549±4		1454±6		1404±5	1248±3	1124±5	1073±4	
Direct	37±2	57±3		63±2		130±4		103±6	100±14	60±5	51±5	
IR	1696±8	1642±7		1561±6		1470±7		1399±7	1248±7	1123±4	1074±4	1083±1
Fractionated	37±3	61±4		38±3		119±4		99±4	104±3	46±5	50±2	14±2
IR+ Omega3	1678±6		1636±5	1550±7		1475±5		1406±8	1255±8	1169±5	1068±6	
Direct	74±3		53±3	66±3		177±4		119±7	100±5	157±5	91±4	
IR+ Omega3	1677±8	1643±7	1638±8	1555±7	1543±8	1441±8		1394±7	1244±10	1113±7	1069±9	
Fractionated	53±4	42±4	66±3	71±3	61±3	99±4		123±4	91±4	62±4	47±3	
IR + GBE	1670±9		1631±9	1597±8	1546±5	1478±5	1459±6	1414±7	1255±8		1073±8	
Direct	62±4		45±4	81±3	62±4	145±7	151±3	103±4	126±4		65±3	
IR + GBE	1664±5		1628±9	1551±5		1465±8		1413±9	1234±9	1146±4	1065±6	1089±5
Fractionated	67±2		45±2	67±3		135±3		96±4	55±4	57±3	56±5	20±1
IR + GSE	1666±5	1641±4		1543±8		1459±9		1410±8	1254±8		1077±8	
Direct	119±2	69±2		55±6		143±7		176±5	124±3		53±4	
IR + GSE	1664±9		1636±9	1565±9	1545±8	1452±7		1400±8	1254±6	1123±8	1075±8	
Fractionated	69±5		57±4	110±7	54±2	121±2		109±3	90±3	22±1	52±3	

The spectra of all groups exposed to IR direct and fractioned 10 minutes, the band around 1630 cm^{-1} disappeared and there is shift in the bands at frequencies 1678, 1460, 1399, 1139, 1068 cm^{-1}. A band at 1081 cm^{-1} appeared in case of fractionated dose that means there is a lack of expression of protein or expression of new stressed protein these results attributed to IR radiation may cause at diverse biological effects of thermal origin [24]. Infrared rays were transmitted throughout the ocular media to the retina and absorbed by the pigment epithelium (PE) of the retina injury where occurs in the neural layers through indirect heating. The enzyme denaturation occurs due to infrared effects on the retina that play important role in the process of energy production of cells, also the protein translation machinery which may affects on the membrane structure [25].

After supplementation with omega-3 at direct exposed to infrared, the band at 1642 cm^{-1} disappeared and the bands at 1547, 1460, 1393, 1248, 1139 cm^{-1} was shifted. There is a new peak appears at 1550 cm^{-1} in fractionated dose with supplemented with omega-3 and shift in bands at 1460, 1113 cm^{-1} (figure 3). DHA affects membrane function by altering permeability, membrane order, thickness, lipid phase properties, and the activation of membrane-bound proteins [26]. When the rabbits supplemented with GBE in direct exposed to IR case the band at 1643 cm^{-1} disappeared, there is two peaks appears at 1597, 1478 cm^{-1}, the bands at frequencies 1393and 1248 cm^{-1} and the band at 1139 cm^{-1} disappeared. When rabbits exposed to fractionated dose with GBE supplementation, the band at 1643 cm^{-1} disappeared and there is shift in all bands and a new band appears at 1089 cm^{-1} (figure 4). The results of our study are in agreement with previous investigations of Baudouin

et al. (1994) [27] concluded that ginkgo biloba minimize the severity of oedema and neovascularization in an experimental retinopathy produced by the intravitreal production of superoxide radicals. In an experimental study, Hirooka et al. (2004) [28] reported the effect of ginkgo biloba extract in rats with unilateral, chronic moderately high intraocular pressure. When compared to their contralateral saline eyes with normal intraocular pressure at 5 months after the induced of an intraocular elevation, the retinal ganglion cell loss in the eyes with chronic.

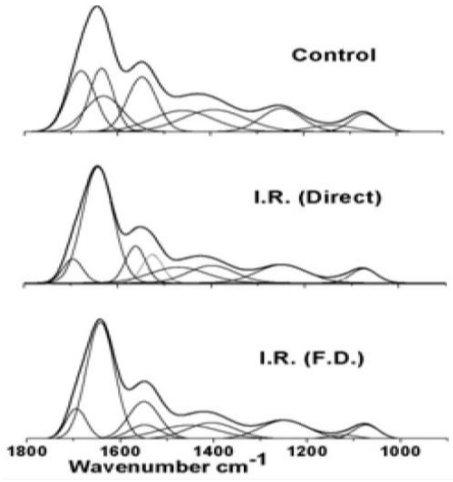


Fig. 2: Fingerprint Region (1800 – 800 Cm^{-1}) Spectral FTIR of Bleached Rhodopsin Extracted from Rabbit's Retina before and after Infrared Radiation Exposure, Direct and Fractionated for 10 Minutes.

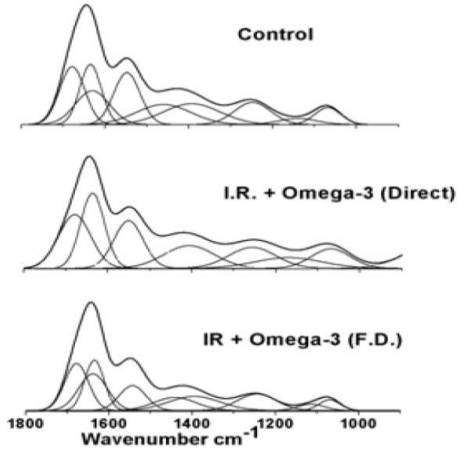


Fig. 3: FTIR Spectra in Fingerprint Region (1800 – 800 Cm^{-1}) of Bleached Rhodopsin Extracted from Rabbit's Retina Supplemented with Omega-3 before and after of Infrared Radiation Exposure, Direct and Fractionated for 10minutes

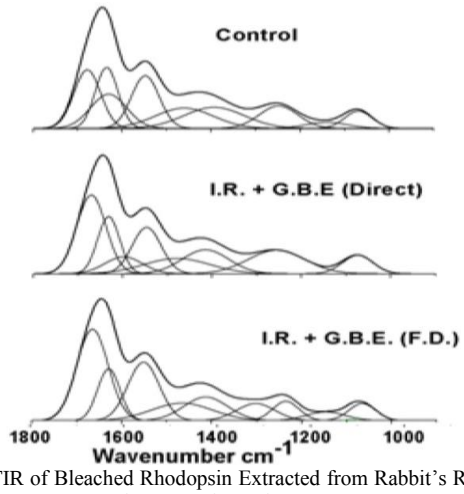


Fig. 4: FTIR of Bleached Rhodopsin Extracted from Rabbit's Retina Supplemented with GBE before and after Infrared Radiation Exposure, Direct and Fractionated for 10minutes, in Fingerprint Region (1800 – 800 Cm^{-1}).

After supplementation with GSE in case of direct dose of IR two bands disappeared at frequencies 1630 cm^{-1} and 1139 cm^{-1}, one peak appears at band 1254 cm^{-1} and there is shift at bands 1678, 1393, 1248, 1068 cm^{-1}. In case of fractionated dose with supplementation with GSE there is one peak disappeared at 1643 cm-1 and one peak appeared at 1565 cm-1, this shift in all bands as shown in figure (5). This agrees with the recent in vitro studies by Kalt et al., (2010) [29] who demonstrated that anthocyanins and other flavonoids act directly with rhodopsin and adjust visual pigment function. The GSE is a rich bioflavonoid which is used for combating free radicals, maintaining capillary health and strengthening the cell membranes. Also it has a control on the permeability and tendency to hemorrhage of retinal vessels. Flavonoids have numerous properties as the prevention and treatment of ocular diseases, mainly those that involve the loss of nerve cells [30].

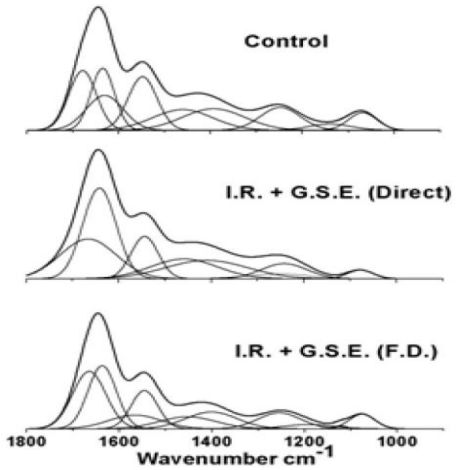


Fig. 5: FTIR of Bleached Rhodopsin Extracted from Rabbit's Retina Supplemented with GSE before and after of Infrared Radiation Exposure, Direct and Fractionated 10minutes, in Fingerprint Region (1800 – 800 Cm^{-1}).

4. Conclusion

Infrared radiation has harmful effect on retinal rhodopsin. This effect increased by direct dose and decrease by fractionated dose. After supplementation with different types of antioxidants (omega-3, GBE and GSE) they play a role in protective on retina damage and reduce the membrane lipid peroxidation. Omega-3 play the best protective role then the GBE at last the GSE.

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