Food-advanced glycation end products aggravate the diabetic vascular complications via modulating the AGEs/RAGE pathway

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ABSTRACT The aim of this study was to investigate the effects of high-advanced glycation end products (AGEs) diet on diabetic vascular complications. The Streptozocin (STZ)-induced diabetic mice were fed with high-AGEs diet. Diabetic characteristics, indicators of renal and cardiovascular functions, and pathohistology of pancreas, heart and renal were evaluated. AGEs/RAGE/ROS pathway parameters were determined. During the experiments, the diabetic mice exhibited typical characteristics including weight loss, polydipsia, polyphagia, polyuria, high-blood glucose, and low-serum insulin levels. However, high-AGEs diet effectively aggravated these diabetic characteristics. It also increased the 24-h urine protein levels, serum levels of urea nitrogen, creatinine, c-reactive protein (CRP), low-density lipoprotein (LDL), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in the diabetic mice. High-AGEs diet deteriorated the histology of pancreas, heart, and kidneys, and caused structural alterations of endothelial cells, mesangial cells and podocytes in renal cortex. Eventually, high-AGEs diet contributed to the high-AGE levels in serum and kidneys, high-levels of reactive oxygen species (ROS) and low-levels of superoxide dismutase (SOD) in serum, heart, and kidneys. It also upregulated RAGE mRNA and protein expression in heart and kidneys. Our results showed that high-AGEs diet deteriorated vascular complications in the diabetic mice. The activation of AGEs/RAGE/ROS pathway may be involved in the pathogenesis of vascular complications in diabetes.

KEY WORDS Advanced glycation end products; Diabetic vascular complications; AGEs/RAGE/ROS pathway; c-Reactive protein; Reactive oxygen species


Introduction

Diabetic mellitus (DM) occurs in 370 million patients around the world [1]. DM can have devastating effects on the vasculature, leading to micro-vascular complications. It is also a leading cause of macro-vascular complications [2-3].

Micro- and macro-vascular complications are the major causes of morbidity and mortality [4]. The vascular structure and function would be changed by the high level of blood sugar, which would affect the heart, kidneys, nervous system, and lower limbs [5-6]. Cardiovascular disorder is important in macro-vascular complications. Cardiovascular complications are also the fatal diseases in non-insulin dependent diabetes mellitus (NIDDM) (65%). Meanwhile, renal involvement is the major microvascular complication. Diabetic nephropathy is the most common cause of end stage renal disease (ESRD), contributing to approximately 45% of new cases, and is an independent risk factor for cardiovascular diseases [7], such as atherosclerosis, hypertension, acute myocardium infarction, chronic heart failure and sudden death, which are the major complications of DM [8-9]. Indeed, cardiovascular complications are the leading cause of morbidity and mortality among...
diabetic patients, accounting for some 50% of all diabetes fatalities [7, 10].

There is growing evidence supporting that production and accumulation of advanced glycation end products (AGEs) are involved in the initiation and development of micro- and macro-vascular complications observed in diabetes mellitus [3, 11-12]. AGEs are formed through a series of reactions (Fig. 1) [13]. They are accumulated quickly in diabetic patients, and their serum levels of AGEs are significantly higher than that in healthy persons. The serum levels of AGEs in diabetes plus coronary disease patients are increased significantly compared to diabetic patients [14]. They are also increased in parallel with the degrees of coronary atherosclerosis [15-16]. The AGEs are more important for in for coronary atherosclerosis with NIDDM than hypertension, hyperlipidemia, or smoking [17]. In addition, the AGEs depositions are discovered in the carotid atherosclerotic plaque and cardiac muscle fibers [18-19]. Above all, AGEs have been indicated as the initial trigger for DN, such as activating a series of intracellular signal-cascade pathways [20-22]. AGEs are accumulated in glomerular basement membranes, mesangial cells, endothelial cells, and podocytes of the patients with DN and/or ESRD failure [22]. What is more, the AGE-receptors (RAGE) could be expressed by mesangial cells, tubular cells, podocytes and endothelial cells [23-26]. The AGEs-RAGE interaction is considered as an initial trigger for DN, such as activating a series of intracellular signal-cascade pathways [20, 27-28].

The AGES/RAGE pathway has been regarded as the pathological activation in diabetic vascular complications. It will activate the base of vascular pathologies. For example, it can interfere the vascular endothelial function in DM. Nevertheless, whether high-AGEs diet could aggravate the diabetic vascular complications is not clear. And mechanisms that possibly enhance pathological changes in diabetic vascular complications by AGES/RAGE pathway are not known. In the present study, we fed the Streptozocin (STZ)-induced diabetic mice with high-AGEs diet. Then deteriorative effects of high-AGEs diet in diabetic vascular complications were observed. Besides, different pathological indicators are detected to verify the pathogenic mechanisms of diabetic vascular complications by the AGES/RAGE/ROS pathway. On this basis, we also aimed to establish this diabetic angiopathy model for the further study in the research and development of natural medicines.

Materials and Methods

Experimental procedures

SPF, male C57BL/6J mice, weighing 18–22 g [SCXK (Su) 2009-0001] were purchased from the Suzhou Industrial Park IMTE Co., Ltd. (Suzhou, China). They were housed in an air-conditioned room at 23 ± 2 °C with a 12/12 h light/dark schedule. The mice were kept under observation for one week prior to the start of the experiments. All the procedures were approved by the Animal Ethics Committee of Nanjing University. The mice were intraperitoneally injected with STZ (100 mg·kg⁻¹) twice on Days 1 and 4, respectively. Fasting blood glucose levels were measured on Day 8, using blood-glucometer (Bayer Contour TS, Leverkusen, Germany). The mice with high fasting blood glucose levels (≥15 mmol·L⁻¹) were selected as the diabetic mice [29-30]. The diabetic mice were randomly divided into STZ group and (AGES + STZ) group. Simultaneously, normal mice were divided into AGES group and normal group. Each group contained 10 mice. AGES group and (AGES + STZ) group were fed with high AGES diet, and the other groups were fed with normal diet. In every 4 weeks, body weights, 24-h food consumption, 24-h water intake, 24-h urine volume, and fasting blood glucose levels were measured. At the end of the intervention, all the mice were fasted overnight and then sacrificed. Fasting blood was collected, and the pancreas, kidneys, livers, and testes were dissected. Tissues and sera were kept at −80 °C until further analysis.

Reagents

The following kits and reagents were used in the present study and the products and sources were as follows: Mouse AGES ELISA kit, Nanjing Jiancheng Bioengineering Institute (Nanjing, China), 20110618; Mouse insulin ELISA kit, BD (Franklin lakes, USA), 14110401; Mouse TNF-α ELISA kit, eBioscience (San Diego, USA), E095081643; Mouse IL-6 ELISA kit, eBioscience (San Diego, USA), E093071637; First antibody, β-Tubulin, genus: rabbit anti mouse, Bioworld Technology (Nanjing, China), AA00111; First antibody, RAGE, genus: rabbit anti mouse, abcam (Cambridge, USA), GR140079-3; β-Tubulin, genus: rabbit anti mouse, Bio-world (Nanjing, China), 101104; Second antibody, horseradish peroxidase labeled, genus: goat anti rabbit, Bio-world (Nanjing, China), 3H293H29; RT-PCR reaction kit, Bao biological Co. Ltd. (Dalian, China), BK2101; Primers, Jie rui Bioengineering Co., Ltd. (Shanghai, China), 13112H29; EB, DEPC, Streptozotocin, TEMED, SDS, Sigma (St. Louis, Franklin lakes, USA), 14110401; Mouse insulin ELISA kit, BD (Franklin lakes, USA), 14110401; Mouse TNF-α ELISA kit, eBioscience (San Diego, USA), E095081643; Mouse IL-6 ELISA kit, eBioscience (San Diego, USA), E093071637; First antibody, β-Tubulin, genus: rabbit anti mouse, Bioworld Technology (Nanjing, China), AA00111; First antibody, RAGE, genus: rabbit anti mouse, abcam (Cambridge, USA), GR140079-3; β-Tubulin, genus: rabbit anti mouse, Bio-world (Nanjing, China), 101104; Second antibody, horseradish peroxidase labeled, genus: goat anti rabbit, Bio-world (Nanjing, China), 3H293H29; RT-PCR reaction kit, Bao biological Co. Ltd. (Dalian, China), BK2101; Primers, Jie rui Bioengineering Co., Ltd. (Shanghai, China), 13112H29; EB, DEPC, Streptozotocin, TEMED, SDS, Sigma (St. Louis,
USA); Trizol reagent, Invitrogen (Carlsbad, USA); 5xTBE buffer, Generay Biotech (Shanghai, China); DNA Marker, ECL light-emitting liquid, Thermo (Waltham, USA); glycine, Tris base, AP, Amresco (Cleveland, USA); and skim milk powder, BD (Franklin lakes, USA). All the other chemicals were of analytical grade.

**High-AGEs diet**

High AGEs diets were produced by baking AIN-93G diet at 160 °C for 60 min \(^1\). The AIN-93G diets were provided by Trophic Animal Feed High-tech Co., Ltd. (Nantong, China). The content of AGEs in the diet was determined by measuring emission at 440 nm with excitation at 370 nm, using a fluorescence spectrophotometer (HIWilliam F-2500, Tokyo, Japan).

**Biochemical analyses of urine and serum samples**

In every 4 weeks, urinary proteins levels in the supernatant were determined after the 24-h urine samples were centrifuged (3 000 r·min\(^{-1}\), 10 min). The levels of urinary proteins, urea nitrogen, creatinine, CRP, and LDL were detected according to the corresponding assay kit instructions designed by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). For light microscopic examination, the renal, pancreatic and cardiac fragments were fixed in 2.5% glutaraldehyde in 0.1 mol·L\(^{-1}\) cacodylate buffer solution (pH 7.4) and fixated in 1% osmium tetroxide phosphate buffer solution. Followed by dehydration using a graded series of ethanol, and embedded in epoxide resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Glomerular endothelial cells, mesangial cells, and podocytes were examined by electron microscope (Hitachi H-800, Tokyo, Japan).

**Morphology Examination**

For light microscopic examination, the renal, pancreatic and cardiac fragments were fixed in 10% neutral buffered formalin and embedded in paraffin, and 5-μm thick sections were cut and stained with hematoxylin-eosin (H&E), and then examined by light microscopy. Photographs at random fields were taken in a blinded fashion. After that, the levels of renal cortex were fixed in 2.5% glutaraldehyde in 0.1 mol·L\(^{-1}\) cacodylate buffer solution (pH 7.4) and fixated in 1% osmium tetroxide phosphate buffer solution. Followed by dehydration using a graded series of ethanol, and embedded in epoxide resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Glomerular endothelial cells, mesangial cells, and podocytes were examined by electron microscope (Hitachi H-800, Tokyo, Japan).

**Western blotting analysis**

Western blot analysis was performed after using Amersham ECL\(\text{TM}\) Western Blotting Detection Reagents (Thermo, Waltham, USA, PA197033), the densities of bands were visualized with a Luminescent image analyzer (GE Healthcare ImageQuant LAS 4000, Fairfield, CT, USA).

**Statistical Analysis**

The data were expressed as means ± standard deviations. Statistical analysis was carried out using standard statistical methods (GraphPad Prism 5.0). Significance of difference between means was determined by unpaired Student’s t-test.
Analysis of variance with the Dunnett’s test for post hoc analysis was used to compare the multiple treatment conditions. \( P < 0.05 \) was regarded statistically significant.

**Results**

*High-AGEs diet aggravates the typical characteristics of STZ-induced diabetic mice*

After being baked in 160 °C for 120 min, the AGEs level was significantly higher in the baked diet compared to other diets (Fig. 2A). Afterwards, more diets were baked on the condition. During the experiment, we fed mice in all groups with the baked diet, except normal group. The typical characteristics of weight loss, polydipsia, polyuria, and polyphagia were observed in the STZ group compared to the normal group, which were exacerbated in AGEs + STZ group (Figs. 2B–E).

![Graph](image)

**Fig. 2** High-AGEs diet exacerbates the symptoms in the STZ-induced diabetic mice (n = 10). (A) Measurement of AGEs levels in baked diets under different roasting conditions. **\( P < 0.01 \) vs 0; \( \Delta \Delta P < 0.01 \) vs 120. (B) Examination of body weight at the 0th, 4th, 8th and 12th weekends. (C) Examination of 24-h water intake at the 0th, 4th, 8th and 12th weekends. (D) Examination of 24-h urine volume at the 0th, 4th, 8th and 12th weekends. (E) Examination of 24-h food consumptions at the 0th, 4th, 8th and 12th weekends. (F) Examination of fasting blood glucose levels at the 0th, 4th, 8th and 12th weekends. The data are expressed as mean ± SD. **\( P < 0.01 \) vs Normal; \( \Delta \Delta P < 0.01 \) vs AGEs; \( \# \# P < 0.01 \), \( \# P < 0.05 \) vs STZ.
Fig. 3 High-AGEs diet exacerbates the renal functions and insufficient insulin secretion in the STZ-induced diabetic mice (n = 10). (A) Examination of 24-h urinary protein excretion at the 0th, 4th, 8th and 12th weekends. (B) Examination of serum urea nitrogen levels. (C) Examination of serum creatinine levels. (D) Examination of serum insulin levels. Data are expressed as mean ± SD. **P < 0.01 vs Normal; ΔΔP < 0.01 vs AGEs; ##P < 0.01 vs STZ

However, the levels of fasting blood glucose were significantly higher in AGEs + STZ group, compared to other groups. In the AGEs + STZ group, these parameters indicated that high-AGEs diet aggravated the typical characteristics of STZ induced diabetic mice.

**High-AGEs diet deteriorates kidney injury and decreases insulin level in STZ-induced diabetic mice**

The renal function indexes were significantly higher in the STZ group, compared with normal group or AGEs group. Among AGEs + STZ group, 24-h urinary protein excretion, serum urea nitrogen and serum creatinine levels were significantly higher in the STZ-induced diabetic mice with high-AGEs diet (Figs. 3A–C). Furthermore, high-AGEs diet also significantly reduced the serum insulin level in the STZ-induced diabetic mice (Fig. 3D). These changes suggested that high-AGEs diet would deteriorate kidney injury and decrease insulin level in the STZ-induced diabetic mice.

**High-AGEs diet intensifies the histology diseases in STZ-induced diabetic mice**

We mainly examined the pancreatic islets and acinus cells in pancreas. Moreover, cardiac muscle, fibrous tissues in heart and glomerulus, tubules, mesangium matrix in kidneys were detected. In the STZ-induced diabetic mice, irregular shape, rough edge, and cell degeneration were found in pancreatic islets. In addition, islet lesion and vacuolization were observed in acinus cells. In addition, interstitial edema and interlobular septa increase were detected in pancreas. In heart, acidophilic degeneration, vacuolization, hypertrophy and necrosis were observed in cardiac muscle cells. Fibrous tissue hyperplasia, disordered arrangement, and breakage were also found in heart. Glomerular atrophy and increased
Fig. 4  High-AGEs diet deteriorates the cardiovascular diseases in the STZ-induced diabetic mice (n = 10). (A) Examination of serum CRP levels. (B) Examination of serum LDL levels. (C) Examination of serum TNF-α levels. (D) Examination of serum IL-6 levels. The data are expressed as mean ± SD. **P < 0.01 vs Normal; △△P < 0.01 vs AGEs; ##P < 0.01 vs STZ

mesangial matrix were observed in kidneys. Loose and litty arrangement and vacuolar degeneration were also found in renal tubules. However, in AGEs + STZ group, high-AGEs diet could exacerbate the pathological manifestations (Fig. 5A). Deeperstudy in endothelial cells, mesangial cells and podocytes was proceeded using a transmission electron microscope. Hyperplasia and swelling of the endothelial cells, cell proliferation, matrix increased in mesangium mesangial cell, lysosomal inclusion, nuclear irregularity and fusion of foot process in podocytes were observed in the STZ group. These pathological changes were deteriorated in the AGEs + STZ group (Fig. 5B). In addition, the nuclear membrane invagination and nuclear irregularity in mesangial cell, degeneration and necrosis in podocytes were observed in the AGEs + STZ group. These results demonstrated that high-AGEs diet intensified the histological diseases in the STZ induced diabetic mice.

High-AGEs diet increases AGEs/RAGE/ROS/SOD levels in the STZ-induced diabetic mice

Numerous studies have described that AGEs, ROS, and SOD are important factors in diabetic vascular dysfunction. Therefore, we hypothesized that the high-AGEs diet could increase the ROS level and decrease the SOD level in the STZ-induced diabetic mice. To test the hypothesis, we detected the AGEs (Figs. 6A1–6A3), SOD (Figs. 6B1–6B3), ROS (Figs. 6C1–6C3), RAGE mRNA (Fig. 6D) and RAGE (Fig. 6E) protein levels in serum and tissue samples. The result indicated that AGEs, ROS, RAGE mRNA and RAGE protein levels in the STZ group were significantly higher, compared to that in the normal group. The SOD levels were significantly decreased in the STZ group. However, high-AGEs diet showed significantly exacerbation in the AGEs + STZ group, compared to that in STZ group. These data suggested that high-AGEs diet could enhance AGEs/RAGE/ROS pathway in diabetes, which contributes to exacerbate the diabetic vascular complications.

Discussion

AGEs, which are formed by non-enzymatic Maillard or “browning” reaction between reducing sugars and amino groups on proteins, lipids or nucleic acids. Heating (100–250 °C), such as cooking and roasting, are very important for AGEs formation in food processing [33]. AIN-93G diet baked at 160 °C for 120 min [31] is customary means to prepare high-AGEs diet for mice. This thermal treatment enhances the formation of food-AGEs significantly, which is consistent with our results. AGEs are considered as the chemically heterogeneous group of compounds with strong biological
Fig. 5 High-AGEs diet deteriorates the pathological changes of renal, heart and pancreas in the STZ-induced diabetic mice (n = 3). (A) Renal, heart and pancreas pathological change assessment with HE staining (×400). (B) Endothelial cells, mesangial cells and podocytes structural examination with transmission electron microscope.

AGEs affect nearly every type of cells and molecules in the body and are thought to be one major risk factor in aging and some age-related chronic diseases [34–36]. They are also believed to play a causative role in the diabetic vascular complications [37]. Studies suggest that, in diabetic patients with micro and/or macro-vascular complications, increasingly higher levels of circulating AGEs are observed. The manifestation indicates that the higher the serum AGE level is, the greater the likelihood of development of diabetic vascular complication is. Earlier gradual increase in serum
Fig. 6 High-AGEs diet increases AGEs/RAGE/ROS/SOD levels in the STZ-induced diabetic mice. (A) Examination of AGE levels in serum, heart and renal (n = 10). (B) Examination of SOD levels in serum, heart and kidneys (n = 10). (C) Examination of ROS levels in serum, heart and kidneys (n = 10). (D) RT-PCR analyses of RAGE mRNA expression in heart and renal (n = 3). (E) Western blot analyses of RAGE protein expression in heart and kidneys (n = 3). The data are expressed as mean ± SD. **P < 0.01 vs Normal; △△P < 0.01 vs AGEs; ^P < 0.05, ^△P < 0.01 vs STZ

AGE-level has been reported with the severity of atherosclerosis in diabetic patients [15-16]. The evidences from animal and human studies support that significant amounts of diet-AGEs are absorbed, and that
diet-AGEs result in the body’s burden of AGEs, and are associated with diseases such as atherosclerosis and kidney diseases [33]. In addition, STZ is a glucosamine-nitrosourea compound which could enter into islet β cells by glucose transporters and destroy the cells [38]. It affects various animals, such as rats, mice, guinea pigs, and rabbits [59]. Of note, STZ will inhibit the synthesis, secretion and sensibility of insulin. It interferes with the glucose metabolism and induces the diabetes mellitus finally. In current study, the diabetic C57BL/6 mice were induced by intraperitoneal injection of STZ. Shortly afterwards, diabetic mice in AGEs + STZ group were fed with high-AGEs diet for 12 weeks. Throughout the experiment, the diabetic mice displayed typical diabetic syndrome: polyphagia, polydipsia, polyuria, and emaciation. However, these indicators of diabetic mice were deteriorative in the AGEs + STZ group, indicating that high-AGEs diet exacerbate diabetic mellitus in the STZ-induced diabetic mice. Compared with the normal group, the fasting blood glucose level was significant higher in STZ group throughout the experiment. However, these indicators of diabetic mice and the fasting blood glucose levels were significant in approximate in the AGEs + STZ group and the STZ group, which indicated that these diabetic indicators were exacerbated by high-AGEs diet exacerbated diabetic mellitus in STZ-induced diabetic mice, which excluded the influence of fasting blood glucose.

Based on these results above, we speculated that the diabetic vascular complications could also be deteriorated by high-AGEs diet. Since the nephropathy and cardiovascular diseases mainly occur in diabetic vascular complications, we focused on the examination of nephritic and cardiac indicators. Generally, the increase of urinary protein, urinary creatinine and urea nitrogen are major manifestations of DN. The persistent proteinuria could be found in primary DN patients. Urinary protein is known as the feature in diagnosing the DN [40-41]. Creatinine is the metabolite of human muscle, excreted with urine through glomerular filtration, which is also known as urinary creatinine. The increase in serum creatinine is in parallel with the augument of the progression of DN [42]. Urea nitrogen is the major end-product of protein metabolism in humans. It causes a subsequent spike in serum urea nitrogen level, which is commonly occurring in glomerulonephritis or DN patients with decreased glomerular filtration rate. Thus, serum urea nitrogen level can be used as a marker in DN for diagnosis [43]. Furthermore, the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and oxidative stress are widely recognized markers of vascular inflammation [44-49]. An increase in circulating levels of TNF-α and IL-6 is known to decrease insulin sensitivity and increase vascular inflammation, leading to the development of cardiovascular diseases [45-51]. Of note, C-reactive protein (CRP) has received considerable attention as a risk marker for cardiovascular disease. Chronic modest elevations in CRP levels have been associated with a greater likelihood of acute cardiovascular syndromes, including myocardial infarction, sudden cardiac death, stroke, and peripheral vascular diseases [52-56]. LDL particles pose another risk for cardiovascular disease when they invade the endothelium and become oxidized, since the oxidized forms are more easily retained by the proteoglycans. Increasing concentrations of LDL particles are strongly associated with increasing amounts of atherosclerosis within the walls of arteries over time, which eventually results in sudden plaque ruptures and triggering clots within the artery opening, or a narrowing or closing of the opening, i.e., cardiovascular disease, stroke, and other vascular disease complications. TNF-α, CRP, and IL-6 are also the main mediators involved in the pathogenesis of DN [58]. Given this understanding, we selected 24-h urinary protein excretion, serum levels of urea nitrogen and creatinine as primary indicators for renal functions. The serum levels of CRP, LDL, IL-6, and TNF-α were also examined as the representative indicators for cardiovascular complications. Among these data, significant changes were found in the STZ group, compared to the normal group. High-AGEs diet aggravated the changes in the AGEs + STZ group. Further histological evidences strengthened the negative role of high-AGEs diet in kidneys, heart and pancreas. These data clearly indicated that high-AGEs diet could deteriorate nephropathy and cardiovascular diseases in diabetic mice induced by STZ.

AGE-RAGE interactions have been suggested to contribute to the pathogenesis of diabetic vascular complications via various mechanisms. RAGE is a 45-kDa transmembrane receptor of immunoglobulin super family and expressed on the cell surface of monocytes, macrophages, endothelial cells, mesangial cells, podocytes, tubular epithelial cells, astrocytes, microglia, and smooth muscle cells [59]. As the RAGE expression enhanced, signaling pathway such as NAD(P) H/ROS, protein tyrosine kinase (PTKs)/P21RAS [60], p38mitogen-activated protein kinase (P38MAPK)extracellular signal-regulated kinase1/2 (ERK1/2), Sphingosine kinase 1 (Sphk-1)/ sphingosine 1-phosphate (S1P) [61] will be activated. Then, they will activate NF-xB, a transcription regulator that regulates the genetic transcription of endothelin-1, cell adhesion molecule-1, and tissue factors. Furthermore, various damage factors (TNF-α, IL-1, TGF-β, VEGF, MCP-1, andIGF-1) and adhesion molecules (ICAM-1, VCAM-1) will be produced [13, 23]. Finally, they will cause the diabetic pathology changes. AGES can decrease the activities of antioxidant enzymes such as SOD [62] or directly stimulate ROS production [63] via receptor-dependent signaling. It will cause the occurrence of oxidative stress [64]. Studies have indicated that oxidative stress plays a pivotal role in the development of diabetes complications, especially cardiovascular complication [65-66]. Our present data could support this notion, because parameters for oxidative stress...
including ROS and SOD could be improved in the diabetic mice. Of note, high-AGEs diet could increase ROS levels and decrease SOD levels in the AGES + STZ group. These parameters are correlated significantly with RAGE expression and demonstrated that AGE-mediated induction of RAGE expression contributes to increased oxidative stress generation. Therefore, it is considered as key stimulus for diabetic vascular complications.

In summary, high-AGEs diet aggravated characteristic symptoms and exacerbated renal and heart complications in the STZ-induced diabetic mice. High-AGEs diet increased AGES levels, RAGE protein/mRNA expressions, and ROS levels, and decreased SOD levels in the diabetic mice. Taken together, the present study revealed that food-advanced glycation end products could aggravate the angiopathies in STZ-induced diabetic mice, which may contribute to the AGE/RAGE/ROS interactions that play a key role in pathogenesis of vascular complications in diabetes. Furthermore, our current data validated the feasibility of using this mouse diabetic angiopathy model for the research and development of natural medicines, based on the modulation of the AGES/RAGE pathway.

References

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