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Antioxidant activity of lutein during non-enzymatic peroxidation of rat renal microsomes and mitochondria

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Abstract Metabolic changes during renal disease may induce increased production of oxygen radicals that play an important role in the progression of renal damage and in the onset of important comorbidities. In the study reported here it was analyzed the effect of lutein on peroxidation-induced chemiluminescence of mitochondria and microsomes isolated from rat kidney. After incubation of mitochondria or microsomes in an ascorbate (0.4 mM)-Fe^{++} (2.15 μM) system (180 min at 37°C) to induced non-enzymatic peroxidation, it was observed that total counts per minute /mg protein originated from the chemiluminiscence light emission was lower in kidney mitochondria or microsomes obtained from the lutein-treated group when compared with the control group (without lutein treatment). Moreover, it was observed that the chemiluminescence was reduced as lutein was raised up to 0.45 mg. Chemiluminescence data indicate that lutein may act as an antioxidant that protects rat kidney mitochondria and microsomes from peroxidative damage.

Keywords lutein, peroxidation, chemiluminescence, mitochondria, microsomes

Introduction

Membranes are invaluable targets for peroxidation with peroxide formation [1-2]. The result of biological membrane peroxidation is severe, rupture and loss of membrane functions, enzymatic inactivation, toxic effects on cell division, etc. [3-4-5]. The microsomes and mitochondria are an interesting system for peroxidation studies [6-7-8]. These organelles are a suitable experimental model for detailed studies of kinetic reaction and peroxidation mechanism, due to of tissue alterations in many pathological processes [9]. The generation of reactive oxygen species has been associated as one of the factors in the etiology of several diseases [10]. *Calendula officinalis* L. is rich in flavonoids, terpenoids and lutein and has both antioxidant and anti-inflammatory [11]. The present study was designed to determine whether mitochondria and micromes obtained from rat kidney could be a target for non-enzymatic peroxidation, as well as to establish the level of protection of such membranes incubated with lutein. The degradation process was monitored by the determination of chemiluminescence [12].

Material and Methods

Experimental

Female Wistar AH/HOK rats (7 weeks-old, 120-137 g) were used. Animals were provided by the Animal Facility Laboratory, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina. All the operations that

included handling and euthanasia of animals were under the supervision of the Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio (CICUAL), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Lutein was kindly supplied by Sigma Laboratory. BSA (Fraction V) was obtained from Wako Pure Chemical Industries Ltd, Japan. L (+) ascorbic acid was from Merck Laboratories. All other reagents and chemicals were of analytical grade from Sigma.

Preparation of lutein stock solution

The stock solution of lutein was prepared by diluting 30 mg in 10 ml of distilled water (final volume) [3 mg / ml]. From this solution, the different concentrations of lutein used in the peroxidation tests were taken. All operations were performed under dim light.

Animals and preparation of microsomes and mitochondria

Female Wistar AH/HOK rats 7 weeks-old, weighing 120-137g were used. All rats were fed commercial rat chow and water *ad-libitum*. The rats were sacrificed by cervical dislocation and kidneys were rapidly removed, cut into small pieces and extensively washed with 0.15 M NaCl. A 30% (w/v) homogenate was prepared in a 0.25 M sucrose solution, 10 mM Tris-HCl pH 7.4 using a Potter-Elvejhem homogenizer. The homogenate was spun at 10,000 x g for 10 min. The supernatant (3ml) obtained was applied to Sepharose 4B column (1.6 x 12cm) equilibrated and eluted with 10mM Tris-HCl pH 7.4, 0.01%NaN_3. The microsomal fraction appearing in the void volume (10-16 ml) was brought to 0.25M sucrose. All operations were performed at 4 °C and under dim light. The quality of this microsomal preparation is of similar composition with respect to concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation [13]. Mitochondria were obtained by method described by [14].

Peroxidation of rat kidney microsomes and mitochondria

Chemiluminicense and peroxidation of six different experiments were initiated by adding ascorbate to microsomes or mitochondria [15]. The microsomes or mitochondria (1 mg of microsomal or 0.5mg mitochondrial protein) with addition of lutein (0.05, 0.25, 0.35 and 0.45 mg of lutein, lutein group) were incubated at 37 °C with 0.01 M phosphate buffer pH7.4, 0.4 mM ascorbate, final vol. 2 ml. Phosphate buffer is contaminated with sufficient iron to provide the necessary ferrous or ferric iron (final concentration in the incubation mixture was 2.15 μM) for peroxidation [16]. Mitochondria or microsomal preparations, which lacked ascorbate, were carried out simultaneously. Membrane light emission was determined over a 180 min period, chemiluminescence was recorded as cpm every10 min and the sum of the total chemiluminescence was used to calculated cpm/mg protein. Chemiluminescence was measured as counts per min in liquid scintillation analyzer Packard 1900 TR equipment with a program for chemiluminescence.

Protein determination

Proteins were determined by the method of Lowry et al. (1951) [17], using BSA as standard.

Statistical analysis

Results are expressed as means ± S.D. of six independent determinations. Data were statistically evaluated by one way analysis of variance (ANOVA) and Tukey's test. The statistical criterion for significance was selected at different p-values, which was indicated in each case.

Results

Light emission of rat kidney mitochondria during peroxidation.

Incubation of rat renal mitochondria in the presence of Ascorbate-Fe^{++} resulted in membrane peroxidation as evidenced by light emission (chemiluminescence). After incubation of mitochondria in an ascorbate-Fe^{++} system at 37 ° C for 180 minutes, the cpm originated from the light emission was lower (concentration dependent) in the lutein group than in the control group. Figure 1 show the light emission obtained from the lutein group and from the

control group. Values were 820.33 ± 26.85 in the control group at 515.67 ± 64.46 cpm with the addition of 0.45 mg of lutein, the significance was $p < 0.04$.

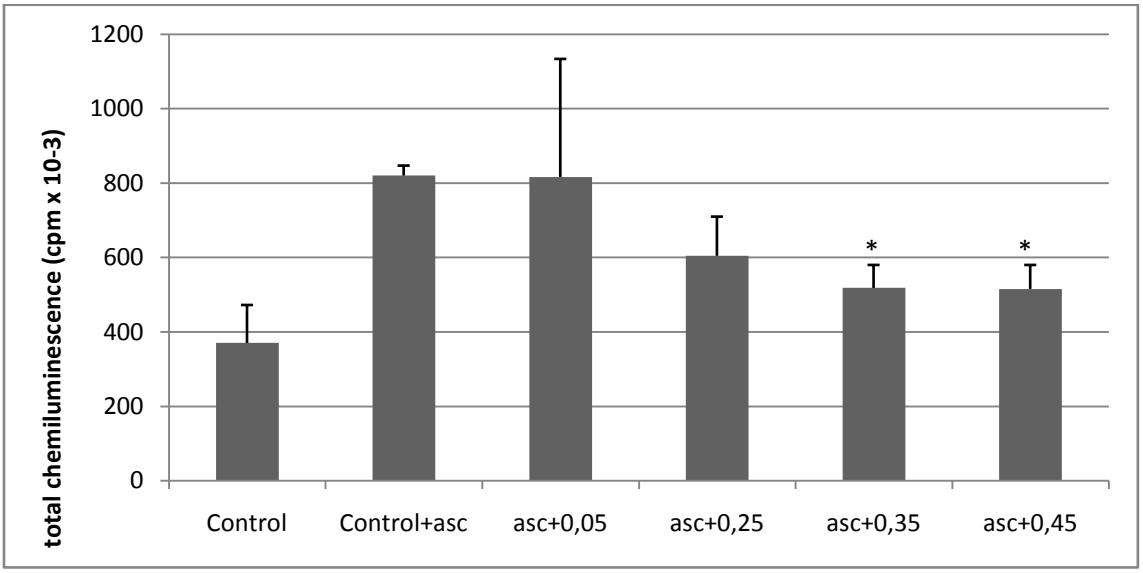

Figure 1: Light emission of rat kidney mitochondria during peroxidation with different concentration of lutein

Non-enzimatic peroxidation ascorbate-Fe^{2+} of rat kidney mitochondria. Chemiluminescence was determined over a 180 min period and recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein (mean ± SD). Statistically significant differences between control + ascorbate and ascorbate 0.35 and 0.45 concentration of lutein are indicated by *$p < 0.04$. Data are given as the mean ± SD of six experiments.

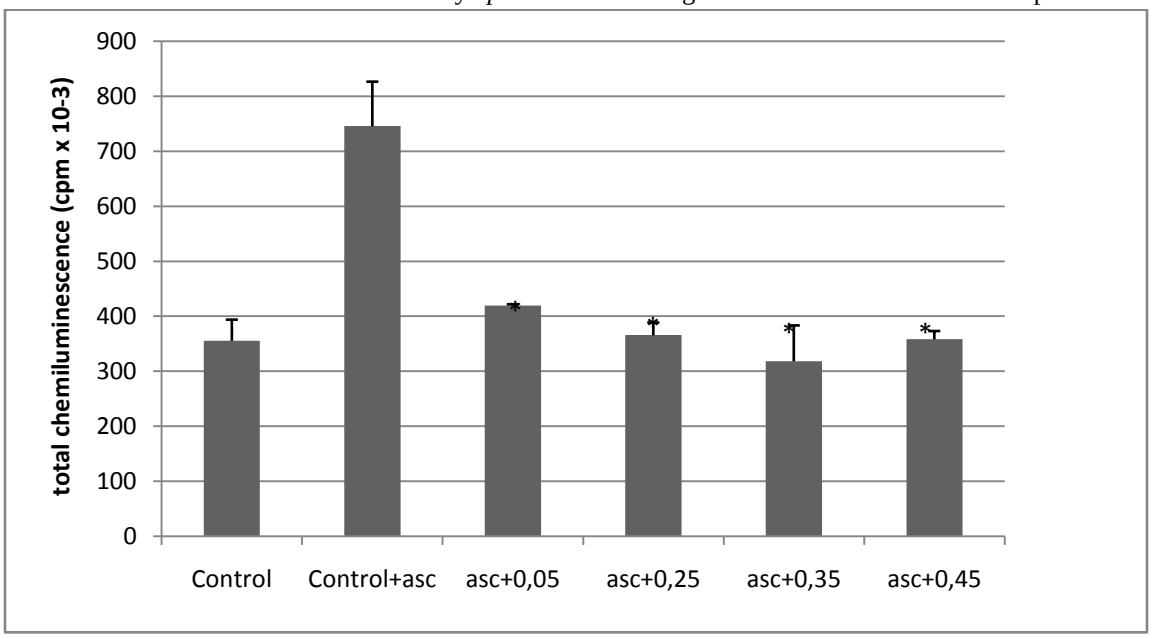

Figure 2: Light emission of rat kidney microsomes during peroxidation with different concentration of lutein

Non-enzimatic peroxidation ascorbate-Fe^{2+} of rat kidney microsomes. Chemiluminescence was determined over a 180 min period and recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein (mean ± SD). Statistically significant differences between control + ascorbate and ascorbate with different concentration of lutein are indicated by *$p < 0.03$. Data are given as the mean ± SD of six experiments.

Light emission of rat kidney microsomes during peroxidation.

Incubation of rat kidney microsomes in the presence of Ascorbate-Fe^{++} resulted in membrane peroxidation as evidenced by light emission (chemiluminescence). After incubation of microsomes in an ascorbate-Fe^{++} system at 37 ° C for 180 minutes, the cpm originated from the light emission was lower (concentration dependent) in the lutein group than in the control group. Figure 2 shows the light emission obtained from the lutein group and from the control group. The values were 745.64 ± 81.27 in the control group at 358.33 ± 15.14 cpm with the addition of 0.45 mg of lutein, the significance was $p < 0.03$.

After incubation of microsomes and mitochondria in an ascorbate-Fe^{++} system (180min at 37°C) it was observed that the percentage cpm/mg of protein originated from light emission (chemiluminescence) was lower in kidney microsomes and mitochondria with addition of lutein. Thus, the percentage of peroxidation inhibition produced by lutein was 83.75% in mitochondria (Fig. 1) and 75.23% in micromes (Fig. 2), when was compared from each Figure control + ascorbate against sample with 0.45 mg of lutein.

Discussion

The object of this study was to measure the ability of lutein to protect renal microsomes or mitochondria against peroxidation. Rat kidney mitochondria incubated with lutein were protected against peroxidation when compared to membranes from the control group, as demonstrated by chemiluminescence results. *In vitro* peroxidation studies are useful for the elucidation of a possible peroxide formation mechanism *in vivo* [18]. These results are in agreement with earlier reports by [19]. *In vitro* research of peroxidation is desirable for the elucidation of possible peroxide formation mechanisms *in vivo* [20], since the membranes composition causes vulnerability to peroxidative degradation [21]. Although important research has been done to characterize changes in structure, composition and physical properties of membranes undergoing oxidation [22-23-24], it is necessary to know how biological compounds with antioxidant properties contribute to the protection of specialized membranes against the harmful effects performed by reactive oxygen species and other free radicals. The evidence leading to the appreciation of anticarcinogenic activity of lutein has been reviewed [25]. Actual evidence show that lutein has an effective antioxidant activity [26], with lutein being able to enhance biochemical parameters and reduce the formation of inflammatory cytokines, thus avoiding oxidative stress. The phytochemical composition of *Calendula Officinalis L*. (Asteraceae) extract indicates that lutein is one of its main components, in agreement with previous work [27-28-29].

Conclusion

Our results are consistent with the hypothesis that lutein may act as a physiological antioxidant in cell membranes.

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