Effect of melatonin versus vitamin D as antioxidant and Hepatoprotective agents in STZ-induced diabetic rats

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Abstract

Background: Diabetes mellitus (DM) is a serious chronic disease, with multiple complications including hepatopathy associated with imbalance of the oxidative status. The purpose of this study is to observe possible protective effects of vitamin-D and melatonin on glucose profile, antioxidant-oxidant status, lipid peroxidation, and histopathological protection of the liver in streptozotocin-induced diabetic rats.

Methods: Eighty three male albino rats were divided into nine groups as follows: G1 (n = 10) Normal control rats; G2 (n = 8) were normal rats treated with melatonin only; G3 (n = 10) were normal rats treated with vitamin D only; G4 (n = 9) were diabetic rats, which received no medications; G5 (n = 8) were diabetic rat treated with insulin only; G6 (n = 10) were diabetic rats treated with melatonin only; G7 (n = 9) were diabetic rats treated with melatonin and insulin; G8 (n = 9) were diabetic rats treated with vitamin D only; G9 (n = 10) were diabetic rats treated with vitamin D and insulin. Two months post treatment, blood was collected to measure: Fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), fructosamine (FA), total antioxidant capacity (TAC), malondialdehyde (MDA). livers were isolated for histopathological study.

Results: As compared to normal rats, our results demonstrate that glucose, fructosamine and HbA1c levels is increased in diabetic groups and declined to lesser levels in treated groups. TAC level of diabetic rats is not significantly changed. Vitamin D administration significantly increased TAC while it is not changed with melatonin either in treated or non-treated groups. The liver of diabetic rats shows only mild focal microvesicular fatty degeneration. The liver of diabetic rats treated with insulin shows degeneration of cell edema in the stroma. The liver of diabetic rats treated with melatonin with or without insulin, exhibited marked improvement. The liver of diabetic rats treated with vitamin D with or without insulin, shows degeneration of cells and edema in the stroma.

Conclusion: Our results demonstrated the beneficial antioxidant effect of vitamin D administration to normal and diabetic rats as compared to melatonin. Nevertheless, melatonin still shows more therapeutic effect on liver cell injury induced by induction of diabetes.

Keywords: Diabetes, Liver, Melatonin, Vitamin D, Oxidative stress

Background

Diabetes mellitus (DM) is a serious chronic disease, which incidence is globally increasing and considered as an epidemic [1]. The prevalence of diabetes is mainly due to an increased prevalence of type 2 diabetes (T2D). The incidence of type 1 diabetes (T1D) is also increasing in parallel to that of T2D worldwide with major health and socio economic impacts [2].

Liver plays vital roles in carbohydrate, lipid and protein synthesis and metabolism [3]. Studies have demonstrated that DM can lead to several liver defects, such as non-alcoholic fatty liver disease (NAFLD) [4], abnormal glyco- gen accumulation, cirrhosis and liver carcinomas [5–7]. The underlying mechanisms that accelerate hepatopathy in patients with diabetes is not fully understood. Several mechanisms have been postulated to explain the damaging effect of DM on the liver. Both hyperglycemia-induced oxidative stress and hyperglycemia-induced inflammatory responses act as hepatocellular damaging factors [8].
Oxidative stress is defined as an imbalance in the oxidant-to-antioxidant ratio, causing the generation of free radicals [9]. Activated Kupffer cells production of free radicals is a central factor to hepatic injuries [10]. Excessive production of reactive oxygen species (ROS) results in various detrimental events, such as irreversible oxidative modification of lipids, proteins and carbohydrates [11]. ROS could activate the release of inflammatory mediators leading to induction of adhesion molecules and infiltration of leukocytes. Moreover, ROS could provoke apoptosis in hepatocytes causing massive destruction of liver tissue [12]. Diabetes is associated with reduced level of glutathione causing accumulation of oxidative stress product such as lipid peroxidation, which subsequently causes substantial increase in malondialdehyde, a marker for oxidative stress [13].

Various antioxidants have been demonstrated to have hepatoprotective effect, such as ginkgo biloba extract, resveratrol, 17β-estradiol, arjunolic acid, α-lipoic acid, L-cysteine and melatonin through anti-oxidative, anti-inflammatory, anti-apoptotic and/or antidiabetic properties [8].

Melatonin is a powerful antioxidant and the only currently available molecule known to block all aspects of the “devil’s triangle” [14]. This pineal gland’s generated hormone has been shown to play various regulatory roles such as regulation of circadian rhythm, sexual behavior, immune function, energy metabolism, regulation of the cardiovascular and the reproductive system. Melatonin also demonstrated a potent antioxidant capability and possessed protective properties against oxidative stress [3]. It has been shown that melatonin ameliorates oxidative damage in hyperglycemia-induced liver injury [13]. Oral melatonin administration reduces liver steatosis and mitochondrial dysfunction in diabetic rats [15]. Melatonin administration partially reduced liver injury in streptozotocin-induced diabetic rats [5]. However, its protective effect on DM-mediated liver dysfunction need further investigation. The role of vitamin D in the pathogenesis of many diseases including DM is growing. The link between vitamin D and various DM-associated disorders such as nephropathy, retinopathy and vasculopathy have been reported [16]. However, the available studies on its beneficial effects on DM-mediated liver dysfunction are limited and controversial [17, 18]. The purpose of this study is to observe possible protective effects of vitamin-D and melatonin on glucose profile, antioxidiant-oxidant status, lipid peroxidation, and histopathological protection of the liver in streptozotocin-induced diabetic rats. Positive observation from these treatments will open new window for better treatment of this chronic disease.

Methods

Induction of DM

Diabetes mellitus in rats was induced by intra-peritoneal administration of nicotinamide (230 mg/kg), 15 min prior to the single dose of streptozotocin (STZ) (65 mg/kg, i.p.) [19]. Control animals were received an equal volume of saline. The STZ was dissolved in saline with a sodium citrate buffer, pH 4.0. The blood glucose levels (by using standard diagnostic kits) were recorded to monitor the degree of diabetes. Confirmation of induction of diabetes was made by measuring blood glucose level prior to further treatment. Rats with established hyperglycemia were used in the study.

Groups and treatments

Eighty three male albino rats (200–250 g) were divided into nine groups as follows: G1 (n = 10) Normal fed diet rats were served as control, which received no medications; G2 (n = 8) were normal rats treated orally with melatonin only (0.3 mg/kg); G3 (n = 10) were normal rats treated orally with vitamin D only (40 mg/kg); G4 (n = 9) were diabetic rats, which received no medications; G5 (n = 8) were diabetic rat treated with insulin only; G6 (n = 10) were diabetic rats treated orally with melatonin (0.3 mg/kg) only; G7 (n = 9) were diabetic rats treated orally with melatonin (0.3 mg/kg) and insulin; G8 (n = 9) were diabetic rats treated orally with vitamin D (40 mg/kg) only; G9 (n = 10) were diabetic rats treated orally with vitamin D (40 mg/kg) and insulin. The duration of the treatment was for 8 weeks and the dose of insulin was calculated according to the weight of each rat and the level of blood glucose.

Biochemical measurements

Two months post treatment, rats were sacrificed and blood was collected for biochemical measurement. Fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), fructosamine (FA), total antioxidant capacity (TAC), malondialdehyde (MDA) were determined using the standard procedures and available commercial kits in a fully automated system. All assays were done by following the recommended procedures for instrument operation, calibration, quality control, and assay guidelines.

Blood samples were drawn in ethylene diamine tetra acetic acid (EDTA)-containing vacominator tubes for measuring glycosylated hemoglobin (HbA1c) in the same day. For serum experiments, samples were obtained following collection of blood in plain tubes and left for 30 min, then centrifuged for 15 min at 3000 rpm. Aliquots (1 ml) were separated in different Eppendorf tubes for the determination of the blood level of FBS, FA, TAC and MDA. Serum samples were then kept in −80 °C for later analysis.

Measurement of TAC, was performed by using rat TAC ELISA Kit from MyBioSource, Inc. The combined antioxidant activities of all vitamins, proteins, lipids, glutathione, uric acid, and others were assessed. MDA was measured by using rat TBARS ELISA assay kit.
As compared to normal rats (G1), our results demonstrate that glucose and fructosamine levels are significantly increased in G3, G4, G5, G6, G7, G8 and G9 (P < 0.05) with no significant change in G2 (Table 1; Figs. 1 and 3; P > 0.05). Figure 2 demonstrate that the percentage of HbA1c is significantly increased in G3, G4, G5, G6 and G8 (P < 0.05) with no significant changes in G2, G7 and G9 (P > 0.05). TAC is significantly increased in G2, G6 and G8 (P < 0.05), and showed no significant changes in G3, G4, G5, G7, and G9 (P > 0.05; Fig. 4). MDA showed non-significant changes in all groups. However, there is a decrease in its level in G3 and G7 but it is non-significant (P > 0.05; Fig. 5).

By comparing G5, G6, G7, G8 and G9 with G4, we found that glucose level is significantly decreased in G5, G7 and G9 (P < 0.05) with no significant changes (P > 0.05) in G6 and G8 (Fig. 1). In comparison, HbA1c showed non-significant changes in G5, G6, G7, and G8 (P > 0.05), but significantly decreased (P < 0.05) in G9 (Fig. 2). Fructosamine showed non-significant changes (P > 0.05) in G5, G6, G7, and G8, but significantly decreased (P < 0.05) in G9 (Fig. 3). TAC is significantly increased (P < 0.05) in G8 and showed non-significant changes (P > 0.05) in G5, G6, G7 and G9 (Fig. 4). However, there is no significant change in the level of MDA in all groups (Fig. 5).

By comparing G8 with G6 we found that glucose, fructosamine and MDA showed no significant changes, while HbA1c and TAC showed significant increase. By comparing G9 with G7, we found that glucose and fructosamine levels are significantly decreased (P < 0.05), while MDA is significantly increased (P < 0.05). However, HbA1c and TAC showed non-significant changes (P > 0.05). By comparing G7 with G6 we found that glucose is significantly decreased, while HbA1c, fructosamine, TAC and MDA showed non-significant changes. By comparing G9 with G8, we found that glucose and fructosamine levels are significantly decreased (P < 0.05), while HbA1c and TAC are significantly decreased (P < 0.05). However, MDA showed non-significant change (P > 0.05).

Histopathological findings
As compared to normal (Fig. 6a), the liver of diabetic rats shows only mild focal microvesicular fatty degeneration (Fig. 6b). The liver of treated diabetic rats with insulin shows degeneration of cell edema in the stroma. The liver exhibited granular degeneration of hepatocytes, including necrosis of individual cells (Fig. 6c). The hepatocytes contained focal fatty vacuoles. The sinusoids are dilated and a progressive loss of general organ structure is seen. Inflammatory changes consistent with steatohepatitis, which are represented by mononuclear inflammatory infiltrates of moderate intensity as observed in periportal spaces. Moreover, the central veins exhibited moderate congestion (Fig. 6c). The liver of diabetic rats treated with melatonin either with insulin or not, exhibited marked improvement (Fig. 6d and e). The liver of diabetic rats taking vitamin D and treated with or without insulin, shows degeneration of cells and edema in the stroma (Fig. 6f and g). The liver exhibited granular degeneration of hepatocytes, including necrosis of...
individual cells. The hepatocytes contained focal fatty vacuoles. The sinusoids are dilated and a progressive loss of general organ structure is seen. Inflammatory changes are consistent with steatohepatitis, which are represented by mononuclear inflammatory infiltrates of moderate intensity as observed in periportal spaces. Moreover, the central veins exhibited moderate congestion (Fig. 6f and g).

Discussion

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [20–22]. Our results do not show any change in TAC of diabetic rats as compared to normal non-diabetic rats. This may be due to the short period of diabetes induced to rats or to the low glucose concentration in diabetic rats. Strangely enough, our results showed that even in non-treated diabetic rats with insulin, vitamin D administration significantly increased TAC while it is not changed with melatonin either in treated or non-treated groups. This may be explained by the hyperglycemia-induced increase in free radicals, and its impairment of the endogenous antioxidant defense system in many ways during diabetes [23].

Table 1 Summary of the effect of vitamin D and melatonin on the level of FBS, HbA1c, FA, TAC and MDA on diabetic rats

| Group | G1   | G2   | G3   | G4   | G5   | G6   | G7   | G8   | G9   |
|-------|------|------|------|------|------|------|------|------|------|
| FBS (mg/dl) | 109.6 | 115.5 | 124.2 | 195.78 | 146.38 | 191.4 | 143 | 181.6 | 127.43 |
| SD    | 14.569 | 23.016 | 15.076 | 34.72 | 9.133 | 35.635 | 5.874 | 8.649 | 10.309 |
| Significance | * | * | *♣ | * | *♣♦ | * | *♣♠♥ | *♣♠♥ | *♣♠♥ |
| HbA1c (%) | 4.137 | 4.0375 | 4.76 | 4.9778 | 4.9 | 4.59 | 4.4111 | 5.4 | 4.1714 |
| SD    | 0.33751 | 0.56553 | 0.6802 | 0.81972 | 0.5757 | 0.46536 | 0.35158 | 0.86023 | 0.4855 |
| Significance | * | * | * | * | *♣♦ | * | *♣♠♥ | *♣♠♥ | *♣♠♥ |
| FA (mmol/l) | 0.491 | 0.479 | 0.872 | 1.043 | 1.166 | 1.037 | 1.139 | 0.908 | 0.694 |
| SD    | 0.0348 | 0.1817 | 0.2271 | 0.2775 | 0.353 | 0.2796 | 0.4198 | 0.0432 | 0.0737 |
| Significance | * | * | * | * | * | * | * | *♣♠♥ | *♣♠♥ |
| TAC (ng/ml) | 7.249 | 7.924 | 7.188 | 7.433 | 7.426 | 7.813 | 7.671 | 8.65 | 7.693 |
| SD    | 0.4189 | 0.6181 | 0.5495 | 0.3024 | 0.7472 | 0.5195 | 0.5182 | 0.3977 | 0.8109 |
| Significance | * | * | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ |
| MDA (nmol/l) | 135.6 | 120 | 111.8 | 127 | 124 | 160.3 | 114.44 | 131 | 154 |
| SD    | 26.416 | 16.036 | 28.871 | 55.794 | 26.431 | 72.207 | 21.066 | 61.417 | 35.581 |
| Significance | * | * | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ | *♣♠♥ |

(FBS) Fasting blood sugar; (HbA1c) glycosylated hemoglobin; (FA) fructosamine; (TAC) total antioxidant capacity; (MDA) malondialdehyde. Symbols represent the followings: (*) statistically significant difference when compared with G1; (♣) statistically significant difference when compared with G4; (^) statistically significant difference when G8 compared with G6; (♠) statistically significant difference when G9 compared with G7; (♦) statistically significant difference when G7 compared with G6; (♥) statistically significant difference when G9 compared with G8.

Fig. 1 Effect of vitamin D and melatonin on the level of FBS on diabetic rats
defense mechanisms involve both enzymatic and nonenzymatic strategies. Vitamin D alone may have a more significant antioxidant effect on diabetic rats or the concentration of melatonin was insufficient to induce any significant change. It is worth mentioning that the doses selected in our study is according to a pharmacological concept of calculating the animal dose of drugs as 1 to 10 of the human dose per Kg. The duration of the treatment is selected for two reasons: firstly, by comparing the life span of humans, one-month duration of treatment in rat (average life span 2 years) is comparable to 30 months duration treatment of a human average life span of 60 years. Secondly, extending the duration of treatment for more than 1 month leads to more loss and dying of rats.

Despite its discovery over 40 years ago, melatonin was not recognized as a free radical scavenger and antioxidant until the last decade. Prior to that time, the circadian rhythm of melatonin in the blood of mammals was known to be functionally linked to the adjustment of 24-h cycles and to circannual rhythm regulation. Additionally, however, melatonin’s actions include modulation of immune function, tumor growth inhibition and influences on retinal physiology. The free radical scavenging property of melatonin was first suggested by Ianas et al. [24]. Although there have been literally hundreds of publications which demonstrate the free radical scavenging [25–27] and antioxidant actions [28–30] of melatonin both in in vitro and in vivo settings, our results showed that vitamin D is more effective antioxidant and free radical scavenger as illustrated by the significantly increased TAC in diabetic rats that received vitamin D as compared to rats receiving melatonin. Insulin treated diabetic rats and receiving either vitamin D or melatonin are comparable. Therefore, it is evident from our study that the antioxidant capacity and free radical scavenging effects of either melatonin or vitamin D do not depend on controlling the diabetic state and they act as antioxidants.
whatever the blood glucose level is. However, the dose and duration of melatonin may not be enough.

NAFLD is accompanied by several predisposing factors such as obesity, diabetes, dyslipidemia, jejunoileal bypass, drugs and parenteral nutrition. Hepatic stellate cells undergo activation, and progression to advanced fibrosis and cirrhosis is possible \[31, 32\]. Several studies have shown that liver injury in the course of NAFLD is mediated by oxidative stress \[33\]. Oxidative stress is especially harmful to mitochondria, causing damage that results in impaired gene expression, alterations in proteins synthesis, decreased mitochondrial content and impaired mitochondrial beta-oxidation. Moreover, in the course of NAFLD, mitochondrial CYP2E1 expression increases and causes a redox state \[34, 35\]. In light of the crucial role of oxidative stress in liver diseases, antioxidants are understandably considered as a good therapeutic strategy for the treatment of liver disorders. To date, the study outcomes remain inconclusive and controversial; however, the therapeutic efficacy of particular antioxidants has been proven \[36, 37\].

Our study shows that the livers of diabetic rats have only mild focal micro vesicular fatty degeneration but no other abnormalities are observed. Even treated diabetic rats with insulin do not affect liver injury as evidenced by the inflammatory changes consistent with steatohepatitis, which were represented by mononuclear inflammatory infiltrates of moderate intensity, observed in periportal spaces. Melatonin administration to diabetic rats with or without insulin exhibited marked liver improvement. On the other hand, vitamin D administration to diabetic rats treated or not with insulin do not affect the inflammatory changes of liver cells consistent with steatohepatitis, which are represented by mononuclear...
inflammatory infiltrates of moderate intensity, observed in periportal spaces.

These discrepancies of our biochemical results to what have been observed in the histopathological studies may be due to the short duration of drug administration to diabetic rats, which may affect either the function of or the liver itself. Despite the numerous studies on humans and animal models, it is extremely difficult to understand and describe the efficacy of antioxidative agents in hepatology [38, 39].

Conclusion

In conclusion, our results demonstrated the beneficial antioxidant effect of vitamin D administration to normal and diabetic rats as compared to melatonin in our rat model. Nevertheless, still melatonin shows more therapeutic effect on liver cell injury induced by induction of diabetes.

Abbreviations

DM: Diabetes mellitus; DS: Standard deviation; EDTA: Ethylene diamine tetra acetic acid; FA: Fructosamine; FBS: Fasting blood sugar; HbA1c: Glycosylated hemoglobin; MDA: Malondialdehyde; NAFLD: Non-alcoholic fatty liver disease; ROS: Reactive oxygen species; STZ: Streptozotocin; T1D: Type 1 diabetes; T2D: Type 2 diabetes; TAC: Total antioxidant capacity

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

All authors participated in the design, study, data acquisition and drafting manuscript except Dr. Esheba, participated only in the histopathological examination and its interpretation. All authors read and approved the final manuscript.

Ethics approval

The study protocol followed the guidelines of National Institute of Health, the USA, and Public health service policy on the use of laboratory animals (NH, 2002). All experimental procedures were approved by Umm Al Qura ethic Board and Institutional Review Board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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