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Molecular Targets of Combined Natural Antioxidants Against Diabetes in Streptozotocin-induced Diabetic Mice

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Abstract Natural antioxidants, due to the different chemical structures and their unique biological protection effects on islet cell, have been received much attention in applied chemistry field for developing a new diabetic drug. However, the molecular targets of the islet cell protection have been remained obscure. For investigating molecular targets of combined natural antioxidant's(CNA) roles in against diabetes, type 1 diabetic mouse model was made by multiple injection of low doses of streptozotocin into C57BL mice. Two combinations of natural antioxidants were orally supplemented to the mice. Blood glucose level, glutathione peroxidase(GSH-Px) and superoxide dismutase (SOD) activities, and malondialdehyde(MDA) contents were measured *via* biochemical assays. Tumor necrosis factor α(TNF-α), interferon γ(IFN-γ) and Interleukin-12(IL-12) in $CD4^+$ Peripheral blood mononuclear cells(PBMC) were evaluated with flow cytometry(FCM). *In situ* hybridization(ISH) and immunohistochemistry(IHC) were used to detect insulin gene expression. The results demonstrate both the combinations significantly: increased GSH-px and SOD, but decreased MDA levels in pancreas(p<0.05—0.01); up-regulated insulin expression at both mRNA and protein levels in islet cells(p<0.05—0.01); down-regulated TNF-α and IL-12 expression in PBMC(p<0.05—0.01). Our data suggest that the CAN may regulate multiple key molecular targets related to β cell function, including reduction of oxidative stress and pre-inflammatory cytokines, and upregulate insulin gene expression to reduce the development of diabetes.

Keywords Combined natural antioxidant; Diabetes; Insulin gene expression; Oxidative stress; Pro-inflammatory cytokine

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1 Introduction

Although the etiology of diabetes is not fully defined, the increased oxidative stress and autoimmune-mediation destruction of pancreatic β-cells are widely accepted as participant in the development and progression of diabetes. Interestingly, pancreatic islets are susceptible to oxidative damage from reactive oxygen species(ROS) and nitric oxide(NO) due to the natural weakness in antioxidative defense system[1—5]. High glucose activates oxidative stress sensitive signaling pathways which further promote the overexpression of pre-inflammatory cytokines. These cytokines in turn cause pancreatic β-cell cytotoxicity. Thus, oxidative stress and overexpression of pre-inflammatory cytokines due to environmental and genetic pathogenic factors might cause slow and progressive pancreatic β-cell lesions, resulting in diabetes onset and development[6—9]. For these reasons, exploring a new antioxidative pathway to protect pancreatic β cells from both oxidative damage and autoimmune-mediation destruction has recently attracted much attention[10—12]. Natural antioxidants, due to the different chemical structures and unique biological protection effects of them on islet cells has been strongly concerned for developing a new diabetic drug in applied chemistry field. Some results have demonstrated that natural antioxidants, such as Se and V_E, may increase the serum insulin level of diabetic rats through the decrease of the oxidative stress on islet[13—15], but the combined effects of multiple antioxidants on improving islet functions in diabetes, and the molecular mechanism has remained obscure. In this study we evaluated the roles of combined natural antioxidants(CNA) in regulating critical gene expressions related to oxidative stress, autoimmune-mediation destruction of islet, and islet function in streptozotocin-induced diabetic mice. On the basis of this study, the united biological roles of CNA and the molecular mechanism for the protection of islet β cells will be further elaborated for improving the prevention and treatment of diabetes and its complications.

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2 Experimental

2.1 Chemicals

Streptozotocin(STZ) was purchased from Sigma-Aldrich Co., Ltd.(Shanghai, China); V_C, V_E, vanadium(V), lipoic acid, and niacinamide from Beijing-Oboxing Biotech. Co., Ltd.(Beijing, China), Se(kappa-selenocarrageenan) from Shanghai Tiansifu Bio. Co., Ltd.(Shanghai, China); chromium-enriched yeast(Cr) from the Institute of High Energy Physics, Chinese Academy of Sciences(Beijing, China); the kits measuring glutathione peroxidase(GSH-px), superoxicle dismutase(SOD), and malondial dehyde(MDA) activity from Nanjing Institute of Biological Engineering Co., Ltd.(Nanjing, China); FITC-conjugated monoclonal antibodies against mouse TNF-α, IFN-γ and IL-12 from Jingmei Biotech Co., Ltd.(Beijing, China), and the kits for insulin *in situ* hybridization(ISH) and immunohistochemistry (IHC) from Wuhan Biological Engineering Co., Ltd.(Wuhan, China).

2.2 Animal Grouping and Type 1 Diabetic Mouse Model

Eighty healthy 7-week-old male C57BL mice[(22±2) g] were obtained from the Experimental Animal Center of the Norman Bethune Medical College, Jilin University(Changchun, China). Mice were stochastically divided into four groups(n=20 for each group): (1) normal control; (2) STZ-induced diabetic model(diabetic control); (3) combined CNA(CNA4) group with Se, V_E, V, Cr; (4) combined CNA(CNA7) group with Se, V_E, V, Cr, V_C, lipoic acid and niacinamide. Diabetic mice models were made by five-day intraperitoneal injection of 0.6% STZ(65 mg/kg body mass) dissolved in an ice-cold citrate buffer(pH=4.2) just before injection[16]. The control group was injected with the same volume of buffer alone. Blood was taken from the tail vein every week for the determination of blood glucose level with a Sentest JPS-3 Blood Glucose Detector(Yicheng Bioelectronic Technology Co., Ltd., China). The mice whose blood glucose levels were higher than 12.0 mmol/L and maintained for at least 2 weeks were selected for the study.

2.3 Animal Feeding and CNA Supplementation

Mice were housed and cared about according to the institution's guidelines in an environment-controlled room[temperature: (22±2) °C; relative humidity: (60±5)%; light/dark cycle: 12 h/12 h]. Mice were fed with mice routine balanced nutrition food(Experimental Animal Centre of Jilin University, China) and had free access to food and water. After the establishment of the diabetic model, CNA4 and CNA7 groups were supplemented by gastric gavage with different CNA combinations. The daily supplement contents of CNA are shown in Table 1. After 7 weeks of CNA supplementation, mice were withheld food for 12 h and then anesthetized with aether. Whole blood samples were taken from blood vessels of the orbital cavity for FCM and biochemical assay. Pancreases were removed for insulin ISH and IHC staining.

Table 1 Animal groups and CNA supplementation(mg·kg^{-1} body mass·d^{-1})

Group	n	Se	V_E	V	Cr	V_C	Lipoic acid	Niacinamide
Normal control	20	—	—	—	—	—	—	—
Diabetic control	20	—	—	—	—	—	—	—
CNA4	20	0.14(16.6)[a]	100	1.785(14)[b]	0.14(127.27)[c]	—	—	—
CNA7	20	0.14(16.6)[a]	100	1.785(14)[b]	0.14(127.27)[c]	100	0.7	70

a. Kappa-selenocarrageenan; *b*. $NaVO_4 \cdot 12H_2O$; *c*. Cr-enriched yeast.

2.4 Biochemical Analysis

Serum glucose levels were measured with a commercial kit as previously described once a week for eight weeks successively[17]. Supernatants from homogenized pancreases were obtained for measuring GSH-Px and SOD activities as well as MDA content with commercial available kits according to the manufacture's instructions. The levels of insulin in serum and pancreatic homogenates were measured *via* RIA with a commercial kit from the Institute of Atomic Energy(Beijing, China).

2.5 ISH and IHC

Pancreases of the mice were removed for insulin ISH and IHC staining after 7 weeks of CNA supplementation.

Mouse insulin ISH: briefly, the 3 μm pancreas sections were deparaffinized, cleared and rehydrated, then incubated with 3%(volume fraction) H_2O_2 for 30 min. After three washes (5 min each) in distilled water, the sections were subjected to 3% pepsin for 30 min at 37 °C, and flowed in 0.2% glycine to stop the reaction. Sections were re-fixed in 4% paraformaldehyde for 10 min, rinsed in distilled water, dehydrated and air-dried. Following this, the sections were covered with 20 μL of pre-hybridization buffer for 2 h at 42 °C and then incubated with hybridization buffer containing DIG-labeled antisense or sense probe overnight at 42 °C. For signal detection, the sections were incubated with biotinylated mouse anti-DIG F(ab) fragments for 60 min, followed by washing in phosphate buffer solution(PBS, 5 min×4) and then incubated for 20 min at 37 °C with the avidin-biotin peroxidase complex. DAB/hydrogen peroxide was used to observe the positive signals.

For testing the immunoreactivity of insulin, IHC staining was performed *via* the avidin-biotin complex(ABC) method. After blocking endogenous peroxidase, the sections were washed in distilled water; incubated with 5%(volume fraction) normal goat serum and with rabbit second antibody, respectively; after washing in 0.1 mol/L PBS, the sections were incubated with biotinylated goat anti-rabbit antibody and washed in 0.1 mol/L PBS and then incubated with the avidin-biotin peroxidase complex. Immunoreactivity of insulin was visualized with DAB/ hydrogen peroxide. The sections were finally rinsed

in distilled water, lightly stained with hematoxylin, dehydrated, cleared, and covered with cover-slips. Sample processed without primary antibody served as negative control.

Pathological image analysis system(HPIAS-1000, Qianping Image Engineering Company, Tongji Medical College, China) was used to quantify ISH and IHC positive staining in pancreatic sections under a light microscope. Averagely gray level of insulin positive staining cells and ratio between staining positive area(the sum of area of insulin-positive staining cells in each islet) and islet area(every islet on the pancreas sections was circled carefully by a light pen and their average area was calculated) were measured. Five random fields of each section and 5 sections of each group(n=5) were analyzed.

2.6 Flow Cytometric Assay

After 7 weeks of CNA supplementation, the mice were withheld from food for 12 h and then anesthetized with aether, from which 100 μL of blood was taken and incubated with a mixture of 5 μL of PMA(5 μg/mL), 10 μL of ionomycin(100 μg/mL) and 2 μL of monensin(1 mg/mL) and then incubated with 10 μL CD4-PC5; after hemolysis, the cells were washed, centrifuged, and then fixed in 100 μL of 1%(volume fraction) paraformaldehyde; after washing, the cell membrane was broken with 100 μL of 0.5 mol/L Triton-100 for 5 min, incubated with 20 μL of PE-conjugated rabbit anti-mouse IgG antibodies against TNF-α, IFN-γ and IL-12, respectively at room temperature for 15 min, in the dark. The cells were washed and then resuspended with 300 μL of 0.1 mol/L PBS. The percentages of $CD4^+$ lymphocytes expressing above cytokines was detected with a flow cytometer(ELITE-II, Coulter Co., USA), respectively.

2.7 Statistical Analysis

Statistical analysis was carried out *via* SPSS 11.0 for Windows(SPSS Co., Ltd., Chicago, USA). One-way ANOVA was used to assess the differences among multiple groups. Results were presented as the mean±SD. Statistical significance was accepted at P<0.05.

3 Results and Discussion

3.1 Body Mass and Blood Glucose Level

With increasing blood glucose levels, excessive thirst, urination and weight loss were observed for the mice in the diabetic group compared to those in the normal control group. Supplementation of CNAs partially restored the mass loss (Fig.1). After 5 continual injections of STZ, the blood glucose level of these mice slowly but significantly increased compared to that in the normal control groups(Fig.2). Around 14 d after the first injection of STZ, the blood glucose level increased to 16—17 mmol/L. After this, there was a slight decline in blood glucose, but a relatively high level still persisted for up to 7 weeks. The shapes of the blood glucose level curves of the CNA groups were similar to that of the model group, but the value at each corresponding time point was significantly lower (P<0.05 or P<0.01) except that in the 2nd week of supplementation of CNA(Fig.2). There are many similarities between human type 1 diabetes and multiple low dose streptozotocin (MLDS)-induced diabetic mice in terms of clinical symptoms, disease processes and pathologic changes of pancreatic islets[18]. Thus, the diabetic mouse model induced by MLDS has been used for a long time to investigate the pathogenesis of T1D. STZ might damage the membrane structure of relevant organelles to induce enzyme diffusion and alter enzyme activity, influencing the structure and function of β cells[16,17]. On the other hand, STZ also causes significant methylation of pancreatic DNA, and if this occurs in the β cells, it may account for their destruction[19].

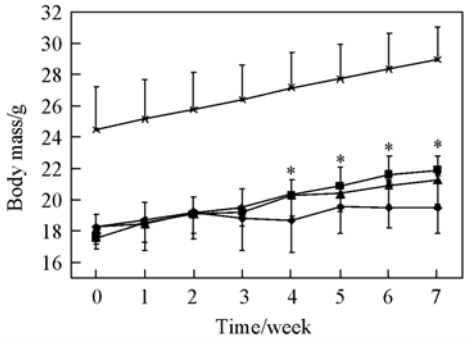

Fig.1 Effects of CNA on body weight of T1D mice

Values are means±SD, n=12. * P<0.05 *vs*. diabetic group. ◆ Diabetic control; × normal control; ■ CNA4 group with Se, V_E, Cr, and V; ▲ CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C.

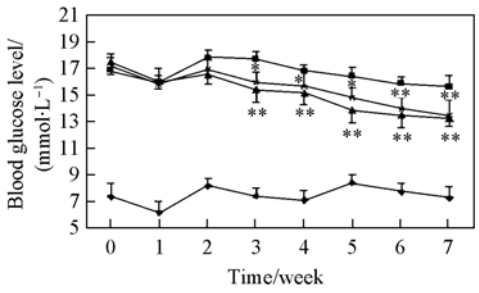

Fig.2 Effects of CNA on blood glucose level of T1D mice

Values are means±SD, n=12. * P<0.05, ** P<0.01 *vs*. diabetic model group. ◆Normal control; ■ diabetic control; ▲ CNA4 group with Se, V_E, Cr, and V; × CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C.

3.2 Effects of CNA on Serum and Pancreatic Insulin Levels

Total serum and pancreatic insulin levels significantly decreased in the diabetic model group compared to those in the normal control group(P<0.01)(Fig.3). Supplementations of CNA4 and CNA7 significantly increased insulin levels, both in serum and pancreas, as compared with the diabetic model group(P<0.05). After the first week of supplementation of CNAs, the blood glucose level of diabetic mice began to decline, and this decline continued up to the 7th week, suggesting that CNAs has relatively strong efficacy against diabetes, which is demonstrated by the decrease of blood glucose level.

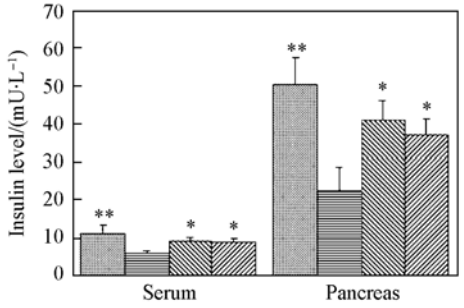

Fig.3 Effects of CNA on insulin levels in serum and supernatants of homogenized pancreatic tissues(1/20 diluted)

Values are means±SD, n=12. * P<0.05, ** P<0.01 *vs*. diabetic model group. ▭Normal control; ▭ diabetic control; ▨ CNA4 group with Se, V_E, Cr, and V; ▨ CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C.

3.3 Effects of CNA on Transcriptional and Translational Expression of Insulin Gene

ISH and IHC were performed to detect insulin expression of pancreatic islets at protein and mRNA levels. In the normal control group, most endocrinal cells within islets were either insulin mRNA or protein exhibiting positive intense dark staining [Fig.4(A) and Fig.5(A)], which was in contrast to that in the diabetic group, with less positive cells and a weaker staining [Fig.4(B) and Fig.5(B)]. The positive cells of islets of the diabetic mice supplemented with CNA4 and CNA7 were increased with relatively deep staining[Fig.4(C) and (D) and Fig.5(C) and (D)]. The quantitative image analysis shows that the average gray level of positive staining area in the diabetic

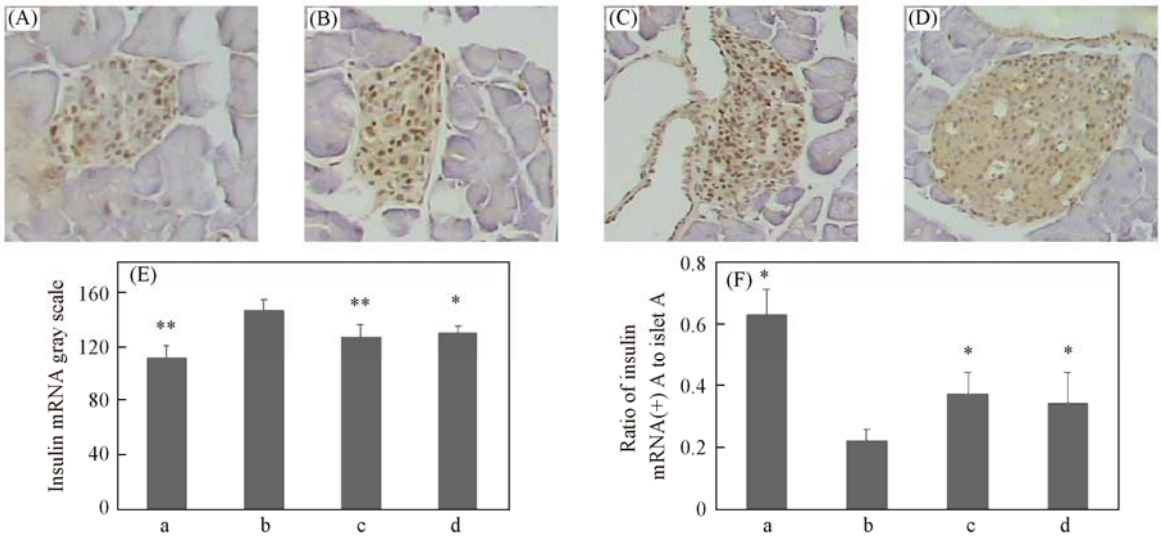

Fig.4 Effects of CNA on insulin mRNA expression in islets β cells

(A)—(D) show typical examples of insulin mRNA signals detected by ISH in pancreatic sections from the normal(A), the diabetic model(B), the CNA4(C) and CNA7(D) groups, respectively(original magnification ×200). (E) and (F) show insulin mRNA expression analyzed by image analysis in gray value (E) and ratio of insulin $mRNA^+$ area to islet area(F). Values are means±SD, n=5. * P<0.05, ** P<0.01 *vs*. diabetic model group. CNA4 group with Se, V_E, Cr, and V; CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C. a. Normal control; b. diabetic control; c. CNA4; d. CNA7.

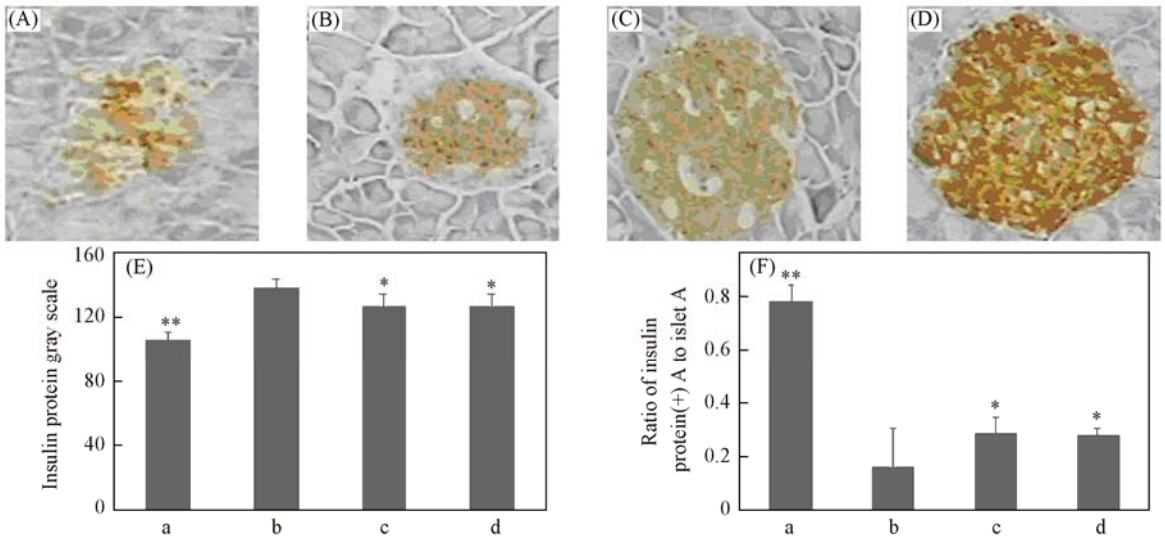

Fig.5 Effects of CNA on insulin immunoreactivity in pancreatic tissues

(A)—(D) show typical examples of insulin immunoreactivity signals detected by IHC in pancreatic sections from the normal(A), the diabetic model(B), the CNA4(C) and CNA7(D) groups, respectively(original magnification ×200). (E) and (F) show insulin immunoreactivity analyzed by image analysis in gray scale(E) and ratio of insulin immunoreactive area and islet area(F). Values are means±SD, n=5. * P<0.05, ** P<0.01 *vs*. diabetic model group. CNA4 group with Se, V_E, Cr, and V; CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C. a. Normal control; b. diabetic control; c. CNA4; d. CNA7.

group was significantly higher than that in the normal control group and those in the CNA4 and CNA7 groups[Fig.4(E), ISH, P<0.01; Fig.5(E), IHC, P<0.01 *vs.* normal, P<0.05 *vs.* CNA4 or CNA7]. The ratio between positive area and overall islet area was lower in the diabetic group compared to those in the other three groups(P<0.01 or P<0.05)[Fig.4(F), ISH; Fig.5(F), IHC]. These results indicate that supplementation of CNAs increases insulin expression of the diabetic mice at both protein and mRNA levels. On the other hand, ROS also affects insulin gene expression. Some studies reported that ROS inhibited insulin gene expression by activating c-Jun amino terminal kinase(JNK) in islet β cells and decreasing the combining activity between insulin gene transcription activation factor-PDX-1 and DNA, which might result in the inhibition of insulin gene expression and reduction of both insulin synthesis and secretion[7,20,21]. Our results also show decreased insulin level in serum and pancreas(Fig.3) and insulin gene expression, both at mRNA and protein levels with the increase of oxidative stress maker, MDA in pancreas in T1D mice(Figs.4—6). Some micronutrients were shown to have antioxidant effects, including vitamins A, C, and E, glutathione, lipoic acid, mixed carotenoids, several bioflavonoids, antioxidant minerals(copper, zinc, manganese, and selenium) and so on[22]. Among these, natural antioxidants, selenium, V_E, V_C, lipoic acid, and niacinamide have better effects on the protection of islet β cells from diabetes.

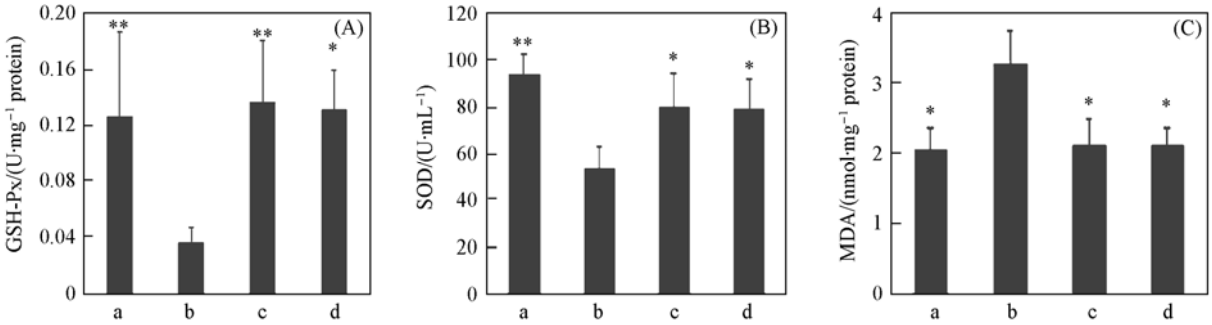

Fig.6 Effects of CNA on activities of GSH-Px(A), SOD(B) and MDA content(C) in supernatants of homogenized pancreatic tissues(1/20 diluted)

Values are means±SD, n=10. * P<0.05, ** P<0.01 *vs*. diabetic model group. CNA4 group with Se, V_E, Cr, and V; CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C. a. Normal control; b. diabetic control; c. CNA4; d. CNA7.

It was shown that combination of these antioxidants increased the activity of antioxidant enzymes, decreased ROS production and improved insulin level of serum and pancreas in diabetic rats[17]. However, it is not clear whether this improvement happens at transcriptional or translational level in diabetic models. Our data show that both the combinations of CNAs, CNA4 and CNA7 upregulated insulin expression transcriptionally at pancreatic tissues level(Fig.4). Furthermore, the effects were exactly mirrored at a protein expression level evidenced by the obervations of more insulin immunoreactive cells in the CNA4 and CNA7 groups than those in the diabetic model group(Fig.5). These data are related to the elevated levels of insulin in serum and pancreatic extracts(Fig.3), suggesting that CNA increases the insulin protein expression in STZ-induced T1D mice at a transcriptional level. Moreover, CNAs also increased the enzymatic activity of antioxidant and decreased ROS in pancreas(Fig.6). However, how the reduction of ROS could increase the transcriptional activity of insulin gene needs further investigation.

3.4 Effects of CNA on Free Radical Metabolism and Enzyme Activity of Antioxidant

There were significant differences in activities of antioxidant scavenger enzymes(GSH-Px and SOD) in pancreas between the normal control and the diabetic model groups[P<0.01, Figs.6(A) and (B)]. In contrast, MDA content in pancreas of the mice in the diabetic control group was significantly higher than that in the normal control group(P<0.05)[Fig.6(C)]. In the CNA4 and CNA7 groups, activities of GSH-Px(P<0.01) and SOD(P<0.05)[Figs.6(A) and (B)] were elevated, while MDA content was decreased compared to that in the diabetic group(P<0.05)[Fig.6(C)]. There were no significant differences in these changes between the CNA and the normal control groups. It was demonstrated that cultured pancreatic islets and insulin-producing cells are extremely sensitive to reactive oxygen species and nitric oxide[23,24]. Additionally, these cells express lower levels of catalase(H_2O_2-inactivating enzyme, cat.), GSH-Px and SOD compared to other cells[25—27].

Moreover, free radicals are toxic to the pancreatic β-cells by targeting polyunsaturated fatty acids(PUFA) which are rich in abundant endoplasmic reticulum membrane system of islet β cells[28,29]. Excess ROS produced in diabetic islets directly attack endoplasmic reticulum membrane and cause the structural and functional dysfunction of pancreatic islets, resulting in the reduction of insulin[30]. Our data show that increased blood glucose and MDA content were acompanied by decreased GPH-Px and SOD activities in T1D mice. This indicates that STZ, as a free radical activator, targets islets β cells and causes the dysfunction of free radicals metabolism in pancreatic islets. This further supports that oxidative stress participates in the pathogenesis of diabetes.

3.5 Effects of CNA on Cytokines Expression in $CD4^+$ PBMC

In the model group, the percentage of $CD4^+$ PBMC expressing TNF-α increased(P<0.01), supplementation of CNA4 decreased its expression(P<0.05). Similar decrease in TNF-α expression was also observed in CNA7 although it was not

statistically significant(Fig.7). Compared to that of the normal control group, IFN-γ expression in $CD4^+$ PBMC of the model group increased(P<0.05), but no significant difference in IFN-γ expression was observed between model group and CNA groups although there was a decreased trend of IFN-γ expression in both CNA4 and CNA7 groups(Fig.7). Compared to that in the normal control, IL-12 expression in $CD4^+$ PBMC was also significantly increased in the diabetic model group (P<0.01). In diabetic mice supplemented with CNA4 and CNA7, IL-12 expression was lower than that in those of the model group(P<0.05)(Fig.7). Recent studies suggest that oxidative stress sensitive signal transduction pathway activated through high glucose induces the overexpression of preinflammatory cytokines, such as TNF-α, IFN-γ, IL-1β and IL-12. Increased expression of these cytokines was observed in blood and spleen lymphocytes, not only in STZ-induced T1D mouse model but also in diabetes onset stage of NOD mice. Co-culture of IL-12，TNF-α，IFN-γ and IL-1β with islet β cell line immediately induces the cell damage and decreases insulin secretion[31,32]. Administration of IL-12 antagonist or exhausting $CD4^+$ T cells delays the diabetic onset of rats[33—35]. Our data also show the increased expression of these cytokines in $CD4^+$ PBMC in STZ-induced T1D mice, while supplementation of CNAs significantly down-regulates the expression of these cytokines(Fig.7). Whether the benefits of CNA against diabetes are due to the intervention of oxidative stress sensitive signal transduction pathway leading to decreased gene expression of proinflammatory cytokines are under futher investigation. On the basis of the relationship between cytokine and T1D, more results of cytokines expression were tested from $CD4^+$ PBMC other than the immune cells infiltrated into the pancreas due to the limited numbers of infiltrated cells in the pancreas of T1D mice although they are a more ideal source to observe the cytokine expression profile.

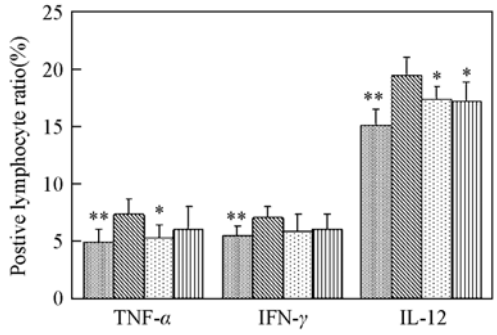

Fig.7 Effects of CNA on expression of cytokines TNF-α, IFN-γ and IL-12 in $CD4^+$ PBMC

Values are means±SD, n=10. * P<0.05, ** P<0.01 *vs*. diabetic model group. Normal control; diabetic control; CNA4 group with Se, V_E, Cr, and V; CNA7 group with Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C.

Mechanisms of CNA improving β cell function are still unclear. These CNAs might work in synergy with each other in objecting different types of free radicals and pathways of oxidative stress. It is well known that V_E suppresses the propagation of lipid peroxidation; V_C with V_E, inhibits hydroperoxide formation; Se increases GSH-Px activity[31]. In a previous study, we showed that combination of Se, V_E, Cr, and V upregulated the expression of Th2 type cytokines, IL-4 and IL-10 in STZ-induced T1D rat model[17]. Thus, IL-4 and IL-10 might play the roles in β cell protection against diabetes. In a mouse model, combined delivery of plasmids encoding IL-4 and IL-10 prevents the development of autoimmune diabetes[36]. Our present results also show CNAs may downregulate the expression of Th1 type cytokines, TNF-α and IL-12 in STZ-induced T1D mice model. That is, CNAs not only upregulate the expression of Th2 type cytokines but also downregulate the expression of Th1 type cytokines against the onset and development of diabetes. Other possible mechanisms include: removing ROS through different ways to increase the activity of insulin gene promoter; maintaining the integrated endoplasmic reticulum structure in β cell through the antioxidative effect of VE located in biomembrane.

In previous studies, we have evaluated the roles of each of Se, V_E, Cr, and V as well as their different combinations in protecting islet β cells from oxidative damage and demonstrated the combination of Se, V_E, Cr, and V was better[13,14]. In the present study, on the basis of the previous results, we further evaluated the effects of combination of seven of natural antioxidants, Se, V_E, Cr, V, lipoic acid, niacinamide, and V_C, on β cell protection in STZ-induced T1D mice to improve the protection of islet cells from diabetes. However, there was no significant difference between the roles of CNA4 and CNA7. It is suggested that each of lipoic acid, niacinamide and V_C, their different combinations, and dose effect relationship in islet protection need to be further evaluated.

4 Conclusions

Supplementation of CNA, with suitable doses and combinations, regulates multiply key molecular targets related β cell function, including reduction of oxidative stress and pre-inflammatory cytokines in STZ-induced T1D mice, which might be useful to prevent the onset and development of diabetes and develop a new drug against diabetes.

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