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Restoring effect of selenium on the molecular content, structure and fluidity of diabetic rat kidney brush border cell membrane

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ABSTRACT

Diabetic kidney disease (DKD) is a dominant factor standing for kidney impairments during diabetes. In this study, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to disclose the diabetes-induced structural changes in the kidney and evaluate the effects of selenium on diabetes. The increase in the area of the olefinic band indicated increased amount of lipid peroxidation end products in diabetic kidney brush border cell membrane. Moreover, saturated lipid content of this cell membrane considerably diminished. DKD was found to disrupt lipid order and cause a decrease in membrane dynamics. However, the administration of selenium at low and medium doses was shown to improve these conditions by changing the lipid contents toward control values, restoring the ordered structure of the lipids and membrane dynamics. Curve-fitting and artificial neural network (ANN) analyses of secondary structures of proteins demonstrated a relative increase in α-helix and reduction in the β-sheet during diabetes in comparison to the control group, which were ameliorated following selenium treatment at low and medium doses. These findings were further confirmed by applying hierarchical cluster analysis (HCA) and principal component analysis (PCA). A clear separation of the experimental groups was obtained with high heterogeneity in the lipid and protein regions. These chemometric analyses showed that the low and medium doses of selenium-treated diabetic groups are successfully segregated from the diabetic group and clustered closer to the control. The study suggests that medium and, more predominantly, low-dose selenium treatment can be efficient in eliminating diabetes-induced structural alterations.

1. Introduction

Chronic diabetes gives way to several complications of the illness by damaging the perivascular and cardiovascular systems. These include retinopathy, nephropathy, neuropathy and cardiovascular disorders [1]. The most common complication of diabetes is a diabetic kidney disease (DKD) frequently leading to end-stage renal disease (ESRD) and eventually to renal transplantation [2]. Although the heterogeneous pathogenesis of DKD is still unclear [3,4], the proposed mechanisms associated directly with diabetic complications include increased polyol pathway flux, increased hexosamine pathway and formation of advanced glycation end products (AGEs), and also activation of protein kinase C (PKC) pathway [5,6]. All these pathways are associated with oxidative stress directly or indirectly [7]. As known, increased concentrations of reactive oxygen species (ROS) in living organisms generate oxidative stress which is an important cause of cell injury during diabetes [8]. Since the existing treatment plans are still inadequate, it is important to search for new treatment approaches for DKD [9]. The great majority of the research focused on oxidative stress and antioxidants [10] and several studies reported that selenium administration improve antioxidant levels and protects the body from diabetes [11,12]. Podocyte loss, thickening of glomerular basement membrane, dysfunction of glomerular endothelium and deposition of extracellular matrix components in the mesangial area are the common structural alterations due to DKD [13,14].

Selenate functions as an integral component of several enzymes such as, glutathione peroxidases, deiodinases and selenoproteins [12]. Glutathione peroxidase was the first determined enzyme protecting cellular membranes from oxidants [15]. In addition to its role in enzyme function, selenate also acts as an antioxidative agent [16]. Selenate prevents the progress of diabetes [17] and it has been suggested as an attractive drug in therapy of diabetes [12].

In the current study, first the diabetes-induced structural and functional changes in rat kidney brush border cell membrane were determined. Then, the possible recovery effects of selenium on structural

Abbreviations: DKD, diabetic kidney disease; ATR-FTIR, attenuated total reflectance-Fourier transform infrared; ANN, artificial neural network; HCA, hierarchical cluster analysis; PCA, principal component analysis; ESRD, end-stage renal disease; AGEs, advanced glycation end products; PKC, protein kinase C; ROS, reactive oxygen species; MDA, malondialdehyde; TBA, thiobarbituric test; GLUT, glucose transporter; TGF-β1, transforming growth factor beta 1; IAPP, islet amyloid polypeptide; hIAPP, human islet amyloid polypeptide-protein.

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parameters such as molecular content, lipid order, protein secondary structure and functional parameters such as lipid fluidity were examined. To achieve this, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used. Finally, we applied chemometric methods such as HCA and PCA in order to obtain information on dissimilarities between experimental groups.

2. Materials and methods

2.1. Animal studies

Twelve- to 14-week-old male adult Wistar rats (250–300 g) were obtained from the Experimental Center of Adnan Menderes University. The ethics committee of Adnan Menderes University approved all experimental procedures. The animals were divided randomly into 5 groups: control ($n = 10$), diabetic ($n = 8$), low dose selenium-treated diabetic ($n = 8$), medium dose selenium-treated diabetic ($n = 8$) and high dose selenium-treated diabetic group ($n = 5$).

Formation of control group: Animals were injected with 0.05 M citrate buffer (pH 4.5) i.p. (i.e. intraperitoneal) as a single dose. In addition, physiological saline solution was injected daily for 5 weeks. All the animals were fed without any restriction for 5 weeks.

Formation of diabetic group: Diabetes was induced with single i.p. injection of streptozotocin (STZ) (50 mg/kg) dissolved in 0.05 M citrate buffer. Animals received 5% dextrose solution in order to prevent death due to hyperglycaemic shock. Four days after STZ injection, blood glucose levels were measured using a glucometer (One Touch Horizon Blood Glucose Monitoring System/Glucometer, USA). Blood glucose levels higher than 250 mg/dl were considered as diabetic. The animals were fed with standard diet without any restriction for 5 weeks.

Formation of selenium-treated diabetic groups: Diabetes was induced with i.p. injection of STZ (50 mg/kg) dissolved in 0.05 M citrate buffer as described previously. Four days after STZ injection and upon selection of diabetic rats, a subset of diabetic rats were divided into three groups. Low dose (1 μmol/kg), medium dose (5 μmol/kg) and high dose (25 μmol/kg) sodium selenate was injected i.p. daily for 5 weeks.

All experimental animals were decapitated at the end of the fifth week; their kidneys were removed and stored at −80 °C for experimental purposes.

2.2. Isolation of rat kidney brush border membrane vesicles

All procedures were carried out at 0 °C–4 °C. The renal brush border cell membranes were isolated using the calcium precipitation method proposed by Evers et al. [18] with some modifications [19]. The fatty tissue surrounding the kidneys was removed, the kidneys were dissected, and the cortex was obtained. The tissue was weighed and homogenized with 10% w/w suspension of mannitol buffer (10 mM mannitol, 2 mM Tris–HCl) for 2 min. The tissue was homogenized using Potter-Elvehjem glass homogenizer packed in crushed ice, coupled with a motor-driven (Black & Decker, V850, multispeed drill) teflon at 2400 rpm for 3 × 20 s. A total of 10 mM $CaCl_2$ was added to the final concentration. After 15 min, the homogenate was diluted 1:1 with a buffer containing 10 mM mannitol, 2 mM Tris–HCl and 10 mM $CaCl_2$. The homogenate was centrifuged at 500*g* for 12 min and the pellet was discarded. The supernatant was centrifuged at 15,000*g* for 12 min. The resulting supernatant was discarded and the pellet was resuspended with 4 ml mannitol buffer (10 mM mannitol, 2 mM Tris–HCl). This procedure was repeated with the exception that the pellet was suspended in 15 ml of buffer containing 10 mM mannitol, 20 mM Tris–HCl and 20 mM of zwitterionic organic chemical buffering agent, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and centrifuged at 48,000 g for 20 min. The supernatant was discarded and the pellet was homogenized with 1.5 ml of buffer containing 10 mM mannitol, 20 mM Tris–HCl and 20 mM HEPES by sucking the suspension 10 times through a steel needle into a plastic syringe. The homogenate was centrifuged at 2000*g* for 5 min, and the pellet discarded. The supernatant was centrifuged at 48,000*g* for 20 min. The resulting pellet contained the final brush border cell membrane vesicles. The pellet was suspended with 0.25 ml of final supernatant and was centrifuged at 14,000 rpm for 10 min. The supernatant was discarded and the pellet was used for ATR-FTIR experiments.

2.3. ATR-FTIR study

The infrared spectra of rat kidney brush border cell membrane samples were collected with a Spectrum 100 FTIR spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA), equipped with a Universal ATR accessory using the single-bounce mode. The isolated FTIR chamber is purged with dry air continuously (24 h) for the elimination of atmospheric water vapour. In addition, before each measurement, a background ATR spectrum is scanned with an empty crystal, which is automatically subtracted from the sample spectrum by the programme, in order to avoid the effect of atmospheric water. The samples (10 μl) were placed on a Diamond/ZnSe crystal plate (Perkin-Elmer) with a micropipette in two steps (5 + 5 μl), according to the absorbance value of the amide A band. In other words, the amide A band absorbance is exploited as an internal reference band. Eventually, similar amide A band absorbance values were ensured in all experiments before scanning the samples. By this way, similar and reproducible spectra were acquired. The samples were scanned from 4000 to 650 cm^{-1} with 200 interferograms at a resolution of 4 cm^{-1} at room temperature. The spectral collections of spectra and the data manipulations were carried out using the Spectrum 100 software (Perkin-Elmer). Since the membrane also contains water (solvent), the last buffer used during membrane isolation was scanned under identical experimental conditions as the samples. These spectra were manually subtracted from the sample spectra using the Spectrum 100 software (Perkin-Elmer). During the subtraction process, the free water band located around 2125 cm^{-1} was flattened using the subtraction procedure of the Spectrum 100 software (Perkin Elmer). The spectral bands resulting from different biomolecules were apparent only after the subtraction procedure. These difference spectra were used for all further spectral analyses. In all of these experiments, three different aliquots were scanned from the same sample and the buffer subtracted from each of these aliquots. Finally, the average (mean) spectra of these aliquots were used for all further spectral analyses.

The second derivative FTIR spectra were used rather than the absorbance spectra, because they provide better resolution during the determination of band positions [20]. The limitation of using the second derivative is that it is highly affected by noise even when a large number of interferograms are scanned. Hence, a 9-point Savitzky–Golay smoothing filter was used. This filter preserves features of the distribution of consecutive data points such as relative maxima, minima, and width [21]. The band area was calculated by quantifying the total area under each spectral band from the smoothed and baseline corrected spectra using the Spectrum 100 software. The spectra were normalized with respect to specific bands only for visual demonstration, easing observation of variations for the readers. It allows a point-to-point comparison to be made. The ratios of the intensities and shifting of the band wavenumbers were examined prior to the normalization process.

2.4. Artificial neural network analysis (ANN)

The effectiveness and accuracy of the neural networks over the other protein secondary structure prediction methods have been well-documented [22–25]. Taking into consideration these clear facts about the application of neural networks to biological systems, this technique has been exploited in the current study for a better understanding of the secondary structural variations in the diabetic kidney cell membrane proteins. In other words, the amide I band between 1700 and 1600 cm^{-1}, assignable to proteins, has been analysed using the